Pharmacological and biochemical interactions between opioids and cannabinoids

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Opioids and cannabinoids are among the most widely consumed drugs of abuse in humans. A number of studies have shown that both types of drugs share several pharmacological properties, including hypothermia, sedation, hypotension, inhibition of both intestinal motility and locomotor activity and, in particular, antinociception. Moreover, phenomena of cross-tolerance or mutual potentiation of some of these pharmacological effects have been reported. In recent years, these phenomena have supported the possible existence of functional links in the mechanisms of action of both types of drugs. The present review addresses the recent advances in the study of pharmacological interactions between opioids and cannabinoids, focusing on two aspects: antinociception and drug addiction. The potential biochemical mechanisms involved in these pharmacological interactions are also discussed together with possible therapeutic implications of opioid–cannabinoid interactions.

New trends have renewed scientists' interest in the pharmacology and biochemistry of cannabinoids due to the recent description of an endogenous cannabinoid receptor system acting in the brain and peripheral tissues. This system comprises at least two types of cannabinoid receptors coupled to well-known signal-transduction mechanisms, as well as endogenous ligands, which could potentially be used as models for the synthesis of new therapeutic drugs. The search for these so-called endocannabinoids has been carried out in a manner similar to that for the endogenous opioids in the 1970s. It is now well-established that plant and synthetic derivatives of both opioids and cannabinoids mimic the effects of endogenous ligands in their ability to bind to membrane receptors. Moreover, it has been shown that opioids and cannabinoids share a variety of pharmacological properties and even putative links in their mechanisms of action, although they also show important pharmacological differences.

Common pharmacological properties of opioids and cannabinoids

Hypothermia, sedation, hypotension, inhibition of intestinal motility and motor depression are just some of the effects elicited by both opioids and cannabinoids[1,2]. However, the most important interactions were found in antinociception[1–5] and, to a lesser extent, in drug reinforcement[6–10].

Antinociception

Data accumulated in the past few years suggest the existence of independent but related mechanisms of antinociception for cannabinoids and opioids. A detailed description of the recent advances in cannabinoid-mediated antinociception research is given in Box 1. These advances point to the existence of distinct cannabinoid antinociceptive pathways[11–14], although a number of studies suggest that opioids might also be involved in the regulation of pain control by cannabinoids.

This hypothesis is supported by a number of studies, which have indicated that opioid receptor antagonists might block cannabinoid-induced antinociception. The first studies used naloxone or other nonselective opioid receptor antagonists to block the antinociceptive effects of cannabinoids, but the results were controversial. Some authors, using mostly tail-flick tests, found that opioid receptor antagonists failed to block the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) or other cannabinoid receptor agonists[11,14–17], whereas others found that low doses of naloxone (1–5 mg kg^{-1}) partially antagonized and, at higher doses (10 mg kg^{-1}), fully blocked the antinociceptive effect of Δ^9-THC. In these latter experiments, tail-flick, paw pressure, hot-plate or other pain tests were used[18–20]. Despite the observation that the results using the hot-plate test more closely resembles a supraspinal response than a spinal response, whereas the tail-flick is mainly under spinal control, and that differences in the supraspinal and spinal location of opioid receptor subtypes have been suggested[21,22], it seems that differences in the pain models used are unable to explain the different responses to naloxone. These differences might be because high doses of this antagonist used in some studies[18–20] are acting at μ-, κ- and δ-opioid receptors. In fact, by using more selective opioid receptor antagonists such as β-funaltrexamine (a μ-opioid selective antagonist) or nor-binaltorphimine (a κ-receptor-selective antagonist; see Fig. 1), several studies have shown that μ- and preferentially κ- but not δ-receptors are involved in the antinociceptive action of Δ^9-THC (Refs 3, 17, 19, 23). In addition, it has been described recently that administration of antisense oligodeoxynucleotides to the κ-receptor blocks the antinociceptive effects of intrathecal (i.t.) Δ^9-THC (Ref. 24) and enhances Δ^9-THC-induced antinociceptive tolerance[25] in rats. The recently available knockout mice for specific opioid receptor subtypes could potentially be used to demonstrate the subtype(s) of opioid receptor involved in the antinociceptive effects of cannabinoids[22]. Moreover, the supraspinal or spinal location of opioid receptor

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Box 1. Cannabinoid-

The hypoalgesic effect of cannabinoids is well documented and the finding is considered to be timely with potential for clinical application[1–3]. However, the delay in the elucidation of molecular sites of action for cannabinoids compared with that for opioids has hampered a rapid clarification of pain-depressing effects of cannabinoids and, hence, their potential uses as analgesics. However, an interesting review by Notcutt and co-workers of the clinical experience using cannabinoids to reduce chronic pain has been recently published[3].

Anatomical sites of cannabinoid-induced antinociception

In recent years, it has been shown that cannabinoids produce antinociception through multiple mechanisms at the spinal and supraspinal levels in the CNS (Refs 4–6). This supports the existence of distinct cannabinoid antinociceptive pathways, which could represent pharmacological targets for pain relief. It has also been proposed that cannabinoids, acting through CB_2 receptors, might regulate pain initiation at sites of tissue injury[7], in a similar way to opioids that have also been shown to be peripherally active[8–10]. This opens the possibility of developing novel peripherally active cannabinoid analgesics devoid of centrally mediated side-effects.

It has been suggested that various brain regions are involved in the centrally mediated antinociceptive effects of cannabinoids. The first site is thought to be located at the spinal level, as direct application of cannabinoids to the spinal cord resulted in antinociception[4,11]. Within the spinal cord, the lumbar rather than thoracic region seems to be implicated[4]. Anatomical data presented by Hohmann and Herkenham[12] showed that only a subpopulation of cannabinoid receptors is located in the rat lumbar spinal cord. On the basis of the efficacy of nor-binaltorphimine when administered intrathecally (i.t.) but not intracerebroventricularly (i.c.v.)[13,14], spinal cannabinoid antinociception has been reported to be opioid-mediated through activation of κ-opioid receptors in mice. Recent studies have suggested that dynorphin peptides, the endogenous κ-opioid ligands, might be acting at the spinal level through non-opioid-mediated mechanisms[15,16]. In this respect, it might be difficult to interpret the results obtained using spinal administration of dynorphin antibodies[13,17,18]. However, the results obtained with κ-receptor antagonists[13,18,19] or antisense oligodeoxynucleotides to these receptors[20,21] strongly suggest a κ-receptor involvement in the antinociceptive effects of cannabinoids. Further studies are required to further address this controversial aspect.

It has been shown that antinociception induced by systemic administration of cannabinoids is attenuated, but not completely blocked, by spinal transection[4,11], thus providing indirect evidence of a role for supraspinal structures in cannabinoid-induced antinociception, particularly in the descending control of nociception[22]. This is consistent with the fact that SR141716A has a much greater efficacy at supraspinal than at spinal sites[23]. It is thought that these supraspinal structures are located mainly in the midbrain[6,24], brainstem[25] and thalamic structures[26]. Walker and co-workers[25] and, more recently, Meng *et al.*[27] demonstrated the existence of a brainstem circuit activated by cannabinoids to reduce pain in the rostral ventromedial medulla. This effect would be independent of the motor-depressing effect of cannabinoids. The periaqueductal grey matter has also been reported to function as an important relay involved in the regulation of cannabinoid antinociception[6,24]. Although cannabinoid receptor binding sites are not abundant in this area[28], local administration of the potent cannabinoid receptor agonist CP55940 into the periaqueductal grey matter produces antinociception[4]. However, for the purpose of this review, it is important to point out that, among other putative areas, this brain region is a common site for pain-suppressing effects of opioids and cannabinoids. Studies using selective opioid receptor antagonists given i.c.v. or i.t. (Refs 13, 14) suggest a major involvement of μ-opioid receptors in supraspinal cannabinoid antinociception.

Taken together, these studies suggest that cannabinoids might act both at the supraspinal level of the descending inhibitory pathways and at the level of the dorsal horn of the spinal cord where intrinsic interneurones containing dynorphin peptides might regulate transmission between primary afferent fibres and spinothalamic tract transmission neurones.

Pharmacological features of cannabinoid-induced antinociception

The high correlation between antinociceptive potencies of a series of cannabinoids, their ability to displace cannabinoid receptor radioligands from binding sites and to inhibit adenylate cyclase suggests that the antinociceptive effects of cannabinoids are primarily mediated by cannabinoid receptors[29]. This has been shown

subtype(s) involved in cannabinoid antinociception needs further clarification, although the involvement of supraspinal μ- and spinal κ-receptors has recently been suggested[23] (see Box 1).

The simultaneous administration of cannabinoid receptor agonists and μ- (Refs 5, 26) or κ- (Refs 3, 25, 27) receptor agonists has provided further support for a cannabinoid–opioid interaction in antinociception. Using chronic treatments, Thorat and Bhargava[26] revealed the existence of bidirectional cross-tolerance between morphine and Δ^9-THC, although this was not related to changes in μ-receptor binding in Δ^9-THC-tolerant mice[26] or to alterations in cannabinoid receptor binding in morphine-dependent mice[28]. Cross-tolerance was also demonstrated for Δ^9-THC and κ-receptor agonists[3,25,27]. However, other authors reported a potentiation of the antinociceptive effects of cannabinoids in morphine-dependent rats in parallel with an increase in cannabinoid receptor binding and mRNA expression in specific brain areas[29]. This contrasts with the cross-tolerance shown by Thorat and Bhargava[26]. These two studies differed in the species used (mice[26] and rats[29]), whereas tail-flick tests, morphine pellets and equivalent doses of Δ^9-THC were used in both cases.

Additive effects of opioids and cannabinoids have also been reported, in studies using acute and subeffective

mediated antinociception

in recent studies using administration of antagonists[30] or antisense oligodeoxynucleotides[31] to the CB_1 receptor. Moreover, the administration of SR141716A produced hyperalgesia by itself[32]. However, there are some discrepancies between anandamide and Δ^9-THC regarding their antinociceptive effects[32–35]. The administration of SR141716A blocked the antinociception induced by Δ^9-THC or by synthetic agonists[13,25,30], but failed to completely antagonize the effects of anandamide[23,33,36]. Welch has recently shown that Δ^9-THC-tolerant mice were cross-tolerant to κ-receptor agonists as well as to anandamide[37]. However, anandamide-tolerant mice were cross-tolerant to Δ^9-THC but not to κ-receptor agonists[37]. These differences suggest either that anandamide does not produce its effects by a direct interaction with the CB_1 receptor or that the existence of a receptor subtype, other than the CB_1, is specifically involved in the effects of anandamide[23,36]. The study by Calignano and co-workers[7] also demonstrated the involvement of peripheral CB_1-like and CB_2-like receptors in the control of pain, as the combined administration of anandamide (which binds to both CB_1 and CB_2 receptors) and palmitoylethanolamide (which binds only to CB_2 receptors) was 100 times more effective in reducing the pain response induced by tissue damage than each compound alone. Specific CB_1 or CB_2 receptor antagonists, SR141716A and SR144528, respectively, prolonged and enhanced the pain[7]. The pain-reducing effects of palmitoylethanolamide have also been described by Jaggar and co-workers[34], indicating a role for CB_2 receptors, whereas Richardson and co-workers[38] reported that cannabinoid-induced reduction of hyperalgesia and inflammation was mediated through CB_1 receptors.

References

1 Dewey, W. L. (1986) *Pharmacol. Rev.* 38, 151–178
2 Holdcroft, A. *et al.* (1997) *Anaesthesia* 52, 483–486
3 Notcutt, W., Price, M. and Chapman, G. (1997) *Pharm. Sci.* 3, 551–555
4 Lichtman, A. H. and Martin, B. R. (1991) *J. Pharmacol. Exp. Ther.* 258, 517–523
5 Martin, W. J., Lai, N. K., Patrick, S. L., Tsou, K. and Walker, J. M. (1993) *Brain Res.* 629, 300–304
6 Martin, W. J., Patrick, S. L., Coffin, P. O., Tsou, K. and Walker, J. M. (1995) *Life Sci.* 56, 2103–2109
7 Calignano, A., La Rana, G., Giuffrida, A. and Piomelli, D. (1998) *Nature* 394, 277–281
8 Stein, C., Schafer, M. and Hassan, A. H. (1995) *Ann. Med.* 27, 219–221
9 Stein, C. and Yassouridis, A. (1997) *Pain* 71, 119–121
10 Nakamura, M. and Ferreira, S. H. (1988) *Eur. J. Pharmacol.* 146, 223–228
11 Smith, P. B. and Martin, B. R. (1992) *Brain Res.* 578, 8–12
12 Hohmann, A. G. and Herkenham, M. (1998) *Neurosci. Lett.* 252, 13–16
13 Reche, I., Fuentes, J. A. and Ruiz-Gayo, M. (1996) *Eur. J. Pharmacol.* 301, 75–81
14 Reche, I., Fuentes, J. A. and Ruiz-Gayo, M. (1996) *Eur. J. Pharmacol.* 318, 11–16
15 Lu, Y., Pirec, V. and Yeomans, D. C. (1997) *Br. J. Pharmacol.* 121, 1210–1216
16 Laughlin, T. M. *et al.* (1997) *Pain* 72, 253–260
17 Pugh, G., Jr, Mason, D. J., Jr, Combs, V. and Welch, S. P. (1997) *J. Pharmacol. Exp. Ther.* 281, 730–737
18 Pugh, G., Jr, Smith, P. B., Dombrowski, D. S. and Welch, S. P. (1996) *J. Pharmacol. Exp. Ther.* 279, 608–616
19 Smith, P. B., Welch, S. P. and Martin, B. R. (1994) *J. Pharmacol. Exp. Ther.* 268, 1381–1387
20 Rowen, D. W., Embrey, J. P., Moore, C. H. and Welch, S. P. (1998) *Pharmacol. Biochem. Behav.* 59, 399–404
21 Pugh, G., Jr, Abood, M. E. and Welch, S. P. (1995) *Brain Res.* 698, 157–158
22 Lichtman, A. H. and Martin, B. R. (1991) *Brain Res.* 559, 309–314
23 Welch, S. P., Huffman, J. W. and Lowe, J. (1998) *J. Pharmacol. Exp. Ther.* 286, 1301–1308
24 Lichtman, A. H., Cook, S. A. and Martin, B. R. (1996) *J. Pharmacol. Exp. Ther.* 276, 585–593
25 Martin, W. J., Tsou, K. and Walker, J. M. (1998) *Neurosci. Lett.* 242, 33–36
26 Martin, W. J., Hohmann, A. G. and Walker, J. M. (1996) *J. Neurosci.* 16, 6601–6611
27 Meng, I. D., Manning, B. H., Martin, W. J. and Fields, H. L. (1998) *Nature* 395, 381–383
28 Herkenham, M. *et al.* (1991) *J. Neurosci.* 11, 563–583
29 Melvin, L. S. *et al.* (1993) *Mol. Pharmacol.* 44, 1008–1013
30 Lichtman, A. H. and Martin, B. R. (1997) *Pharmacol. Biochem. Behav.* 57, 7–12
31 Edsall, S. A. *et al.* (1996) *NeuroReport* 7, 593–596
32 Richardson, J. D., Aanonsen, L. and Hargreaves, K. M. (1997) *Eur. J. Pharmacol.* 319, R3–R4
33 Smith, F. L., Fujimori, K., Lowe, J. and Welch, S. P. (1998) *Pharmacol. Biochem. Behav.* 60, 183–191
34 Jaggar, S. I., Hasnie, F. S., Sellaturay, S. and Rice, A. S. (1998) *Pain* 76, 189–199
35 Welch, S. P., Dunlow, L. D., Patrick, G. S. and Razdan, R. K. (1995) *J. Pharmacol. Exp. Ther.* 273, 1235–1244
36 Adams, I. B., Compton, D. R. and Martin, B. R. (1998) *J. Pharmacol. Exp. Ther.* 284, 1209–1217
37 Welch, S. P. (1997) *Drug Alcohol Depend.* 45, 39–45
38 Richardson, J. D., Kilo, S. and Hargreaves, K. M. (1998) *Pain* 75, 111–119

doses of both compounds[5,30–32]. Synergism is commonly observed among analgesic agents, and this might not be directly related to receptor–signalling interactions. However, Smith and co-workers[32] showed recently that the enhancement of morphine-induced antinociception by Δ^9-THC was antagonized by SR141716A and concluded that it was mediated through cannabinoid receptors. They demonstrated that nor-binaltorphimine or naltrindole, a δ-receptor antagonist, was also able to block the antinociceptive effect caused by the combination of Δ^9-THC and morphine[30]. They suggested that the antinociceptive effects of morphine, which are predominantly mediated by μ-receptors, might be enhanced by Δ^9-THC through activation of κ- and δ-receptors[30]. This fact could be potentially useful in the treatment of pain.

Drug addiction

Drug addiction is another relevant issue with regard to cannabinoid–opioid interactions. One consideration is whether long-term marihuana consumption alters the steady-state of the endogenous opioid system, so potentially increasing an individual's susceptibility to opioid addiction. In a recent paper, Tanda and co-workers[9] reported that cannabinoids might activate mesolimbic dopaminergic neurotransmission, which is an important component of the reward system. The effect observed

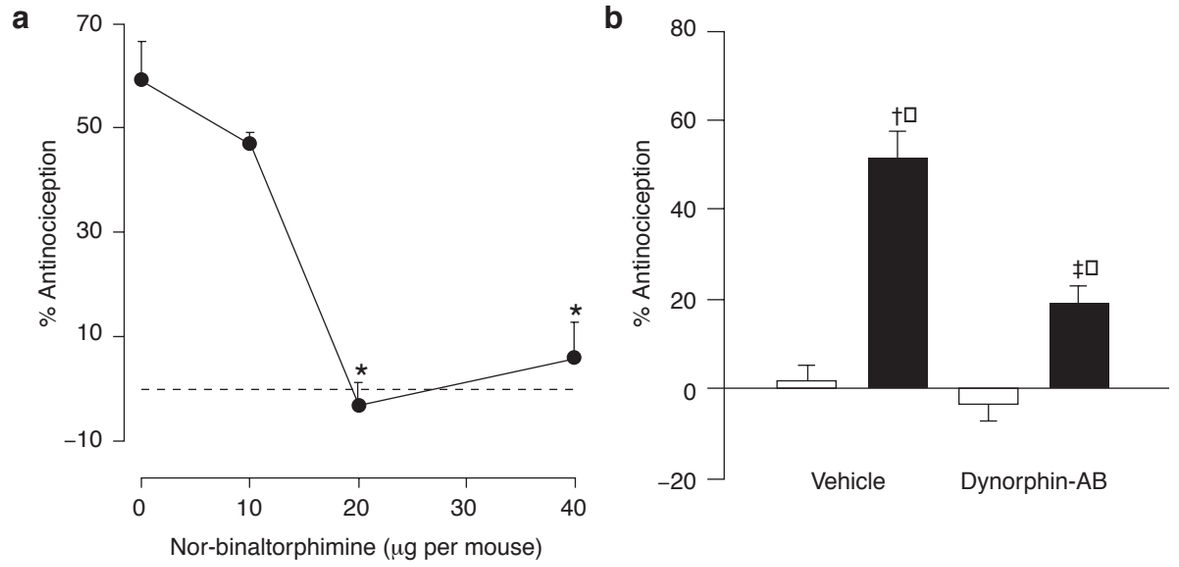

Fig. 1. Effects of the intrathecal administration of **a:** the κ-opioid receptor antagonist nor-binaltorphimine or **b:** dynorphin A-(1–8) rabbit antiserum on the antinociceptive effect of Δ^9-tetrahydrocannabinol, measured using the tail-flick test. In a, * indicates statistical significance. In b, † indicates statistical significance compared with their corresponding vehicle-injected group, whereas ‡ indicates statistical significance compared with their corresponding vehicle-injected group and also with the group treated with Δ^9-tetrahydrocannabinol but not with the dynorphin A-(1–8) rabbit antiserum. White bars, vehicle; black bars, Δ^9-tetrahydrocannabinol. Data reproduced, with permission, from Ref. 19.

was similar to that caused by heroin and probably mediated through a μ_1-receptor mechanism common to both. This is consistent with the fact that, as for the antinociceptive effect and for other cannabinoid-mediated effects[2], opioid receptor antagonists blocked, at least partially, some effects of Δ^9-THC that are related to alterations in the reward substrates[6,33]. They were detected by electrical brain stimulation reward[7] and *in vivo* microdialysis[6]. These authors proposed that cannabinoids act in a similar way to most drugs of abuse in that their increase in brain-reward facilitation is also naloxone-reversible, at doses which by themselves have no effect on brain-stimulation reward[34]. By contrast, other authors were unable to show a reversal by naloxone of the Δ^9-THC-induced increased firing of dopaminergic neurones in the ventral tegmental area[35], which was, in turn, blocked by SR141716A, a specific cannabinoid receptor antagonist. This suggests the involvement of a CB_1 receptor-mediated mechanism.

Mimicking the effect observed with naloxone in morphine-dependent animals, administration of SR141716A to Δ^9-THC-tolerant rodents also precipitated several somatic signs of withdrawal[36–38], but these were of a smaller magnitude than those shown in the case of opioids. For example, neither dominant behavioural signs (e.g. jumping) or autonomic signs (lacrimation, diarrhoea and others), which are both considered to be indicative of the severity of the withdrawal response, are observed in the SR141716A-induced withdrawal syndrome in Δ^9-THC-tolerant animals[36–38]. However, Δ^9-THC-tolerant rodents develop an opioid-like withdrawal syndrome after acute administration of naloxone[8], which suggests that prolonged exposure to cannabinoids could lead to profound alterations in opioid neurotransmission.

This latter effect has also been observed using developmental models. It has been reported recently that rats exposed to Δ^9-THC during crucial periods of brain development also exhibited a naloxone-induced withdrawal syndrome at immature ages[39]. The response was mild compared with that of morphine-dependent animals, but this could indicate a cannabinoid-induced alteration in the maturation of opioid peptide-containing neurones. In fact, when these animals reached adult age, they were more susceptible to the reinforcing properties of morphine, as revealed by either self-administration studies[40] (see Fig. 2) or by conditioning place-preference studies[41]. These behavioural signs run in parallel with an increase in the density of μ-receptor binding in areas related to drug reinforcement[40]. In this respect, a reduction in the levels of endogenous opioids might be expected to occur. This anticipated outcome was confirmed by the decrease observed in the expression of proenkephalin gene in some of these areas[42] (see Fig. 2). The ratio of enkephalin content to μ-receptor density might be a crucial factor for self-administration of morphine in rats. In this sense, by using different rat strains, it has recently been shown that these rats were more vulnerable to morphine when having lesser basal proenkephalin mRNA levels[43]. However, other studies have shown that the absence of μ-receptors in mutant mice led to a loss of reinforcing properties of morphine[22].

Despite these data, the hypothesis that marihuana consumption might enhance the reinforcing potential of opioids needs to be more extensively addressed. Two novel methodological tools, which have been developed very recently, could provide further insight. Using the synthetic cannabinoid receptor agonist WIN552122, Martellota and co-workers[44] have observed self-administration of this agonist in mice; such self-administration had previously

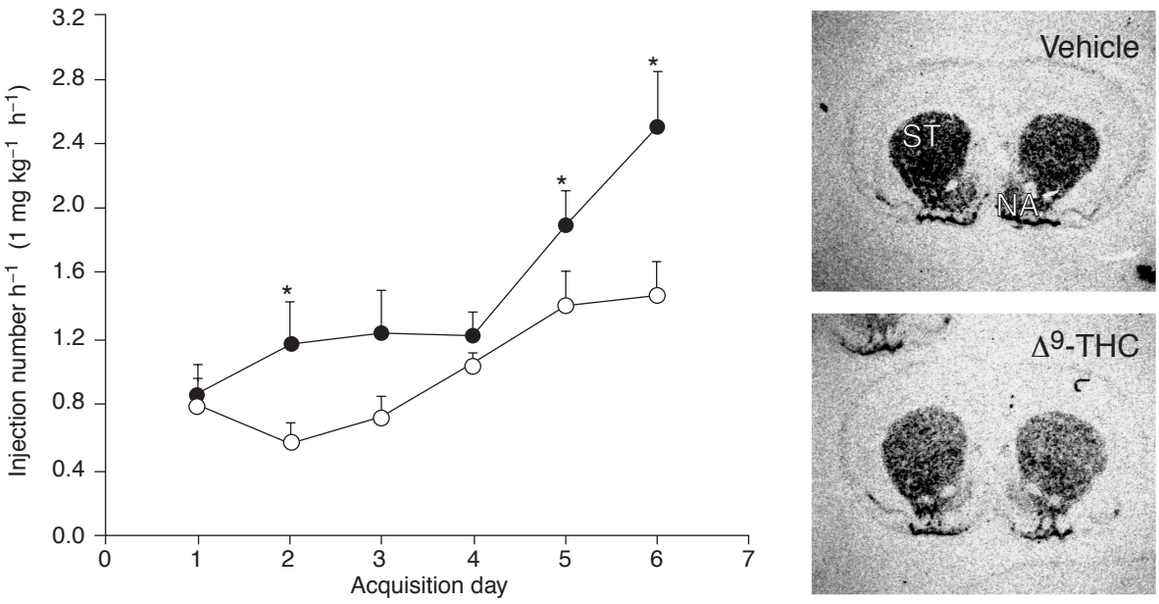

Fig. 2. Basal mRNA levels for proenkephalin in the forebrain of adult rats that had been perinatally exposed to Δ^9-tetrahydrocannabinol (Δ^9-THC) (right panel) and number of injections in these animals when subjected to morphine self-administration studies (left panel). * Indicates statistical significance. Open circles, vehicle; closed circles, Δ^9-THC. ST, striatum; NA, nucleus accumbens. Data reproduced, with permission, from Refs 40 and 42.

been extremely complicated using Δ^9-THC or other classic cannabinoids in rats and monkeys[45,46]. Mice lacking CB_1 receptors have also been produced by gene targeting and provided an additional molecular tool to study the role of these receptors in drug addiction. Using these mutant mice, Ledent and co-workers[47] have shown that the acute effects of morphine were unaffected, but their reinforcing properties and, consequently, the severity of the withdrawal syndrome were reduced. This extends the concept of an interconnected role of CB_1 and opioid receptors in brain regions mediating addictive behaviours and, additionally, suggests that long-term administration of CB_1 receptor antagonists might be considered as tools for preventing the development of opioid dependence[47]. Other studies have also provided evidence of a possible beneficial effect of cannabinoids in the treatment of opioid withdrawal, although this evidence was based on the use of cannabinoid receptor agonists.

Thus, although still anecdotal, there is clinical evidence to suggest that the use of marihuana might attenuate the magnitude of the withdrawal syndrome in heroin addicts[48]. In fact, Δ^9-THC, as well as anandamide, the main endogenous cannabinoid receptor ligand, ameliorates the magnitude of withdrawal signs observed in morphine-dependent rodents[10,49,50]. This suggests that CB_1 receptor agonists might be effective in the treatment of opioid withdrawal rather than in the development of dependence. As both states are behaviourally and biochemically opposite and pharmacologically dissociable, the use of antagonists to prevent the development of dependence and of agonists during withdrawal appears reasonable. This could have clinical implications because these compounds can be used as a potential therapy in the treatment of opioid abusers.

Biochemical mechanisms involved in opioid–cannabinoid interactions

Despite data that support an interaction between the opioid and the cannabinoid systems in antinociception and drug addiction, as well as in other less-studied pharmacological effects such as hypothermia[2], the nature of such interactions remains unclear. Several authors have suggested that both drugs share common links in their molecular mechanisms of action, although this has been a matter of controversy. In this respect, it is important to consider that the nature of cannabinoid–opioid interactions might differ in brain circuits mediating reward and in those mediating other pharmacological properties, such as antinociception[34]. Two hypotheses have been formulated and these are discussed below.

Signal-transduction interactions between cannabinoids and opioids

Despite the existence of bidirectional cross-tolerance, Thorat and Bhargava[26] in 1994 showed an absence of changes in opioid receptor binding in Δ^9-THC-tolerant mice. These authors suggested that cannabinoids and opioids might interact at the level of their signal-transduction mechanisms. Receptors for both types of drugs are coupled to similar intracellular signalling mechanisms, mainly to a decrease in cAMP production through activation of G_i proteins[51,52]. Previous studies have shown that animals chronically exposed to morphine exhibited adaptive changes in adenylate cyclase-coupled G proteins[53–55], thus affecting the efficiency of other receptors also coupled to G_i and G_o proteins such as cannabinoid receptors. A requirement for this hypothesis is that, in order to be able to compete for the same pools of G proteins, receptors for both compounds should co-localize

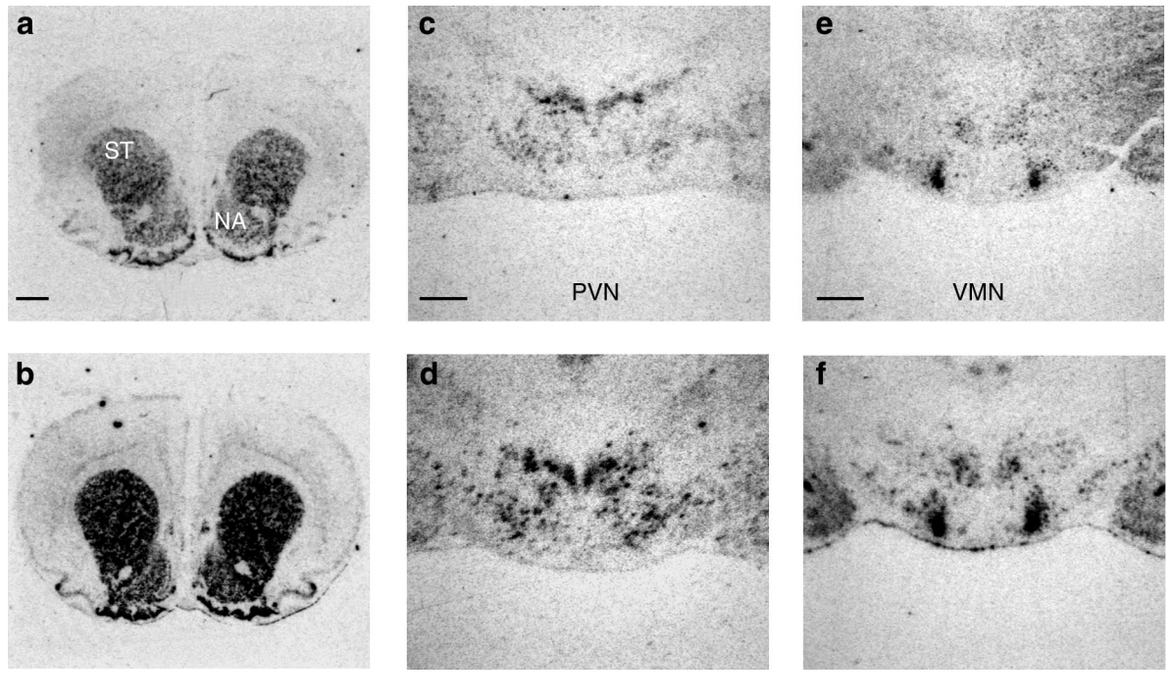

Fig. 3. Effects of 18-day treatment with vehicle (panels **a**, **c** and **e**) and CP55940 (panels **b**, **d** and **f**) on proenkephalin mRNA levels measured by *in situ* hybridization. NA, nucleus accumbens; PVN, paraventricular hypothalamic nucleus; ST, striatum; VMN, ventromedial hypothalamic nucleus. Scale bar, 1 mm. Data reproduced, with permission, from Ref. 65.

in the same neurones. Unfortunately, double-labelling experiments have not been carried out yet but should be considered, given the recent preparation of specific antibodies for CB_1 receptors. There are several brain structures, such as the caudate-putamen, the dorsal hippocampus and the substantia nigra that are rich in both cannabinoid and opioid receptors[21,56,57] and where co-localization of both types of receptors would be feasible. Other brain structures, such as the periaqueductal grey and the medial basal hypothalamus, contain more moderate levels of cannabinoid and opioid binding sites[21,56,57], but play an important role in cannabinoid-mediated antinociception[58] and in neuroendocrine effects of cannabinoids[59,60], respectively.

The hypothesis of an interaction at the level of signal-transduction mechanisms might serve to explain the attenuating effect of Δ^9-THC on naloxone-precipitated withdrawal signs in morphine-dependent animals[49,50], as well as on the phenomena of mutual potentiation or cross-tolerance in antinociception[3,5,25–27,30–32] (see earlier). By using cultures of cell lines from tumours of the CNS, more recent studies have provided new elements to support this hypothesis. Thus, Shapira and co-workers[61] observed that δ- and cannabinoid receptors activate different pools of G proteins, although these authors[61] and others[62] observed the phenomenon of cross-desensitization in cannabinoid or opioid agonist-activation of G proteins. Nevertheless, this assumption does not appear to agree with recent results of a lack of change in both cannabinoid receptor binding and agonist-induced activation of signal-transduction mechanisms in brain structures of morphine-dependent mice[28].

Cannabinoid-induced synthesis and release of endogenous opioids

Recent studies have provided evidence for another hypothesis for opioid–cannabinoid interactions, which states that cannabinoids might increase the synthesis or release of endogenous opioids, or both[63–66]. This would be particularly relevant for the antinociceptive effects of cannabinoids and could also explain the ability of some opioid receptor antagonists to block some effects of Δ^9-THC (Refs 6, 19, 23, 33), as well as to induce withdrawal signs in Δ^9-THC-tolerant rats[8].

The block of Δ^9-THC-induced antinociception by intrathecal administration of antisera to κ-receptor endogenous ligands such as dynorphin A(1–17), dynorphin A(1–8) or α-neoendorphin[19,30] points to a cannabinoid-induced activation of the spinal dynorphin system. This is also supported by the fact that, using spinal catheterization of rats, the administration of the cannabinoid receptor agonist CP55940 has been reported to produce a significant release of dynorphin B concurrent with the production of antinociception[31]. Similarly, it has been shown recently that opioid-degrading enzyme inhibitors potentiate Δ^9-THC-induced antinociception[67]. The involvement of released dynorphin-related peptides in the antinociceptive effect of Δ^9-THC in the spinal cord is concordant with the above-described pharmacological interactions between Δ^9-THC and κ-opioid receptor agonists[17,19,24,25,27]. However, some recent studies have argued against the notion that dynorphin release at spinal level is acting through κ-receptors to produce antinociception[68,69]. (This has been discussed in more detail in Box 1.) It is possible that other opioid peptides are alternatively involved, given

that a five-day administration of Δ^9-THC produced a marked increase in prodynorphin and proenkephalin gene expression in the spinal cord[64]. Chronic cannabinoid administration was also found to increase proopiomelanocortin gene expression (its post-translational product is β-endorphin) in the arcuate nucleus of the hypothalamus[63] and to enhance proenkephalin gene expression in the periaqueductal grey matter[65]. β-endorphin is known to be a more potent analgesic than morphine itself. All of these brain areas are closely related to spinal and supraspinal circuits regulating nociceptive pathways. Despite some controversial results discussed in Box 1, this is consistent with the suggestion of an involvement of both supraspinal μ- and spinal κ-receptors in the interaction between opioid and cannabinoid systems that regulate nociception in mice[67].

Hence, the cannabinoid-induced increase in the synthesis or release of endogenous opioids, or both, might explain the antinociceptive effects of cannabinoids. Additional data suggest that cannabinoid-induced upregulation of opioid gene expression occurs not only in pain-related brain and spinal cord areas, but also in regions that regulate motor behaviour, pituitary hormone secretion and reward. This means that other pharmacological interactions between opioids and cannabinoids, including addiction, could also be explained through this mechanism. Thus, it has been shown recently that prolonged administration of different cannabinoid receptor agonists to rats increases proenkephalin gene expression in the caudate-putamen, nucleus accumbens, paraventricular and ventromedial hypothalamic nuclei and medial mammilary nucleus[65] (Fig. 3). On the basis that these areas are directly or indirectly related to drug reinforcement, cannabinoid-induced increase in proenkephalin gene expression might be interpreted as part of a molecular integrative response to behavioural and neurochemical alterations that might occur in cannabinoid drug abuse.

Concluding remarks

According to the results discussed in this review, it appears that opioids and cannabinoids share a number of pharmacological actions that could be relevant in understanding the therapeutic potential of cannabinoids, particularly as analgesics. There is also an interconnected role for cannabinoid and opioid receptors in brain regions mediating addictive behaviours that might be of clinical interest. Although some hypotheses have been presented and discussed here on the basis that they are not mutually exclusive, the precise nature of the interaction between both cannabinoid and opioid system remains to be more clearly determined. With this purpose, these data suggest a cannabinoid-induced increase in the synthesis or release, or both, of opioid peptides as a potential mechanism to explain some of these interactions. In the near future, elucidation of the molecular substrates of these interactions might represent a crucial contribution regarding the usefulness of cannabinoids as therapeutics and the role of these compounds in the production of addiction and dependence.

References

1 Dewey, W. L. (1986) *Pharmacol. Rev.* 38, 151–178
2 Bloom, A. S. and Dewey, W. L. (1978) *Psychopharmacology* 57, 243–248
3 Smith, P. B., Welch, S. P. and Martin, B. R. (1994) *J. Pharmacol. Exp. Ther.* 268, 1381–1387
4 Tulunay, F. C., Ayhan, I. H., Portoghese, P. S. and Takemori, A. E. (1981) *Eur. J. Pharmacol.* 70, 219–224
5 Welch, S. P. and Stevens, D. L. (1992) *J. Pharmacol. Exp. Ther.* 262, 10–18
6 Chen, J. *et al.* (1990) *Psychopharmacology* 102, 156–162
7 Gardner, E. L., Paredes, W., Smith, D. and Zukin, R. S. (1989) *Adv. Biosci.* 75, 671–674
8 Kaymakçalan, S., Ayhan, I. H. and Tulunay, F. C. (1977) *Psychopharmacology* 55, 243–249
9 Tanda, G., Pontieri, F. E. and Di Chiara, G. (1997) *Science* 276, 2048–2050
10 Vela, G., Ruiz-Gayo, M. and Fuentes, J. A. (1995) *Neuropharmacology* 34, 665–668
11 Calignano, A., La Rana, G., Giuffrida, A. and Piomelli, D. (1998) *Nature* 394, 277–281
12 Lichtman, A. H. and Martin, B. R. (1991) *Brain Res.* 559, 309–314
13 Martin, W. J., Tsou, K. and Walker, J. M. (1998) *Neurosci. Lett.* 242, 33–36
14 Meng, I. D., Manning, B. H., Martin, W. J. and Fields, H. L. (1998) *Nature* 395, 381–383
15 Martin, B. R. (1985) *Life Sci.* 36, 1523–1530
16 Vivian, J. A. *et al.* (1998) *J. Pharmacol. Exp. Ther.* 286, 697–703
17 Welch, S. P. (1993) *J. Pharmacol. Exp. Ther.* 265, 633–640
18 Welch, S. P. (1994) *Pharmacol. Biochem. Behav.* 49, 929–934
19 Reche, I., Fuentes, J. A. and Ruiz-Gayo, M. (1996) *Eur. J. Pharmacol.* 301, 75–81
20 Smith, F. L., Fujimori, K., Lowe, J. and Welch, S. P. (1998) *Pharmacol. Biochem. Behav.* 60, 183–191
21 Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H. and Watson, S. J. (1988) *Trends Neurosci.* 11, 308–314
22 Kieffer, B. L. (1999) *Trends Pharmacol. Sci.* 20, 19–26
23 Reche, I., Fuentes, J. A. and Ruiz-Gayo, M. (1996) *Eur. J. Pharmacol.* 318, 11–16
24 Pugh, G., Jr, Abood, M. E. and Welch, S. P. (1995) *Brain Res.* 698, 157–158
25 Rowen, D. W., Embrey, J. P., Moore, C. H. and Welch, S. P. (1998) *Pharmacol. Biochem. Behav.* 59, 399–404
26 Thorat, S. N. and Bhargava, H. N. (1994) *Eur. J. Pharmacol.* 260, 5–13
27 Welch, S. P. (1997) *Drug Alcohol Depend.* 45, 39–45
28 Romero, J. *et al.* (1998) *Drug Alcohol Depend.* 50, 241–249
29 Rubino, T., Tizzoni, L., Vigano, D., Massi, P. and Parolaro, D. (1997) *NeuroReport* 8, 3219–3233
30 Pugh, G., Jr, Smith, P. B., Dombrowski, D. S. and Welch, S. P. (1996) *J. Pharmacol. Exp. Ther.* 279, 608–616
31 Pugh, G., Jr, Mason, D. J., Jr, Combs, V. and Welch, S. P. (1997) *J. Pharmacol. Exp. Ther.* 281, 730–737
32 Smith, F. L., Cichewicz, D., Martin, Z. L. and Welch, S. P. (1998) *Pharmacol. Biochem. Behav.* 60, 559–566
33 Gardner, E. L. and Lowinson, J. H. (1991) *Pharmacol. Biochem. Behav.* 40, 571–580
34 Gardner, E. L. (1992) in *Marijuana/Cannabinoids. Neurobiology and Neurophysiology* (Murphy, L. and Bartke, A., eds), pp. 275–335, CRC Press
35 French, E. D. (1997) *Neurosci. Lett.* 226, 159–162
36 Tsou, K., Patrick, S. L. and Walker, J. M. (1995) *Eur. J. Pharmacol.* 280, R13–R15
37 Aceto, M. D., Scates, S. M., Lowe, J. A. and Martin, B. R. (1995) *Eur. J. Pharmacol.* 282, R1–R2
38 Hutcheson, D. M. *et al.* (1998) *Brit. J. Pharmacol.* 125, 1567–1577
39 Vela, G., Fuentes, J. A., Bonnin, A., Fernández-Ruiz, J. J. and Ruiz-Gayo, M. (1995) *Brain Res.* 680, 142–147
40 Vela, G. *et al.* (1998) *Brain Res.* 807, 101–109
41 Rubio, P. *et al.* (1998) *Pharmacol. Biochem. Behav.* 61, 229–238
42 Corchero, J. *et al.* (1998) *Life Sci.* 63, 843–850
43 Martin, S. *et al.* (1999) *Brain Res.* 821, 350–355
44 Martellota, M. C., Cossu, G., Gessa, G. L. and Fratta, W. (1998) *Neuroscience* 85, 327–330
45 Mansbach, R. S., Nicholson, K. L., Martin, B. R. and Balster, R. L. (1994) *Behav. Pharmacol.* 5, 219–225
46 Leite, J. R. and Carlini, E. A. (1974) *Psychopharmacologia* 36, 133–145
47 Ledent, C. *et al.* (1999) *Science* 283, 401–404
48 Hollister, L. E. (1986) *Pharmacol. Rev.* 38, 1–20
49 Bhargava, H. N. (1976) *Eur. J. Pharmacol.* 36, 259–264
50 Hine, B., Friedman, E., Torrelio, M. and Gershon, S. (1975) *Science* 187, 443–445

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51 Bidaut-Russell, M. and Howlett, A. (1989) *Adv. Biosci.* 75, 165–168
52 Childers, S. R. *et al.* (1992) *Ann. New York Acad. Sci.* 654, 33–51
53 De Vries, T. J. *et al.* (1991) *Eur. J. Pharmacol.* 208, 97–104
54 Nestler, E. J., Erdos, J. J., Terwilliger, R., Duman, R. S. and Tallman, J. F. (1989) *Brain Res.* 476, 230–239
55 Nestler, E. J. (1992) *J. Neurosci.* 12, 2439–2450
56 Herkenham, M. *et al.* (1991) *J. Neurosci.* 11, 563–583
57 Mailleux, P. and Vanderhaeghen, J-J. (1992) *Neuroscience* 48, 655–668
58 Lichtman, A. H., Cook, S. A. and Martin, B. R. (1996) *J. Pharmacol. Exp. Ther.* 276, 585–593
59 Fernández-Ruiz, J. J. *et al.* (1997) *Biochem. Pharmacol.* 53, 1919–1927
60 Romero, J., Wenger, T., de Miguel, R., Ramos, J. A. and Fernández-Ruiz, J. J. (1998) *Life Sci.* 63, 351–356
61 Shapira, M., Gafni, M. and Sarne, Y. (1998) *Brain Res.* 806, 26–35
62 Di Toro, R., Campana, G., Sciarretta, V., Murari, G. and Spampinato, S. (1998) *Life Sci.* 63, PL197–PL204
63 Corchero, J., Fuentes, J. A. and Manzanares, J. (1997) *Eur. J. Pharmacol.* 323, 193–195
64 Corchero, J., Avila, M. A., Fuentes, J. A. and Manzanares, J. (1997) *Life Sci.* 61, PL39–PL43
65 Manzanares, J. *et al.* (1998) *Mol. Brain Res.* 55, 126–132
66 Kumar, M. S. *et al.* (1990) *Brain Res.* 525, 78–83
67 Reche, I., Ruiz-Gayo, M. and Fuentes, J. A. (1998) *Neuropharmacology* 37, 215–222
68 Lu, Y., Pirec, V. and Yeomans, D. C. (1997) *Br. J. Pharmacol.* 121, 1210–1216
69 Laughlin, T. M. *et al.* (1997) *Pain* 72, 253–260

Chemical names

CP55940: (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl) phenyl]-*trans*-4-(3-hydroxy-propyl)cyclohexanol

SR141716A: *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)4-methyl-3-pyrazolecarboxamide

SR144528: *N*-([1s]-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide

WIN552122: R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl) methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-yl]-(1-naphtalenyl)methanone

7TM receptors: the splicing on the cake

Gavin J. Kilpatrick, Frank M. Dautzenberg, Graeme R. Martin and Richard M. Eglen

Within a given family of seven transmembrane domain (7TM) receptors, functional diversity is most often afforded by the existence of multiple receptor subtypes, each encoded by a distinct gene. However, it is now clear that the existence of introns in genes encoding some members of a receptor family provides scope for additional diversity by virtue of splicing events that result in the formation of different receptor mRNAs and consequently distinct receptor isoforms. A large number of 7TM receptor splice variants have now been shown to exist. In this article, the current data on alternatively spliced variants for hormone and neurotransmitter 7TMs are reviewed, their potential physiological importance considered and some of the issues pertaining to the classification and nomenclature of receptor isoforms produced in this way are addressed.

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Seven transmembrane domain (7TM) receptors comprise a large family of cell-surface proteins whose main role is to bind ligands such as neurotransmitters and hormones and to transduce their signal intracellularly. There is a large diversity within the family and frequently several 7TM receptors recognize the same endogenous ligand. For example, 13 unique 7TM receptors have been identified for 5-HT, eight for glutamate, five for dopamine and five for acetylcholine[1]. This complexity has made difficult the assignment of clear physiological roles to each 7TM especially as highly selective agonists and antagonists for most 7TM receptors are unavailable. This situation is made even more complex by translational and post-translational modifications of the RNA encoding some of these receptors, which results in isoforms that might exhibit quite different properties. In this review, one of the most important mechanisms leading to physiological diversity among 7TM receptors, namely variation in splicing events, is considered.

Many 7TM receptor genes contain introns, which introduce the possibility of variants due to alternative splicing, exon skipping and intron retention. Variations in gene-product splicing occur at the level of processing of heteronuclear-RNAs (hn RNAs or pre-mRNAs) in the cell nucleus. In genes with multiple exons, the introns are removed. Alternative splicing pathways vary the particular introns to be deleted (Fig. 1) and this is an important process for many gene families. A notable example, for a non-7TM receptor family, is the neurexin family, where three genes are thought to produce, in a combinatorial fashion, thousands of alternatively spliced gene products, many of which are functionally distinct[2]. The first 7TM receptor for which splice variants were identified was the dopamine D2 receptor. Monsma and colleagues[3] showed that this receptor exists as two isoforms, either with or without an additional 29 amino acids in the putative third intracellular loop. There are now >30 7TM receptors for which splice variants have been identified and their potential physiological relevance is discussed in this review. For the sake of brevity, the 7TM receptors considered are limited to those for hormone or neurotransmitter ligands, and only splice variants where the basic

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