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Review article

Endogenous opiates: 1999

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Abstract

This paper is the twenty-second installment of the annual review of research concerning the opiate system. It summarizes papers published during 1999 that studied the behavioral effects of the opiate peptides and antagonists, excluding the purely analgesic effects, although stress-induced analgesia is included. The specific topics covered this year include stress; tolerance and dependence; learning, memory, and reward; eating and drinking; alcohol and other drugs of abuse; sexual activity, pregnancy, and development; mental illness and mood; seizures and other neurologic disorders; electrical-related activity; general activity and locomotion; gastrointestinal, renal, and hepatic function; cardiovascular responses; respiration and thermoregulation; and immunologic responses.

Keywords: Stress; Tolerance; Dependence; Learning; Memory; Reward; Eating; Drinking; Alcohol; Mental illness; Depression; Activity; Cardiovascular responses; Temperature; Respiration; Epilepsy; Sex; Immunology; Opiate; Peptide

1. Introduction

In 1999, as in previous years, interest in the role of endogenous opiates in mediating behavior remained high. Although much of the research focused on characterizing the role of opiate receptor types, there was still a great deal of interest in the interactions between opiate and nonopiate systems. This paper will review work published in 1999 that studied the behavioral and nonanalgesic activity (except stress-induced analgesia) of endogenous opiate systems. This represents the twenty-second installment of the series of reviews that attempts to summarize the developments in the field during the past year.

Stress-induced activation of endogenous opiate systems, and the changes that occurred because of stress, continued to be of interest in 1999. Much of the research focused on stress-induced analgesia, and as had been reported in previous years, the parameters of the stressor influenced both its behavioral effects and physiological consequences. There was continued concern regarding opiate tolerance, dependence, and abuse. Chronic administration of opiates produced a number of changes within opiate systems that were complex, including long-lasting molecular and cellular adaptions, as well as interactions between opiate and nonopiate systems. Among these changes, receptor internalization was described as a possible mechanism to explain functional desensitization after chronic administration of opiates. The clinical application of various pharmacological treatments to treat opiate dependence also were assessed, including the use of various opiate agonists and antagonists. Interest in the role of endogenous opiates in learning, memory, and reward remained high. In particular, the reinforcing and discriminative properties of opiates were used to assess both their motivational and subjective effects, findings that have important clinical relevance for understanding opiate abuse. Research in the role of endogenous opiates in eating and drinking remained high, but it is becoming increasingly apparent that the motivational state is important in determining the outcome, and that the palatability of foods is a dynamic feature that can change as a function of the choices presented. The role of opiates systems in the modulation of alcohol consumption also were examined, and opiate antagonists were used as a means of modifying its motivational and rewarding properties. However, this was not without controversy as opiate antagonist tended to decrease consumption in general, thus raising doubts about the specificity of their effects. Furthermore, in recent years there has been growing interest in the opiate modulation of other drugs of abuse, including stimulants, cannabinoids, and tobacco.

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Studies of endogenous opiates in sex and development remained high, including the ontogeny of opiate systems during development. The effects of prenatal exposure to opiates on development continued to be addressed, and had obvious clinical implications for opiate use and abuse during pregnancy. There was more interest this year than in previous ones regarding the role of endogenous opiates in some mental illnesses, in particular anxiety and depression. However, attempts to link opiate systems to other mental illnesses were for the most part inconclusive. The role of opiates in mediating seizure activity was examined, and it was generally the case that opiate agonists were proconvulsive and antagonists anticonvulsive. The involvement of opiates in ischemic brain damage were elucidated and research indicated that κ-agonists may have neuroprotective effects. Animal models also were used to examine opiate modulation of other neurologic disorders, including the Parkinson's and Alzheimer's disease. Interest in opiate involvement in electrical-related activity remained high in 1999, and used various in vitro preparations to examine opiate modulation of neural events in both the spinal cord and supraspinal structures. As in previous years, locomotor activity was influenced by opiate agonists, but the results were generally inconsistent and depended on the paradigm that was used.

Research continued to focus on the inhibition of gastrointestinal functions by opiate agonists and on the potential therapeutic benefits of the use of opiate antagonists to treatment gut motility and transit. However, the involvement of endogenous opiates in renal and hepatic functions is still not entirely clear. The role of endogenous opiates in mediating cardiovascular function continued to be studied, including the potential benefits of using κ-opiate receptor agonists to treat hypertension. Research also examined the possible role opiate receptors located in the heart to protect against infarct after ischemia, and arrhythmia. Research continued to address opiate modulation of respiratory functions, but interest in opiate involvement in thermoregulation was once again low. Research on the relationship between opiate and immune function remained high, revealing that opiates can both suppress and enhance immune functions, determined in part by whether they were tested in vitro or in vivo. Furthermore, because of the high incidence of human immunodeficiency virus (HIV) among intravenous drug users, the role of opiates in the progression of this disease received much attention. Besides having obvious clinical implications, the results also highlight the importance of specifying whether or not opiate analgesic are used in studies that examine immunologic processes.

2. Stress

Many stressors interact with endogenous opiate systems. In 1999, the physiological and behavioral effects of a variety of stressors were further elucidated, including forced swim [159,174,603], foot-shock [14,122,168,174,341,342, 343,352,515,602], exposure to animals exposed to foot-shock [174], tail-shock [161,215], restraint/immobilization [13,77,269,505,559,603], overcrowding/confinement [26, 122,602], isolation [602], ether [592], air exposure [26], water or food deprivation [26,602], whole body vibration/ shake [122,602], maternal deprivation [124], handling [159, 424], soiled cages [602], cage tilt [602], parturition [235], biting flies [268,269], hypothermia [503], bright light [122, 559,602], blood sampling [206], noxious heat [212,213, 559], lithium chloride injections [352], forced walking [387, 421], physical exercise [331], intracerebroventricular (ICV) injections of saline [368], white noise [122,602], and social conflict and threat [131,146,394,495,533]

As in previous years, there was continued interest in stress-induced analgesia (SIA). Based on their sensitivity to opiate agonists and antagonists, some stressors are known to activate endogenous opiate systems whereas others do not. Exposure to a local hypothermic stress (immersion of leg in 6°C water for 10 min) produced analgesia in frogs that was both opiate- and nonopiate-mediated because it was only partially antagonized by doses of naltrexone and naloxone that completely blocked morphine-induced analgesia [503]. However, there is now increasing evidence that stressors that had previously been defined as opiate-mediated, including intermittent immobilization [505] and foot-shock [14], interact with nonopiate systems as well. Early exposure to stress may have long-lasting effects on endogenous analgesic systems, as mice deprived of maternal/nest odor for 15 min/day during the first 2 weeks of life showed decreases in pain sensitivity and morphine analgesia in the formalin and tail-flick assays when tested as adults [124].

The opiate receptor subtypes that mediate SIA were elucidated. Analgesia induced by 6 h of forced walking in mice is mediated by the ε-opiate receptor because it is blocked by ICV injection of the ε-opiate receptor antagonist β-endorphin (1–27), but not by the μ-, δ-, and κ-opiate antagonist naloxone [387]. It likely that increased β-endorphin levels within the periaqueductal gray matter and/or the arcuate nucleus areas are involved, since increases in β-endorphin content was observed in these areas after forced walking [387], and ICV β-endorphin produces analgesia blocked by β-endorphin (1–27) [387] and naloxone [497]. In contrast, subanalgesic doses of β-endorphin (0.03 nmol) reduced the analgesia produced by repeated exposure to a noxious heat source (hot-plate), suggesting that β-endorphin may have antianalgesic properties as well [212]. Similarly, the endogenous antiopiate peptide Tyr-MIF-1 also has antianalgesic effects that are centrally mediated because it prevented the analgesic effects of foot-shockshock and forced swim when injected intraperitoneal (IP) or ICV [174]. It is possible that Tyr-MIF-1 attenuates the emotional factors associated with SIA, as it also prevented analgesia produced by watching other animals exposed to foot-shock [174]. Free hand ICV injections of saline alone can be stressful depending on the strain of mice used as it produced

analgesia in AKR, BALB/c, CBA, and Swiss-Webster strains of mice, but not in CD-1 and C3H strains [368]. The opiate-mediation of SIA after ICV injections of saline also is strain-dependent as the nontraditional opiate orphanin FQ/nociceptin (OFQ/N), which is distributed in various brain areas involved in stress and analgesia [390], blocked the analgesia in BALB/c and Swiss-Webster strains, but not in the AKR and CBA strains [368].

Stress can modulate the analgesic actions of opiates, as repeated exposure to a noxious heat source (hot-plate) reduced analgesia produced by β-endorphin [212] or morphine [213] injected into the ventrolateral periaqueductal gray [212], or by morphine given systemically [213]. Therefore, although stress has analgesic properties given alone, it may have antianalgesic properties when combined with opiates [212,213]. In contrast, defeat stress did not alter the analgesic effects of morphine administered into the ventrolateral periaqueductal gray, as sensitivity to morphine analgesia was similar between defeated and inexperienced rats [533]. The reasons for these differential effect of stress on opiate analgesia are not clear but may be related to the stressors used, as repeated exposures to the hotplate produces nonopiate analgesia [212], whereas defeat stress is opiate-mediated [533].

Conditioned SIA, in which an auditory signal is paired with a foot-shock, is mediated by μ-opiate receptors within the rostral ventromedial medulla because injection of the μ-opiate antagonist CTOP, but not by the κ-opiate antagonist nor-BNI, into that brain region blocked the analgesia [168]. However, conditioned SIA depended on both the stressor and pain test used because exposure to a context previously paired with a foot-shock elicited analgesia in the tail-flick test, whereas exposure to an environment paired with an injection of the emetic agent lithium chloride produced hyperalgesia in the tail-flick test but analgesia in the hot-plate and formalin tests [352]. It is likely that the analgesic and hyperalgesic effects of the context paired with lithium chloride injections are mediated by opiate and nonopiate systems, respectively, because naloxone blocked the analgesia in the formalin test but did not block hyperalgesia in the tail-flick test [352]. Conditioned SIA also can be produced in deer mice exposed to biting flies, as exposure to biting flies that have had their mouth parts removed produced analgesia in mice that had been exposed to biting flies but did not produce analgesia in fly naive mice or mice that were exposed to restraint stress [269]. However, although naloxone blocks SIA after exposure to biting flies and thus is opiate-mediated, the acquisition of conditioned analgesia to the altered biting flies in not opiate-mediated because naloxone did not affect it [269]. Since the development of such a conditioned response facilitates the activation of analgesic and defense mechanisms before biting occurs, it probably serves an adaptive function [268,269].

Besides analgesia, stress also affects reward systems that are opiate-mediated because restraint stress decreased sucrose preference in morphine-treated rats that naloxone blocked [603]. Since stress did not affect sucrose preference without morphine pretreatment, however, it suggests that morphine facilitated the occurrence of stress-induced anhedonia [603]. In contrast, a single restraint stress enhanced amphetamine-induced conditioned place preference (CPP), but does not involve opiate systems as naloxone did not block the enhancement [77]. Stress also is important in the development of ethanol reward as ethanol produced a CPP in rats exposed to an environment that was previously paired with electric foot shock, but not in nonstressed rats [341,342,343]. It is likely that μ- and δ-opiate receptors mediate the effects of stress on ethanol reward, as ethanol-induced CPP was blocked by naloxone, the μ-opiate antagonist β-FNA, and the δ-opiate antagonist naltrindole [343]. Furthermore, doses of morphine or the δ-opiate agonist TAN-67 that did not produce CPP when given alone enhanced ethanol-induced CPP [341,342,343] that was blocked by β-FNA and naltrindole, respectively [341]. However, nonopiate systems are likely involved as well, as dopaminergic [342] and serotonergic antagonists [341] also attenuated the enhancement of ethanol-induced CPP by morphine and TAN-67. In contrast, κ-opiate receptors may negatively modulate the rewarding effects of ethanol during stress, as the selective κ-opiate antagonist nor-BNI enhanced ethanol CPP under conditions of stress, whereas the κ-opiate agonist U50488H attenuated it [343].

Stress also affects locomotor activity that is opiate-mediated, as restraint increased immobility during forced swim and naloxone blocked it [603]. Social conflict altered locomotor activity in crickets that depended on social rank, as it increased jumping responses to tactile stimulation in dominant males, but decreased it in subordinate males. Both effects are opiate-mediated because the μ-opiate agonist DAMGO reversed the effects in dominant crickets, and naloxone reversed the effects in subordinates [146]. In socially defeated rats, however, morphine injections into the ventral periaqueductal gray decreased autogrooming, rearing, and inactivity, and increased crouching and locomotion that was not opiate-mediated because naloxone had no effect on its own, nor did it not modify the effects of morphine [533].

Previous exposure to inescapable tail-shock enhanced freezing behavior and produced deficits in escape behavior that opiate receptors in the dorsal raphe nucleus mediate because injection of naltrexone into that brain area prevented both the escape deficits and enhanced freezing [197]. Conversely, when combined with systemic morphine, subthreshold inescapable shocks that do not by themselves have any effects also induced deficits in escape behavior and enhanced conditioned freezing that naltrexone injections into the dorsal raphe nucleus blocked [197]. In contrast, the dorsolateral periaqueductal gray is probably not involved in opiate-mediated conditioned fear responding after inescapable shocks because injections of naltrexone into that area had no effect [197]. Chronic variable stress for 7 days that included horizontal shaking, white noise, crowding, isola-

tion, water deprivation, cage tilting, soiled cages, and bright lights, facilitated the onset of escape-avoidance deficits [602] and release of dopamine from the frontal cortex [122] in rats pre-exposed to inescapable shock. It is likely that endogenous opiates are involved because pretreatment with naloxone blocked both the enhanced escape failures [602] and dopamine release [122] after chronic stress, whereas morphine increased escape failures [602]. Furthermore, because the antidepressant, desipramine, blocked both the sensitization of escape failures produced by the chronic variable stress and its enhancement by morphine, these results suggest that unavoidable stress may contribute to depression and endogenous opiate systems are involved [602].

Restraint stress decreased the frequency of entries into an open arm maze and introductory activity to an unfamiliar opponent, suggesting that the stressor also increased anxiety-related behaviors [13]. However, the nature of opiate involvement is not clear, as restraint stress increased β-endorphin content in the anterior pituitary, but decreased it in the neurointermediate lobe [13]. The effects of stress on pituitary β-endorphin may depend on HPA activity because the corticotropin releasing hormone (CRH) antagonist, α-helical $CRH_{9-41,}$ prevented both the increases in the anterior pituitary and decreases in the posterior lobe observed after restraint stress [13]. In contrast, neonatal handling may reduce anxiety and decrease fearfulness to novel environments because it increased locomotion and rearing behavior in an open field test and the number of entries and total time spent in open arms of an elevated plus maze in male handled rats as compared with non-handled controls [424]. It is likely that the effects of neonatal handling on locomotion-related behaviors are opiate-mediated because handling also was correlated with an up-regulation of dynorphin A and B in various brain areas [424]. Furthermore, because κ-opiate agonists can modulate hypothalamic-pituitary-adrenal (HPA) activity, it is possible that the decreased response to stressful environments after handling is related to an inhibitory effect of dynorphins on HPA activity during stress [424]. However, the effects of neonatal handling on the stress response are sex-dependent, as manipulation (daily injections of saline) from birth to postnatal day 19 decreased basal levels of corticosterone in females but not males, and prevented corticosterone increases after a 3-min forced swim in males but not females [159]. It is likely that δ-opiate receptors are involved in the stress reactivity of females because naltrindole treatment inhibited the stress-induced rise in corticosterone [159].

Increased endogenous opiate activity during labor may reflect response to pain and discomfort, as plasma β-endorphin concentrations increased during labor in goats, and in heifers that needed assistance during labor [235]. In contrast, resting levels of β-endorphin were lower in pregnant female rats during the third trimester, as compared with nonpregnant rats, and repeated stress during the third trimester of pregnancy did not change plasma β-endorphin immunoreactivity but did increase other stress-related hormones, including corticosterone and adrenocorticotropic hormone (ACTH) [559]. Similarly, no differences in plasma β-endorphin were found in the offspring of stressed females, as compared with offspring of nonstressed females [559]. Endogenous opiate activity during stress modulate the release luteinizing hormone (LH), as exposure to footshock in female ewes increased hypothalamic β-endorphin after the first and third day of exposure, whereas LH was increased after the first day but suppressed after the third day [515].

As in previous years, there was continued interest in the cellular and molecular changes produced by stress. Social defeat increased μ-opiate receptor mRNA in the ventral tegmental area, but not in the substantia nigra [394]. Because the ventral tegmental area is involved in addictive processes, increased μ-opiate receptor mRNA after stress may be involved in sensitization to morphine after prolonged exposure [394]. Tail-shock produced increased enkephalin mRNA in the paraventricular nucleus that was similar whether the shock was controllable or uncontrollable, suggesting that the effects of stress on opiate gene expression depend on the physical but not psychological characteristics of the stressor [215]. Neonatal handling induced long-term changes in opiate peptide content that may be related to its anxiolytic effects, including an up-regulation of dynorphin A in the hypothalamus, pituitary, and striatum, and of dynorphin B in the hypothalamus, pituitary, hippocampus, medulla oblongata, and midbrain [424].

Peripheral tissue trauma alters neuronal excitability, as inflammation-induced by complete Freund's adjuvant increased spinal cord preprodynorphin [239,317] and preproenkephalin [317] mRNA. Endogenous opiates likely modulate the effects of peripheral tissue injury on neuronal excitability because naloxone increased c-Fos mRNA expression induced by formalin injections [460]. Similarly, naloxone enhanced the expression c-Fos immunoreactivity after experimental tooth movement in ipsilateral neurons of the trigeminal subnucleus caudalis [7]. In contrast, endogenous opiates do not modulate increased spinal cord c-Fos immunoreactivity after carrageenin injections, as the μ-opiate antagonists CTOP [82] and β-FNA [294], the κ-opiate antagonist nor-BNI [82,83], and the δ-opiate antagonist naltrindole [82] had no effect. Although the reasons for the differential effects of opiate antagonists on injury-induced c-Fos expression are not clear, it may be related to sensitivity of the technique to measure c-Fos activity, as the former study used c-Fos mRNA assays that may provide more sensitive measures than Fos proteins [460].

Blood-sampling is stressful, as plasma β-endorphin levels were increased in response to 4 blood samples drawn from the tail vein in rats within a 2-h period [206]. Furthermore, because repeated blood sampling produced only transient increases in corticosterone, levels of β-endorphin may therefore be more sensitive in reflecting states of moderate stress than corticosterone [206]. Similarly, exposure to air

or confinement in sea bream produced sustained increases in β-endorphin, but only a transient increase in corticosterone [26]. In contrast, walking stress in sheep caused a sustained increase in corticosterone, but only a transient decrease in α-neoendorphin [421]. Ether induced increases in plasma β-endorphin in rats that was blocked by clonidine, suggesting that patients suffering from hypertension and treated with clonidine may have altered stress responses [592].

The effects of stress in humans also were examined. Strenuous physical exercise has been shown to interact with endogenous opiates, as plasma β-endorphin levels were increased in healthy males 2 min after a maximal cycling exercise [331]. Furthermore, although the synthetic corticosteroid dexamethasone decreased plasma β-endorphin levels and is sometimes used by athletes to presumably increase performance, it did not alter any subjective or objective parameters of performance [331]. Stress may be involved in the expression of self-injurious and stereotyped behavior in mentally retarded subjects, but endogenous opiates are likely not involved as no differences in plasma β-endorphin levels were found between mentally retarded subjects with or without stereotyped and/or self-injurious behaviors [528].

3. Tolerance and dependence

As in 1998, much of the research on opiates dealt with the induction of tolerance and dependence after chronic administration. A better understanding of the biologic mechanisms that underlie these phenomena could help in the identification of pharmacological targets for treatment. However, as was evident in previous years, the physiological mechanisms that underlie tolerance and dependence are complex, involving long-lasting changes in opiate signal transduction mechanisms, and interactions between opiate and nonopiate systems [302,351,552]. Furthermore, it also should be stressed that, particularly for addiction, chronic opiate use produces behavioral changes as well, thus requiring treatment approaches that include both behavioral and pharmacological interventions [121,302,547].

Chronic administration of opiate agonists was associated with changes in endogenous opiate systems, as dynorphin and preproenkephalin mRNA in the caudate and nucleus accumbens was decreased after 7 days of morphine [184]. In the mollusk pedal ganglia, μ-opiate receptor transcript was down-regulated to almost zero after chronic morphine, indicating that μ-opiate receptors are well developed in invertebrates [73]. Furthermore, because the mollusk μ-opiate receptor reveals 95% identity with the human μ-opiate receptor [73], it is possible that these and perhaps other opiate receptors first appeared in invertebrates and were retained during evolution [73,126,129,500]. Prolonged in vitro exposure to μ-opiate agonists morphine or DAMGO, or the δ-opiate receptor agonist DPDPE for 24 h down-regulated opiate receptor binding of the nonselective opiate-antagonist [^{3}H]Diprenorphine in μ- and δ-opiate receptor-expressing SH-SY5Y cells [286]. However, the agonists did so with differences in potency because at the highest concentration tested DAMGO produced a 58.3% decrease, morphine a 37% decrease, and DPDPE only an 8.8% decrease [286]. Furthermore, although SH-SY5Y cells express both μ- and δ-opiate receptors, the effects of DAMGO on opiate-receptor binding were probably μ-opiate-mediated because no changes were found in δ-opiate receptor density after DAMGO [286]. Repeated morphine produced changes in δ-opiate receptor density that was both site- and time-dependent, as it decreased both δ_1- and δ_2-opiate receptor binding in the nucleus accumbens at 3, 24, and 48 h after administration, and in the striatum at 24 and 48 h [521]. The finding that changes in δ-opiate receptor density were most pronounced later suggests their possible role in dependence [521]. Chronic morphine decreased spinal dynorphin A (1–17) immunoreactivity that returned to normal levels after electroacupuncture, indicating that electroacupuncture may be effective in treating tolerance and dependence [563]. In contrast, repeated injections of increasing doses of 20–100 mg/kg morphine twice daily for 10 days did not produced changes in brain μ-opiate receptor density in rats [521], and no changes in μ-opiate receptor density in the parieto-occipital cortex were found after prolonged consumption of the μ-opiate etonitazene [344].

Chronic exposure to morphine up-regulated [^{125}I-Tyr^{14}] OFQ/N-immunoreactivity in the superficial layers of the spinal cord after 3, 5, and 7 days of morphine infusion, but not after 2 days [194]. OFQ/N-density also was up-regulated supraspinally after chronic morphine that depended on the length of morphine exposure, as 1, 3, and 5 days of morphine injections produced an increase of [^{125}I-Tyr^{14}]OFQ/N-immunoreactivity in the periaqueductal gray of 17%, 48%, and 81%, respectively, and in the amygdala a 36% increase was noted after 3 days, and a 55% increase after 5 days [582]. Similarly, OFQ/N-immunoreactivity in cerebroventricular perfusate increased 25% after 3 days of morphine and 52% after 5 days of morphine, as compared with control [582]. Taken together, these results suggest that continuous infusions of morphine may accelerate the release and biosynthesis of this antiopiate peptide, and may be involved in the induction of compensatory responses after chronic morphine [194,582]. However, in various in vitro cell preparations, chronic morphine also increased enzymatic activity responsible for the biotransformation of OFQ/N into its metabolites, which is opiate-mediated because naloxone blocked it [535]. Therefore, although chronic morphine up-regulates OFQ/N-immunoreactivity [194,582], it also increases the biotransformation of OFQ/N into its fragments, raising the possibility that elevated levels of OFQ/N fragments during chronic morphine also may have antiopiate effects [535].

As shown in previous years, chronic morphine produced long-lasting molecular and cellular adaptions including changes in cAMP pathways and signaling proteins [302,

481]. DPDPE and deltorphin I inhibited adenylyl cyclase activity in human SK-N-BE cells that was opiate-mediated because naloxone blocked their effects [12]. However, by 30 min desensitization of adenylyl cyclase inhibition was greater for DPDPE and deltorphin I than for etorphine, indicating that the agonists interacted differentially with δ-opiate receptors [12]. This is supported further by the finding that etorphine pretreatment totally blocked DPDPE- and deltorphin I-inhibition adenylyl cyclase, whereas pretreatment with DPDPE and deltorphin I did not block etorphine-induced inhibition [12].

Supersensitization of the adenylyl cyclase system occurs after chronic morphine, as forskolin-stimulated cAMP accumulation was elevated above control levels after naloxone in μ-opiate receptor-expressing HEK293 cells exposed to morphine [544]. The naloxone-induced overshoot in cAMP after chronic morphine is likely μ-opiate-mediated because naloxone had no effect in morphine-treated HEK293 cells that did not express the μ-opiate receptor [544]. Similarly, forskolin-induced cAMP accumulation was elevated beyond control levels in Chinese hamster ovary cells expressing μ- or κ-opiate receptors treated with morphine or U69593 and challenged with naloxone or nor-BNI, respectively [386,402]. Since pertussis toxin blocked cAMP overshoot after chronic treatment, it is likely that $G_{i/o}$-proteins are involved [386,402]. However, opiate receptors also have the capacity to interact with pertussis toxin-insensitive G_z- and $G\alpha_{i2}$ -proteins because the effects of sustained agonist treatment on cAMP accumulation were unaffected by pertussis toxin in Chinese hamster ovary cells co-expressing μ- or κ-opiate receptors and the α_z subunit [402], or in cells co-expressing κ-opiate receptors and the α_{i2} subunit [386].

Protein kinase C (PKC) contributes to μ-opiate receptor regulation after chronic opiates because the onset of opiate-receptor down-regulation after DAMGO parallels PKC translocation to the plasma membrane, with both effects blocked by naloxone but not by the δ-opiate receptor antagonist naltrindole [286]. Furthermore, DAMGO-induced down-regulation of μ-opiate receptors in μ-expressing Chinese hamster ovary cells is mediated by G-protein-coupled receptor kinase dependent phosphorylation that Thr^{394} regulates because it is partially reduced by pertussis toxin or by mutagenesis of Thr^{394} [405]. However, a G-protein-independent pathway also is involved that is dependent upon tyrosine kinase because the tyrosine kinase inhibitor, genistein, partially blocked μ-opiate down-regulation, and inhibited receptor down-regulation after mutagenesis of Thr^{394} [405]. Alterations in signal transduction in the parieto-occipital cortex were found after consumption of etonitazene, including changes in the maximum efficacy in basal [^{35}S]GTPγS that was higher 2 days, but not 6 weeks after etonitazene intake [344], and GTPγS, which decreases [^{3}H]DAMGO binding affinity in opiate-naive rats, did so to a lesser extent after etonitazene consumption [344]. Levels of opiate stimulated [^{35}S]GTPγS binding in the mouse and rat midbrain were greater after DAMGO than after DPDPE or DELT II, which correlates with the relative distribution densities of these receptor subtypes [520]. However, the levels of agonist-stimulated [^{35}S]GTPγS binding may not always be a valid indication of receptor density because in the opiate-dense striatum and forebrain the level of G-protein activation was relatively low for all 3 agonists [520].

Functional desensitization after prolonged agonist exposure may be related to receptor internalization, as prolonged exposure to the δ-opiate agonist, DADLE, produced a time-dependent redistribution of δ-opiate receptors in neuronal cells, decreasing δ-opiate receptor staining in the cell surface throughout the 24-h exposure period while gradually increasing intracellular receptor concentration up to 4-h after exposure [275]. Furthermore, although recycling of internalized receptors occurred after short-term agonist treatment, prolonged exposure to the agonist results in a lysomal-dependent degradation and/or decreases in receptor synthesis because intracellular δ-opiate receptor immunoreactivity gradually decreased after 4-h that was up-regulated by chloroquinine, a lysosomotropic agent [275]. However, the effects of agonists treatment on receptor δ-opiate internalization depended on the agonists studied because the nonselective opiate agonist etorphine promoted internalization in δ-expressing HEK293 cells, but morphine did not [593]. The inability of morphine to cause δ receptor internalization is correlated with its inability to promote G-protein-coupled receptor kinase-dependent phosphorylation of the receptor, as etorphine but not morphine triggers δ receptor phosphorylation and β-arrestin membrane translocation [593].

Internalization of the κ-opiate receptor after agonist treatment depended on the species studies because U50488H, U69593, ethylketocyclazocine, and tifluadom, promoted internalization in human but not rat κ-opiate receptor-expressing Chinese hamster ovary cells [306]. Furthermore, since naloxone, but not pertussis toxin, blocked U50488H-induced internalization, it suggests that κ-opiate receptor internalization requires receptor activation, but does not require pertussis toxin-sensitive receptor/G-protein coupling [306]. However, receptor activation in of itself is not sufficient to induce receptor internalization for all agonists because etorphine activates κ-opiate receptors but does not promote internalization [306]. U50488H-induced internalization of human κ-opiate receptors involve G-protein-coupled kinase-, β-arrestin-, and dynamin-dependent processes because internalization was reduced in Chinese hamster ovary cells transfected with dominant negative mutants GRK2-K22OR, β-arrestin (319−418), or dynamin I-K44A [306]. Similarly, the rate κ-agonist-induced desensitization can be facilitated by co-expression of GRK2 or GRK5 along with β-arrestin, thus further demonstrating the role of G-protein-coupled kinase and β-arrestin [22].

DAMGO, endomorphin-1, and endomorphin-2 activate μ-opiate receptors to induce receptor internalization because in μ-opiate transfected neurons of the guinea-pig ileum incubated with these agonists, μ-opiate receptors are

concentrated in endosomes in the soma and neurites, but not in the plasma membrane [347]. Since pre-incubation with naloxone prevented both DAMGO- [338,347] and endomorphin-2 [347]-induced endocytosis, receptor activation is apparently necessary. The Thr^{394} phosphorylation site influences the rate and extent of internalization of the μ-opiate receptor-1 isoform, however, as mutagenesis of the Thr^{394} accelerates both internalization after DAMGO exposure and resensitization after its removal [561]. It is possible that rapid internalization and resensitization functions to counteract DAMGO-induced desensitization because the rate of desensitization is slower after mutagenesis of the Thr^{394} [561]. Furthermore, although morphine fails to internalize μ-opiate receptors in NG 108–15 cells, it does internalize them when β-arrestin is overexpressed in these cells, suggesting that internalization depends on β-arrestin-dependent uncoupling of the receptor from its G-protein complex [470].

Long-lasting cellular changes after chronic morphine also were shown by morphine-induced c-Fos expression in the brain as rats given repeated doses of morphine twice daily for 10 days showed increased c-Fos expression in limbic and limbic-related structures, including the dorsomedial and lateral striatum, lateral septum, medial mammillary nuclei, anterior thalamus, and the cingulate cortex [152]. In contrast, a single dose of morphine produced only minimal c-Fos expression [152]. Furthermore, sensitization to c-Fos expression occurred after repeated morphine as the c-Fos response was induced in the dorsal caudate, cingulate cortex, mammillary nuclei, and anterior thalamus by a single injection of morphine when given 4 weeks after the cessation of morphine injections [152]. Although the functional significance of the sensitized c-Fos response is not entirely clear, retrograde labeling studies showed that these areas receive input from limbic and motor areas, suggesting a possible relationship to morphine-induced sensitization of locomotor responses and relapse in opiate addicts [152]. Chronic morphine also produces changes in long-term potentiation (LTP) that may be related to addiction, as rats given morphine in their drinking water for 20–30 days showed enhanced orthodromic population spike delays and amplitude in hippocampal slices, as compared with rats given no morphine or only short-term morphine [327].

Chronic treatment with opiate antagonists generally produced an up-regulation of opiate receptors [132]. Continuous infusion of naloxone via osmotic minipumps for 7 days increased μ- and κ-opiate receptor density in the cerebral cortex, caudate, nucleus accumbens, medial habenulla, thalamus, basolateral amygdala, and hippocampus, as compared with controls [236]. Similar increases in δ-opiate receptor density was found after chronic naloxone, except in the hippocampus where no changes were noted [236]. This up-regulation of μ-opiate density after chronic naloxone is functional because it also increased G-protein activation in all the brain areas studied, as measured by binding of [^{35}S]GTPγS in response to the μ-agonist DAMGO [236]. However, the functional significance of the up-regulation of δ- and κ-opiate receptor binding after naloxone may be small, as [^{35}S]GTPγS binding was increased only in the cerebral cortex and caudate in response to the δ-opiate agonist, DPDPE, and no changes in [^{35}S]GTPγS binding were found in response to the κ-opiate agonist, U-50488H [236]. The effects of opiate antagonists on opiate receptor up-regulation are independent of basal receptor density because naltrexone produced similar increases in whole brain μ- and δ-opiate receptor density and $G_{i\alpha 2}$ protein levels in Swiss Webster mice, as compared with μ-opiate receptor deficient CXBK mice [145].

Antiopiate peptides may tonically regulate opiate receptor density and thus modulate the effects of opiates, as continuous ICV infusion of anti-dynorphin A IgG and anti-α-MSH IgG increased δ-opiate receptor labeling in the caudate, nucleus accumbens, and cingulate cortex, whereas anti-NPFF IgG decreased δ labeling in the caudate, nucleus accumbens, claustrum, olfactory tubercle, and cingulate cortex [193]. Chronic activation of the opioid receptor-like receptor with the endogenous antiopiate peptide OFQ/N produces changes in cAMP pathways, as chronic treatment with OFQ/N enhanced forskolin-induced adenylyl cyclase activity in HEK293 and SK-N-SH cells expressing the opioid receptor-like receptor [91]. Since adenylyl cyclase supersensitivity after chronic treatment with OFQ/N was blocked in both cell lines by pertussis toxin, it is likely that G_i/G_o-protein are involved [91]. Repeated administration of morphine decreased and naloxone increased galanin binding in the nucleus accumbens, indicating that endogenous opiates tonically regulate galanin binding which may have antiopiate properties [583]

Behaviorally, chronic administration of opiates generally produces tolerance [10,18,43,52,118,125,131,133,174,222, 229,279,284,295,304,314,315,316,430,463,466,491,540, 544, 591,598]. Tolerance after chronic morphine was sex-dependent, as the ED_{50} for morphine analgesia was increased 6.9-fold in male rats after repeated morphine, as compared with only 3.7-fold in females, an indication that males developed more tolerance than females [118]. The nutritive value of a solution also affected tolerance because rats that drank a sucrose or polysucrose solution showed less tolerance than rats that drank a saccharin-based solution [125]. Although tolerance was typically demonstrated after systemically administered opiates, tolerance developed to topical morphine as well, as immersion of the tail in a DMSO solution containing morphine produced a local analgesic effect that tolerated after repeated exposures [279].

Stress modified tolerance because concurrent exposure to foot-shock in mice, or exposure to mice being shocked, suppressed the development of tolerance to morphine analgesia [174]. The prototypic antiopiate peptide Tyr-MIF-1 is probably involved because it prevented the stress-induced suppression of morphine tolerance [174]. Pain also influenced the development of tolerance to morphine analgesia as tolerance to the analgesic effects of intravenous (IV)

infused morphine in the tail-flick test developed more slowly in rats that received abdominal surgery, as compared with unoperated controls [222]. Furthermore, since no differences were found between groups in plasma morphine concentrations during the 8-h infusion period, the appearance of tolerance after morphine infusion is likely not pharmacodynamic in nature [222]. Taken together, these results show that tolerance is not an inevitable response to chronic morphine, especially when given in the presence of pain and stress [174,222]. This also may translate clinically, as the development of tolerance to spinally administered morphine does not greatly limit its use for pain control, as successful pain relief was achieved in 50% of chronic nonmalignant pain patients after 24 months [18]. Furthermore, although 30% of the patients developed some tolerance to morphine, switching to another opiate could manage the pain effectively because of incomplete cross tolerance [18]. However, physicians should be cautious when switching opiates as the degree of cross tolerance may change as opiate doses are increased [357].

The opiate receptor subtypes that mediate tolerance were elucidated and involve phosphorylation of the μ-opiate receptor because IBMX both inhibits basal μ-opiate receptor phosphorylation and prevents tolerance [544]. A role for δ-opiate receptors also was demonstrated as δ-opiate receptor knockout mice did not develop tolerance to morphine analgesia [598]. In contrast, although the knockout mice showed reduced spinal analgesia after DPDPE, supraspinal analgesia was retained without tolerance, indicating that a second δ-opiate-like analgesic system may exist [598]. It is important to recognize, however, that inactivation of one opiate receptor subtype gene may result in the compensatory expression of other opiate receptor subtypes [64,488] and should be considered when interpreting the results of these procedures.

The endogenous μ-opiate receptor agonists, endomorphin-1 and endomorphin-2, produced transient analgesic and antihyperalgesic effects that are probably related to acute tolerance because a 10 ug dose of endomorphin-2 was more potent in the tail-flick test when applied in the first intrathecal (IT) injection than in the third injection [229]. In contrast, tolerance was not observed in the acetic writhing test after repeated ICV injections of 6d, a naloxone-derived compound with mixed μ-agonist/δ-antagonist properties [17]. Similarly, the mixed μ-agonist/δ-antagonist DIPP-$NH_2[\Psi]$ produced more potent analgesia and less tolerance than morphine in the tail-flick test [463]. Therefore, because the δ-antagonist component of these compounds could diminish tolerance, compounds that possess mixed μ-agonist/δ-antagonist properties may have great therapeutic value [17,140,463].

Typically, cross-tolerance occurs between opiate agonists with affinity for the same receptor. When tested for analgesia to a relatively low temperature stimulus (tail-withdrawal from 50°C water), tolerance developed to the analgesic effects of butorphanol, that was cross-tolerant with morphine, etorphine, levorphanol, dezocine, pentazocine, and nalbuphine [491]. It is likely that butorphanol possesses μ-opiate agonist activity under these conditions because it increased the acute analgesic effects of these opiates when tested for analgesia at this temperature of water. However, cross-tolerance differed between opiates that was inversely related to their relative efficacy to the μ receptor, as cross-tolerance was greater for the low efficacy opiates (dezocine, pentazocine, and nalbuphine), as compared with the high-efficacy ones (morphine, etorphine, levorphanol) [491]. Interestingly, although butorphanol has antagonistic properties when tested for analgesia at higher temperatures (55°C water) because it antagonized the effects of morphine, etorphine, levorphanol, and dezocine, cross-tolerance was still conferred between butorphanol and these opiates [491]. This finding is in contrast to the typical effects of 'pure' opiate antagonists, which enhance sensitivity to opiates when administered chronically, and may reflect differential effects on endogenous opiate systems, as naloxone and naltrexone generally up-regulate μ-opiate receptors [132], whereas chronic butorphanol usually down-regulates them [491].

Cross-tolerance did not occur between morphine and the peripherally acting κ-opiate agonist asimadoline in rats with peripheral nerve injuries, indicating that this peripherally selective κ-agonist has potential for the treatment of neuropathic pain [540]. Administration of dynorphin A (1–13) enhanced analgesia in morphine-treated chronic pain patients that may be due to its direct analgesic effects and/or a reversal of morphine tolerance [428]. An interaction between δ and μ receptors was demonstrated, as chronic neonatal treatment with the δ-opiate antagonist naltrindole blocked subsequent analgesia in the tail-flick withdrawal test to the μ-agonist alfentanil, but not the κ-agonist CL-977 [160]. In contrast, chronic treatment with naltrindole did not block the inhibitory effects of morphine on the vocalization discharge to a painful stimulus [161]. Since vocalization discharge is related to the affective component of pain that involves supraspinal mechanisms, whereas the tail-withdrawal test is spinally mediated, it suggests that there is no μ-δ interaction in the modulation of the affective component of pain [161]. Tolerance did not develop to the analgesic effects of the mixed μ-agonist/antagonist, Tyr-W-MIF-1, indicating its potential for pain treatment [43]. Interestingly, however, although rats pretreated with Tyr-W-MIF-1 showed cross-tolerance with morphine, cross-tolerance with Tyr-W-MIF-1 was not conferred in morphine-pretreated rats [43]. The reasons for the lack of symmetrical cross-tolerance between Tyr-W-MIF-1 and morphine are not clear, but may be related to the action of Tyr-W-MIF-1at its own receptor or to a differential activity at μ-opiate receptor subtypes [43].

Besides analgesia, tolerance to other opiate effects was examined. Methadone increased immobility in mice after an acute injection, but not after chronic injections, indicating the development of tolerance [131]. However, tolerance

does not develop to the antiaggressive effects of methadone because it decreased attack behaviors between male mice similarly after acute and chronic injections [131]. Tolerance to the rewarding effects of morphine also was not demonstrated as mice chronically treated with morphine in one environment were still able to acquire morphine CPP when subsequently paired with a different environment [110]. In contrast, tolerance develops to the subjective, but not the physiological effects of buprenorphine, as positive mood decreased during buprenorphine maintenance in opiate-experienced human volunteers, whereas buprenorphine-induced miosis and respiratory depression did not [466]. Tolerance does not occur to the rate decreasing effects of mirfentanil, that is not explained by to low opiate activity, because cross-tolerance was conferred with the effects of morphine [180]. Conversely, morphine induced ipsilateral turning behavior in rats with nigostriatal lesions that showed sensitization, as daily injections of morphine progressively increased turning over 13 days [536]. The morphine-induced sensitization to turning behavior is long-lasting as a 10 mg/kg dose of morphine increased ipsilateral turning 71 days after the last morphine-injections [536]. Long-lasting sensitization also occurs to morphine-induced oral stereotypy, as daily injections of morphine progressively increased biting behaviors over 4 days, and a challenge dose of morphine given up to 6 weeks later still produced biting behaviors [550].

As was the trend in previous years, in 1999 there was continued interest in the role of the glutamate receptor in the development of morphine tolerance. The *N*-methyl-D-aspartate receptor (NMDA) receptor plays a functional role in the development of tolerance to morphine analgesia as the NMDA antagonist MK-801 [284] and the NMDA receptor/glycine site antagonist ACEA-1328 [315] given concurrently with repeated morphine prevented the development of tolerance. Furthermore, co-administration of ACEA-1328 with morphine for 7 days in morphine-tolerant mice also prevented tolerance, indicating that once established ACEA-1328 can reverse tolerance [315]. However, it did not prevent the expression of tolerance because an acute injection of ACEA-1328 did not reinstate analgesia in morphine-tolerant mice [315]. In contrast, although the NMDA receptor antagonist LY235959 also prevented the development of tolerance to morphine analgesia, the same doses of LY235959 could not reverse pre-established tolerance [10]. Peripheral NMDA receptors mediate tolerance to the local analgesic effects of topical morphine, as the NMDA antagonist MK-801 prevented tolerance when given topically or systemically, but not when given IT [279].

The type of NMDA antagonist used was important because MK-801 and LY235959 prevented tolerance to the suppressive effects of morphine on carrageenin-induced c-Fos expression, whereas the glycine site antagonist HA-966 did not [295]. This is in contrast to the effects of ACEA-1328 which prevented tolerance [315], indicating that differences exist between ACEA-1328 and HA-966 in their allosteric modulation of the glutamate site [295,315]. The type of NMDA antagonist used also was important in the development of tolerance to the discriminative stimulus effects of morphine, as eliprodil and D-CPPene prevented tolerance, but MK-801 and HA-966 did not [52]. In contrast, all the antagonists prevented the induction of tolerance to morphine's rate decreasing effects [52]. The role of the NMDA receptor in the development of tolerance to κ-opiate agonists is not clear because ACEA-1328 partially (but not significantly) restored analgesia in U50488H-tolerant mice that was confounded by a decrease in U50488H analgesia after chronic treatment with ACEA-1328 [316].

It is possible that tolerance is related to a NMDA-mediated enhancement of pain sensitivity because acute or repeated injections of heroin enhanced pain sensitivity that MK-801 prevented [291]. Similarly, MK-801 reversed naloxone-induced hyperalgesia after morphine or fentanyl injection [86], and repeated injections of heroin produced an immediate analgesic response that ketamine enhanced and a long-lasting hyperalgesic response that ketamine prevented [86]. Taken together, these results suggest that besides tolerance, decreases in analgesia after chronic opiate treatment also may be related to increases in pain sensitivity triggered by opiate treatment, both of which are sensitive to NMDA antagonism [86,87,291,351].

Morphine tolerance may be related to an up-regulation of NMDA receptors because chronic administration of morphine up-regulated NMDA receptors, whereas MK-801 given concomitantly with morphine down-regulated them [284]. Changes in gene expression in the NR1 subunit if the NMDA receptor also may be involved because increases in the NR1, but not the NR2A and NR2B subunit, mRNAs was found in the locus coeruleus and the hypothalamic paraventricular nucleus after 3 days of ICV morphine infusions [597]. Activation of the NMDA receptor can lead to the production of the second messenger nitric oxide (NO) that also is involved in morphine tolerance because administration of the NO synthase (NOS) inhibitor L-NAME attenuated both the development and expression of tolerance to morphine analgesia [430]. Similarly, cyclo-oxygenase inhibitors, ketorolac and ibuprofen, also prevented tolerance, possibly due to their NMDA antagonistic actions [430].

In humans, the potential therapeutic benefit of combining NMDA antagonists with morphine treatment was suggested as post-operative morphine consumption after laparotomy was lower in patients given simultaneous IV ketamine with patient controlled morphine analgesia, as compared with those given only morphine [6]. Furthermore, there was a trend toward fewer total side-effects in the ketamine-treated patients, including a significant decrease in the incidence of nausea [6]. Similar beneficial effects of combined opiate and ketamine treatment were reported in the case studies that included an 80-year-old man with prostate cancer and skeletal metastasis, a 23-year-old man with a spinal neurectodermal tumor, and a 67-year-old woman with squamous cell carcinoma of the lung and skeletal metastasis [42]. The

beneficial effects of combining opiates and ketamine are possibly explained by their additive or synergistic actions at different receptors, as naloxone did not block ketamine-induced sedation or its effects on hyperalgesia [362].

Interactions between opiates and dopamine receptors were noted after chronic morphine that depended on the brain area studied because morphine-induced dopamine transmission was increased in the core of the nucleus accumbens and the caudate-putamen after repeated morphine injections, but decreased in the shell of the nucleus accumbens [74]. Furthermore, although acute intrastriatal morphine injections decreased extracellular dopamine release, they did so less in rats chronically treated with morphine, indicating that tolerance develops to this response [418]. Both μ- and δ-opiate receptors interact with mesolimbic dopamine receptors because intra-accumbens administration of the μ-agonist DAMGO, the δ_1-agonists DPDPE, and the δ_2-agonist deltorphin II enhanced nucleus accumbens dopamine release [576], and 39% of δ-opiate receptors in the nucleus accumbens either contained dopamine transporter or apposed dopamine transporter-immunoreactive terminals [509]. Fentanyl also increased extracellular dopamine in the nucleus accumbens that can be explained by its actions at both μ- and δ_2-opiate receptors because naloxone, the μ-antagonist CTOP, the δ-antagonist naltrindole, and the δ_2-antagonist naltriben blocked it, whereas the δ_1-antagonist BNTX did not [576]. However, GABAergic systems also are likely involved because the GABA-transaminase inhibitor, γ-vinyl GABA blocked heroin-induced increased nucleus accumbens dopamine release [185]. Mesolimbic dopamine receptors likely mediate sensitization to morphine's locomotor effects because doses of caffeine that decrease the firing of dopamine neurons in the ventral tegmental area also prevent morphine-induced sensitization to ambulatory activity [548]. Since the adenosine receptor antagonist PACPX also prevents morphine-induced sensitization, it is possible that the effects of caffeine are mediated by the adenosine receptor [548]. However, behavioral sensitization to morphine can occur independently of sensitization to psychomotor stimulants because cross-sensitization between morphine and amphetamine or cocaine did not occur [74,536].

Serotonergic systems are likely involved in both opiate tolerance and sensitization because serotonin re-uptake blockers prevented both the development of tolerance to sufentanil-induced analgesia [314] and the expression of sensitization to morphine-induced oral stereotypy [550]. Although opiate-cholecystokinin (CCK) interactions have been shown [552], tolerance to morphine analgesia can occur without changes in CCK synthesis or release because no differences in extracellular CCK were found in the spinal cord of tolerant and nontolerant rats [133]. Sex differences were found in the interaction between δ-opiate and α_2-adrenergic receptors as chronic treatment with naltrindole during the neonatal period prevented clonidine-induced analgesia in females, whereas it allowed the appearance of analgesia in males [8]. Adrenergic compounds may be effective in managing pain in morphine tolerant patients, as IT morphine and clonidine produced analgesic effects that were synergistic in morphine-tolerant mice [155]. Agmatine prevented tolerance to morphine analgesia related to its actions at imidazoline receptors because the imidazoline receptor antagonist idazoxan blocked it, but the α_2-adrenoceptors antagonist yohimbine did not [304]. An interaction between opiate and nicotinic receptors was shown because tolerance developed to both morphine- and nicotine-induced analgesia in mice treated chronically with either morphine or nicotine [591].

Chronic administration of opiates usually results in dependence as measured by the appearance of withdrawal symptoms after cessation of the drug, or when an opiate antagonist is delivered. In animals, withdrawal symptoms included abnormal posture/writhing/stretching [76,78,118,147,157,308,309,463,469,512,527,563], attenuated gait [76], aggression [443], body tremors [76,297], body grooming [76,133,157,512], changes in operant responding [78,147,181,301,469], chromodacryorrhea [76], contractures of the guinea-pig ileum [311,444], diarrhea [78,118,133,157,297,308,309,469,512,527,590], digging/burrowing [157,527], ejaculation [78,469,563], excessive eye blinks [469], freezing [76], hyperalgesia [291], hyperlocomotion/exploration [118,308,309], irritability [78,118,133,147,469,527], leaning [76], lying [76], nose irritability [133], jumping/escape [33,61,76,118,157,284,297,304,305,308,309,463,469,512,527,544,563,589,590], paw tremors [157,297], penile erections/licking [76,78,469,527], piloerection [512], ptosis [43,78,118,133,297,308,309,469,512,527], rearing [308,309,512,563], rhinorrhea [76,527], salivation [76,147,469,527], scratching [308,309], sniffing [76,297,308,309,512], swallowing [78,147,469], teeth chattering/mastication/chewing [43,76,78,118,133,297,308,309,512,463,469,527,563], vocalization [76,563], weight loss [76,78,118,147,284,297,304,305,469,527], wet dog shakes [43,78,118,133,147,297,308,309,463,469,527,563], and yawning [512].

In humans, withdrawal symptoms included abdominal distention/cramps [54,189,200,336,356,523], backaches [200,356], changes in pupil diameter [149,356,414,523], changes in heart rate/blood pressure/perspiration/skin temperature [11,189,200,356,415,523], depression/sadness [54,356], clammy/damp skin [189,200,356], diarrhea [189,336], drooling [336], nausea/vomiting [189,336,356,523], goose flesh/piloerection [54,189,200,356,523], sluggishness [200], hot/cold flashes [54,189,200,356,523], hyperphagia [336], insomnia [54], irritability [54,200,356], muscle cramps/pain [54,189,200,356,523], painful joints [54,200], poor appetite [54], restlessness [54,189,200,356,523], runny nose/nasal congestion [54,189,200,356,523], sleepiness [356], sneezing [54,356], stomach sickness [54,200,356], sweating [54,189,200,356,523], tenseness [54,356], tremors [54,189,356,523], watery eyes [54,189,200,356,523], week knees [54], yawning [189,200,356,523], and other symptoms recorded

on observer- and self-rating scales [41,93,105,149,336,414, 523].

Chronic morphine produced dependence as shown by withdrawal symptoms precipitated by naloxone [11,33,43, 61,76,78,118,149,157,189,297,304,305,311,356,415,443, 444,456,463,469,512,527,544,563,589,590], nalmefene [189], naltrexone [41,284,415,523], and abstinence [54,76,93,105, 184,200,291,336,356,414,502]. Sex-differences were noted, however, as naloxone precipitated greater withdrawal symptoms in morphine-dependent male rats than females, especially with respect to wet-dog shakes [118].

Increased sensitivity to naloxone may provide an index of dependence, as naloxone doses ranging from 0.003–0.03 mg/kg affected heroin-self administration rates in morphine-dependent rats, whereas self-administration rates were modified only by the highest dose (0.03 mg/kg) in placebo controls [78]. Dependence is likely progressive, as naloxone-precipitated withdrawal occurred after a single injection of morphine, and in rats chronically infused with morphine greater signs of withdrawal were precipitated by lower doses of naloxone that increased as a function the amount of morphine infused [147]. Similarly, naloxone suppressed operant response rates in rats after a single injection of morphine that was potentiated with repeated morphine presentations given at 24-h, 1-week, 3-week or 6-week intervals [469]. Naloxone-precipitated somatic withdrawal signs, however, were not increased when morphine was given at intervals of 1 week or more, suggesting that operant responding is a more sensitive index of dependence than the appearance of physical withdrawal symptoms [469]. A similar dissociation between affective and physical signs of withdrawal also was demonstrated by the finding that a low dose of naloxone (0.003 mg/kg) that increased heroin self-administration in morphine-treated rats, did not elicit somatic signs of withdrawal [78].

Drug discrimination paradigms were used as a means of assessing subjective effects of naloxone associated with opiate withdrawal. In morphine-dependent monkeys trained to discriminate between SC injections of naltrexone from saline under a fixed ratio 5 schedule of shock termination, SC administration of naltrexone or naloxone was more potent than oral administration in producing naltrexone-appropriate responding [181]. However, there was little difference between the potency of the two antagonists when given by either route, indicating that the preferred use of oral naltrexone over naloxone in the treatment of abuse is not supported by differences in bioavailability [181]. The discriminative stimulus effects of naltrexone could be demonstrated after an acute injection of morphine as rats could discriminate between naltrexone that was preceded by a single injection of morphine or saline [147]. Furthermore, naloxone-appropriate responding was observed under conditions in which somatic withdrawal signs were minimal [147], supporting further the notion that the affective and physical signs of withdrawal can be dissociated [78,469].

The opiate receptor subtypes that mediate dependence were examined and, like tolerance, involve phosphorylation of the μ-opiate receptor because inhibition of μ-opiate receptor phosphorylation with IBMX attenuated naloxone-induced jumping [544]. It is likely that μ-opiate receptors in the medullary nucleus paragigantocellularis participate in withdrawal-induced discharge activity of the locus coeruleus because μ-opiate receptors are distributed along plasmalemmal sites of neurons in the nucleus paragigantocellularis that project to the locus coeruleus [524]. Furthermore, electrical stimulation of the nucleus paragigantocellularis produces withdrawal-like behaviors in opiate-naive rats that is mediated by μ- and δ- but not κ-opiate receptors because ICV injections of naloxone, β-FNA, and naltrindole, but not nor-BNI, blocked the withdrawal-like behaviors [309]. In contrast, electroacupuncture suppressed naloxone-precipitated withdrawal in morphine-dependent rats that involves κ-opiate receptors because nor-BNI and dynorphin A (1–13) antibodies blocked it [563]. The δ-opiate receptor is likely involved in dependence as chronic morphine decreased δ-opiate receptor density that was most pronounced later in withdrawal [521]. As opposed to morphine, however, the mixed μ-agonist/δ-antagonist DIPP-$NH_2[\Psi]$ does not produce dependence as measured by naloxone-precipitated withdrawal symptoms, indicating that this novel analgesic may have low abuse potential [463]. Interestingly, although tolerance did not develop to the mixed μ-agonist/antagonist Tyr-W-MIF, it showed similar levels of dependence as morphine, indicating that different mechanisms may mediate tolerance and dependence, and this peptide may have abuse potential [43].

As with tolerance, interactions between opiate dependence and nonopiate mechanisms were shown [302]. Dopaminergic systems are involved as the selective D_1 antagonist SCH 23990, the selective D_2 antagonist raclopride, and the mixed D_1/D_2 antagonists haloperidol equally decreased the occurrence of aggressive encounters after spontaneous withdrawal in morphine-dependent male mice. However, when withdrawal was precipitated with naloxone, the antiaggressive effects of SCH 23990 and haloperidol but not raclopride were more pronounced, indicating that D_1- and D_2-dopamine receptors are differentially involved in spontaneous and naloxone-precipitated withdrawal [443]. In contrast, neither haloperidol nor the atypical neuroleptic clozapine modified naloxone-precipitated jumping [33]. Withdrawal is associated with a down-regulation of dopamine receptors, as both D_1 and D_2 receptor mRNA are decreased 1 day after abstinence in morphine-dependent rats, that gradually increased by the second and third day [184].

Noradrenergic transmission in the locus coeruleus is not involved in opiate withdrawal because lesions of noradrenergic neurons in that area did not affect naloxone-precipitated and spontaneous withdrawal [76]. Furthermore, the lesions did not prevent suppression of naloxone-precipitated withdrawal by clonidine, indicating that the effects of clonidine are mediated by post-synaptic α_2-adrenoceptor

stimulation [76]. In contrast, the ability of clonidine to suppress withdrawal symptoms was not demonstrated in another study, as it did not modify naloxone-induced jumping [33]. Agmatine also prevented naloxone-induced jumping and loss in body weight but is related to its actions at imidazoline receptors because idazoxan blocked it and yohimbine did not [304].

A role of CCK in dependence was shown as CCK-8 enhanced naloxone-induced contractures of isolated guinea pig ileum preparations exposed to μ- and κ-agonists [444]. Furthermore, both the nonselective CCK agonist caerulein and the CCK_B receptor agonist CCK-4 reduced naloxone-precipitated jumping after acute morphine, although CCK-4 was less potent that caerulein [61]. Since the CCK_A antagonist devazepide reversed the effects of caerulein and CCK-4, whereas the CCK_B antagonist LY 288,513 blocked the effects of CCK-4 but not caerulein, it is likely that CCK_A receptors are involved [61]. Interactions between GABAergic systems and withdrawal also were demonstrated as the $GABA_A$ agonist, baclofen, and the $GABA_B$ agonist, muscimol, reduced naloxone-precipitated jumping, whereas the $GABA_A$ antagonist CGP35348 increased naloxone-precipitated jumping and decreased the effects of baclofen [589]. A role for neuropeptide FF was demonstrated because it potentiated naloxone-precipitated withdrawal, and the neuropeptide FF antagonist dansyl-PQR-amide attenuated withdrawal [512]. Changes in cholinergic systems occurred after chronic morphine that dependent on the length of abstinence, as morphine decreased acetylcholine release in the nucleus accumbens during the early phase of abstinence, but increased it during the later phase when no withdrawal symptoms were present [167]. An interaction between brain cannabinoid systems and dependence was noted as cannabinoid receptor knockout mice showed fewer naloxone-precipitated withdrawal symptoms than wild-type controls [297].

Chronic opiates produce long-lasting changes in signal transduction mechanisms that may contribute to dependence, including alterations in cAMP pathways [302]. Changes in PKC activity occur after chronic butorphanol that may contribute to dependence as binding of the phorbol ester ligand [^{3}H]PDBu was increased in multiple brain areas after withdrawal from butorphanol [243]. In contrast to morphine, however, withdrawal from chronic butorphanol treatment produced only minor alterations in adenylyl cyclase activity, indicating that the mechanisms that mediate morphine and butorphanol dependence are different [243]. A role for endogenous adenosine in dependence was demonstrated, as higher levels of adenosine metabolites were noted in the striatum and nucleus accumbens in morphine-dependent rats after naloxone-precipitated withdrawal [456]. Consistent with the role of adenosine in dependence, the adenosine A_1 agonist CHA decreased naloxone-precipitated jumping and diarrhea, and the A_1 antagonist DPCPX blocked the effects of CHA but increased jumping and decreased diarrhea when given alone [590]. However, although the A_2 agonist CPCA also decreased naloxone-precipitated jumping and diarrhea, the A_2 antagonist DMPX only decreased the CPCA-inhibition of diarrhea, suggesting that A_1 receptors are involved in naloxone-precipitated jumping, whereas A_1 and A_2, which are present in the gastrointestinal tract, are involved in naloxone-precipitated diarrhea [590]. The involvement of Ca^{2+} in dependence was demonstrated as Ca^{2+} channel blockers inhibited naloxone-precipitated withdrawal contractures of the ileum of morphine-dependent guinea pigs [311].

As with tolerance, excitatory amino acids play a functional role in opiate dependence as reduction of glutamate release by the metabolically active glutamate receptor agonist LY354740 attenuated both naltrexone-precipitated activation of locus coeruleus neurons and behavioral signs of withdrawal [527]. Similarly, opiate withdrawal-like behaviors induced by electrical stimulation of the nucleus paragigantocellularis are correlated with increases in extracellular glutamate in the locus coeruleus, and these withdrawal-like behaviors are reduced by injection of the glutamate antagonist kynurenic acid injected into the locus coeruleus [308]. Glutamate receptors within the nucleus accumbens also are involved in dependence because chronic morphine produced changes in NMDA receptor-mediated synaptic transmission in that area that persisted for 1 week after withdrawal [333]. However, the interactions between opiate and NMDA receptors is probably more complex that originally thought, as the dopamine agonist apomorphine attenuated decreased naloxone-precipitated withdrawal symptoms after dextromethorphan, indicating an opiate-NMDA-dopamine interaction [157]. NOS also is involved in dependence as naloxone-induced jumping in morphine dependent mice was correlated with increases in NOS activity in the cerebellum, forebrain, and thalamus [305]. Furthermore, agmatine prevented both naloxone-induced withdrawal and NOS activity, that was mediated by its actions at the imidazoline receptor because idazoxan antagonized the effects of naloxone [304,305].

Pharmacotherapies for the treatment of opiate abuse and dependence included the use of both opiate agonists and antagonists. The long-lasting μ-agonist methadone may have potential for the treatment of opiate addiction that depends on the dosage used, as opiate addicts maintained on high doses of daily oral methadone (80–100 mg) had lower rates of opiate-positive urine test and lower self-reported opiate use than those maintained on moderate doses (40–50 mg) [502]. However, although methadone dose may be an important treatment characteristic, in 1994 44% of methadone treatment programs in Spain used daily methadone doses below the acceptable limit (60 mg) [139]. Since the drop-out rate increases when doses below 60 mg/kg are used [545], a need for improved treatment in that country is indicated [139]. Hyperphagia is commonly found in infants withdrawing from methadone, as 56% of infants born to mothers maintained on methadone during pregnancy had oral intakes of greater than 190 cc/kg/day by post-natal day

16 [336]. However, its relationship to dependence is not clear because those born with hyperphagia did not have higher withdrawal scores than those born without hyperphagia [336]. Abnormal brain metabolism and phospholipid balance were found in short-term methadone-maintained subjects, which improved after prolonged methadone treatment, an indication that chronic opiate use can lead to altered brain neurochemistry that may be ameliorated after prolonged methadone-maintenance [267].

The synthetic μ-opiate agonist *levo*-α-acetylmethadol (LAAM) also is used to treat opiate dependence and may be more potent that methadone because it produced greater physiological and subjective effects [149]. Furthermore, since naloxone fully reversed pupil constriction after methadone but only partially reversed it after LAAM, LAAM overdose may require greater antagonist treatment [149]. Heroin maintenance also may be effective in treating opiate dependence as the retention rate was 75% after 18 months in patients that had previously failed in methadone treatment, although various methodological constraints confound interpretation of these results [142].

As in previous years there was continued interest in the use of the partial μ-agonist/κ-antagonist buprenorphine for the treatment of opiate abuse [247]. Although buprenorphine can be delivered by both tablet and liquid, the doses may have to be adjusted as an 8 mg tablet produced lower plasma concentration than the liquid [467]. Biodegradable sustained release preparations to deliver buprenorphine also were examined, indicating that by the first week cumulative drug release was 2–3% and by 45 days between 63–64% [324]. Plasma concentrations of buprenorphine 24 h after administration of 16 mg/70 kg buprenorphine daily were comparable to plasma levels 72 h after 44 mg/70 kg buprenorphine and 48 h after 34 mg/kg buprenorphine in subjects given buprenorphine 3-times a week [93]. Since withdrawal symptoms were low across all dosing regimens and no differences were found in heroin use, this supports the use of higher doses of buprenorphine given 3-times a week as opposed to daily dosing, as it may increase compliance with treatment [93]. Similarly, effective buprenorphine treatment can be accomplished by administration of higher than standard doses twice-weekly, as no differences were found in agonist or withdrawal effects or percentage of drug-positive urine samples in opiate-dependent subjects given either a maintenance dose every 24 h, a doubled dose every 48 h, a tripled dose every 72 h [54,414], or a quadrupled dose every 96 h [414]. Furthermore, although some withdrawal effects were found to increase as a function of time since the last injection, they were low and therefore should not limit its clinical use [54,414]. Buprenorphine attenuated the subjective and physiological effects of hydromorphone in opiate-experienced volunteers that depended on the dose, as 8 mg blocked most effects of hydromorphone, whereas a 2 mg dose was only partially effective [466]. However, individual differences existed in the ability of buprenorphine to attenuate opiate reinforcement as buprenorphine attenuated both heroin use and hydromorphone's reinforcing and subjective effects in some subjects, but did not do so in others [200].

Opiate detoxification with opiate antagonists continued to be used, but there are some risks associated with it [207,514], especially in non-compliant relapse patients given naltrexone without an appropriate opiate-free period [514]. Furthermore, adaptational changes occur in opiate systems after antagonist treatment that could contribute to adverse side-effects, including receptor up-regulation that could enhance opiate agonists responses and possibly contribute to overdose [132]. Rapid opiate detoxification with high doses of opiate antagonists given under general anesthesia were used but yielded results that were inconsistent, and indicated that risks may be associated with it. Detoxification was completed in 20 opiate addicts given naloxone followed by nalmefene over a 2–3 h period under general anesthesia, as defined by minimal post-treatment withdrawal responses to naloxone [189]. Although the rate of abstinence was high it was still comparable to traditional detoxification techniques, as 3 of 17 patients remained abstinent after 18 months, and 4 others had relapses that were brief [189]. However, one patient died on the second day after the procedure of unknown causes, indicating the possible risks of this type of treatment and the need for continuous monitoring [189]. In contrast, detoxification was not completed within 48 h in opiate addicts detoxified with naloxone and naltrexone under general anesthesia, and no differences in withdrawal symptoms were found in a small sample of dihydrocodeine-addicted patients treated with this method as compared with a sample previously treated with conventional detoxification methods [415]. Furthermore, the risks associated with this method may be high, as the study was terminated because of renal and respiratory failure, although the cause of these side-effects was not determined [415]. Electrocardiographic abnormalities occur during recovery from rapid detoxification under general anesthesia that are reversible but should still be monitored, including bradycardia [11]. Rapid detoxification under minimal sedation may be effective and safe, as 60% of the patients reported that the procedure was 'quite' acceptable, 60% required only one night of hospitalization, and 80% later accepted naltrexone, although the relapse rate was high, as only 23% remained abstinent after 3 months, and one patient died of an overdose [41]. However, treatment compliancy might be improved if incentives are provided, as opiate-addicts given vouchers for each naltrexone dose ingested had a longer treatment retention period and consumed more naltrexone as compared with controls [431].

When combined with buprenorphine, naltrexone may shorten detoxification as withdrawal symptoms were abolished after 5 days in subjects maintained on buprenorphine for 4 days combined with naltrexone [523]. In contrast, those maintained on buprenorphine alone for 4 days showed significant withdrawal symptoms when challenged with naltrexone on day 8 [523]. Since the peak withdrawal symp-

toms were only of moderate intensity, these results suggest that combined buprenorphine and naltrexone treatment is an acceptable alternative procedure [523]. Furthermore, although buprenorphine itself may have a potential for abuse [584], buprenorphine and naloxone combinations may have low abuse potential because they decreased the pleasurable effects and estimated street value, and increased withdrawal symptoms, as compared with buprenorphine or morphine alone [356]. However, the dose combination ratios are important because buprenorphine with naloxone ratios of 2:1 and 4:1 produced moderate to high withdrawal symptoms, whereas a dose ratio of 8:1 produced only mild symptoms [356]. Detoxification with clonidine could be improved when combined with benzodiazepines, as 59.7% of opiate addicts detoxified with clonidine left treatment against medical advice, as compared with 22.6% in those detoxified with combined clonidine and diazepam treatments [28].

Significant twin resemblance for opiate use, abuse, and dependence was found for monozygotic female twins, but not for dizygotic ones, an indication that genetics may contribute to opiate addiction [271]. An association between the proenkephalin gene and opiate dependence was demonstrated because 66% of opiate dependent subjects carried the 81 bp allele of the proenkephalin gene, as compared with 49% in controls and 40% in subjects with nonopiate substance abuse [106]. In contrast, an association between genetic variation of the δ-opiate receptor 921T/C allele and heroin dependence was not demonstrated because no differences were found between heroin-dependent subjects and controls, nor was there a preferential transmission of the C allele from parent to offspring [169]. Similarly, no differences were found between opiate-dependent and nondependent subjects in the allele frequency of two polymorphic variations, Ala6Val and Asp40Asn, that affect coding of the μ-opiate receptor [183]. Furthermore, no association was found between opiate dependence and the dopamine D_3 receptor Bal1 or serotonin transporter region 5-HTTLPR polymorphisms, as no differences in allele or genotype frequencies were found between heroin addicts and controls [283].

HIV is linked to iv drug use as 60% of patients receiving methadone treatment in Spain also were HIV-positive [139]. Similarly, in Northern Thailand the incidence of HIV was dramatically higher in iv opiate users as compared with those who smoked or inhaled opiates [85], indicating that increased access to sterile needle may help limit the spread of HIV [85,165]. Furthermore, opiate drug use also may influence the progression of AIDS, as higher levels of plasma viral load was associated with continued drug use in HIV-infected patients with a long history of opiate use [79].

Many factors can trigger relapse after extended periods of opiate abstinence, including reexposure to both positive and negative states that become associated with drug environment [302]. Furthermore, long-lasting changes in brain reward systems and stress response after chronic opiates may provide motivation for drug-seeking behavior and increase vulnerability to relapse [302,496]. Besides exposure to cues associated with opiate-use, exposure to opiates, such as morphine, meperidine, hydromorphone, fentanyl, and codeine, may reinstate drug-craving and thus contribute to relapse in abstinent opiate-addicts undergoing surgery [452, 584]. However, although this should be of concern to both anesthesiologists and abstinent addicts, a survey revealed that less than 50% of abstinent opiate-addicts expressed such concerns, and 50% of anesthesiologists would try to use opiate-free perioperative drugs in patients that expressed concerns of relapse [452]. Similarly, a survey of physicians in Sweden revealed that although it presented them with a dilemma, very few denied prescribing opiates to drug-seeking patients [45]. This may in part be because of the dichotomy presented in the pain management and chemical dependency communities, as the former advocates the safe use of opiates, whereas opiates are the problem to the latter [165].

4. Learning, memory, and reward

In 1999, as in previous years, there was interest in the role of endogenous opiate systems in learning, memory, and reward. In particular, research continued to emphasize that the reinforcing and discriminative properties of opiates provide a means of assessing their motivational and subjective effects, thus evaluating their potential for abuse. A review of the literature indicates that both the primary and conditioned reinforcing effects of opiates, and their role in cognition, involve interactions with both opiate and nonopiate mechanisms [162,346,358].

The role of opiates in mediating classically conditioned responses was examined. In particular, the CPP paradigm was used to evaluate the reinforcing properties of morphine by pairing morphine with a distinctive environment, and a placebo with another environment. The reinforcing properties of morphine were demonstrated because when tested in a drug-free state the animals chose the side paired with morphine [53,110,346,380,396,426,506,583]. However, testing an animal in a drug-free state may not always reflect whether conditioning occurred because mice conditioned to morphine that did not show a CPP when tested in a drug-free state 2 and 3 days later, did when given morphine before testing for place preference [53]. Conversely, conditioned place aversion (CPA) produced by naloxone was demonstrated when animals were primed with naloxone on the test day, but not when tested in a drug-free state [53]. Furthermore, since heroin, fentanyl, cocaine and pentobarbital given on the test day did not facilitate morphine CPP, it is likely that the ability of morphine to cue CPP is explained by state-dependent learning thus reflecting morphine's unique pharmacological profile [53]. Although maximum CPP is demonstrated after 4 pairing with morphine, CPP could be demonstrated in the same animals when additional pairings with a new environment were

made, thus showing that morphine maintains its rewarding properties and tolerance does not occur in this paradigm [110]. The ability of morphine to produce CPP is influenced by pain and inflammation that involves activation of κ but not δ receptors, as an injection of formalin into the hind paw of rats attenuated CPP, that was blocked by nor-BNI but not naltrindole [506].

The antiopiate peptide OFQ/N modulates the acquisition of morphine-induced CPP that depended on the dose used because 3 and 10 nmol, but not 30 nmol, of OFQ/N injected ICV prevented it [380]. In contrast, doses of Tyr-W-MIF (25–100 μg, ICV) suggested to have μ-opiate antagonistic properties did not prevent the development of morphine-induced CPP [396]. Since at low doses, Tyr-W-MIF preferentially binds to both μ_1 receptors and a nonopiate Tyr-MIF-1 site, these findings suggest that either μ_1-opiate receptors are not involved in morphine CPP, or that Tyr-W-MIF modifies the analgesic but not rewarding properties of morphine [396]. However, the former possibility was not supported because the μ_1-opiate agonist etonitazene produced CPP and the μ_1-opiate antagonist naloxonazine blocked it, suggesting the μ_1 receptors are involved in opiate reward [419]. Furthermore, at higher doses Tyr-W-MIF may have μ-opiate agonist properties that are reinforcing because 200 μg of the peptide produced CPP when given alone [396].

The reinforcing properties of morphine are mediated in part by nonopiate systems including mesolimbic NMDA receptors, as injection of the NMDA antagonist NPC 17742 into the nucleus accumbens and ventral tegmental area attenuated the expression of morphine-induced CPP [426]. However, NMDA receptors in the caudate-putamen also may be involved in mediating the rewarding effects opiates because μ and NMDA receptors are highly colocalized in that area [542]. Mesolimbic dopamine systems also are involved as naloxonazine prevents both etonitazene-induced CPP and increases striatal and nucleus accumbens dopamine transmission [418]. Furthermore, CPP produced by the μ-opiate agonist dihydroetorphine involves D_1 but not D_2 receptors because it was attenuated by systemic or intra-accumbens injections of the D_1/D_2 antagonist haloperidol, the D_1 antagonist SCH-23390, but not the D_2 antagonists *l*-sulperide and spiperone [312]. An interaction between morphine reward and galanin receptors in the nucleus accumbens was demonstrated as galanin attenuated morphine CPP, which was correlated with a decrease in galanin binding in the nucleus accumbens after morphine, and an increase after naloxone [583].

Endogenous opiates also modulate CPP produced by other drugs as ethanol-induced CPP in stressed rats was attenuated by β-FNA and naltrindole, indicating a role of both μ- and δ-opiate receptors [343]. Similarly, doses of ethanol (75 mg/kg) that did not produce CPP in nonstressed animals, did so when combined with nonreinforcing doses of morphine and the δ-agonist TAN-67 [341,342,343], that was blocked by β-FNA and naltrindole, respectively [341, 343]. OFQ/N also mediates the reinforcing properties of ethanol because it too blocked ethanol CPP [99]. In contrast, κ-opiate receptors may negatively modulate ethanol reward during stress because ethanol-induced CPP in stressed animals was enhanced by nor-BNI and attenuated by U50488H, and conversely the CPA produced by U50488H was enhanced by stress [343]. Endogenous opiates are not involved in the stress-induced enhancement of amphetamine-induced CPP, however, because naltrexone did not affect it [77].

Conditioned taste aversion (CTA) is another classical conditioning paradigm that occurs when a novel and detectable flavor (conditioned stimulus or CS) is avoided after it has been paired with an aversive stimulus (unconditioned stimulus or US). Naltrexone is aversive because it decreased the intake of a novel Kool-Aid® solution when paired with it [556]. When lithium chloride was used as the aversive US, pretreatment with morphine dose-dependently increased the acquisition of CTA, indicating that morphine can serve as a CS [244,245]. Furthermore, morphine itself may have aversive properties because when given alone at the highest dose (10 mg/kg) it also suppressed fluid intake [245]. In contrast, endogenous opiates are probably not involved in conditioned flavor preferences as naltrexone did not modify the preference for a novel cherry or grape flavored solution (CS) previously paired with a sucrose solution (US) [578].

Analgesic responses to stress can be classically conditioned and involve endogenous opiates because an auditory signal (CS) that was previously paired with lithium chloride (US) produced analgesia (conditioned response or CR) and naloxone blocked it [352]. Furthermore, conditioned analgesia induced by a white noise (CS) that was previously paired with a foot-shock (US) is mediated by μ- but not δ- or κ-opiate receptors in the rostral ventromedial medulla because injections of CTAP, but not naltrindole or nor-BNI into that area prevented it [168]. In contrast, the conditioned analgesia produced by exposure to flies that have had their mouth parts removed in mice that had been exposed to biting flies was not blocked by naloxone and thus is not opiate-mediated [269]. Respiratory depression induced by opiates also can be classically conditioned as a placebo saline injection (CS) given after 2 days of buprenorphine treatment (US) produced a respiratory depressant effect (CR) that was opiate-mediated because naloxone blocked it [46]. Furthermore, it is unlikely that cognitive or motivational factors were involved because the verbal instructions used by the experimenter did not suggest any expectation of respiratory depression, nor did the subjects notice any overt signs of respiratory discomfort [46].

Besides classical conditioned, operant conditioning paradigms also were used to evaluate the rewarding properties of opiates [105,346], including self-administration of morphine [15,335,453], heroin [78,105,298], and methadone [543]. Naloxone increased self-administration of both IV heroin [78] and oral methadone [543], which is suggested to

reflect a compensatory response to a decrease in their opiate-reinforcing value [78]. However, the dose of naloxone was important because low doses increased methadone responding, whereas high doses decreased it [543], and in morphine-dependent rats 0.01 mg/kg naloxone increased heroin self-administration and 0.03 mg/kg decreased it [78]. Although the training dose and time of access did not affect the rate of heroin responding, the naloxone-dose response curve shifted when the training dose and access time was reduced, thus reflecting a decrease in potency [78].

When allowed to self-administer heroin under a progressive ratio schedule, morphine-dependent animals were more motivated to obtain heroin than non-dependent rats as demonstrated by a higher heroin break-point value [78]. Furthermore, in opiate addicts self-administering heroin under a progressive-ratio schedule of reinforcement, although IV heroin was 4-times more potent than intranasal (IN) heroin, their reinforcing efficacy and potential for abuse may not be different because the heroin break-point value was similar for both routes [105]. Therefore, since IN heroin can be self-administered more conveniently than IV heroin, it may become an increasing popular route of administration to abuse [105]. Sex-differences also were noted in morphine self-administration as female rats had higher break-points than males when responding under a progressive ratio schedule of reinforcement [15].

The neural mechanisms that mediate opiate reward were elucidated, and a review of intracranial self-administration and place conditioning studies indicated a critical role of the ventral tegmental area, nucleus accumbens, central gray, hippocampus, and possibly the lateral hypothalamus [346]. Strain differences related to differences in basal opiate activity existed as Lewis rats responded to higher morphine breaking points than Fischer rats that was correlated with differences in basal proenkephalin mRNA levels in the dorsal striatum and nucleus accumbens [335]. However, the role of the nucleus accumbens in mediating drug-seeking behavior is not entirely clear as only 20% of the neurons in that area exhibited naloxone-reversible inhibitory responses after heroin-self administration [298]. There are some indications that the medial prefrontal cortex may be involved in opiate reinforcement because 40% of neurons in that area were inhibited after heroin self-administration, although the small number of neurons studied ($n = 5$) make any conclusions premature [298].

$GABA_B$ receptors are involved in opiate reward because systemically administered baclofen suppressed both the acquisition and maintenance of heroin self-administration, and when injected into the ventral tegmental area baclofen increased it [566]. Furthermore, heroin reinforcement also is related to nucleus accumbens dopamine release that is mediated by $GABA_B$ receptors in the ventral tegmental area, as intra-ventral tegmental area baclofen blocked heroin-induced nucleus accumbens dopamine release that was attenuated by intra-ventral tegmental area injections of the $GABA_B$ antagonist, 2-hydroxysaclofen [566]. A role for the non-traditional opiate OFQ/N was demonstrated in opiate reward because it decreased nucleus accumbens dopamine release that a GABA antagonist injected into the ventral tegmental area attenuated [381]. Adenosine receptors are involved in morphine self-administration because the adenosine antagonists theophylline and DMPX reduced self-administration when given before the test session and increased it when given during the training period, whereas the adenosine agonists NECA and CGS21680 increased self-administration when given before the test session and decreased it when given during the training period [453]. It is likely that these effects are due to interactions with adenosine A_2 receptors because adenosine A_1 receptor agonists and antagonists had no effect [453]. A role for Δ^9-tetrahydrocannabinol (THC) was not shown, however, as maternal exposure to THC did not modify morphine self-administration in adult male and female rats [15].

Endogenous opiates mediate operant responding to other types of reinforcement, including ethanol [55,177,178,218, 231,256,361,442,556,557,558], cocaine [80,114,115,355, 462], phencyclidine [442,558], sucrose [558,603], saccharin [256,442], milk [486,487], food [70,442,469,539], and water [361,557]. In general, opiate antagonists reduced ethanol-maintained responding that was independent of the species studied, as they reduced it in monkeys [442,556,557, 558], rats [55,177,231,256], and mice [361]. The effects of naltrexone on ethanol-maintained responding depended on the regimen of administration because an acute injection did not alter responding, whereas repeated treatment with the same dose progressively suppressed responding [55]. The motivation to consume alcohol also was an important factor because an acute injection of naltrexone suppressed ethanol-maintained responding for longer periods in ethanol-experienced rats that were ethanol-deprived as compared with non-deprived ones [231]. Similarly, acute naltrexone inhibited the reinstatement of ethanol responding produced by non-contingent delivery of ethanol, and increased extinction when ethanol was substituted with water [55].

The alcohol content was important because naloxone had a greater effect on operant responding for beer containing 4.5% ethanol v/v as compared with beer containing less than 0.5% ethanol v/v [177]. However, naltrexone only decreased ethanol-responding when it was the preferred solution as naltrexone decreased responding to a 2% ethanol concentration that was preferred to water, but did not decrease responding to a 32% ethanol solution that was not preferred to water [557]. When the rats were presented with an ethanol concentration (8%) and water that were equally preferred naltrexone decreased both [557]. Similarly, naltrexone decreased ethanol responding when it was preferred to water in low thirsty rats, although selectivity for ethanol reward may exist because in thirsty mice a dose of naltrexone (1.25 mg/kg) that reduced ethanol-reinforced responding did not reduce water-reinforced responding [361]. It is important to recognize, however, the effects of naltrexone on operant responding also could be related to an aversive

introceptive state produced by the antagonist that is incompatible with consummatory behaviors because it also produces a CTA [556] and CPA [53].

It is likely that opiate receptors in the amygdala and nucleus accumbens are involved in ethanol-reinforced responding because injection of methylnaloxonium into those areas reduced it [218]. A role for μ-opiate receptors was suggested as both naltrexone and quadazocine reduced ethanol-maintained responding in rhesus monkeys at doses that correlate with their selectivity for the μ-opiate receptor [558]. However, the effects were not entirely selective for ethanol because the antagonists also reduced responding for sucrose and phencyclidine in the same animals [558]. In contrast, when rhesus monkeys were presented with progressive ratio schedules of reinforcement, naltrexone attenuated operant responding and decreased break point values for both ethanol and saccharin, but did not alter responding maintained by phencyclidine and food [442]. A role of δ-opiate receptors in mediating ethanol reward was shown as the δ_2 antagonist naltriben attenuated ethanol-responding in alcohol-preferring rats, which was specific for ethanol because doses that suppressed ethanol responding did not affect the responding maintained by saccharin [256].

Morphine and dezocine suppressed food-maintained responding that was μ-mediated because both naltrexone and β-FNA antagonized their effects [539]. Food-maintained responding under a fixed ratio 20 schedule involves κ-opiate receptors because U69593 decreased responding and quadazocine blocked that decrease [70]. However, although the endogenous κ-opiate dynorphin A (1–17) also decreased responding, it was not because of interactions with μ or κ receptors because quadazocine did not block its effects [70]. Decreases of food-maintained responding that occurred after naloxone may serve as an index of opiate-dependence because the suppression of operant responding progressively increased with repeated morphine injections [469].

The reinforcing properties of cocaine involve μ-opiate receptors as injections of DAMGO into the ventral tegmental area [114] and pedunculopontine tegmental nucleus [115] altered IV cocaine self-administration. A role of κ-opiate receptors also was demonstrated because U69593 decreased responding to low doses of cocaine and attenuated cocaine-induced reinstatement of drug-seeking behavior, suggesting that κ-opiate antagonists may have potential for relapse prevention [462]. In contrast, injections of U50488H and nor-BNI into the ventral tegmental area did not alter cocaine self-administration, indicating that κ-opiate receptors in that area are not involved [114]. However, there are indication that κ-opiate receptors in the nucleus accumbens may modulate cocaine reinforcement, as the sites for κ-opiate receptor activation are mainly on axon terminals containing dopamine [508]. Self-administration of 'speedball' combination of heroin and cocaine was reduced by combinations of quadazocine and flupenthixol, but not by the antagonists given alone, suggesting that treatment of speedball abuse may require antagonists that target both opiate and dopamine receptors [355].

Opiate agonists and antagonists have been used as stimuli in drug discrimination tasks, in which animals trained to respond differentially to a test dose of the drug from vehicle. As in previous years, drug discrimination paradigms were useful to both identify the neurobiological mechanisms of drug action, and to evaluate the abuse liability of opiates [104]. When morphine was used as the discriminative stimulus (S^D) against saline, the S^D effects of morphine generalized to fentanyl and naltrexone antagonized it, suggesting that the stimulus properties of morphine are μ-opiate-mediated [423]. In contrast, κ and δ receptors are probably not involved because the κ-agonists U50488H [423] and U69593 [119], and the δ-agonists SNC 80 and BW 373U86 [423], did not substitute for morphine.

Sex differences existed in the potency of morphine as a S^D because the ED_{50} for morphine discrimination was lower in female than in males trained to discriminate 3.0 mg/kg morphine from saline [119]. Furthermore, the ED_{50} for morphine discrimination was higher in gonadectomized rats than intact ones, and although S^D effects of morphine generalized to fentanyl in both males and females, the partial μ-agonists buprenorphine and nalbuphine substituted for morphine in females and intact males but in only four of seven gonadectomized males [119]. The mechanisms that mediate these differences, however, are not clear because hormone replacement did not reverse the effects of gonadectomy, and although naltrexone differentially antagonized the stimulus effects of morphine, no group differences found in opiate receptors that mediate the S^D effects of morphine [119]. The potency of morphine as the S^D did not depend on the strain of rats studied because the ED_{50} for morphine discrimination against water was the same in F344, Sprague-Dawley, Long-Evans, and Lewis rats [374]. Similarly, the S^D effects of morphine generalized to levorphanol, buprenorphine, butorphanol, and nalbuphine equally across strains [374]. Since strain differences in the analgesic effects of the agonists were related to their intrinsic activity at the μ-opiate receptor, it is likely that different neural systems mediate the analgesic and S^D effects of opiates [374].

Whether or not opiates could substitute for the S^D effects of morphine varied with the training dose as morphine generalized to nalorphine in rats trained with a low dose (5.6 mg/kg) of morphine but not in those trained with a high dose (10 mg/kg) [196]. An opiate mechanism is indicated because naloxone blocked both the S^D effects of morphine, and the ability of nalorphine to substitute for morphine in the low dose group [196]. In contrast, methadone generalized to morphine in rats trained at higher doses of morphine as well, thus reflecting differences between the methadone and nalorphine in their intrinsic activity at the opiate receptor [196]. In pigeons trained to discriminate between morphine, nalbuphine, and saline, nalbuphine only produced nalbuphine-like responding, whereas low doses of morphine

produced saline-like responding, intermediate doses produced nalbuphine-like responding, and high doses produced morphine-like responding [538]. It is likely that these differences in substitution patterns reflect differences in μ-opiate efficacy between morphine and nalbuphine, rather than differences in receptor selectivity, because the same dose of naltrexone reversed the S^D effects of both morphine and nalbuphine [538]. Opiate agonists also produce discriminative stimulus effects in humans, as six of eight nondependent heroin users could discriminate between saline and different doses of hydromorphone [253]. Consistent with the animal studies showing that generalization from high training doses requires opiates with higher efficacy than do lower training doses, pentazocine, butorphanol, nalbuphine, and buprenorphine produced generalization with low but not high training doses, whereas hydromorphone produced generalizations with both training doses [253].

The S^D effects of butorphanol are primarily μ-mediated because naloxone was more potent than naltrindole in antagonizing the stimulus effects, and the μ-opiate agonists morphine, methadone, fentanyl, buprenorphine, pentazocine, nalbuphine, metazocine, and nalorphine substituted completely for butorphanol [417]. Furthermore, although the potency of the agonists decreased as the training dose of butorphanol increased, differences in intrinsic activity existed because the rank order of potency for the agonists was the similar across all doses tested [417]. In contrast, various κ-agonists only partially substituted for butorphanol in the low dose group that was not reversed by naloxone, and δ-agonists completely substituted for butorphanol in the low dose group but only partially in the medium and high training dose groups. The ability of δ-agonists to produce butorphanol-like responding is likely explained by its actions at the δ receptor because naltrindole but not naloxone blocked the effects [417]. Taken together, these results suggest that μ-, κ-, and δ-agonists share stimulus properties with low training doses of butorphanol, whereas only μ-agonists share stimulus properties with higher training doses [417]. However, although butorphanol did not substitute for the S^D effects of the κ-agonist, EKC, it did so when challenged with an insurmountable amount of the μ-opiate antagonist clocinnamox, suggesting that κ-agonist-like stimulus effects for butorphanol can be demonstrated when its μ-agonist properties are abolished [534].

In studies that used naloxone or naltrexone as the S^D, morphine-dependent animals could discriminate the antagonists against saline, and was used to measure the subjective effects associated with opiate withdrawal [147,181,301]. In morphine-deprived monkeys, morphine [181,301], hydrocodone, and hydromorphone [301] reversed naltrexone-lever responding that was opiate-mediated because naltrexone shifted the discriminative effects of the agonists to the right [181, 301]. The discriminative effects of hydrocodone may in part be dependent upon its conversion to hydromorphone because budipine, which diminishes the conversion of hydrocodone to hydromorphone, also attenuated the effects of the hydrocodone [301]. However, more research is necessary to address this issue because budipine itself may have opiate antagonist properties as it also diminished the effects of hydromorphone [301].

There was continued interest in the interactions between the discriminative stimulus effects of opiates and dopaminergic compounds, primarily because of the increasing use of opiate/stimulant combinations (i.e. speedballs). When cocaine was used as the S^D, cocaine-opiate interactions depended on the training dose and opiate agonist studied as morphine substituted for cocaine in the low but not high training dose condition, whereas U50488H did not produce cocaine-like responding in either condition [262]. In the low dose condition, pretreatment with morphine enhanced the S^D effects of cocaine and U50488H attenuated it, suggesting that μ and κ receptors have opposing effects on the S^D effects of cocaine, and that κ-agonists may have therapeutic potential for the treatment of cocaine abuse [262]. However, opiate-stimulant interactions may be related to individual differences because the S^D effects of morphine generalized to cocaine and amphetamine in three of five squirrel monkeys, which the dopamine antagonist flupenthixol but not naltrexone blocked [423]. Similarly, pretreatment with cocaine or amphetamine increased the S^D effects of morphine in these three monkeys, but attenuated or did not affect it in the other two monkeys [423]. This raises the possibility that similar individual differences exist in humans such that some people combine stimulants and opiates to enhance euphoria, whereas others combine them to attenuate the unwanted side effects [423]. Furthermore, since no individual differences were found in the S^D effects of morphine, it is likely that the neural mechanisms that mediate the S^D effects of morphine and the morphine-like effects of cocaine and amphetamine are different [423]. The involvement of opiates in mediating the S^D effects of ethanol also was shown because both naltrexone [282,361] and morphine [282] attenuated the S^D effects of ethanol. The stimulus properties of noribogaine, a metabolite of ibogaine, are not explained by its interaction with the κ receptor because it did not substitute for the S^D effects of U50488H [599].

Morphine produces state-dependent learning as it impaired retrieval of an operant task when the task was acquired in a drug-free state, but not when morphine was given during acquisition [486,487]. Conversely, when tested in a drug-free state, retrieval was impaired only when morphine was present during acquisition [486,487]. It is likely that μ-opiate receptor activation is involved because naloxone, but not naltrindole, antagonized the effects of morphine, and retrieval partially occurred in rats trained with morphine and then tested with heroin or (-)-cyclazocine, but not when tested with U50488H, (+)-cyclazocine, and SNC80 [487]. Furthermore, since retrieval was not complete when higher or lower doses of morphine were given than those during acquisition, and bell-shaped responses similarly occurred with heroin and (-)-cyclazocine, the degree of receptor activation is important [487].

Naloxone may be effective in ameliorating cognitive deficits associated with electroconvulsive therapy because when given before therapy it reduced anterograde amnesia, and increased verbal fluency and attention [433]. OFQ/N is involved in learning and memory as OFQ/N-knockout mice performed better in a variety of cognitive tasks, as compared with wild-type mice [385], and normal mice treated with OFQ/N were impaired [220,221,323,385]. However, a traditional opiate receptor site does not mediate the memory impairment produced by OFQ/N because naloxone and U50488H had no effects [323]. Rather, a naloxone benzoylhydrazone recognition site may be involved because OFQ/N impaired performance on a passive avoidance task that was blocked by naloxone benzoylhydrazone [323]. Furthermore, nocistatin, a biologic active peptide isolated from the same precursor as OFQ/N, also may play a modulatory role in learning and memory because it too attenuated OFQ/N-induced memory impairments [221]. Dynorphin A (1–8) may be involved in age-related memory deficits as a decrease in peptide immunoreactivity was noted in the hippocampus of aged rats that showed memory impairment in the water maze [120].

5. Eating and drinking

In 1999, there was interest in the opiate modulation of eating and drinking. In particular, research continued to emphasize the interactions between opiate systems, motivational states, and palatability of foods. However, it is becoming increasingly evident that palatability is both a dynamic and relative characteristic because when given concurrent choices between foods of different palatability opiate agonists and antagonists selectively influence the intake of the preferred one [187,361,442,557].

Opiate agonists stimulated feeding, including the μ-agonist DAMGO which increased food (lab chow) but not water intake when injected into the paraventricular nucleus [187]. However, when presented with a choice between lab chow and 10% sucrose solution, DAMGO only increased the intake of the 10% sucrose solution [187]. Therefore, these results indicate that endogenous opiates mediate the palatability of foods, and that the rewarding value of a food changes as a function of the choices presented [187]. Rat chow did not alter morphine analgesia even when levels of protein, minerals, or vitamins were manipulated, suggesting that the enhancement of morphine analgesia observed after long-term intake of a palatable sucrose solution is explained by an activation of endogenous opiate systems that is not related to levels of protein or vitamins and minerals [259]. Furthermore, endogenous opiates are not involved in the modulating the energy needs of an organism because although a sucrose solution has less calories than the rat chow, DAMGO-treated rats derived 85% of their caloric intake from the sucrose solution [187].

Not all opiate agonists increased feeding, however, as the κ-opiate agonist U69593 decreased food-maintained responding that was opiate-mediated because quadazocine blocked it [70]. In contrast, although IV dynorphin A (1–17) also decreased food-responding, it did so to a lesser extent that was not blocked by quadazocine, an indication that nonopiate mechanisms are involved [70]. The reasons for the differential effects of U69593 and dynorphin A (1–17) are not clear, but may be related to their differential ability to cross the blood-brain barrier after systemic injection and access κ-opiate receptors located in the brain that mediate feeding [70], or to possible nonopiate actions of dynorphins [511]. Indeed, a number of differences have been noted in the ability of peptides to cross the blood-brain barrier, indicating that it is a dynamic process and cannot be predicted solely based on size, lipophilicity, and receptor binding characteristics [265].

The effects of opiate antagonists were inconsistent as they sometimes decreased eating and drinking [44,103,231, 236,330,361,442,472,556,557,558], or had no effect [117, 170,442,557]. However, because opiate antagonists are known to produce both CTA [556] and CPA [53], it is possible that they also may have nonspecific effects related to nausea and aversion. Naltrexone suppressed saccharin- but not food-maintained responding in rhesus monkeys that is not explained by differences in their reinforcing effectiveness because naltrexone similarly decreased break point values for saccharin but not food when monkeys were presented with a progressive ratio schedule of reinforcement [442]. When presented with a choice between two solutions, naltrexone preferentially decreased responding to the preferred, more palatable solution as it suppressed water intake when it was preferred to a 32% ethanol [557] or saccharin solution [442], and preferentially decreased ethanol when it was preferred to water [361,557]. Similarly, when presented with a choice between a sucrose solution that was preferred to a saccharin solution, naltrexone selectively decreased the intake of the sucrose solution in freely fed rats, that was not explained by differences in post-ingestion actions because the rats had open gastric fistula [578]. The motivational state of the animal also may be important because naltrexone equally decreased the intake of both the sucrose or saccharin in hungry rats, and naltrexone preferentially decreased ethanol responding in thirsty mice even though it was equally preferred to water [361]. Selectivity may exist, however, because when presented with a choice between equally preferred solutions of ethanol and saccharin, higher doses of naloxone and the δ_2 antagonist naltriben were required to suppress saccharin responding as compared with ethanol [256]. A role for the antiopiate peptide OFQ/N was not demonstrated in food intake, although there was a slight but not significant trend to increase it [99].

Besides mediating food-preferences based on the taste of the food, endogenous opiates mediate flavor preferences that are socially transmitted because mice ate more of a cocoa-flavored solution when exposed to another mouse that had previously eaten a cocoa diet, and naltrexone

blocked it [369]. Furthermore, although naltrexone blocked the preference when given before or after the interaction, it is likely that opiates are involved in the expression but not acquisition of socially acquired food preferences because naloxone, which is shorter lasting than naltrexone, had no effect when given before the interaction [369]. Although naltrexone reduces the intake of sweet flavored solutions, endogenous opiates are probably not involved in conditioned flavor preferences as naltrexone did not modify the preference for a novel cherry or grape flavored solution previously paired with a sucrose solution [578].

Both naltrexone and quadazocine reduced sucrose intake in rhesus monkeys that is probably related to their μ-opiate activity because the doses correlated with their selectivity for that receptor [558]. However, whether the effects of the antagonists on sucrose intake are related to palatability or are because of nonspecific aversive effects produced by the antagonists is not clear as they also reduced intake of PCP which has a taste that is similar to quinine [558]. In contrast, although acute naloxone treatment reduces both water and food intake, intake eventually returns to normal after prolonged naloxone treatment and surpasses control levels after the antagonist is removed [236]. Although the overshoot after antagonist removal may be explained by a compensatory response to restore body weight and nutrient balance, a concurrent up-regulation of opiate receptors suggests that changes endogenous opiate systems also may be involved [117,236].

The neural mechanisms that mediate opiate-induced feeding have been further elucidated. Naloxone suppressed food intake in rats that was potentiated by knife cuts placed ventral to the dorsomedial hypothalamic nucleus, suggesting that this brain area is part of an opiate-mediated feeding system [44]. In contrast, feeding was not related to basal opiate activity in the dorsal striatum and nucleus accumbens as food-reinforced behavior was similar between Fischer and Lewis rats, even though basal proenkephalin mRNA levels are higher in the Fischer strain [335]. However, although the strains were not compared for responding to more pleasurable foods, there are indications that the two strains might be different in that respect because differences were noted between Fischer and Lewis rats in the reinforcing actions of morphine [335]. Parabrachial nucleus μ-opiate receptors are localized on amygdaloid projection neurons, indicating that ability of μ-antagonists to attenuate eating when injected into the parabrachial nucleus may be explained by projections to the amygdala [90]. Similarly, the ability of μ-agonists to increase eating when injected into the nucleus of the solitary tract also may be modulated by the amygdala as μ-opiate receptors are localized in targets of efferent projections from the central nucleus of the amygdala to the nucleus of the solitary tract [416].

There is evidence that endogenous opiates are involved in obesity because leptin-receptor deficient obese rats had lower levels of hypothalamic proopiomelanocortin (POMC) and β-endorphin mRNA than lean rats [281], and systemic administration of naltrexone decreased daily food intake and body weight in obese Zucker rats that was reversed by morphine pretreatment [330]. Overconsumption of palatable foods can alter endogenous opiates systems and may contribute to obesity by increasing the pleasurable response to foods because naloxone suppression of sucrose intake was greater in rats that had been given free access to sucrose for 3 months as compared with sucrose-naive controls [472]. Similarly, the dietary history is important in determining the effects of opiate antagonists as μ-, δ_2- and κ_1-antagonists produced greater weight loss in rats given a high-fat diet relative to those given a high-carbohydrate diet [103]. The opiate-receptor subtypes that mediate weight loss for high-fat or -carbohydrate diets are likely different because the μ-antagonist was more potent than δ_2- and κ_1-antagonists in the high-carbohydrate group, whereas both the μ- and δ_2-antagonists were more potent than the κ_1-antagonist in the high-fat group [103]. However, since the differential effects of the antagonists on weight loss could not be predicted by differential effects on food intake, the effects of the antagonists are probably related to differences in gastrointestinal transit and absorption [103]. Hyperphagia is commonly found in infants withdrawing from methadone, but is likely related to higher metabolic demands in these infants because it was not associated with abnormal weight gains [336].

Acute ICV injections of zinc decreased water intake in dehydrated rats that involved endogenous opiates because naloxone attenuated it at doses that when given alone had no effect [170]. Acute fasting decreased hypothalamic POMC mRNA and lipopolysaccharide up-regulated preprodynorphin mRNA in fed rats but not fasted ones, an indication that fasting impairs endogenous opiate systems [182]. Furthermore, although preprodynorphin mRNA was not affected by fasting, it increased 42% in animals re-fed after fasting, which may reflect an increase in the rewarding properties of the food [182]. Endogenous opiates are involved in thyrotropin-stimulating hormone secretion during starvation because when combined with the cholinergic agonist pyridostigmine, naloxone restored the blunted thyrotropin-stimulating hormone response to thyrotropin-releasing hormone after prolonged starvation [102]. Although the role of endogenous opiates in mediating age-related decreases in eating remains to be clarified, a review of the literature indicates that impairments in opiate function may be a contributing factor [58,376]. Stress also decreased feeding that involved endogenous opiate systems because restraint reduced sucrose intake in morphine- but not vehicle-treated rats that was blocked by naloxone [602]. However, although interleukin-1 (IL-1) influence the expression of opiates [225], endogenous opiates are not involved in illness-induced hypophagia as IL-1 decreased intake of a sweetened milk solution that was unaffected by naloxone [510].

6. Alcohol and other drugs of abuse

In 1999, as in previous years, there was continued interest in the modulation of alcohol intake by endogenous opiates. A number of different paradigms were used to study the effects of opiate antagonist on alcohol intake, including limited alcohol access [218,236,401], two-bottle free choice [99,116,117,202,218,383,236,401], and ethanol-reinforced responding [55,177,178,231,256,282,361,442,556,557,558]. However, although opiate antagonists generally decreased alcohol consumption that was independent of the paradigm used, a number of issues still remain to be clarified regarding their mechanism of action. First, since opiate antagonists also decreased consumption of nonalcoholic solutions [137], it is unclear to what extent their effects are specific to alcohol consumption or on consumption in general. Secondly, because opiate antagonists produce CPA [53] and CTA [556], their effects on ethanol consumption could be related to an aversive introceptive state produced by the antagonist that is incompatible with consummatory behaviors [556]. However, selectivity for ethanol consumption apparently exists because doses of naltrexone that reduced ethanol consumption did not reduce water [32,361] or saccharin [442] responding. Similarly, a dose of naltrexone (3 mg/kg) that did not modify water-reinforced responding, decreased ethanol responding during extinction and inhibited reinstatement of ethanol-responding produced by a non-contingent delivery of ethanol [55], or after a period of abstinence [32]. Furthermore, when presented with a progressive ratio of reinforcement, naltrexone decreased break point values for ethanol but not food [442], and naloxone decreased the break point values more for beer containing 4.5% ethanol v/v than to the same brand of "near-beer" containing only 0.5% ethanol v/v [177,178]. Since lower break point values reflect lower reinforcing effectiveness it suggests that naloxone interacts with brain reward systems to decrease ethanol intake [177,178,442]. Therefore, although opiate antagonist have nonspecific effects that should be considered when interpreting their effects on alcohol consumption, it also is apparent that opiate antagonist can decrease consumption by modulating the reinforcing properties of alcohol as well.

Both naltrexone and quadazocine reduced ethanol reinforced-responding, but the effects were not specific for alcohol because the same doses reduced responding for sucrose and PCP, thus indicating that ethanol, sucrose, and PCP produce similar degrees of opiate activation [558]. The effects of naltrexone on ethanol responding, however, depended on both the concentration used and whether alternative solutions were available, as it decreased responding to a 2% ethanol concentration that was preferred to concurrently available water, but did not decrease responding to a 32% ethanol solution that was not preferred to water [557]. These results suggests that naloxone modulates responding to the preferred solutions rather than interacting specifically with ethanol's pharmacological effects [557]. In contrast, although ethanol-responding decreased as a function of concentration, total ethanol intake constantly remained above 1 g/kg, raising the possibility that at high concentrations the taste of the ethanol was aversive, and the increased water intake served to dilute that taste [557]. Furthermore, the interaction between ethanol and naltrexone was not typical of opiate antagonist effects because the suppressive effects were not overcome by increasing the concentration of the ethanol [557], and the doses of naltrexone and quadazocine required to reduce ethanol consumption were higher than those needed to antagonize exogenous opiate effects [558]. Interestingly, although opiate agonists would be expected to increase ethanol consumption because antagonist typically decreased it, comparable to naltrexone morphine also decreased ethanol-responding in a drug-discrimination task [282]. It is possible that their similar effects are related to a common non-opiate pharmacokinetic and pharmacodynamic mechanism because both naltrexone and morphine decreased plasma levels of ethanol and delayed the time of peak concentrations [282]. An interaction between endogenous opiates and non-opiate systems was demonstrated as morphine increased dopamine metabolism and release more in the caudate-putamen of alcohol-preferring rats than in non-preferring ones [228], and may be important in mediating the reinforcing properties of ethanol [116].

The effects of chronic opiate antagonist treatment also was examined, and the results are important to evaluate the clinical benefits of long-term antagonist treatment for alcohol dependence. In high-alcohol drinking rats, continuous infusion of naloxone via osmotic minipumps decreased alcohol drinking that was independent of the dose used because infusions of 0.3 and 3.0 mg/kg/h produced similar decreases in a 1-h limited access procedure during the entire 7 days of treatment [236]. Consistent with this finding, continuous infusion of the 3.0 mg/kg/h dose regimen also suppressed ethanol consumption with a continual 24-h access procedure [236]. The clinical significance and specificity of this procedure is questionable, however, because naloxone also suppressed water- and food-intake, and up-regulated μ-, δ-, and κ-opiate receptor binding that could lead to a rebound increase in alcohol craving when the antagonist is removed [117,236,401]. Furthermore, tolerance may develop to continuous opiate receptor blockade because infusion of naloxone [401] or naltrexone [117] via osmotic minipumps produced an initial suppression in both limited and continuous consumption of alcohol that gradually returned to baseline. It is possible that this tolerance is related to opiate receptor up-regulation because concurrent increases in opiate receptor density were noted in the various brain areas after the antagonist treatment [117,401].

The effects of prolonged naloxone treatment depended on the route of delivery as daily SC injections of 0.5 or 2.5 mg/kg naloxone before a 1-h limited access to alcohol suppressed intake for up to 14 days, although similar to continuous infusion tolerance developed in rats given continuous access to alcohol [401]. Differences in the route of administration were even more evident when motivation to drink alcohol was high as continuous naltrexone infusion

for 3 days increased both ethanol intake and preference in ethanol-experienced rats deprived of alcohol, whereas intermittent treatment with a comparable dose of naltrexone (5 mg/kg) suppressed it [231]. Similarly, in alcohol-preferring rats, daily injections of naloxone suppressed alcohol intake for up to 30 days, and inhibited reinstatement of ethanol-responding after a period of abstinence [32]. Although acute ICV injection of the antiopiate peptide OFQ/N increased ethanol intake, subchronic treatment for 7 days progressively reduced ethanol but not food intake, that gradually returned to baseline levels when OFQ/N treatment ended [99]. Since OFQ/N also blocked ethanol-induced CPP, it suggests that the increase after an acute injection reflects a compensatory response to the reduction in ethanol reinforcement, that is followed by an extinction of ethanol drinking after chronic treatment [99].

The brain areas involved in the opiate-mediation of alcohol consumption were elucidated. A role for nucleus accumbens μ-opiate receptors was demonstrated as antisense oligodeoxynucleotide directed against the μ-opiate receptor, but not the corresponding mismatch antisense, injected into the nucleus accumbens dose-dependently decreased free-access alcohol drinking in high-alcohol preferring rats [383]. Similarly, injections of methylnaloxonium into both the nucleus accumbens and amygdala reduced ethanol but not water self-administration when concurrently available [218]. However, although the dissociation between the effects of methylnaloxonium on ethanol and water responding suggests that the effects were ethanol-specific, it is important to note that since baseline responding for ethanol was higher than the concurrently available water, the antagonistic may have modulated responses to the preferred solution [218].

Alcohol intake is associated with changes in endogenous opiate systems that depended on the brain area studied, as ethanol consumption up-regulated μ-opiate receptor density in the caudate, island of callega, nucleus accumbens, olfactory tubercle, and septum, but down-regulated receptor density in the substantia nigra in ethanol-preferring Fawn-Hooded rats [117]. However, the effects of ethanol on endogenous opiate systems also are strain-dependent as μ-opiate receptor density in outbred Wistar rats was down-regulated in the nucleus accumbens and striatum after long-term exposure to ethanol [521]. Although δ-opiate receptors may be involved in alcohol abuse [293], their role is not clear because both naloxone and the δ_2-antagonist naltriben reduced ethanol-reinforced responding in alcohol-preferring rats [256], whereas chronic ethanol treatment did not change δ_1 or δ_2 receptor density in the nucleus accumbens or striatum [521]. The reasons for these discrepancies are not known, but suggests that either the δ-opiate receptors that mediate ethanol consumption are located in other brain areas, or that chronic ethanol treatment affects the affinity rather than the density of δ-opiate receptors [521]. Prenatal exposure to alcohol also can produce changes in endogenous opiate systems as proenkephalin mRNA was increased in the nucleus accumbens and striatum in rats exposed to ethanol in utero [143]. However, although the increases in the nucleus accumbens persisted up to postnatal day 19, its functional significance is not clear because no change in proenkephalin content was found in that brain area [143].

Rodent lines that were bred for low or high alcohol preference were used to examine the contribution of genetics to alcohol consumption. However, it is important to note that although studies have demonstrated that differences in endogenous opiate systems exist between lines of rats that differ in alcohol preference, the modifications are not always the same but are discrepant, an indication that some of the differences are not directly related to alcohol preference [154]. Genetic modification in endogenous opiate systems may account for differences in alcohol consumption in rats selectively bred for alcohol preference because, as compared with Sardinian alcohol-nonpreferring rats, Sardinian alcohol-preferring rats had reduced μ- and δ-opiate receptors binding in the caudate-putamen [154]. Furthermore, [^{3}H]naloxone binding also was reduced in the nucleus accumbens of Sardinian alcohol-preferring rats that may or may not be because of its affinity for the κ-opiate receptor. [154]. Enkephalin mRNA also differed between the strains that depended on the brain areas studied as the levels were lower in the ventral caudate and higher in the cerebral cortex of alcohol-preferring rats as compared with alcohol-nonpreferring rats [154]. Since the rats were alcohol-naive, these differences are not a consequence of alcohol intake and suggests that alcoholism may be related to a genetic predisposition [154].

Differences in alcohol consumption between C57BL/6 (alcohol-preferring) and DBA/2 (alcohol-avoiding) mice also is related to differences in endogenous opiated systems because as compared with DBA/2 mice, C57BL/6 mice had a higher content of POMC mRNA in the arcuate nucleus and higher proenkephalin mRNA in the nucleus accumbens and caudate-putamen [242]. In contrast, Met-enkephalin content in the amygdala, hippocampus, ventral tegmental area, and periaqueductal gray was lower in C57BL/6 mice as compared with the DBA/2 strain [242]. Although no differences in β-endorphin content were found in any brain region between C57BL/6 and DBA/2 mice [242], a role for β-endorphin was demonstrated because β-endorphin deficient C57BL/6 mice (knockouts) drank less alcohol in a two-bottle free choice paradigm, as compared with wild-type controls [202].

Genetic variation in μ-opiate receptor may contribute to alcohol dependency in humans, as the +118A/G polymorphism within the μ-opiate receptor gene was associated with alcoholism, as compared with nonalcoholic controls [518]. The functional significant of the +118A/G polymorphism is not clear, and although its differential affinity for β-endorphin suggests that β-endorphin is involved [518], no differences were found in cerebrospinal fluid levels of β-endorphin between early- and late-onset alcoholics and controls [413]. Furthermore, the genetic variation may not

be specific to alcohol dependency because the nonalcoholic controls were not selected out for other abuse or psychiatric disorders [518]. In male subjects with substance abuse including alcohol, genetic variations of the enkephalinase gene were associated with a low amplitude P300 wave [107]. Since hereditary alcoholism has been linked to low amplitude P300 waves, reduced enkephalin activity may contribute to a genetic predisposition to abuse alcohol [107]. An association between genetic variation of the δ-opiate receptor 921T/C allele and alcohol dependence was not demonstrated because no differences were found between alcohol-dependent subjects and controls, nor was there a preferential transmission of the C allele from alcoholic parent to offspring [169].

Altered opiate control over the HPA axis may contribute to alcoholism as naltrexone produced a significant rise in ACTH and cortisol in alcohol-dependent subjects as compared with placebo controls [156]. Similarly, nonalcoholic male sons with a family history of alcohol dependence had higher ACTH responses to naloxone as compared with males without a family history of alcoholism, and to females with or without a family history of alcoholism [541]. Although it is not conclusive that stress is related to alcoholism [484], or that an altered HPA responses stress contributes to both alcoholism [156] and the high incidence of alcoholism in male children of alcoholics [541], it is interesting to note that in animals stress enhances the rewarding effects of ethanol [341,342,343].

Besides alcohol consumption, the role of endogenous opiates in modulating alcohol's motivational effects was examined in the CPP paradigm. The rewarding effects of ethanol depended on stress because ethanol produced CPP in the presence of foot-shock, but not in the absence of the stressor [341,342,343]. It is likely that ethanol-induced CPP in stressed rats is mediated by both μ- and δ-opiate receptors because it was attenuated by β-FNA and naltrindole [343]. Conversely, nonreinforcing doses of ethanol produced CPP in nonstressed animals when combined with nonreinforcing doses of morphine and the δ-agonist TAN-67, that was blocked by β-FNA and naltrindole, respectively [341,342,343]. In contrast, ethanol-induced CPP in stressed animals was enhanced by nor-BNI and attenuated by U50488H, and U50488H produced a CPA that was enhanced by stress, indicating that κ-opiate receptors negatively modulate ethanol reinforcement [343]. Ethanol also may affect sexual function that is opiate-mediated because chronic ethanol exposure suppressed testosterone and naltrexone blocked the suppression [150].

Considering that opiate antagonists reduce alcohol consumption in animals, naltrexone has been evaluated clinically for the treatment of alcoholism [484]. A review of the literature indicated that although naltrexone may decrease drinking and the risk of relapse in heavy drinkers, it may not substantially increase the incidence of complete abstinence [179,223,484]. Naltrexone decreased the incidence of relapse that may be related to a decrease in cue-elicited cravings, as fewer abstinent alcohol-dependent subjects given naltrexone for 1 week reported the urge to drink when exposed to alcohol, as compared with placebo controls [372]. In contrast, endogenous opiates do not mediate alcohol effects in healthy alcohol-nondependent subjects, because naltrexone did not modify the acute subjective effects of ethanol or ethanol-impaired performance [450]. In a randomized, double-blind crossover study, heavy beer drinkers given naltrexone for 7 days consumed less beer, had fewer urges to consume alcohol, took longer to finish each glass of beer, and were more likely to stop drinking earlier than placebo control [130]. However, it is not clear why some subjects drank more beer after naltrexone, as it was not related to a family history of alcohol dependence or to differences in blood levels of the active metabolite β-naltrexol [130].

Although naltrexone decreased positive mood states, suggesting a decease in ethanol reward [130], it also increased the incidence of nausea and headache [130,223] that may have contributed to the observed reductions in alcohol intake [130]. In fact, in social drinkers naltrexone reduced alcohol consumption, but similar to the studies in animals [557,558] it also decreased intake of a nonalcoholic placebo beverage, thus calling into question the specificity of its effect [137]. Naltrexone treatment may benefit from concurrent cognitive behavioral therapy, as abstinent alcohol-dependent patients treated with 12 weekly sessions cognitive behavioral therapy drank less, took longer to relapse, had more time between relapse, and had fewer urges to drink alcohol when given naltrexone than when given placebo [21]. However, since both naltrexone- and placebo-treated subjects received cognitive behavioral therapy, the specific contribution of the therapy cannot be determined. The neurophysiological mechanisms that mediate naloxone's effects in alcoholics is not understood but may be related to metabolic activity in the basal ganglia and mesial temporal lobe because regional cerebral blood flow in these areas was decreased after naltrexone in abstinent alcoholic patients [81]. This may play a role in naltrexone's effect on drug craving, as these areas are also involved in emotional memories and obsessive-compulsive disorder [81].

In addition to alcohol, endogenous opiates are involved in the reinforcing actions of cocaine [80,114,115,259,423]. Morphine modified the reinforcing effects of cocaine that depended on the training dose used as it enhanced the stimulus effects of a low training dose of cocaine (3.0 mg/kg) but not a high dose (10 mg/kg) [259]. This result is consistent with the notion that stimulant and opiates are often abused together to enhance euphoria [259]. However, pretreatment with cocaine or amphetamine increased the stimulus effects of morphine in some monkeys, but attenu ated it in the others, suggesting that although some individuals combine stimulants and opiates to enhance euphoria, others combine them to attenuate the unwanted side effects [423]. The treatment of speedball abuse may require antagonists that target both opiate and dopaminergic systems

because the opiate antagonist quadazocine did not reduce self-administration of heroin and cocaine combinations, whereas combinations of quadazocine and the dopamine antagonist flupenthixol did [355].

It is likely that μ-opiate receptors participate in cocaine reinforcement as injection of DAMGO into the ventral tegmental area decreased self-administration during the descending limb of the cocaine dose-response curve, whereas self-administration of a dose of cocaine on the ascending limb of dose-response curve was increased by DAMGO [114]. Furthermore although injections of DAMGO into the pedunculopontine tegmental nucleus also decreased cocaine self-administration [115], injection of a μ-antagonist had no effect, indicating that a tonically active opiate system is not involved in cocaine reinforcement [114,115]. A role of κ-opiate receptors was demonstrated that indicated their potential in treating cocaine abuse and preventing relapse, as U50488H attenuated the discriminative effects of low dose of cocaine [262], and U69593 decreased responding to low doses of cocaine and attenuated cocaine-induced reinstatement of drug-seeking behavior in 'abstinent' rats primed with cocaine [462]. The neural mechanisms that mediate these effects are not fully understood, but may involve the ventral striatum because amphetamine-induced rearing, sniffing, and hole-poking behaviors, as well as amphetamine-induced dopamine and glutamate release from the ventral striatum, were attenuated by U69593 and nor-BNI antagonized the effects of U69593 [199]. In contrast, the ventral tegmental area is probably not involved because injections of κ-agonists and -antagonists into that area did not alter cocaine self-administration [114].

Changes in endogenous opiate systems were noted after administration of amphetamine as both acute and chronic administration of methamphetamine up-regulated prodynorphin mRNA in the hypothalamus but not the hippocampus, whereas chronic but not acute administration increased dynorphin A content in the hypothalamus and striatum [445]. Similarly, repeated administrations of cocaine increased dynorphin A-like immunoreactivity in the striatum, substantia nigra, and nucleus accumbens, indicating that κ-opiate receptors in these areas also are involved in cocaine-reward [9]. Changes in δ_1-opiate receptors also occurred as chronic administration of cocaine decreased binding of the selective δ-antagonist [^{3}H]TIPP[Ψ] in the nucleus accumbens and striatum 24 and 48 h later, but had no effects on binding of the δ_2-antagonist [^{3}H]DELT [521]. Similarly, μ-opiate receptor density was down-regulated, but only in the nucleus accumbens and only at 24 h after administration, thus obscuring its functional significance [521]. Prenatal exposure to cocaine affected endogenous opiate systems that depended on the brain areas studied, as μ-opiate receptor mRNA was down-regulated in the diencephalon, but not the frontal cortex, striatum, and midbrain in rhesus macaque fetuses exposed to cocaine from days 22–70 of gestation, as compared with controls [89]. Interactions between Δ^9-tetrahydrocannabinol (Δ^9-THC) and endogenous opiates were demonstrated [15,100,111,112,113,173,178,297,329,339,340,501,549], indicating that some of the behavioral and physiological effects of marihuana and hashish are opiate-mediated. Repeated administration of Δ^9-THC produced an increase in proenkephalin mRNA in both the paraventricular and ventromedial nucleus of the hypothalamus, and an increase in POMC mRNA in the arcuate nucleus of the hypothalamus and anterior lobe of the pituitary [112,113]. Since increases in ACTH and corticosterone also were noted after Δ^9-THC that was blocked by naloxone [329], it is possible that endogenous opiates are part of a stress-response associated with cannabinoid abuse [112,113]. Proenkephalin mRNA was increased after administration of Δ^9-THC in the caudate-putamen that was functional because DAMGO-stimulated [^{35}S]GTPγS binding also was increased [111]. Both of these effects were blocked by naloxone and thus were opiate-mediated [111]. Although the functional significance of this finding is not clear, the involvement of the caudate-putamen in the regulation of motor activity suggests that endogenous opiates may be involved in changes in motor behavior that occur after chronic exposure to cannabinoids [111]. This is further supported by the finding that cannabinoid-receptor knockout mice show both reduced locomotor activity and reduced levels of dynorphin and enkephalin in the striatum, as compared with wild-type controls [501]. Cannabinoids produce analgesia that may involve spinal κ-opiate receptors as Δ^9-THC and the synthetic cannabinoid CP55,940 enhanced spinal dynorphin A (1–17) release [339,340], and the cannabinoid levonantradol increased release of dynorphin B [549]. In contrast, Δ^9-THC enhanced the analgesic effects of both morphine and codeine that was blocked by naltrindole but not nor-BNI, indicating a role of δ-opiate receptors [100].

Cannabinoid receptors may be involved in morphine-dependence and abuse, as both naloxone-precipitated morphine withdrawal and morphine self-administration was decreased in cannabinoid receptor knockout mice as compared with wild-type controls [297]. However, prenatal exposure to THC may not increase the likelihood morphine abuse because it did not modify morphine self-administration in rats exposed to THC in utero and tested as adults [15]. Cannabinoid receptors also may modulate the reinforcing actions of alcohol that is opiate-mediated because CP55,940 increased beer-reinforced responding that was blocked by naloxone [178] Cannabinoid receptors may mediate the dysphoric effects of κ-opiate agonist because U50488H-induced CPA was higher in cannabinoid receptor knockout mice as compared with wild-type controls [297].

Endogenous opiates likely mediate smoking behavior and reinforcement because smokers given a nicotine patch smoked fewer cigarettes and had fewer smoking-induced cravings as compared with placebo controls that was blocked by naltrexone [66]. In contrast, when given alone naltrexone did not affect cigarette cravings, the number of cigarettes smoked, or the aversive effects of the first ciga-

rette of the day, although it decreased measures of wakefulness produced by smoking [66]. Nicotine dependence is opiate-mediated because, as compared with nonsmokers and placebo controls, naloxone dose-dependently increased withdrawal symptoms in nicotine-dependent subjects, and low doses of naloxone increased their urge to smoke and tiredness [287]. Furthermore, since smokers also had lower levels of cortisol than nonsmokers, and naloxone produced a greater cortisol response in nonsmokers than smokers, it suggests that altered opiate control over the HPA axis may contribute to nicotine-dependence [287]. Naltrexone in combination with nicotine replacement may have therapeutic potential in abstinent smokers as those maintained on a transdermal nicotine patch showed fewer urges to smoke, less negative effects, and a decrease in withdrawal symptoms when given naltrexone than when given placebo [234]. Nicotine produced changes in endogenous opiate systems as rats given nicotine 3 times a day for 14 days at doses representative of human smoking showed an up-regulation of μ-opiate receptor binding in the striatum and a decrease in Met-enkephalin levels [551]. Although the functional significance of these changes are not clear, they may be related tolerance to nicotine-induced analgesia because the changes correlated with the development of tolerance to nicotine-induced analgesia [551], and cross-tolerance between morphine- and nicotine-induced analgesia was conferred [591]. Smoking-induced changes in respiratory function involves μ- but not δ- or κ-opiate receptors because cigarette smoke increased plasma exudation in the guinea pig bronchi that was inhibited by morphine and DAMGO, but not by DPDPE or U50488H [300].

7. Sexual activity, pregnancy, and development

As in previous years, there was continued interest in the opiate modulation of sexual and reproductive function, and prenatal and postnatal development. In general, endogenous opiates appear to play an inhibitory role in sexual behavior [27,35] as opiate agonists decreased sexual behaviors [354, 429] and antagonists increased them [439]. A tonically active opiate-mediated inhibition of male sexual behavior was demonstrated as central injections of naloxone increased copulation in castrated, testosterone-treated Japanese quail as compared with placebo controls [439]. Such an opiate-mediated inhibition of sexual responses may have an adaptive function by allowing an individual to avoid dangers that might result from excessive sexual activity, including incest, population overcrowding, and a lowering of available sperm stores [35]. Sperm production is mediated by μ-opiate receptors located in the vas deferens as morphine inhibited electrically evoked contraction of the vas deferens in non-monogamous deer mice [429]. Since competitive interactions can affect levels of endogenous opiates, it is possible that μ-opiate receptors located in the vas deferens play a role in the regulation of sperm delivery in species that must compete for a common female [429]. This is further supported by the finding that morphine did not inhibit contractions in monogamous deer mice in which there is no adaptive advantage to regulating sperm delivery [429].

Male sexual behaviors appear to involve μ-opiate receptors in the paraventricular nucleus of the hypothalamus as morphine injected into that area prevented penile erections in male rats placed in the presence of an unattainable receptive female, and this was opiate-mediated because naloxone blocked it [354]. In contrast, κ-opiate receptors are probably not involved because injections of U69595 into the paraventricular nucleus had no effect [354]. Furthermore, the effects of morphine are likely mediated by the prevention of NO production in the paraventricular nucleus as NO production increased during noncontact erections that was prevented by morphine and the effects of morphine were antagonized by naloxone [354]. These results may help explain the high incidence of penile dysfunction in opiate addicts, and the common occurrence of penile erections during withdrawal [354]. The altered sexual function in opiate addicts may be related to decreased levels of testosterone as chronic morphine administration decreased plasma testosterone in male rats, indicating that sexual function in male opiate addicts may be restored with testosterone supplementation [573]. Furthermore, since plasma levels of LH, which stimulates testosterone production, were decreased after morphine without affecting testicular weight and morphology, it is likely the effects of morphine on testosterone production is by way of the hypothalamo-hypophyseal-gonadal axis rather than a local testicular mechanism [573]. In contrast, although chronic ethanol exposure also suppressed testosterone that was opiate-mediated because naltrexone blocked the suppression, the effect possibly is mediated at the gonadal level as no changes in hypothalamic LH-releasing hormone mRNA were observed [150]. However, the contribution of endogenous opiates in mediating LH release depends on the seasonal reproductive cycle, as naloxone-induced stimulation of LH release in sheep was higher during sexually active short days, as compared with long days [517]. Testosterone does not influence endogenous opiates during maturation because opiate receptor density in the brain was not different in prepubertal, pubertal, and postpubertal mice even though plasma levels are known to fluctuate during stages of development [204]. Therefore, although testosterone is known to suppress LH, the role of endogenous opiates in mediating the suppression is not clear [204,219]. In that respect, it is ultimately important to recognize that nonopiate mechanisms also may participate in the opiate regulation of LH [36].

Sexual behavior in females involves endogenous opiates as ICV naloxone inhibited lordosis in estrogen-primed Sprague-Dawley female rats [516]. However, although ICV injection of β-endorphin facilitated lordosis when given at the time of estrogen priming, it inhibited lordosis behavior

when given 12 h later [516]. Therefore, two different opiate systems appear to mediate steroid-primed sexual behavior, an early facilitatory mechanism that is followed by an inhibitory one during the final stages of estrogen-induced sexual behavior [516]. Similarly, systemic administered naltrexone increased lordosis and proceptivity behavior in obese Zucker rats when given 22 h after estradiol treatment, and this is μ-mediated because injection of morphine prevented the effects of naltrexone [330]. Since obese Zucker female rats are sterile and show abnormally low levels of reproductive behavior, these results suggests that a tonically hyperactive endogenous opiate system may contribute to the observed abnormalities in sexual-related behaviors in this strain [330].

Endogenous opiates are involved in pregnancy and reproductive functions [29,51,230,250,299,411,515,574,575]. Exposure of bovine anterior pituitary cells to estradiol-17β increased both the LH response to gonadotropin-releasing hormone (GnRH) and spontaneous release of prodynorphin-derived peptides [250]. However, the functional link between prodynorphin-derived peptides and the effects of estradiol-17β on LH secretion is not clear because, as compared with controls, antisense oligodeoxynucleotide directed against bovine prodynorphin decreased the LH response to GnRH, without affecting basal release or synthesis of LH [250]. Furthermore, although some β-endorphin neurons in the arcuate nucleus make direct connections with GnRH neurons, the ability of estrogen to influence these neurons is questionable as only 4% of these neurons express the estrogen receptor protein [482].

The effects of various opiate agonists and antagonists on the anticipated afternoon LH surge in female rats were examined, but the results were inconclusive as plasma levels of LH were low or undetectable in all animals that may have been because of the ketamine anesthesia used [574,575]. Endogenous opiates may mediated abnormal fluctuations in LH secretion in a subset of women with hypothalamic amenorrhea as naloxone increased LH pulse frequency in some women but did not in others [411]. Since LH provides an index of GnRH secretion it is possible that endogenous opiates mediated abnormal GnRH in some individuals with this disorder [411]. Growth hormone (GH) also modulates reproductive function that involves endogenous opiates as naloxone increased GH release in ovary-intact mares, but not ovariectomized mares, an affect that was most pronounced during the breeding season [29]. In contrast, although THC [379] and nicotine [458] suppresses LH secretion in ovariectomized rats, an opiate mechanisms is not involved because naloxone or naltrexone did not antagonize their effects [379].

Endogenous opiates may mediate prolactin secretion during the first half of pregnancy in rats as diurnal rhythms of β-endorphin activity in the mediobasal hypothalamus were correlated with daily surges in prolactin secretion [299], and the nocturnal prolactin surge on day 8 of pregnancy was decreased by naloxone [230]. However, although placental lactogens suppress prolactin secretion and maintain the second half of pregnancy, the role of endogenous opiates in mediating this effect is not known because injection of placental lactogens-secreting cells into the lateral ventricle suppressed prolactin secretion without altering β-endorphin activity [299]. Prolactin secretion in androgenized female rats also is opiate-mediated as prolactin secretion was increased in androgenized rats and was antagonized by naloxone and increased by the μ-agonist DAMGO, the κ-agonist U50488H, but not the δ-agonist DPDPE [492]. Endogenous opiates may suppress prolactin secretion in patients with Addison's disease (hypoadrenalism) as naloxone increased it in these patients, although its physiological role may be minor as it was similar during periods of normocortisolism and hypercortisolism [210]

Prolonged stress can disrupt reproductive function by opiate mechanisms as exposure to foot-shock for 3 days increased hypothalamic β-endorphin-like immunoreactivity and decreased LHRH-like immunoreactivity in female ewes [515]. High levels of cortisol also can disrupt reproductive function in women as dexamethasone decreased the LH response to GnRH that is opiate-mediated because naltrexone prevented the suppression [289]. In contrast, repeated stress during the third trimester of pregnancy did not change plasma β-endorphin immunoreactivity, but did increase other stress-related hormones including corticosterone and ACTH [559]. Furthermore, although resting levels of β-endorphin were lower in pregnant female rats during the third trimester, as compared with nonpregnant ones [559], plasma β-endorphin concentrations in goats increased during labor and may reflect a response to pain and discomfort [235]. Endogenous opiates also may function to inhibit HPA activity during parturition to protect the offspring from stress-related hormones, as ACTH and corticosterone decreased during and after delivery in rats, but were enhanced by naloxone [553]. In contrast, although exposure to cortisol in fetal sheep decreases epinephrine synthesis thus limiting adrenal responses to stress, endogenous opiates were not involved as adrenal proenkephalin A mRNA was not affected [5].

Maternal behavior involves endogenous opiates as morphine increased the latency to express full maternal behavior on postpartum days 5 and 6 [366]. However, it is likely that nonopiate systems are involved as well because CCK antagonists potentiated the effects of morphine [366]. Suckling suppresses LH secretion in sows as plasma LH increased in zero-weaned sows by 48 h postpartum but not in suckled sows [136]. Furthermore, since morphine decreased LH secretion in both suckled and zero-suckled sows, it suggests that although endogenous opiates mediate the inhibitory effect in suckled rats, not all the receptors are occupied by endogenous opiates and exogenous opiates were able to increase the inhibition [136]. Maternal deprivation can alter endogenous opiate systems as mice deprived of maternal/nest odor for 15 min/day during the first 2 weeks of life showed decreases in pain sensitivity and

morphine analgesia when tested as adults [124]. Neonatal handling produces anxiolytic effects that may be related to long-term changes in opiate peptide content as dynorphin A in the hypothalamus, pituitary, and striatum, and of dynorphin B in the hypothalamus, pituitary, hippocampus, medulla oblongata, and midbrain was up-regulated after handling [424].

Endogenous opiates may be involved in menopause as POMC mRNA in the infundibular nucleus of the hypothalamus was reduced by 65% in postmenopausal women as compared with premenopausal women [1]. Given the role of endogenous opiates in mediating various physiological and behavior processes, alterations of this system during menopause can have important consequences for the health of elderly women [1], including alterations in thermoregulation [208]. However, the loss of POMC-containing neurons in postmenopausal women may be caused by factors other than ovarian failure, as hormone replacement therapy did not alter hypothalamic POMC mRNA in ovariectomized monkeys that were used as a model of postmenopausal women [2].

Prenatal exposure to opiates can alter development and behavior. The ability of morphine to cross the human placenta is not affected by naloxone as the maternal-to-fetal transfer rate was similar in term human placental cotyledons treated with morphine and naloxone, as compared with those treated with morphine alone [280]. Therefore, naloxone antagonism of fetal distress during maternal exposure to opiates is explained by direct interactions with fetal μ-receptors, and not because of a change in rate of transfer of morphine from mother to fetus [280]. Prenatal exposure to morphine alters seizure susceptibility that in female rats is age-related and transient as exposure to morphine from gestational days 11–18 decreased seizure thresholds at postnatal day 15 but not at postnatal day 25, as compared with saline controls [529]. Prenatal exposure to morphine affected social- and play-related behaviors that depended on the dose used as prenatal exposure to 10 mg/kg morphine daily from gestational days 8–21 increased pinning and social grooming as compared with controls, whereas 1 mg/kg morphine increased pinning behavior only [392]. Since morphine did not affect sensory motor development in the offspring or the gestation of the pregnant mother, the effects are probably not because of global deficits in development or to maternal toxicity [392]. However, there appears to be a critical time in which in utero exposure to morphine can differentially affect play and social behavior because pinning was increased only in offspring exposed to morphine from gestational day 16 to parturition, whereas social behavior was increased only in offspring exposed to morphine from gestational days 8–15 [392]. This differential effect of prenatal morphine on play and social behavior may reflect the differential involvement of endogenous opiates systems [392]. Furthermore, naltrexone given at the time of testing reduced play behavior in controls, but not in prenatally exposed juveniles, indicating that prenatal exposure to morphine may have altered the sensitivity of endogenous opiate receptors [392].

Prenatal exposure to opiates affects eating behavior in newborns as 56% of infants born to mothers maintained on methadone during pregnancy had abnormally high oral intakes by post-natal day 16 that was related to higher metabolic demands in these newborns [336]. The observation that infants born to opiate-addicted mothers have a lower incidence of respiratory distress as compared with nonaddicted newborns may be related to an opiate-mediated acceleration of lung maturation as heroin, morphine, and methadone increased the development of type II pneumocytes and lamellar bodies per alveolar lining cell in isolated fetal rat lungs [186]. In contrast, changes in respiratory-control brainstem nuclei are not involved as DAMGO and naloxone had no effect neuronal development in these areas [364]. Newborns are not at risk for early onset hearing loss as the rate of severe bilateral hearing impairment was not higher in newborn infants of opiate-addicted mothers as compared with controls [201]. Prenatal exposure to nonopiate drugs also affected endogenous opiates systems, as proenkephalin mRNA was increased in the nucleus accumbens and striatum in rats exposed to ethanol in utero [143], and in rhesus macaque fetuses exposed to cocaine from days 22–70 of gestation μ-opiate receptor mRNA was down-regulated in the diencephalon, as compared with controls [89]. However, prenatal exposure to THC did not have long-lasting effects on endogenous opiate systems as morphine self-administration was not different in adult rats exposed to THC in utero as compared with controls [15].

As in previous years, interest in the ontogeny of endogenous opiate systems continued. Audioradiographic localization of [^{3}H]DAMGO revealed that μ-opiate receptors are present in the human fetal spinal cord by week 12–13 of gestation with a high density in the superficial laminae I-II [436]. However, a biphasic increase was noted that included an increase in μ-receptor density the dorsal horn between weeks 12–17 that corresponded with neurogenesis, followed by a second increase by week 24–25 that was similar to the adults pattern of distribution [436]. In mice, the developmental onset of the preprodynorphin gene, which encodes for six different dynorphin peptides, starts at embryonic day 12.5 and then dramatically increases between days 12.5 and 14.5, indicating that dynorphins are involved in early developmental events [473]. Similarly, κ-opiate receptors appear early in development and may be involved in cell growth, as the κ-opiate receptor gene is expressed in the mantle areas of the midbrain, medulla oblongata, hindbrain, cranial ganglion, and vagus nerve at embryonic day 12.5 and 13, and in the eye, ear, neopallial cortex, caudate putamen, lateral ventricle, thalamus, hypothalamus, and pons at embryonic day 15.5 and 17.5 [232].

The endogenous μ-opiate agonist endomorphin-2 appears later in development than other opiate peptides as dynorphin- and enkephalin-like staining was noted in the spinal cord by postnatal day 3, whereas endomorphin-2-like

immunoreactivity was not [37]. By postnatal day 7 limited and faint endomorphin-2-like immunoreactivity was found that increased to adult levels by postnatal day 21 [37]. The late appearance of endomorphin-2-like immunoreactivity suggests that this peptide is not involved in early opiate-mediated events, but may be involved in opiate-mediated stress-induced analgesia which also appears later in development [37]. Changes in POMC mRNA in the central portion of the arcuate nucleus were noted in male and female juvenile rats from 21 to 33 days of age, including an increase in POMC mRNA at 30 days of age [325]. Given that opiates can inhibit parental behavior [366], and 20% of POMC neurons in the arcuate nucleus express μ receptor mRNA [60], is possible that increases in POMC mRNA contribute to the inhibition of parental responses observed at that time [325]. In respiratory-related brainstem regions, δ-opiate receptor density was minimal in the brainstem of young piglets that increase with age, whereas μ-opiate receptor density exceeded δ-opiate receptor density at all ages, and remained unchanged with age [290]. These results demonstrate that the development of δ receptors lags behind that of μ receptors, indicating that the influence of μ- and δ-opiate systems on breathing changes during development [290]. Age- and sex-related differences were noted in μ- and κ-opiate receptor densities in song bird vocal control brain areas, indicating their possible role in song learning or sexual differentiation [203].

The effects of aging on endogenous opiate systems also were studied, and may be important in understanding age-related changes in sensory, cognitive and motor functions [58,59,188,176,376,600]. Met-enkephalin but not dynorphin A (1–17) content was decreased in both the ventral and dorsal horns of 22-month-old rat spinal cords as compared with 3-month-old ones [188]. Furthermore, potentiation of nerve growth factor with the neurotrophic ganglioside GM1 restored deficits in the ventral horns, suggesting that age-related deficits in sensory and motor function may be related to decreases in spinal cord opiate peptides, and GM1 can restore these deficits [188]. Positron emission tomography revealed both age- and gender-related differences in μ-opiate receptor binding as μ-opiate receptor binding potential increased with age in the neocortex and putamen, and more binding was found in the brain of women than men [600]. However, although women had higher binding than men during the reproductive years, after menopause it decreased to levels below those observed in men an indication that changes in hormonal status can influence endogenous opiate systems [600]. Although the functional significance of these changes are not well understood, they clearly highlight the importance of age and gender when interpreting the role of endogenous opiates in mediating various functions. The role of endogenous opiates in mediating age-related anorexia remains to be clarified, but a review of the literature indicates that age-related impairments in opiate function may be one of the factors that contribute to it [58,376]. Leucine aminopeptidase activity, which is involved the metabolism of endogenous opiates, increased in rat brains during the first month postnatal and then decreased in 5- and 24-month-old rats, indicating its possible role both in early neural development and later neuronal loss [25].

Endogenous opiates can inhibit cell proliferation as estradiol increased cell densities in myometrial cells that was inhibited by the opiate agonist D-Met^2-Pro^5-enkephalinamide, and naloxone blocked that inhibition [285]. It is likely that these effects are μ-opiate-mediated because DAMGO also inhibited cell proliferation, whereas DPDPE and dynorphin-A did not [285]. These results indicate that endogenous opiates are involved in the regulation of uterine growth and provide a better understanding of the mechanisms that mediate estradiol-related disorders, including leiomyoma and endometrial cancer [285]. Exposure of estrogen receptor-transfected neuroblastoma cells to estradiol induced neuronal differentiation that may involve endogenous opiates because a concurrent down-regulation of opiate receptor density also was noted [322], and may provide a physiological basis for the sex-related differences in opiate-mediated analgesia [288,322].

Opiates are involved in embryonic development as Met-enkephalin decreased and naloxone increased DNA synthesis in embryonic ectoderm, mesoderm, and endoderm cells [586]. Since Met-enkephalin and its ζ-receptor site also were identified in human fetal tissue, it is possibly involved in human organ development and the pathogenesis of birth disorders [586]. In contrast, endogenous opiates also interact with nerve growth factor to promote neuronal survival as Leu-enkephalin, Met-enkephalin, and DAMGO increased neuronal survival in chick embryonic dorsal root ganglion exposed to nerve growth factor that was blocked by naloxone [455]. These effects are likely μ-mediated because dynorphin A (1–13), DPLPE, and U50488H had no effect [455]. The role of endogenous opiates in mink hair growth was not demonstrated as no changes in β-endorphin levels were noted during the onset of winter hair growth [447].

8. Mental illness and mood

Unlike the declining trend that had been observed in recent years, interest in the role of opiate systems in mental illness and mood increased in 1999, particularly in the modulation of anxiety [13,20,30,65,172,174,278,398,424,528] and depression [16,122,138,226,433,477,588,602]. In addition, the involvement of endogenous opiates in autism [84,296,519], aggression [480,495], kleptomania [127], and Tourette's syndrome [526] also was examined.

Morphine has anxiolytic actions that are opiate-mediated as it increased exploratory behavior in the elevated-plus-maze, and naloxone [278] or naltrexone [20] blocked it. However, nonopiate systems also are likely involved as the CCK_B agonist BOC-CCK-4 reversed the anxiolytic effects of morphine and the CCK_B antagonist L-365,260 potentiated them [278]. The neural mechanisms that mediate the

anxiolytic effects of morphine were elucidated. The nucleus accumbens is involved as morphine injections into that area increased the entries into the open arms of an elevated-plus-maze, and this was opiate-mediated because naloxone blocked it [20]. Since morphine also increased general locomotor activity, μ-opiate receptors in the nucleus accumbens are involved in the motor output for motivational processes [20]. In contrast, when injected into the dorsal periaqueductal gray morphine has differential effects that depend on the dose used as 10 nmol increased entries into the open arms but 30 nmol decreased entries. [20]. It is likely that these differential effects are related to a differential activation of opiate receptor subtypes as naloxone blocked the low dose morphine effect and κ-antagonist nor-BNI blocked the high dose effect of morphine [20]. The endogenous antiopiate peptide Tyr-MIF-1 also has anxiolytic properties in mice as it increased the time spent in the open arms of an elevated-plus-maze as compared with controls [174]. Furthermore, since Tyr-MIF-1 also blocked behavioral responses to socio-psychological stress it may function to regulate anxiety-related responses to stress [174].

Neonatal handling reduced anxiety that involved endogenous opiates as it increased locomotion and rearing behavior in an open field test, and the number of entries and total time spent in open arms of an elevated plus maze; this was correlated with an up-regulation of dynorphin A in the hypothalamus, pituitary, and striatum, and of dynorphin B in the hypothalamus, pituitary, hippocampus, medulla oblongata, and midbrain [424]. Stress, on the other hand, may increase anxiety as it decreased the frequency of entries into an open arm maze and introductory activity to an unfamiliar opponent [13]. The effects are likely mediated by endogenous opiates that are under HPA axis control because changes in pituitary β-endorphin content after stress were prevented by ICV injection of a CRH antagonist [13]. High levels of anxiety exhibited in enkephalin-deficient mice (knockouts) may be related to their alterations in endogenous opiate systems as they have increased μ-and δ binding sites in limbic-related areas, as compared with wild-type controls [64]. In contrast, although aminopeptidase gene-deficient mice spend less time and make fewer entries into open arms of a plus maze than controls, a role for endogenous opiates was not demonstrated as no differences in distribution or intensity of Met- and Leu-enkephalin immunohistochemistry was found in the brain of mutant mice as compared with controls [398].

The anxiolytic effects of benzodiazepines were opiate-mediated but only in some strains as chlordiazepoxide increased the proportion of open-arm entries in Swiss, C57BL/6, and BALB/c mice, but naloxone blocked those effects in Swiss and C57BL/6 mice and potentiated the effects in BALB/c mice [30]. The lack of naloxone antagonism in the BALB/c mice suggests that opiates do not mediate the anxiolytic effects of benzodiazepines in this strain that may be explained by their low stress-induced opiate activity [30]. Furthermore, the species used was important as chlordiazepoxide increased both the time spent in the open arms of an elevated-plus-maze and social interactions in rats, and naltrexone potentiated those effects [172]. In contrast, when rats were previously exposed to the test apparatus naltrexone had no effect on the anxiolytic effects of chlordiazepoxide, suggesting that endogenous opiates are involved in the anxiolytic effects of benzodiazepines in novel environments [172]. Clearly, the interaction between opiate and benzodiazepine systems is very complex, requiring additional research to address the role of novelty and genetic factors [30,172,498].

Opiates may be involved in depression as patients with ‘endogenous’ and ‘nonendogenous’ depression had lower plasma levels of β-endorphin than normal volunteers, with the lowest levels observed in those with ‘endogenous’ depression [138]. Furthermore, after a 4-week treatment with the antidepressant fluvoxamine, β-endorphin levels were increased and depressive symptoms were decreased in all depressed patients, indicating that β-endorphin serum levels could be used in the diagnosis of depression [138]. The neural mechanisms that mediate these effects are not known, but may involve the nucleus accumbens and arcuate nucleus as extracellular β-endorphin levels in these areas were increased 2–3 fold by fluoxetine [588]. Similar results also were observed after application of serotonin, indicating that the actions of antidepressant drugs involve interactions between serotonergic and opiate systems [588]. Furthermore, the caudate nucleus, dentate gyrus, lateral septum, and frontal, parietal and piriform corticies also are involved because fluoxetine increased μ-opiate receptor staining in those areas [135]. Such decreased β-endorphin levels in depressed patients may lead to other symptoms that are opiate-mediated, including changes in pain threshold [477]. In a case study of a 57-year-old women with major depression that did not respond to various antidepressants, paroxetine was found to completely alleviate the depression when augmented with 50 mg/day of naltrexone [16]. However, although this case report indicates that naltrexone may augment the effects of antidepressants, a controlled clinical study is obviously necessary to further evaluate its therapeutic potential [16]. Naloxone also may be effective in ameliorating cognitive deficits associated with electroconvulsive therapy that is sometimes used to treat depression, as it reduced anterograde amnesia, and increased verbal fluency and attention when given before therapy [433].

Stressful life events may enhance vulnerability to depression that involves endogenous opiate as exposure to chronic variable stress facilitated the onset of escape-avoidance deficits in rats pre-exposed to inescapable shock that was blocked by naloxone and enhanced by morphine, and the antidepressant, desipramine, blocked both the sensitization of escape failures produced by the stress and its enhancement by morphine [602]. It is possible that nonopiate systems also are involved, however, as chronic variable stress increased the release of dopamine from the frontal cortex in

rats pre-exposed to inescapable shock that was blocked by naloxone [122]. Preproenkephalin mRNA was increased in the olfactory tubercle in rats after bilateral olfactory bulbectomy that may be associated with the behavioral and physiological alteration observed these animals that resemble human depression [226].

A role for endogenous opiates in kleptomania was indicated as 2 kleptomaniac patients showed improved behavior after naltrexone administration [127]. The mechanisms that mediate these effects of naltrexone are not known, but may be related to an attenuation of poor impulse control because it also controlled compulsive gambling behavior in one of the subjects [127]. The possible role of endogenous opiates in impulse control is further supported by the finding that disinhibitory behaviors in serotonin-depleted rats was reversed by naloxone [495]. Similarly, naloxone also reversed aggressive attacks and acts in these animals, indicating a possible role of endogenous opiates in aggressive acts related to impulse control disorders [495]. A review of the literature also indicates that endogenous opiates are involved the regulation of feline aggressive behavior, in particular μ-opiate receptors in the periaqueductal gray [480]. Atypical antipsychotic drugs may interact with endogenous opiates as the neuroleptic clozapine produced analgesia that was blocked by naloxone, β-FNA, naloxonazine, and nor-BNI, although the role of opiates in mediating its antipsychotic effect was not addressed [465]. A role for opiates in Tourette's syndrome was not demonstrated as cerebrospinal fluid levels of dynorphin A (1–8) and β-endorphin were not elevated in these patients as compared with controls [526].

Autism may involve alterations endogenous opiate systems that can be maternally inherited as plasma levels of β-endorphin were higher in autistic subjects, and in 53% of the mothers of autistic subjects, as compared with age- and sex-matched controls [84,296]. Furthermore, long-term treatment with naltrexone improved autistic symptoms in 11 children, and reduced β-endorphin levels in 7 of the children [84]. In contrast, naloxone increased β-endorphin levels in 4 of the children, and although they also showed improvements none were noted in areas involving social behavior [84]. The involvement of endogenous opiates in the expression of self-injurious and stereotyped behavior in mentally retarded subjects was not demonstrated, as no differences in plasma β-endorphin levels were found between mentally retarded subjects that did or did not show stereotyped and/or self-injurious behaviors [528]. In contrast, self-mutilation in patients with Lesch-Nyhan syndrome may involve an altered opiate system as Met-enkephalin immunoreactivity was increased in the caudate, although no changes were noted in the periaqueductal gray or spinal trigeminal nucleus [454]. A review of the literature suggests although that the effects of naloxone on autism and self-injurious behaviors have been inconsistent, the role of opiates and efficacy of naloxone in treating these illnesses may merit further evaluation in patients that do not respond to other medications [519].

Exogenous opiates such as morphine, hydromorphone, LAMM, methadone, and meperidine produced subjective effects, including 'liking the effects', 'floating', 'coasting', and 'feeling good' [23, 200, 149, 253, 466, 537], that are opiate-mediated because naltrexone blocked the mood-altering effects of hydromorphone [466]. However, differences were noted in their relative effects on mood as morphine produced milder effects than hydromorphone and meperidine, with the effects of meperidine rated the highest [537], and LAAM was more potent than methadone [149]. Furthermore, although some of the subjects reported liking the effects of morphine, hydromorphone, and meperidine, others also reported that they disliked them [537]. Therefore, although 'drug liking' is associated with abuse potential, in healthy subjects who might be prescribed opiates for pain relief μ-agonists do not consistently produce these abuse-related effects [537]. The μ-agonists remifentanil and alfentanil produced 'drug liking' effects that were consistent with their reported short duration of action as their effects on mood was similar to placebo 60 min after the infusion ended [57]. In contrast, their effects on physical performance was still present after 60 min indicating overt behavioral measures are more sensitive than mood measures in evaluating duration of action, and that they may have longer durations of action than originally thought [57].

The effects of heroin on mood depended on the route of administration, as IV heroin was 4-times more potent than IN heroin in producing 'good drug effects' and 'drug liking' in heroin-dependent subjects [105]. However, although these differences in potency exist between IV and IN routes, the potential for abuse may be the same as no differences were found between routes in progressive ratio break point values during self-administration [105]. The subjective effects of the mixed opiate agonists/antagonists pentazocine, nalbuphine, and buprenorphine in nondependent heroin users reflected their μ-agonist activity because, similar to hydromorphone and morphine, they all produced 'drug liking' effects [253, 356, 466]. In contrast, butorphanol produced feelings of dysphoria and 'bad effects' that may reflect its κ-agonist activity [253]. Similarly, the κ-agonist dynorphin A (1–13) produced negative mood effects, although some positive drug effects also were noted [273]. The effects of 'pure' opiate antagonists on mood were conflicting and depended on whether or not the subjects were opiate-experienced as naloxone produced negative mood effects in healthy subjects [585], but naltrexone did not affect mood in opiate-experienced subjects [466]. The effects of NO on mood are not opiated-mediated because naloxone did not reverse it's mood-altering effects [585].

9. Seizures and other neurologic disorders

In 1999, interest in the role of endogenous opiates in seizure activity continued, that included research on the long term changes in endogenous opiate systems after sei-

zures [158,248,440,446,490,594], as well as the effects of opiate agonists [40,292,325,529,601] and antagonists [384] on seizure activity. In general, opiate agonists had proconvulsive effects, as both IV fentanyl and alfentanil increased epileptiform spike activity in patients undergoing surgery for intractable temporal lobe epilepsy [325]. However, although both fentanyl and alfentanil increased activity that was most pronounced in the amygdala or hippocampus, alfentanil was more potent than fentanyl and had a shorter duration of action [325]. Since general anesthesia can suppress electrical brain activity, induction of seizure activity with opiates during surgery can be useful in localization of the epileptogenic focus [325]. Prenatal exposure to opiates can affect seizure activity as exposure to morphine from gestational days 11–18 decreased the thresholds for seizures induced by flurothyl at postnatal day 15, as compared with saline controls [529]. However, the effects are transient as no differences in seizure activity were observed on postnatal day 25 [529].

Seizure activity also was produced in mice after ICV injection of codeine-6-sulfate at doses below its maximal analgesic potency, indicating that the use of this opiate for pain control is limited because of its ability to induce seizures [601]. In contrast, morphine-6-sulphate did not produce seizure activity [601]. Since the opiate binding profiles of codeine-6-sulfate and morphine-6-sulphate are different, it suggests that the mechanisms that mediate the seizure-inducing activity of codeine-6-sulfate is opiate-receptor specific or explained by possible non-opiate actions [601]. Interestingly, although morphine-6-sulphate has an affinity for κ_3 receptors, codeine-6-sulfate does not [601], raising the possibility that seizure activity after morphine-6-sulphate is suppressed by its κ-agonist actions. In fact, there is evidence that κ receptors are involved as the κ-agonist enadoline suppressed the strength of pentylenetetrazol-induced seizure activity in rats [40]. This effect was long-lasting because seizure activity was still suppressed in enadoline-treated animals when given a challenge dose of pentylenetetrazol 8 days after kindling completion [40]. Furthermore, it is possible that glutaminergic systems are involved as kindling resulted in increased glutamate binding in the hippocampus that was prevented by enadoline [40]. Opiate antagonists have anticonvulsive effects as injections of naloxone into the periaqueductal gray suppressed audiogenic seizures in a strain of genetically epilepsy-prone rats [384]. However, the effects of naloxone were delayed and incomplete, indicating that nonopiate systems also are involved [384]. An interaction between opiate and GABA systems was suggested as opiate release from the amygdala, but not the hippocampus, was increased above basal levels after administration of the GABAergic agent gabapentin, and may account for the antiepileptic actions of this drug [441]

Alterations in endogenous opiate systems were observed after seizures that depended on the brain area studied as a single sub-convulsive dose of pentylenetetrazol increased opiate levels in the amygdala, up-regulated μ-opiate receptor binding in the cortex and amygdala but not in the striatum or hippocampus, and enhanced proenkephalin mRNA in the dentate gyrus, CA1-CA3 hippocampus, and various cortical areas [440]. These results indicate that the progressive susceptibility to seizure activity after daily subconvulsive doses of pentylenetetrazol (i.e. kindling) is related to progressive changes in endogenous opiate systems that occur after the first injection [440]. Long-lasting changes in opiate systems in the hippocampus after seizures induced by a single injection of kainic acid is related to the Sp-1 transcript factor as enkephalin-Sp-1 DNA-binding activity was increased for up to 5 months after treatment [158]. Kindling induced by sub-threshold electrical stimulation of the amygdala produces time-dependent changes in dynorphin systems that occur differently in different brain areas, as prodynorphin mRNA in the hippocampus was increased 1 h after kindled seizures that returned to baseline by 2 h, and dynorphin A levels in that brain area decreased at 1 h, increased at 2 h, and returned to baseline by 6 h [446]. In contrast, in the temporal and frontal cortex, both prodynorphin mRNA and dynorphin A levels decreased at 2 h and stayed decreased for up to 24 h [446]. However, although these results suggests that in the hippocampus dynorphin release is followed by an activation of prodynorphin genes that synthesis new peptides, whereas dynorphin synthesis in the cortex is arrested after kindling, the functional significance of these changes in epilepsy is not known [446]. In humans with temporal lobe epilepsy, reorganization of mossy fibers is often found in granule cells of the dentate gyrus that show alterations of dynorphin-immunoreactivity [248,594]. Since dentate granule cells isolated from epileptic patients with mossy fiber sprouting showed reduced dynorphin-inhibition of voltage-dependent Ca2+ channels, as compared with those without mossy fiber sprouting, dynorphins may act as endogenous anticonvulsants thus limiting excitability in epilepsy [248].

Endogenous opiates are involved after focal cerebral ischemia as preproenkephalin mRNA was down-regulated in the ipsilateral infarcted striatum at 1.5 and 3 h after a 90-min cerebral artery occlusion [171]. However, at 24 h preproenkephalin mRNA was up-regulated and no cell death was noted, indicating that changes in preproenkephalin gene expression occur before significant cell loss in regions undergoing infarct [171]. Opiate receptor density in the frontal parietal corticies was affected after a middle cerebral artery occlusion that was time-dependent as decreases were noted in δ receptor density between 1h and 3 h post-ischemia, in μ receptor density between 6 and12 h, and in κ receptor density between 6 h and 24 h [62]. This differential time course suggests that the early the changes in δ receptor density may serve as markers for early neuronal death, whereas the delayed changes in κ receptor density indicate their possible neuroprotective function [62]. In rats, preproenkephalin mRNA was increased for at least 3 days even after a small cortical infarct, thus caution-

ing researchers to take these long-lasting changes into account when electrodes and probes are placed into rat brains [525].

The effects of opiates on ischemic brain damage was examined and the results are relevant because cerebral ischemia sometimes occurs during surgery in patients maintained on opiates [277,494]. However, the results were inconsistent as fentanyl increased neuronal death in one study [277], but did not affect it in another [494]. In rats given a 50 μg/kg loading dose of fentanyl followed by a 2 μg/kg/min maintenance dose for 30 min (110 μg/kg total), or 800 μg/kg loading dose followed by a 32 ug/kg/min maintenance dose for 30 min (1760 μg/kg total), 15 min before a 12-min artery occlusion, neuronal injury was exacerbated in the caudate-putamen at both 6 and 18 h post-ischemia, and in many brain areas at 18 h, as compared with controls [277]. In contrast, no change in neuronal damage was reported 7 days after a 90-min focal ischemia in rats given 50 μg/kg of fentanyl loading dose and then maintained on 50 μg/kg/h (150 μg/kg total), as compared with controls [494]. The reasons for these discrepancies are not clear but may be related to changes in brain temperature as the former study noted an increase in brain temperature after fentanyl [277], whereas the latter study did not [494]. Morphine prevented peroxynitrate-induced cell death in human neuroblastoma SH-SY5Y cell, but was not opiate-mediated because it was not antagonized by naloxone, and DAMGO, DPDPE, and U-50488 did not prevent cell death [260]. However, morphine also may have pro-oxidant effects that could render the central nervous system susceptible to damage as cancer patients undergoing treatment with ICV morphine for intractable pain showed depleted levels of the antioxidant glutathione [195]. β-endorphin also may contribute to ischemic brain damage as CRH-induced increases in cerebrospinal fluid β-endorphin were greater after a subarachnoid hemorrhage as compared with controls [216].

Changes in endogenous opiates were noted after spinal cord injury that may help explain the inability of morphine to treat allodynia after spinal injury [577], as μ-opiate receptor density was down-regulated in the dorsal horns 2 days after spinal cord ischemia [577] and from 2 to16 days after dorsal rhizotomy [224]. Increases in dynorphin A after spinal cord injury may contribute to cell death as embryonic spinal cord neuron treated with dynorphin A (1–13) showed neurodegeneration that was exacerbated by naloxone and the κ-antagonist nor-BNI, and prevented by the NMDA antagonists MK-801, AP-5, and CKA [211]. Since nor-BNI and naloxone had no effect when given alone, these results suggests that dynorphin may activate κ-opiate receptors to impart a neuroprotective effect, but also activate NMDA receptors that leads to neurodegeneration [211].

In previous years there was interest in the role of the opiate system in Parkinson's disease, and it continued in 1999. Changes in endogenous opiate systems may contribute to treatment responsiveness and disease progression as positron emission tomography indicated bilateral decreases in opiate receptor density in the caudate, thalamus, anterior putamen, and amygdala in an animal model of Parkinson's disease produced by unilateral administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [101]. Abnormalities in endogenous opiate systems in patients with Parkinson's varied with severity as reduced Met-enkephalin immunoreactivity in the caudate nucleus was only found in a subset of patients and was correlated to higher disease and gait scores [134]. Preproenkephalin mRNA was up-regulated in the caudate and putamen of monkeys after MPTP as compared with untreated control that was decreased by the dopamine D_2 agonist cabergoline, but was increased by dopamine D_1 agonist SKF-82958 in 2 monkeys that had improved Parkinson's symptoms and developed dyskinesia [375]. These results suggest, therefore, that dopamine D_1 and D_2 receptors have opposite effects on MPTP-induced preproenkephalin mRNA expression, and that increases in preproenkephalin mRNA may be involved in the development of dyskinesia in Parkinson's patients [375]. The effects of MPTP on preproenkephalin gene expression, however, depended on the duration of treatment as preproenkephalin mRNA was increased in the striatum of monkeys that were acutely symptomatic after receiving large doses of MPTP over short periods of time, but not in monkeys that were symptomatic for long periods of time after receiving escalating doses of long periods or large doses over short periods [464]. Since the level of symptomology and degree of dopamine denervation was similar between these groups, the results call into question the role of increased enkephalin activity in the expression of parkinsonian symptoms [464].

Preproenkephalin gene expression in the globus pallidus is regulated by dopamine D_2 but not D_1 receptors because preproenkephalin mRNA was increased in that area in rats that received 6-OHDA lesions or repeated D_2 antagonist treatment, but not after repeated injections of a D_1 antagonist [468]. Striatal prodynorphin gene expression may involve FosB-related transcription factor as chronic L-dopa gradually induced abnormal body movement in rats with unilateral 6OHDA lesions that was paralleled with the induction of FosB-like immunoreactivity in neurons that showed regional and cellular colocalization with prodynorphin mRNA [19]. It is possible that this relationship is causal as intrastriatal infusion of *fos*B antisense inhibited the dyskinesia and down-regulated prodynorphin mRNA [19]. Morphine may be effective in reducing L-dopa-induced dyskinesia in Parkinson's patients as low doses of morphine reduced it in 2 patients, although at high doses morphine produced an increase in akinesia [48].

Patients with Alzheimer's disease have altered HPA activity that may reflect changes in endogenous opiate systems as naloxone-induced increases in cortisol were blunted in Alzheimer's patients as compared with age-matched controls [513]. The potential benefits of naloxone in the treatment of pathophysiological conditions, such as senile dementia in Alzheimer's disease, may be explained by

increases in cerebral functional activity, as it increased regional cerebral metabolic rate for glucose in the cerebral cortex, midbrain, cerebellum, and medulla [507].

10. Electrical-related activity

Interest in the ability of opiates to modulate neural activity remained high in 1999, and that included measurement of their effects on specific neurons, structures, and pathways. The opiate modulation of neural events in the spinal cord were further elucidated, and provided valuable information about the role of opiates in spinal nociceptive processes. In the isolated adult rat spinal cord, DAMGO, but not DPDPE or U50488H, inhibited slow depolarization of substantia gelatinosa neurons evoked by repetitive electrical stimulation of dorsal roots at C-fiber intensity, and this was μ-opiate-mediated because naloxone reversed the inhibitory effect of DAMGO [367]. Similarly, fentanyl inhibited wind-up in single motor neurons produced by electrical or mechanical stimulation [345]. It is likely that μ-opiate receptors modulate nociceptive transmission in the newborn spinal cord as DAMGO, and the endogenous μ-opiate agonists endomorphin-1 and endomorphin-2, reduced slow ventral root potentials in the isolated spinal cord of 1- to 6-day-old rats [504]. Furthermore the effects of endomorphin-1 and endomorphin-2 were blocked by CTOP, demonstrating their μ-opiate receptor selectivity [504]. A role of endomorphins in modulating nociceptive transmission also is supported by the finding that endomorphin-2-like immunoreactivity is increased in the dorsal horns during electrical stimulation of the dorsal root entry zone [555]. It is likely that phospholipase C mediates the opiate regulation of voltage-sensitive Ca^{2+} channels in dorsal root ganglion neurons as greater DAMGO-inhibition of whole cell Ca^{2+} currents was found in phospholipase C-deficient mice, as compared with controls [567]. Since these differences were correlated with the behavioral effects of DAMGO, it is possible that differences among individuals in opiate sensitivity is related to similar genetic factors [567]. Both μ- and κ-opiate receptors modulate rat dorsal horn neurons that is dependent upon PKC as activation of PKC with 4β-phorbol 12-myristate 13-acetate attenuated morphine-, dynorphin-, and U50488H-inhibition of N-type calcium currents [274].

A role for κ-opiate receptors in the modulation of spinal cord nociceptive activity was demonstrated as IV U50488H and fedotozine inhibited dorsal horn neurons that showed sustained excitatory activity after colorectal distention, and nor-BNI partially reversed that inhibition [391]. In contrast, when administered IT, U50488H had no effect, whereas fedotozine inhibited the evoked activity that was partially blocked by nor-BNI and completely blocked by naloxone [391]. Taken together, these results suggest that U50488H and fedotozine have a peripheral site of action, but fedotozine may additionally have a site of action within the spinal cord that is mediated by non-κ-opiate receptors [391]. Dynorphin A (1–17) increased K^+-evoked substance P release in trigeminal nucleus caudalis slices and may be related to its antianalgesic and allodynic activities [24]. However, this effect is not opiate-mediated because naloxone, β-FNA, naloxonazine, and nor-BNI did not block it, but the NMDA antagonist MK-801 did [24]. It is likely that δ-opiate receptors modulate voltage-dependent Ca^{2+} currents in rat primary sensory neurons as the δ enkephalin analog DADLE inhibited Ca^{2+} influx in cultured dorsal root ganglion neurons that was prevented by naltrindole but not β-FNA [3]. Endogenous opiates modulate nucleus reticularis gigantocellular-evoked inhibitory postsynaptic potential (IPSPs) in cat spinal cord motoneurons during active sleep, as the amplitude of carbachol-induced IPSPs in lumbar motoneurons was reduced by naloxone when compared with controls [565].

In recordings from slices of rat periaqueductal gray, DAMGO activated inwardly rectifying K^+ currents in the periaqueductal gray that involves G-proteins because the effects of DAMGO were modified by intracellular application of the guanine nucleotide analogs GDPβs and GTPγs [209]. Thus, the DAMGO-induced activation of G-protein-coupled inwardly rectifying K^+ currents would result in hyperpolarization and inhibit periaqueductal gray activity [209]. However, although DAMGO induced hyperpolarization and inhibited excitatory postsynaptic potentials (EPSPs) in ventrolateral periaqueductal gray, it increased cell firing in many of the neurons [95]. The ability of DAMGO to increase cell firing under these paradoxical conditions may be related to its ability to also block the inhibitory drive of GABAergic inputs that can overcome DAMGO-induced hyperpolarization and EPSPs, as DAMGO inhibited EPSPs by 35.5% while also inhibiting GABA-induced IPSPs by 60.7% [95]. Such flexibility would provide dynamic control of periaqueductal gray function, allowing the actions of opiates to vary depending on the state of the neurons [95]. Met-enkephalin, endomorphin-1, and DAMGO also modulate Ca^{2+} channels in the mouse periaqueductal gray that is μ-opiate-mediated because the agonists inhibited Ca^{2+} channel currents that was prevented by CTAP [108]. The μ-opiate modulation of Ca^{2+} channel currents is further supported by the finding that DPDPE, deltorphin II, and U-69593 had no effect, and in μ-opiate deficient mice, Met-enkephalin, endomorphin-1, and DAMGO did not inhibit Ca^{2+} channel currents [108].

Endogenous opiates mediate electrical activity in the heart as Met-enkephalin increased intracellular Ca^{2+} levels in rat intracardiac neurons [490]. Furthermore, κ receptors also modulate neuronal excitability in the heart because U50488H [409,564,579,595] and bremazocine [595] blocked forskolin- and electrically induced Ca^{2+} transients, that was blocked by nor-BNI and quadazocine [595]. Given the role of κ receptors in arrhythmia and ischemia, these results suggest that intracellular Ca^{2+} is involved in the antiarrhythmia [409,579,595] and cardioprotective [564] effects of endogenous κ-agonists.

Both OFQ/N and DAMGO reduced the magnitude of depolarization-induced Ca^{2+} channel transients in rat dorsal raphe nuclei [448]. Furthermore, although many more neurons responded to OFQ/N than DAMGO that is consistent with the rare occurrence of μ-opiate receptors on the perikarya of dorsal raphe nuclei neurons, both effects were attenuated by a neuropeptide FF analog, indicting its anti-opiate activity [448]. Enkephalin, OFQ/N, endomorphin-1 and endomorphin-2 all inhibited spontaneous discharge of ventrolateral medullary neurons, but did so with different potencies as the EC_{50} was 3.17 nM for endomorphin-1, 3.17 nM for OFQ/N, 10.1 nM for endomorphin-1, and 150 nM for enkephalin [97]. It is likely that only the effects of endomorphin-1 and endomorphin-2 are μ-opiate-mediated because naloxone prevented the effects of the endomorphins and enkephalin but not OFQ/N, whereas β-FNA only blocked the effects of the endomorphins [97].

The nucleus ventroposterolateralis of the thalamus is involved in visceral nociception as 59% of the neurons responded to colorectal distention, that was attenuated by morphine and naloxone blocked the attenuation [571]. The role of opiates in mediating circadian rhythms, however, was not demonstrated as basal or NMDA-evoked firing rates in the hamster suprachiasmatic nucleus were not profoundly affected by Met-enkephalin, Leu-enkephalin, or morphine [123]. Interestingly, a rebound excitatory response was observed after washout of the agonists, or when naloxone was applied to morphine-treated neurons, and thus may have implications for circadian function during opiate withdrawal [123].

Postsynaptic μ-opiate and OFQ/N receptors modulate neuronal activity in both the ventromedial hypothalamus and arcuate nucleus as DAMGO and OFQ/N activated inwardly rectifying K^+ currents in both structures, whereas DPDPE and U69593 did not [151]. In contrast, presynaptic modulation of excitatory transmission in the arcuate nucleus occurs through μ, κ, and OFQ/N receptors as DAMGO, U69593, and OFQ/N depressed the amplitude of electrically evoked EPSPs and decreased the magnitude of pair-pulsed depression [151]. On the other hand, presynaptic modulation of the ventromedial hypothalamus only involves μ receptors because only DAMGO depressed EPSPs and decreased the magnitude of pair-pulsed depression [151]. Therefore, since the overlap of presynaptic and postsynaptic opiate receptors is partially, and the localization of receptors differs between the ventromedial hypothalamus and arcuate nucleus, it is possible that these opiate receptor subtypes have distinct modulatory functions [151]. The ability of DAMGO to hyperpolarize guinea pig hypothalamic neurons is modulated by estrogen because DAMGO-induced increases in inwardly rectifying K^+ currents in that area were attenuated by 17β-estradiol [270]. It is likely that activation of PKA and PKC pathways are involved because the selective PKA antagonists Rp-cAMP and KT5720 and the selective PKC inhibitor calphostin C blocked the effects of 17β-estradiol [270].

Neuronal activity of rat supraoptic nucleus vasopressin cells (i.e. phasic cells) is regulated by an endogenous κ-opiate agonist as U50488H decreased burst duration [68] and suppressed inwardly rectifying K^+ currents that was blocked by nor-BNI [382]. Excitatory activity in the supraoptic nucleus also is modulated by μ-opiate receptors as DAMGO reduced spontaneous glutamate-induced EPSPs that was blocked by CTOP [310], and increased inwardly rectifying K^+ currents in oxytocin magnocellular neurons that was blocked by naloxone [382]. Taken together, these results suggest that both μ and κ receptors modulate oxytocin magnocellular neurons via voltage-dependent K^+ currents, whereas κ receptors differentially modulate K^+ currents in vasopressin magnocellular [68,310,382]. A role for OFQ/N also was shown in the modulation of the supraoptic nucleus as it hyperpolarize all the magnocellular neurons tested, with half of them identified as vasopressin neurons [489]. However, since the κ_3-opiate antagonist naloxone benzoylhydrazone was without effect, it is unknown whether OFQ/N tonically modulates activity within the supraoptic nucleus [489].

In hippocampal slices, morphine decreased the spontaneous firing rate of CA1 hippocampal interneurons, and blocked formalin-induced excitation of CA1 interneurons, an effect that naloxone prevented [596]. Dynorphin also modulates the excitability of CA1 hippocampal neurons as dynorphin A (1–17), but not DPDPE, deltorphin II, DAMGO, or DADLE, increased voltage-dependent K+ currents that was blocked by naloxone and nor-BNI [320]. In contrast, dynorphin inhibited voltage-dependent Ca^{2+} channels in dentate granule cells isolated from epileptic patients, that was reduced in those patients with mossy fiber sprouting, suggesting that dynorphins may act as endogenous anticonvulsants [248]. Although the synthetic estrogen diethylstilbestrol modulates hippocampal activity, it is unrelated to its actions at opiate receptors, as it increased the amplitude of population spikes in the CA1 pyramidal layer that was not antagonized by naloxone [457].

Endogenous opiates modulate LTP in mossy fibers in the CA3 region of the hippocampus as naloxone inhibited LTP, and DAMGO enhanced mossy fiber LTP and paired-pulse facilitation that was blocked by the μ-opiate antagonist CTOP [251]. However, nonopiate systems additionally are involved as the $GABA_B$ receptor antagonists phaclofen, and SCH 50911 also inhibited the effects of DAMGO [251]. This finding is complemented by the observation that many μ-opiate-labeled neurons in the hippocampus also contained GABA-like immunoreactivity [141]. The opiate modulation of LTP, however, is modified by chronic morphine as orthodromic population spike delays and amplitude in hippocampal slices were enhanced in rats given morphine in their drinking water for 20–30 days, as compared with rats given no morphine or only short-term morphine [327].

In contrast, both morphine and Met-enkephalin modulate Ca^{2+} channel currents in the locus coeruleus that is not affected by chronic morphine pretreatment, indicating that

functional μ-opiate coupling is not altered in the locus coeruleus of morphine-dependent rats [109]. The opiate peptides endomorphin-1, endomorphin-2, morphiceptin, hemorphin-4, Tyr-MIF-1, Tyr-W-MIF-1, TAPS, and DALDA inhibited spontaneous firing in all locus coeruleus neurons tested that was μ-opiate-mediated as CTAP reversed their inhibitory effects [572]. However, differences were noted in the potency of the opiates to inhibit spontaneous firing in the rank order of: TAPS > endomorphin-1 and endomorphin-2 > DALDA > morphiceptin > Tyr-W-MIF-1 > hemorphin-4 > Tyr-MIF-1 [572]. In the nucleus accumbens, heroin suppressed neuronal activity in freely moving rats that was naloxone-reversible, but the results were confusing as it only did so when self-administered by the rat and not when administered non-contingently by the experimenter [298]. The reasons for these differential effects are not known, but indicates a complex interaction between behavioral, pharmacological, and electrophysiological processes. Glutamate receptor agonists inhibit the NMDA-evoked excitatory postsynaptic currents in core nucleus accumbens neurons that are potentiated by chronic exposure to morphine, suggesting that interactions between NMDA and metabotropic glutamate receptors may be involved in opiate tolerance and dependence [333,334]. In the globus pallidus, endogenous μ-opiates modulate $GABA_A$ receptor mediated IPSPs elicited by stimulation of the striatum that is presynaptic in nature as DAMGO and Met-enkephalin reduced IPSCs and increased paired-pulse facilitation of evoked IPSCs [499].

The role of endogenous opiates in mediating K^+-evoked release of L-glutamate and GABA from rat cerebrocortical synaptosomes depended on the opiate subtype studies as L-glutamate and GABA release was inhibited by the κ-agonist U50488H that nor-BNI blocked, and the μ-agonist endomorphin-1 that naloxone blocked, but was not affected by the δ-agonist deltorphin or OFQ/N [461]. Similarly, κ but not δ receptors modulate glutamate release from striatal synaptosomes as Ca^{2+}-dependent glutamate release was suppressed by U-69593 that nor-BNI antagonized, whereas the δ-agonist DPDPE was without effect [435]. Although the functional significance of these results is yet to be determined, it may help clarify the mechanisms underlying the opiate modulation of cortical functions, including pain, motivation, reinforcement, learning, and anxiety [461]. Endogenous opiates may regulate P300 wave amplitude as genetic variations of the enkephalinase gene were associated with low amplitude of the P300 wave in male subjects with substance abuse [107]. Given the association of low amplitude P300 waves to alcoholism, it is possible that such alterations in endogenous opiate systems may contribute to a genetic predisposition to substance abuse, particularly alcoholism [107].

In neuroblastoma SK-N-SH cells, endogenous opiates modulate Ca^{2+} channels as the κ-agonists U50488H and U60593, and the μ-agonist sufentanil, inhibited depolarization-induced Ca^{2+} influx, whereas OFQ/N produced dual excitatory and inhibitory effects [233]. However, it is likely that these effects are mediated by the OFQ/N receptor as an OFQ/N antagonist prevented their effects of U50488H, sufentanil, and OFQ/N, whereas nor-BNI was without effect, and naltrexone and naloxone were only minimally effective [233]. These results suggest, therefore, that some opiate responses that are defined as nonopiate because they are not sensitive to naloxone or naltrexone antagonism, may in fact be mediated by OFQ/N receptors [233]. Endogenous opiates also modulate voltage-dependent Ca^{2+} channels to potentiate dopamine release from human neuroblastoma cells as U50488H increased both basal and evoked dopamine release [272]. In contrast, DAMGO had minimal stimulatory effects that is probably related to the small number of μ receptors on these cells, whereas the δ-agonists DPDPE and DSLET had both inhibitory and stimulatory effects in human neuroblastoma cells [272]. Furthermore, when the stimulatory effect of U50488H and DAMGO were blocked by the N-type Ca^{2+} channel antagonist Ω-conotoxin an inhibitory effect was revealed, thus indicating that within the same cell opiates can have both stimulatory and inhibitory effects on transmitter release [272]. Morphine simulates intracellular Ca^{2+} transits in both human endothelial cells [163] and invertebrate immunocytes [393] that naloxone blocked [163,393], thus demonstrating opiate involvement. Specifically, it appears that μ_3-opiate receptors are involved because DAMGO, DADLE, DAMA, β-endorphin, and dynorphin (1–17) were without effect in both types of cells [164,393].

11. General activity and locomotion

In 1999, as in previous years, interest in the opiate modulation of general activity, in particular locomotion, continued. However, the effects of opiate agonists and antagonists were somewhat inconsistent, thus making generalizations difficult. Morphine generally increased locomotion [74,459,533,536,548,550], although in some circumstances it was decreased [533,603]. An acute injection of morphine (10 mg/kg) increased locomotion in mice [459,548] that was blocked by a dose of glucose (100 mg/kg) that did not by itself modify locomotion, thus demonstrating that glucose can inhibit opiate functions [459]. Antihistamines also modulate morphine-induced locomotion as the histamine H1-receptor antagonist tripelennamine potentiated spontaneous locomotor activity in morphine-treated mice that also was antagonized by glucose [459]. Since antihistamines are sometimes combined with opiates to enhance euphoria, the ability of glucose to modulate their locomotor effects may have important implication for drug abuse [459]. In contrast, co-administration of caffeine or the adenosine receptor antagonist PACPX did not alter locomotion, suggesting that dopamine and adenosine receptors are not involved [548].

The neural mechanisms that mediate some of the locomotor effects of morphine were elucidated. Morphine injec-

tions into the caudal ventrolateral periaqueductal gray also increased locomotor activity in rats that was blocked by naltrexone, suggesting that opiate receptors in this area are involved [533].However, under conditions of stress morphine differentially affected activity depending on the behavioral measure, as it produced explosive locomotion and decreased inactivity, but also decreased exploration, rearing, autogrooming, and increased explosive arrested behaviors in rats that were placed under the threat of attack by an intruder [533]. Although it is not clear why morphine decreased exploratory behavior during threats of attack, it is probably related to the increased time spent in arrested and explosive locomotor behaviors [533]. However, none of these behaviors were opiate-mediated because naltrexone did not antagonize the effect of morphine in threatened rats [533]. Previous opiate activation is an important factor in regulating the behavioral response to stressful events, as previous exposure to morphine increased immobility in rats in a forced swim test that was opiate-mediated because naloxone blocked it [603]. Since behavioral inactivity is considered a defensive response to a threatening stimulus, these results indicate that when exposed to a novel and stressful environment previous opiate activation sensitizes the expression of this adaptive response [603]. Indeed, similar to morphine, previous exposure to restraint stress also increased immobility that naloxone blocked [603].

Repeated injections of morphine produced sensitization to the stimulation of locomotor responses to an acute challenge of morphine [74,536,548,550], that may be explained by interactions with dopaminergic systems [74,536,548, 550]. Sensitization to morphine-induced locomotion was demonstrated as mice injected with 10 mg/kg morphine every other day for 9 days showed a progressive increase in locomotion on days 3, 5, 7, and 9, and also when challenged with morphine 2 days later [548]. Furthermore, although caffeine and PACPX did not block the acute locomotor effects of morphine, they prevented the sensitization to it, suggesting that dopamine receptors mediate sensitization to morphine's locomotor reactivity that involves adenosine receptors [548]. It is likely that dopamine transmission in the nucleus accumbens and caudate-putamen is involved as sensitization to both morphine-induced locomotion and stereotypy was accompanied by sensitization to dopamine transmission in the those areas when rats were challenged with morphine 15 days after being injected with morphine twice daily for three days [74]. However, cross-sensitization did not occur to amphetamine or cocaine in morphine-treated rats, indicating that behavioral sensitization to morphine can occur independently of sensitization to psychomotor stimulants [74]. Similarly, in rats with nigral-striatal lesions, repeated morphine injections produced a progressive increase in turning behavior over 13 days, but did not show cross-sensitization when challenged with amphetamine or cocaine, but rather a decrease in cocaine-induced turning was observed [536]. Serotonergic systems also are involved in sensitization because the serotonin re-uptake blocker fluoxetine blocked the expression sensitization to morphine-induced oral stereotypy [550]. Morphine-induced behavioral sensitization is long-lasting as sensitization to morphine-induced turning in rats with nigral-striatal lesions was still presented 71 days after the last morphine-injections [536], and sensitization to morphine-induced oral stereotypy was present after 6 weeks [550].

Besides morphine, the effects of other opiate agonists on locomotor activity were examined but the results were inconsistent. In rats, injections of DAMGO into the ventral pallidum increased locomotor activity that involves efferent projections to either the medial dorsal nucleus of the thalamus or the midbrain extrapyramidal area because injection of a local anesthetic into those areas prevented the increases in locomotion [98]. It is likely that planaria (flatworm) possess κ-opiate receptors, but not μ- or δ-opiate receptors, as exposure to DAMGO or DPDPE did not increase motor activity in these organisms, whereas U50488H and bremazocine increased motor activity that was blocked by naloxone and nor-BNI [408]. Furthermore, since the effects of U50488H and bremazocine also were blocked by the dopamine D_1 antagonist SCH-23390 and D_2 antagonist sulpiride, dopamine systems are involved [408]. In 17-day-old preweanling rats U50488H also increased locomotion [348, 389], but the involvement of dopamine systems is confusing because U50488H-enhancement of locomotor activity was attenuated by the D_2-like agonist NPA [348,389] and the dopamine antagonist flupenthixol [389], whereas the indirect dopamine agonists cocaine [348,389], methylphenidate, and amphetamine [348] augmented the effects. It is not clear why opposite effects were obtained by direct (NPA) and indirect (cocaine, methylphenidate, and amphetamine) dopamine agonists, but this may be related to their differential activation of the D_1 receptor [348].

Although κ-agonists increase locomotion in young preweanling rodents [348,389] they decrease activity in adults as U50488H produced dose-dependent sedative effects in adult (>6-weeks-old) CXBK and C57/BL mice [237]. However, differences were noted between these two strains as CXBK mice showed lower baseline locomotor levels and fewer sedative effects of U50, 488H than C57/BL mice, indicating lower endogenous opiate levels in CXBK mice [237]. In rhesus monkeys, U50488H produced dose-dependent sedative effects that were centrally mediated because they antagonized for up to 35 days by intracisternal but not by SC injection of nor-BNI [276]. In contrast, although the bremazocine also produced sedation, neither SC or intracisternal. nor-BNI treatment affected it, suggesting that at least two κ-opiate receptor subtypes exist that can cause sedation when activated [276]. Indeed, the existence of multiple κ-opiate receptors was demonstrated in another model as nor-BNI was about 50-times more potent at inhibiting U69593-stimulated [^{35}S]GTP-γ-S binding than tifluadom-stimulated [^{35}S]GTP-γ-S binding in the guinea pig caudate [217].

When injected SC the peripherally selective κ-agonist

ICI204,488 and the dynorphin A (1–8) analog E-2078 were relatively ineffective at producing sedation in rhesus monkeys as compared with the reference κ-agonist spiradoline, indicating a central site of action [71]. However, at the highest dose both ICI204,488 and E-2078 produced sedation, suggesting that ICI204,488 can indeed have central effects, and that E-2078 can cross the blood-brain barrier [71]. The partial μ-agonist/κ-antagonist buprenorphine interacts with dopaminergic systems as a single administration of buprenorphine shortly after 6-OHDA lesions potentiated L-DOPA-induced turning 3 weeks later, as compared with animals not given buprenorphine [4]. Although when given alone naltrexone [533], naloxone [408], and nor-BNI [276,408] did not affect locomotor activity, ICV injection of anti-β-endorphin reduced self-grooming in rats it, suggesting that a tonically active opiate system may modulate some behavioral activities [427].

Endogenous opiates may be involved in circadian rhythms as c-Fos is spontaneously expressed in hypothalamic β-endorphin neurons 1 h after dark onset in rats, but is undetectable from late morning until 30 min after light offset [241]. In contrast, neural activity in the hamster suprachiasmatic nucleus were not greatly modified by Met-enkephalin, Leu-enkephalin, or morphine, although a rebound excitatory response was observed that may have implications for circadian function during opiate withdrawal [123]. Opiates inhibit rapid eye movements (REM) during sleep that may be related to cholinergic systems as morphine decreased acetylcholine in the medial pontine reticular formation, an area that contributes to REM sleep [377]. Morphine also produces changes in pupil diameter, and therefore can be used as an objective criterion to measure drug effect [403].

Research continued to address the role of opiates during strenuous exercise and physical motor activity. Running in rats produced changes in endogenous opiate systems, as preproenkephalin mRNA in the striatum was increased 90% in rats 2 h after running a treadmill for 20 min [307]. It is likely that interactions with both dopaminergic and glutaminergic systems are involved because increases in preproenkephalin mRNA after running were blocked by the dopamine D_1-antagonist SCH-23390 and the NMDA antagonist MK-801, and the dopamine D_2-antagonist eticlopride enhanced it [307]. Plasma β-endorphin levels were increased in males 2 min after a maximal cycling exercise, that was decreased by the synthetic corticosteroid dexamethasone [331]. However, although dexamethasone is sometimes used by athletes, it did not alter any subjective or objective parameters of performance, thus not supporting its assumed performance-enhancing 'doping' effects [331]. β-endorphin levels also are increased in the periaqueductal gray matter and arcuate nucleus in rats after forced walking, and this may be stress-related because it also produced analgesia [387].

12. Gastrointestinal, renal, and hepatic functions

As in 1999, there was continued interest in the role of endogenous opiates systems in gastrointestinal (GI), renal, and hepatic function. Consistent with previous years, morphine [67,214,303,399,425,451,554,570] and other opiate agonists [67,347,399,410,425,451,522,532,570,580,581], inhibited GI functions, including transit and gastric emptying, and these effects were reversed by opiate antagonists [67,303,347,399,410,570,580,581]. In patients with chronic pancreatitis, morphine increased orocecal and colonic transit times, and the patients felt more constipated, reported more strain on defecation, had harder stool consistency, and more bloating after 5 days of morphine, as compared with the first day [554]. In contrast, the μ-agonists tramadol did not interfere with orocecal transit times, produced fewer GI symptoms than morphine, and fewer patients had prolonged colonic transit times than with morphine [554]. Since tramadol produced similar degrees of analgesia as morphine in these patients, it may be preferred over morphine because it has fewer GI side-effects [554]. In newborns, fentanyl may have therapeutic advantages over morphine, as both opiates produced similar degrees of analgesia, whereas decreases in GI motility were less frequent after fentanyl than after morphine [451]. The effects of morphine on GI function should be of concern at low doses as well, since duodenal motility was increased within 15 min in 9 of 10 subjects infused with 40 μg/kg/h of morphine, and this was opiate-mediated because IV naloxone blocked the effects in 5 of 6 subjects [303].

Low doses of methylnaltrexone were highly effective in treating gut motility and transit changes in opiate addicts maintained on methadone as IV administration of the antagonist (0.05–0.45 mg/kg) twice daily reversed methadone-induced constipation and delay in gut transit time, as compared with placebo controls [581]. Therefore, since methylnaltrexone does not cross the blood-brain barrier, it may prove to be effective in treating constipation in pain patients maintained on opiates by acting on peripheral opiate receptors in the GI tract, while sparing the centrally mediated opiate analgesic effects [581]. The gastric effects of opiates may involve the nucleus tractus solitarius as DAMGO applied to the gastric compartment inhibited neuronal activity in this area that was antagonized by both methylnaltrexone and naloxone [580]. It is likely that κ-opiate receptors also are involved as U50488H inhibited nucleus tractus solitarius neurons, an effect that was blocked by naloxone, although much higher doses of methylnaltrexone were required [580]. In contrast, methylnaltrexone given alone activated nucleus tractus solitarius neurons, indicating that endogenous gastric opiate tonically modulate brainstem and gastric responses [580].

Morphine and fentanyl inhibited both GI transit and permeability of the small intestine in mice; this involves central μ-opiate effects because when administered ICV the agonists were more potent than when given SC, and the ICV

effects were decreased by ICV μ-opiate antibodies or antisense oligodeoxynucleotides to μ-opiate receptors [425]. A central μ-opiate site of action is further supported as SC injections of low doses of morphine and PL017, a peripherally acting μ-agonist, inhibited GI transit and permeability that was unaffected by μ-opiate antibodies, whereas high SC doses of morphine that should bind to both central and peripheral intestinal opiate receptors inhibited GI transit and permeability that was partially blocked by the μ-opiate antibodies [424]. However, morphine and fentanyl were more potent in inhibiting of GI transit than permeability, suggesting that the inhibition of permeability may have contributed to their antitransit effects [425]. In contrast, although the δ-agonist DPDPE was less potent than the morphine and fentanyl, it had greater effects on permeability than GI transit that was not modified by μ-opiate antibodies or antisense oligodeoxynucleotides to μ-opiate receptors [425]. OFQ/N has both peripheral and central actions that occur independently of traditional opiate systems as ICV injections OFQ/N inhibited GI transit and contracted isolated colon preparations, actions that were unaffected by naltrexone [399]. However, when doses of morphine, DAMGO, and DPDPE that inhibited GI transit were co-administered with OFQ/N, a subadditivity was observed with morphine and DPDPE, but not DAMGO, indicating a possible functional interaction between central δ and OFQ/N receptors [399].

Stimulation of the sciatic nerve inhibits cholera toxin-induced secretion in the small intestine that is primarily centrally opiate-mediated as IV naloxone blocked the effect, whereas IV naloxone methiodide, which does not cross the blood-brain barrier, only partially antagonized it [254]. The enkephalinase inhibitor, racecadotril, also has antidiarrheal effects that are opiate-mediated because it decreased cholera toxin-induced hypersecretion of water, sodium, and potassium that naloxone blocked [432], and reduced acute diarrhea in both children [522] and adults [532]. Since racecadotril has no effect on GI motility, it may be safer to use than the μ-agonist loperamide which reduces diarrhea, but also increases GI transit time leading to constipation and abdominal distention [522,532]. Endogenous opiates are involved in vomiting as high doses of naloxone induced emesis, and fentanyl inhibited nicotine-induced emesis in house musk shrews [449]. The antiemetic actions of fentanyl are likely mediated by μ_2-opiate receptors within the brain because its effects were antagonized by naloxone, naltrexone, the δ/μ- antagonist M8008, the κ/μ-antagonist MR2266, but not by the μ_1-antagonist naloxonazine, the κ-antagonist DIPPA, the δ-antagonist naltrindole, or the peripherally acting antagonists naloxone methylbromide and naloxone methyliodide [449]. However, since doses of naloxone, naltrexone, and M8008 that blocked the antiemetic effects of fentanyl did not by themselves induce vomiting, it is unlikely that emesis is regulated by a tonically active opiate system [449].

Inflammation of the colon may produce an up-regulation of peripheral κ-opiate receptors as κ-opiate agonists inhibited visceromotor responses to colonic distention more so in rats with inflamed colons than those with uninflamed colons [471]. However, μ-opiate receptors are probably not affected because morphine was equally potent in rats with inflamed or uninflamed colons [471]. Colonic irritation can modify pain responses that involve endogenous opiates as colonic distention decreased pain responses to IP acetic acid injections which naloxone reversed [337]. In rat model of inflammatory bowel disease produced by intracolonic installation of trinitrobenzene sulfonic acid, morphine improved performance in a discrimination task and naloxone blocked it, thus providing a valuable paradigm to evaluate analgesics in this pain model [359].

The classic guinea pig ileum perpetration has been used to determine opiate modulation of contractions of the ileum as a means of assessing selectivity for opiate receptors. The inhibitory effect of mitragynine pseudoindoxyl, an alkaloid synthesized from mitragynine used in Thai folk medicine, on electrically induced contractions of the guinea pig ileum is μ-opiate-mediated because it was prevented by naloxone and the μ receptor agonist naloxonazine [570]. Endomorphin-1, endomorphin-2 [347], and DAMGO [347,410] also caused a concentration-dependent inhibition of electrically induced contractions of the guinea pig ileum that was blocked by naloxone, thus further supporting the hypothesis that endomorphins are endogenous opiate ligands [347]. The selectivity of various enkephalin analogs for opiate receptors were analyzed in the guinea pig ileum perpetration, and it was found that dalargin, dalarginamide, and N-Me-[L-phe^4-dalarginamid, all inhibited electrically evoked contractions that naloxone blocked, although dalarginamide, and N-Me-[L-phe^4-dalarginamid were 2 and 20-times respectively more potent μ-agonists than dalargin [410].

Opiate receptors are involved in renal excretory functions, as naloxone decreased urine flow and sodium excretion in response to plasma volume expansion in conscious rabbits [479]. Urinary function in spinally transected guinea pigs involves endogenous opiates systems as IT morphine completely blocked the micturition reflex, and inhibition of volume-induced bladder contractions by IT capscaicin was prevented by IT naloxone [406]. Neutral endopeptidase, which has been shown to inactivate enkephalins, is involved in renal function as it was down-regulated in the plasma of rats after ischemia-induced acute renal failure, that was preceded by a decrease in kidney cortical neutral endopeptidase mRNA [388]. Given that neutral endopeptidase is distributed in the glomerulus and proximal tubule of the rat and rabbit kidney [148], these results suggest that down-regulation of this enzyme may contribute to acute renal failure [388], perhaps by affecting enkephalin activity. A down-regulated endogenous opiate system may explain the extreme polydipsia and polyuria in inbred STR/N mice as morphine did not produce analgesia in these mice, but when the opiate system was up-regulated by repeated administra-

tion of naltrexone analgesia was produced by morphine and the animals drank less water [257].

In 1999, research indicated that OFQ/N may play an important role in renal function. In rats, ICV injection of OFQ/N decreased urinary sodium excretion [263] and renal sympathetic nerve activity [263,478], and increased urine flow [263]. Furthermore, when combined with a dose of OFQ/N (10 nmol) that had no effect, naloxone decreased renal sympathetic nerve activity that may reflect an attenuation of hypotension-induced increase in nerve activity, thus unmasking an inherent decrease of nerve activity by OFQ/N [478]. Although the mechanisms by which OFQ/N produces diuresis and antinatriuresis is not clear, a pathway that is independent of the renal nerves is probably involved because renal-denervation had no effect on urine flow and sodium excretion [263]. Interestingly, ICV injections of the OFQ/N antagonist [FG]OFQ/N (1–13)-NH_2 also produced decreases in sodium excretion and urine flow, that were of longer duration than OFQ/N, and low doses of [FG]OFQ/N (1–13)-NH_2 did not block the effects of OFQ/N [264]. Therefore, although [FG]OFQ/N (1–13)-NH_2 is presumed to be an antagonist of the OFQ/N receptor in vitro, the present results suggest that in vivo it has central agonists effects similar to OFQ/N in vivo [264]. The κ receptor also is involved in renal function as EKC [534], U69593 [72, 534], and the dynorphin A (1–8) [534] analog E-2078 caused diuresis in rhesus monkeys that was blocked by quadazocine [72]. Furthermore, although the butorphanol did not alter urine output, it did when challenged with the μ-opiate antagonist clocinnamox, suggesting that κ-agonist-like diuretic effects for butorphanol can be demonstrated when its μ-agonist properties are antagonized [534].

Endogenous opiates are involved in liver disease, in particular the pruritus associated with it, as naloxone [252], naltrexone [360], and nalmefene [49] reduced itching in patients with cholestatic liver disease. It is possible that itch associated with cholestatis is mediated by cutaneous serotonin receptors as injection of serotonin into the backs of mice elicited scratching in the injected area that was blocked by naloxone [569]. Pruritus associated with morphine injections [205,255,266] also can be effectively treated with opiate antagonists as prophylactic administration of nalmefene reduced the need for antipruritic medications and recall of itching in pain receiving IV morphine, as compared with placebo control [255]. In rats, intracisternal administration of the opiate peptide analog D-Ala-Met-enkephalinamide decreased bile flow that was blocked by naloxone, indicating that impaired bile flow in patients with cholestatis may involve an endogenous opiate system [50].

13. Cardiovascular responses

As in previous years, interest in endogenous opiate systems and cardiovascular function continued. In particular, there was interest in the role of κ-opiate systems in cardiovascular function as κ-agonists consistently reduced blood pressure [347,474,475,476] and heart rate [353,437,474, 475,476], and therefore may be valuable in the treatment of hypertension. However, the effects of κ-agonists depended on the dose used as systemic administration of BRL52626, which freely crosses the blood-brain barrier, decreased heart rate and blood pressure at low doses but produced hypertension at higher doses in a strain of spontaneously hypertensive rats [474]. In contrast, BRL52974, a κ-agonist that has limited access to the brain, produced hypertension at low doses but hypotension at higher doses [474]. Since at low doses BRL52974 would have selective peripheral actions, whereas high doses may have central effects as well, it is reasonable to conclude that κ-agonist have centrally mediated hypotensive effects but peripherally mediated hypertensive actions [474]. This is further supported by the finding that continuous infusion of BRL52626 (0.2 mg/kg/day) decreased heart rate and blood pressure over the 14-day period, whereas the same dose of BRL52974 had no effect, except for a decrease in heart rate after 7 days of treatment [474].

Isolation induced hypertension that is κ-mediated as 7 days of isolation in normotensive rats increased heart rate and blood pressure that was returned to control levels after systemic injection of the κ-agonist U62,066E [475]. It is likely that hippocampal κ-opiate systems are involved in cardiovascular regulation as intrahippocampal administration of U62,066E decreased heart rate and blood pressure in isolated [475,476] and spontaneously [476] hypertensive rats, and normotensive group-housed controls [475,476], that was blocked by nor-BNI [476]. Conversely, intra-hippocampal microinjection of antisense oligodeoxynucleotide to the κ-opiate receptor increased systolic and mean blood pressure in normotensive and spontaneously hypertensive rats, as compared with missense controls [562]. In transgenic mice expressing Ro1 in the heart, an engineered receptor that can only be activated by a synthetic ligand, the κ-agonist spiradoline caused an immediate bradycardia ($<$1 min) that lasted for several hours [437]. Since Ro1 receptors cannot be activated by their endogenous ligands, this technique can be used to activate G-protein signaling systems in a controllable fashion, thus allowing researchers to further delineate their role in cardiovascular function [437].

There also was interest in the role of OFQ/N in cardiovascular function because, similar to the κ-agonists [347, 474,475,476], OFQ/N consistently reduced blood pressure [96,263,264,353,478] and heart rate [96,263,264,353,478]. In mice, bolus IV injections of OFQ/N reduced both heart rate and blood pressure that was blocked by the OFQ/N-antagonist [F/G]NC (1–13)NH_2 [353]. Similarly, hypotension and bradycardia also was produced by the OFQ/N fragments NC (1–17)NH_2 and NC (1–13)NH_2, although the NC (1–13)OH and NC (1–9)NH_2 fragments were without effect [353]. It is likely that the lack of effect observed after the NC (1–13)OH fragment is because of its high susceptibility to enzymatic degradation because it produced hypo-

tension and bradycardia when pretreated with the protease inhibitor thiorphan, which also enhanced the hypotensive and bradycardic effects of OFQ/N [353]. OFQ/N and its fragments also produced hypotension and bradycardia when administered centrally as ICV injections of OFQ/N [263, 264,478] and NC (1–13)NH_2 [264] reduced blood pressure and heart rate; however the OFQ/N (2–17) was without effect [264]. Furthermore, although the [F/G]NC (1–13)NH_2 blocked the effects of OFQ/N when administered peripherally and thus has antagonists actions [353], when administered ICV [FG]OFQ/N (1–13)-NH_2 also produced hypotension and bradycardia, and low doses of [FG]OFQ/N (1–13)-NH_2 did not block the effects of OFQ/N [264]. Taken together, these results suggest that [FG]OFQ/N (1–13)-NH_2 has peripheral OFQ/N-antagonist properties [353], whereas centrally it possesses agonists properties that are similar to OFQ/N [264]. The neural mechanisms that mediate the effects of OFQ/N were elucidated and probably involve the rostral ventrolateral medulla because injection of OFQ/N into that area reduced blood pressure and heart rate, effects blocked by [FG]OFQ/N (1–13)-NH_2 but not naloxone [96]. This finding that [FG]OFQ/N (1–13)-NH_2 blocked the effects of intra-rostral ventrolateral medulla OFQ/N [96] is in direct contrast to the agonist-like effects observed after ICV injections [264]. Clearly, more research is necessary to further evaluate the potential OFQ-agonists/antagonist properties of [FG]OFQ/N (1–13)-NH_2.

Met-enkephalin, which is highly concentrated in the heart and alters neuronal excitability [313], reduced bradycardia induced by vagal stimulation that was opiate-mediated because the opiate antagonist diprenorphine blocked it [75]. Since vagal influences are important in maintaining a balance between sympathetic and parasympathetic influences, these results suggest that elevated levels of endogenous opiates may reduce vagal control of the heart, and possibly contribute to heart disease by increasing sympathetic activity [75]. Similarly, increased sympathetic activity after leptin may elevate endogenous opiates as ICV injections of leptin increased blood pressure and naloxone blocked it [144]. In contrast, endogenous opiates also decrease blood pressure as Met-enkephalin injections into the rostral ventrolateral medulla reduced blood pressure and heart rate that was opiate-mediated because naloxone blocked it [97], and. electroacupuncture inhibited bradykinin-induced hypertension in cats that was opiate-mediated because it was blocked by naloxone given IV or microinjected into the rostral ventrolateral medulla [92].

A role for μ- and δ-opiate receptors was demonstrated as the μ-agonist endomorphin-1 induced a hypotensive response and the δ-agonist Leu-enkephalin induced bradycardia [353]. However, intra-hippocampal injections of DAMGO, DADLE, and DPDPE had no effect on heart rate and blood pressure, indicating μ- and δ-opiate receptors in that area are not involved [476]. In patients treated with opiates during surgery, the effects depended on the agonist used as alfentanil decreased heart rate but not blood pressure [326], remifentanil decreased heart rate but increased blood pressure [422], and fentanyl [326] and morphine [400] had no effect. In opiate-dependent patients maintained on opiates, administration of morphine [356], heroin [105], methadone [149], hydromorphone [200, 253], and LAAM [149] all failed to affect blood pressure and heart rate. However, chronic morphine produced an up-regulation of cAMP in the right ventricle of the rat heart, indicating that adaptive changes also can occur in the heart of opiate-dependent subjects [363].

When given alone, opiate antagonists typically did not affect basal heart rate or blood pressure [47,75,97], although a slight increase in heart rate over time was observed after naloxone in one study [479]. In contrast, in opiate addicts detoxified with naltrexone under general anesthesia, heart rate was lowered after detoxification, indicating that bradycardia should be of concern when this procedure is used [11]. The cardiovascular effects of the partial μ-agonist/κ-antagonist buprenorphine in opiate-dependent subjects were examined, but the results were inconsistent as it sometimes increased blood pressure [253] and other times had no effect [200,356,466]. However, when combined with naloxone, buprenorphine increased blood pressure and heart rate, indicating that withdrawal symptoms were present in these subjects [356].

The endogenous opiate system is involved in hypotension resulting from hemorrhage as plasma levels of β-endorphin are elevated during hemorrhage in rats [370]. It is likely that endogenous opiates are involved in the regulation of hepatic arterial blood flow during hemorrhage as naloxone prevented increases in hepatic vascular resistance and decreases in hepatic arterial blood flow during hypotension induced by stepwise bleeding from the right femoral artery of cats [47]. Since hemorrhage hypotension was kept constant (mean arterial pressure = 50 mmHg), the increases in hepatic arterial blood flow after naloxone were probably explained by a restoration of systemic vasoconstriction [47].

Arrhythmia may involve peripheral endogenous opiates, as systemic administration of methylnaltrexone, an opiate antagonist that does not cross the blood-brain barrier, reduced the incidence of ventricular fibrillation and arrhythmia during coronary artery occlusion, and naltrexone similarly reduced ventricular fibrillation [378]. Furthermore, although morphine had no effect on its own, it attenuated the antiarrhythmic effects of methylnaltrexone, thus further supporting a peripheral opiate mechanism of action [378]. Therefore, methylnaltrexone may have clinical potential in treating patients with myocardial ischemia by decreasing ventricular arrhythmia, while sparing the central analgesic effects of morphine [378]. A role for κ-opiate receptors also was demonstrated as U50488H abolished norepinephrine-potentiation of arrhythmia in the isolated rat heart that was blocked by nor-BNI [579]. It is likely that the antiarrhythmic actions of U50488H are mediated by cAMP-dependent pathways because it also decreased forskolin- [579] and

electrically- [409,564,595] induced $[Ca^{2+}]_i$ transients and norepinephrine- [579] and forskolin- [595] induced elevation of cAMP in the heart [579], that were blocked by nor-BNI and quadazocine [595]. Furthermore, it is likely that both κ_1 and κ_2 receptors are functional in the heart as bremazocine, which preferentially binds to κ_2 receptors, also blocked electrically induced $[Ca^{2+}]_i$ transients and forskolin-induced elevation of cAMP in the heart, and nor-BNI and quadazocine blocked it [595].

Ischemic preconditioning produced by a 5-min coronary artery occlusion given15 min before a 30-min occlusion in rabbits produced a decrease in infarct size that is mediated by peripheral opiate receptors because naloxone methiodide given 1 min before ischemic preconditioning blocked it [94]. Specifically, it is likely that opiate receptors in the heart are involved as ischemic preconditioning in isolated rabbit hearts before a 45-min occlusion also reduced infarct size that was blocked by naloxone [94]. The opiate receptor subtypes in the heart that mediate the protective effects of ischemic preconditioning also were examined. In the isolated rat heart, preconditioning with a sublethal metabolic inhibition for 30 min given 20 h before severe metabolic inhibition reduced cell death that was attenuated by the κ-antagonist nor-BNI, but not by the μ-antagonist CTOP or the δ-antagonist naltrindole [564]. The role of κ-opiate receptors in mediating the cardioprotective effects of ischemic preconditioning was demonstrated further as U50488H for 30 min also inhibited cell death produced by severe metabolic inhibition that nor-BNI blocked [564]. However, there may two critical time windows for κ-opiate-mediated cardioprotection as U50488H reduced cell death when given 1, 16, and 20 h before severe metabolic inhibition, but not when given 6 h before the ischemia [564]. Furthermore, since both ischemic preconditioning [564] and U50488H [409,564,595] blocked electrically induced $[Ca^{2+}]_i$ transients, and the PKC inhibitor chelerythrine attenuated the effects of ischemic preconditioning and U50488H, it is likely that PKC and $[Ca^{2+}]_i$ are involved in κ-opiate-mediated cardioprotection [564].

Taken together, these results suggest that opiate receptors, in particular κ-opiate receptors, are expressed in the heart and may function to protect the heart from arrhythmia [378,409,579,595] and ischemic infarct [94,564]. Indeed, cardiomyocytes in the heart synthesize opiate peptides as POMC mRNA transcripts were found to be present in both atrial and ventricular tissue of the rat heart, and β-endorphin mRNA was localized in atrial myocytes [365]. Therefore, the heart can be viewed as a neuroendocrine organ capable of synthesizing and releasing opiate peptides [365], and may play an integral role in the interaction between sympathetic and parasympathetic control of the heart [38].

Interactions between the opiate regulation of cardiovascular function and pain sensitivity have been documented, and may help explain the reported abnormalities in pain thresholds in patients with coronary heart disease [69]. Indeed, in patients with stable angina, stimulation of pituitary β-endorphin release with ketoconazole increased peripheral pain thresholds as compared with placebo controls [246]. However, it is unlikely that the presence of anginal symptoms depends on levels of β-endorphin because the onset of anginal symptoms after treadmill exercise in these patients was unaffected by ketoconazole [246]. These results suggest, therefore, that different systems mediate the perception of somatic and cardiac pain [246], and elevated levels of β-endorphin during exercise-induced silent ischemia may not suppress anginal symptoms.

14. Respiration and thermoregulation

Although endogenous opiates are involved in respiration and thermoregulation, research on this topic, in particular thermoregulation, remained low in 1999, following a similar trend in recent years. In general, respiratory depression was produced by opiate agonists, including morphine [205, 400,434,485], hydromorphone [466], heroin [105], LAAM [149], remifentanil [328], sufentanil [530, 531], buprenorphine [46, 466], and sameridine [400], although there were some exceptions [200, 356]. Opiate-induced respiratory depression is opiate-mediated because naloxone blocked morphine-induced respiratory depression [205], and naltrexone blocked respiratory depression after hydromorphone [466].

It is likely that μ-opiate receptors are involved as hypoxia and hypercapnia induced by the IT or IV injections of the μ-agonist sufentanil in rats was reversed by both naloxone and naloxonazine given IV 5 min after the opiate [531]. However, when the antagonists were administered 24 h before the opiate, the effects depended on the antagonist used as naloxonazine blocked both IT and IV sufentanil-induced hypoxia and hypercapnia, whereas naloxone given 24 h before had no effect on IT sufentanil, and only partially blocked hypoxia after IV sufentanil [531]. Since naloxonazine irreversibly blocks μ_1 receptors, whereas its antagonism of μ_2 receptors is reversible and short-lasting, these time-dependent results suggest that either μ_2 receptors are not selectively involved in sufentanil-induced respiratory depression as originally thought, or that naloxonazine is not selective for μ_1-opiate receptors [531]. Besides μ-opiate receptors, δ_2-opiate receptors are involved as hypoxia and hypercapnia induced by sufentanil was reversed by both the δ-antagonist naltrindole and the δ_2-antagonist naltrindole 5′-isothiocyanate [530].

Placebos can induce respiratory depression as a saline injection given after 2 days of buprenorphine treatment produced a respiratory depressant effect that was blocked by naloxone and thus is opiate-mediated [46]. Since the verbal instructions used by the experimenter did not suggest any expectation of respiratory depression, these results suggest that the placebo respiratory depression is not explained by cognitive or motivational factors, but rather a conditioning mechanism that is mediated by endogenous opiates [46]. There are indications that pain could antagonize the venti-

latory depressant effects of morphine as revealed in case study of a 59-year-old man given large doses of morphine to control severe pain because of spinal cord compression, who developed acute respiratory depression after the pain suddenly improved [434]. Thus, the high dose of morphine may have induced respiratory depression once the pain was relieved, an indication that caution should be practiced when a change in disease status or pain-relief occurs in patients maintained on high doses of opiates [434].

In opiate-dependent subjects maintained on buprenorphine respiration was depressed, which is consistent with its partial μ-agonist properties [466]. Conversely, the same dose of buprenorphine also blocked the respiratory depressant effects of hydromorphone, thus indicating opiate receptor blockade as well [466]. Furthermore, although opiate antagonists typically did not affect respiration when given alone [46, 466, 530, 531], in opiate dependent subjects naloxone increased respiration when combined with a dose of buprenorphine (2 mg) that was without effect, indicating that the response to the antagonist was a manifestation of withdrawal [356]. Since respiratory depression occurs after heroin [105] and thus can contribute to overdose, buprenorphine and naloxone combinations may be safer than continued heroin use [356]. Methadone-maintenance may be safer than LAAM-maintenance as 3 of the 9 opiate-dependent subjects that participated in the study had to withdraw because of clinically significant respiratory depression after 60 mg of LAAM [149]. Although sameridine, a new drug with both μ-agonist and local anesthetic properties, produced more respiratory depression at high doses (0.73 mg/kg) than did morphine, a clinical dose (0.15 mg/kg) of this drug did not affect ventilation, indicating that sameridine is safe for patients undergoing surgery [400].

Neutral endopeptidase, which is responsible for the degradation of various peptides including enkephalins, is involved ventilatory responses to hypoxia as neutral endopeptidase-knockout mice showed increased respiration and minute ventilation, and decreased inspiratory and expiratory times, after exposure to 8% oxygen, as compared with wild-type controls [198]. Similarly, exposure C57BL/6J mice to the neutral endopeptidase inhibitor thiorphan caused a greater ventilatory response to hypoxia, as compared with untreated controls [198]. However, although enkephalins have been shown to be involved in hypoxia [153] and respiratory processes [34], the specific contribution of endogenous opiates to this ventilatory response cannot be concluded, as neutral endopeptidase also is involved the breakdown of other peptides, including substance P [198].

Endomorphin-1 and endomorphin-2 inhibited electrical field stimulation-induced tachyinergic contractions of the guinea-pig isolated bronchus, indicating their possible role in modulating lung function [166]. However, although the effects of endomorphin-1 are μ-mediated because naloxone blocked it but naltrindole and nor-BNI did not, the effects of endomorphin-2 in this situation are possibly nonopiate-mediated because none of the antagonists affected it [166]. Endogenous opiates may play a role in the pathophysiological processes that leads to allergic asthma as a reduction in plasma Leu-enkephalin hydrolysis was noted in patients suffering from seasonal allergies, as compared with controls [175]. The functional significance of these changes is not clear, however, as more research is necessary to determine whether these observed modifications in opiate peptide hydrolysis are involved in the pathogenesis of asthma, or are a consequence of it [175].

Endogenous opiates are localized in lung tissue and thus may play a role in its development. Rat fetal lung tissue exposed in vitro to heroin, morphine, and methadone showed increased development of type II pneumocytes and lamellar bodies per alveolar lining cell, an indication that opiates can directly accelerate lung development [186]. This finding may help explain why infants born to opiate-addicted mothers have a lower incidence of respiratory distress as compared with nonaddicted newborns [186]. The contribution of opiate systems to breathing during postnatal development may change as δ-opiate receptor density in respiratory-related brainstem regions was minimal in young piglets but increased with age, whereas μ-opiate receptor density remained constant through development and exceeded δ-opiate receptor density at all ages [290]. However, the role of endogenous opiates in mediating developmental changes in respiratory-control brainstem nuclei is not clear as DAMGO and naloxone had no effect on neuronal development in these areas [364].

Interest in the role of endogenous opiates in thermoregulation was low in 1999, following a similar decline in recent years. Morphine induced hypothermia in mice that was opiate-mediated because naloxone blocked it [438]. In contrast, opiate agonists produced variable results on temperature in opiate addicts, as methadone and LAAM had no effect on skin temperature [149], and hydromorphone either decreased it [253], increased it [466], or had no effect [200]. In opiate-dependent subjects given buprenorphine skin temperature was not altered [200, 356, 466], although it did block increases in skin temperature produced by hydromorphone, thus demonstrating opiate receptor blockade [466]. Although naltrexone [466] and naloxone [356] did not alter skin temperature, naloxone decreased it when combined with buprenorphine, indicating a possible withdrawal-response to the antagonist [356].

OFQ/N also has thermoregulatory functions as it induced hypothermia when injected into the hypothalamus of rats [568]. Furthermore, in an vitro model of hypothalamic control of body temperature, OFQ/N produced concentration-dependent effects as low doses (1 ηM) increased temperature sensitivity of warm-sensitive neurons, whereas a high concentration (100 ηM) decreased temperature sensitivity in both warm- and cold-sensitive neurons [568]. Furthermore, CTOP, nor-BNI, and naltrindole did not modify the effects of OFQ/N, suggesting that a traditional opiate receptor (μ, κ, or δ) is not involved [568]. However, although [FG]OFQ/N (1–13)-NH_2 is proposed to be a selective

OFQ/N antagonist it produced concentration-dependent effects similar to OFQ/N and did not antagonize the effects of OFQ/N, suggesting that it may have agonist-like properties as well [568]. Indeed, whether or not [FG]OFQ/N (1–13)-NH_2 is an antagonist at the OFQ/N receptor is not without controversy as a similar agonist effect was reported in another model after ICV injections [264].

15. Immunologic responses

There was continued interest in the opiate modulation of immune function in 1999. It appears that opiate have dual roles, suppressing immune function under some circumstances [88,191,192,395,397,404,407,412,420,483] and facilitating it in other [63,240,321,373,395,397,407,546]. Furthermore, it was often the case that opiates enhanced immune function when tested in vitro [240,321] and suppressed it when tested in vivo [191,192,404,420], although there were many exception to that rule [240,349,350,373, 397,483,587]. Morphine tended to suppress the immune system in vivo [191,192,420], and may help explain the increased susceptibility of opiate addicts to infectious diseases [79,85,139,191,192,420,483]. It is likely that opiate receptors in the periaqueductal gray are involved as injections of morphine into that area reduced natural killer cell (NKC) activity, IL-2 production, mitogen-induced T lymphocytes proliferation [192], NO and TNF-α production, and phagocytosis by splenic macrophages [191]. Similarly, when injected peripherally both fentanyl and buprenorphine dose-dependently decreased TNF-α production, an indication that the immune system was compromised [420]. Morphine may suppress immune function by enhancing apoptosis of T lymphocytes as it dose-dependently promoted apoptosis of Jurkat cells and isolated T lymphocytes [483]. This effect is probably μ-opiate-mediated because DAMGO also promoted apoptosis of Jurkat cells and naltrexone blocked the effects of morphine [483].

In patients receiving epidural morphine analgesia for thoracic pain, IL-8 levels were lower in these patients as compared with those using patient-controlled analgesia [373]. Since increased levels of IL-8 are linked to infectious and inflammatory complications, these results suggest that epidural morphine could improve immune function and potentially lower the incidence of some infections [373]. Similarly, in healthy human subjects who received fentanyl, plasma NKC numbers increased as compared with placebo controls, thus demonstrating an enhancement of immune function [240]. Furthermore, since fentanyl had no effect on NKC function in vitro, it is possible that the enhancement of NKC circulation after fentanyl is mediated by a central mechanism, rather than a direct effect of the opiate on NKCs [240]. Interestingly, naturally occurring antibodies also may exert their immunomodulatory effects by functionally interacting with μ-opiate receptors as natural human IgG inhibited [^{3}H]DAMGO binding similar to morphine and also inhibited adenylyl cyclase activity by a G-protein-mediated mechanism [318,319]. Morphine-stimulated NO production may be involved in cancer progression as its production was increased and more prolonged in lung carcinoma than in normal lung [164]. Furthermore, μ_3-opiate receptors were found to be present in lung carcinoma, and may play a role in cancer progression via NO release [164].

There was interest in the role of opiates in HIV, in part because of the high incidence of infection among iv drug users [79,85,139]. Besides contributing to the spread of HIV because of sharing of contaminated needles [85,165], the progression of HIV has been linked to iv drug use as higher levels of plasma viral load was associated with continued opiate use in HIV-infected patients [79]. In an vitro model of HIV-1 brain infection, endomorphin-1 potentiated viral expression in HIV-1-infected brain cell cultures, an effect that was μ-opiate-mediated because β-FNA blocked it [412]. In contrast, neither morphine nor DAMGO affected viral expression, indicating that the effects of endomorphin-1 were because of interactions with an atypical μ-opiate receptor [412]. However, morphine may have differential effects in humans that are coinfected with human T-cell leukemia virus type 1 (HTLV-1) and HIV as it enhanced levels of HTLV-1, an effect attenuated by naltrexone in vitro, while at the same time reducing HIV-1 infection that naloxone also diminished but less consistently [397]. Therefore, it is apparently important to consider whether concurrent HTLV-1 and HIV-1 infections exist when planning treatment programs, as some therapies used to treat HIV infection may not be as effective in treating patients that are coinfected [397].

Much attention focused on the modulation of immune responses by endogenous enkephalins, in particular Met-enkephalin or opioid growth factor (OGF) [249]. Met-enkephalin depressed the proliferation of human renal cancer cells within 12 h in vitro, a receptor mediated event because naloxone and antibodies to Met-enkephalin blocked it [249]. Similarly, Leu-enkephalin, and some related peptides also suppressed renal cancer cell proliferation, but not as potently as Met-enkephalin [249]. Furthermore, removal of OGF with antibody to this opiate and persistent opiate-receptor interaction with naltrexone increased cell proliferation, indicating that Met-enkephalin tonically inhibits renal cancer cell growth [56]. The possibility of a tonically active Met-enkephalin system that modulates renal cancer growth is further supported by the finding that both OGF and its receptor were located in these cells, and suggests that the production of OGF (or alternately a defect in production) may contribute to cancer cell proliferation [56]. Consistent results also were obtained in the regulation of head and neck cell carcinoma [350], human pancreatic cancer [587], and human neuroblastomas [349]. In all these instances OGF inhibited cancer cell proliferation, naloxone and OGF-antibodies blocked it, naltrexone and OGF-antibodies increased it, and OGF and its receptors were identified in the cells

[349,350,587]. Furthermore, a number of synthetic and natural agonists related to μ-, δ-, κ-, σ-, ϵ-opiate receptors were without effect, an indication that Met-enkephalin was indeed the OGF involved in cancer cell proliferation [349, 350,587]. Low levels of enkephalin may contribute to impaired immune function in the elderly as the activity of enkephalin-degrading plasma enzymes was higher in elderly individuals (mean age = 78.3 years) as compared with controls (mean age = 31.5 years) [59]. Met-enkephalin also affects lymphocytes in the skin as it increased the number of lymphocyte-positive cells in normal human skin, but decreased them in tuberculin-treated skin [395]. These results suggest that Met-enkephalin can either suppress immune responses or reverse immunosuppression, thus operating to 'fine-tune' the immune system [395]. Met-enkephalin also increased interferon-γ production in splenocytes infected with Friend leukemia virus, an effect that was enhanced when combined with AZT treatment, and thus may have implications for treatment of HIV [63,261].

β-endorphin is found in immune tissue, including the thymus [128], and also may have immunomodulatory functions. Both β-endorphin and Met-enkephalin may prime human neutrophils as physiologically relevant concentrations of these opiates (10^{-8} and 10^{-6} M, respectively) enhanced chemiluminescence and chemotactic responses in vitro which were opiate-mediated because naloxone attenuated their effects [406]. Similarly, phagocytic and chemotaxis activities of kidney leucocytes were increased in rainbow trout after injection of this opiate, as compared with controls [546]. In contrast, higher concentrations of β-endorphin and Met-enkephalin (10^{-3} and 10^{-5} M, respectively) may be immunosuppressive as they deceased chemiluminescence [406]. The increased susceptibility of young rats to malignant tumor growths may be related to NKC activity as 36-day-old rats had a higher lung tumor cell retention and decreased NKC toxicity as compared with 100-day-old rats, and depletion of NKC increased tumor retention in all rats [404]. Although the contribution of endogenous opiates to this response is not known, it may be related as plasma levels of β-endorphin were higher in the young rats than the old [404]. However, since β-endorphin typically increases NKC activity, it is unlikely that these differences in β-endorphin levels contributed to the differences in lung tumor cell retention [404]. Acupuncture has been suggested to enhance immune function that may be related to changes in endogenous opiates, as it also has been reported to increase the release of β-endorphin and Met-enkephalin [190]. Plasma morphine-like immunoreactivity was increased in immune tissue after physical trauma in snails and mussels as compared with controls, and may be involved in immunologic responses to trauma [493]. However, the relationship between physical trauma, endogenous opiates, and immune responses is not clear as decreases in plasma hydrolysis and modifications in immune response were found in humans after severe physical trauma, but no quantitative relationship was observed between the reduction in hydrolysis and modifications in the immune system [31].

Binding studies revealed that OFQ/N has a uniquely higher affinity for human B-cell lymphoblastic cell lines derived from Burkitt's lymphoma (i.e. Raji cells) than traditional opiates agonists and antagonists including morphine, DAMGO, U69593, DPDPE, naltrindole, naloxone, naltrexone, diprenorphine, dynorphin A, Met-enkephalin, Leu-enkephalin, β-endorphin, and pentazocine [227]. Although the functional significance of these receptors is not clear, its location on these cells suggests a possible immunomodulatory role [227]. A role of κ receptors in modulating immune function also was suggested as they too are expressed on cancer cells [258,332]. It is possible that κ receptors are involved in bone marrow regeneration as bone marrow macrophages express these receptors, the immunoenhancing properties of melatonin were blocked by nor-BNI, and the colony stimulating activity of dynorphin A was nullified by antisense oligodeoxynucleotide to the κ-opiate receptor [321].

Although it is well established that opiates participate in immune responses [31,39,56,63,88,163,164,191,192,227, 238,240,249,258,261,318,319,321,332,371,373,395,397, 406,412,420,483,493,546,560,587], a review of the literature indicated that most scientific papers describing immunologic experiments did not specify whether or not opiate analgesic were employed [420]. Conversely, while some researchers may refrain from using opiate analgesics in their experiments because they can modify immune responses and thus confound the results, the stress associated with omitting analgesics also may contribute to changes in immune response [420]. Therefore, it is important that scientific papers specify whether or not opiates were used for pain control [420], and employ the appropriate controls to allow the influence of these factors to be properly evaluated.

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