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Original investigations

Δ^9-Tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis

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Abstract. This study examined the effects of acute administration of delta-9-tetrahydrocannabinol (Δ^9–THC), the psychoactive ingredient in marijuana, on extracellular efflux of dopamine (DA) and its metabolites as measured by in vivo microdialysis in nucleus accumbens of conscious, freely-moving rats. Δ^9–THC, at low doses (0.5–1.0 mg/kg), which significantly enhance brain stimulation reward (intracranial self-stimulation), significantly increased DA efflux in nucleus accumbens. Augmentation of DA efflux by Δ^9–THC was abolished by removal of calcium (Ca^{++}) ions from the perfusion fluid, indicating a Ca^{++}-dependence of Δ^9–THC's action. Augmentation of DA efflux by Δ^9–THC was either totally blocked or significantly attenuated by doses of naloxone as low as 0.1 mg/kg. Given the postulated role of mesocorticolimbic DA circuits in mediating and/or modulating brain stimulation reward, the present data raise the possibility that marijuana's rewarding effects, and hence its euphorigenic effects and abuse potential, may be related to pharmacological augmentation of presynaptic DA mechanisms. Additionally, the DA mechanisms enhanced by marijuana appear to be modulated by an endogenous opioid peptide system.

Key words: Marijuana – Δ^9-Tetrahydrocannabinol – Naloxone – Dopamine – Microdialysis

Although marijuana is the most widely used illicit drug in North America, with significant public health consequences (Petersen 1980; Glantz 1984), the neuropharmacological basis for its widespread recreational use and abuse liability have remained obscure. The hypothesis that drugs of abuse derive a significant portion of their abuse liability from direct neuropharmacological augmentation of brain reward circuits, and that this augmentation involves a portion of the mesotelencephalic dopamine (DA) projections of the medial forebrain bundle, has become a central and seminal conception in recent years (Kornetsky et al. 1979; Wise 1980; Bozarth and Wise 1981; Spyraki et al. 1983; Kornetsky 1985; Wise and Rompre 1989). Congruent with this conception, we have recently shown: (1) that delta-9 tetrahydrocannabinol (Δ^9–THC), the psychoactive ingredient in marijuana, augments brain stimulation reward (electrical intracranial self-stimulation) in the rat medial forebrain bundle at low doses believed pharmacologically relevant to moderate human use of marijuana (Gardner et al. 1988); and (2) that low doses of Δ^9–THC augment potassium (K^+)-stimulated presynaptic DA efflux in rat neostriatum as measured by both in vivo voltammetric electrochemistry and in vivo brain microdialysis (Ng Cheong Ton et al. 1988). However, for technical reasons relating to the sensitivities of the electrochemical and microdialysis paradigms, our previous work (Ng Cheong Ton et al. 1988) was done with anesthetized rats, with measurements of DA efflux in the neostriatum, and with K^+-evoked rather than basal DA efflux. The present study was undertaken to determine whether Δ^9–THC's augmenting effect on DA efflux could be demonstrated under more physiologically and pharmacologically meaningful conditions, i.e., in conscious freely-moving animals, and on basal rather than K^+-evoked DA efflux. Also, since the mesocorticolimbic portions of the mesotelencephalic DA systems appear far more implicated in mediating or modulating the effects of drugs of abuse on brain reward mechanisms than the mesostriatal portions (Wise and Rompre 1989), the present study focused on DA efflux in the nucleus accumbens, a

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crucial forebrain anatomic convergence of reward-relevant DA circuitry (Wise and Rompre 1989). In addition, since drug-induced facilitation of brain stimulation reward is naloxone reversible for most well-studied drugs of abuse (e.g., Esposito et al. 1980; Nazzaro et al. 1980, 1981; Wise and Bozarth 1984; Wise and Rompre 1989), including Δ^9–THC (Gardner et al. 1989), at doses of naloxone which themselves have no effect on brain-stimulation reward, we sought to determine whether naloxone has any effect on augmented mesocorticolimbic DA efflux induced by Δ^9–THC.

Materials and methods

Male Lewis rats (Charles River) weighing between 300 and 350 g were used for these experiments. They were housed individually on ad libitum food and water in an animal colony room kept at 72° F with a 12 h light-dark cycle. Before experiments started, all rats were implanted under pentobarbital anesthesia with microdialysis probes in the nucleus accumbens by standard surgical and stereotaxic technique. The probes were implanted at stereotaxic coordinates A+2.0 (from bregma), L 1.2 (from midline), and V–7.0 (from dura), according to the brain atlas of Paxinos and Watson (1982). Loop-shaped microdialysis probes (Sharp et al. 1984) were used; they were implanted longitudinally parallel to the mid-sagittal line and positioned in the midline of the medial-lateral extent of the nucleus accumbens. Each dialysis probe had a 5 mm exposed tubular dialysis membrane which covered essentially the vertical extent of the nucleus accumbens. Prior to implantation, each dialysis probe was tested in vitro in beakers containing perfusate together with known concentrations of monoamines and monoamine metabolites, and per cent recoveries for DA and its metabolites established; any probe not yielding satisfactory recovery in vitro was discarded. As determined by these in vitro tests, relative recovery rates for the probes used in these experiments were approximately 6% for DA, 8% for DOPAC, and 9% for HVA. Immediately following implantation, intracerebral dialysis was begun and continued for the duration of the experiment, the animals living in individual test chambers with food and water available ad libitum. Drug experiments were begun when animals had recovered from the implantation surgery for 20–24 h. The perfusate was a modified Ringer's solution [189 mM NaCl, 3.9 mM KCl, 3.37 mM $CaCl_2$ (pH = 6.0)], perfused through the dialysis probes at a flow rate of 2 µl/min. When Ca^{++}-free Ringer's solution was used to study the Ca^{++} dependence of Δ^9–THC's effect, $CaCl_2$ was removed and 0.1 mM EGTA (Sigma Chemical Company) was added (Kalén et al. 1988).

Dialysis samples (40 µl) were collected every 20 min and immediately assayed for DA and for the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by high performance liquid chromatography (HPLC) with electrochemical detection (EC). The compounds were separated by reverse phase chromatography with a Brownlee Velosep RP–18 (3 µm particles) 100 × 3.2 mm column and a mobile phase, pH 3.0, containing 0.1 M chloroacetic acid, 0.1 mM EDTA, 0.2 mM sodium octyl sulfate, and 6.5% methanol. The mobile phase flow rate was 1.0 ml/min. Electrochemical measurements of the eluting species were made with a glassy carbon working electrode (Bioanalytical Systems model TL–5A; spacer thickness 0.051 mm) coupled to a LC–4B potentiostat (Bioanalytical Systems) set at +0.7 V. The minimum detection limit was approximately 6–16 fmol for all chemicals measured.

Δ^9–THC (National Institute on Drug Abuse, US Public Health Service) was prepared for in vivo administration by an adaptation of methods we have previously described (Gardner et al. 1988; Ng Cheong Ton et al. 1988). Briefly, the Δ^9–THC was dissolved in a 20% solution of polyvinylpyrrolidone (PVP) (Aldrich Chemical Company) prepared in ethanol. This mixed solution was then evaporated under nitrogen and the dried, concentrated Δ^9–THC–PVP solution was suspended in saline at a concentration of either 0.5 or 1.0 mg/ml. A 15% PVP solution was used for control injections. Naloxone (Sigma Chemical Company) was dissolved in saline at concentrations of 5.0 and 0.1 mg/ml. Animals were randomly assigned to the Δ^9–THC and PVP-control groups. Within the Δ^9–THC group, animals were randomly assigned to the 0.5 mg/kg and 1.0 mg/kg dose groups. Within each group, two out of five rats were given Δ^9–THC on the first test day and then given Δ^9–THC together with naloxone on the second test day while the other three rats received Δ^9–THC together with naloxone at 5 mg/kg on the first test day and Δ–THC alone on the second test day. A 24 h drug wash-out period was always interspersed between test days. All drugs and control solutions were given by intraperitoneal injection (IP). Naloxone was tested at both 5.0 mg/kg and 0.1 mg/kg, the latter dose chosen to be within the range of high specificity for opiate receptors (Di Chiara and Imperato 1988). Ca^{++}-dependence of Δ^9–THC's effect on basal DA efflux was tested by switching a Ca^{++}-free perfusate for the standard Ringers solution perfusate by means of a liquid switch (Carnegie Medicin) after a stable baseline was established with the standard Ringers solution perfusate, with 1 mg/kg Δ^9–THC administered 1 h after the start of Ca^{++}-free perfusion.

Dialysis samples were collected until levels of DA, DOPAC, and HVA were stable. Then, three 20 min baseline pre-injection dialysis samples were collected, drug or vehicle injections were administered, and 20 min dialysis sampling continued. The mean level of DA, DOPAC, and HVA for the three 20 min dialysis samples immediately preceding drug or vehicle administrations were used as baselines and analysis was performed on per cent changes from baseline values after drug or vehicle injections. The data were expressed as means ± the standard error of the mean (SEM). Statistical comparisons were made by analysis of variance for repeated measures (Winer 1962; Kirk 1982), with individual comparisons within each data set tested for significance by the Tukey-Kramer a posteriori statistical procedure (Kirk 1982).

In view of the fact that the animals were fully conscious and freely moving, behavioral observations were made of all animals throughout all dialysis experiments.

After the completion of experiments, animals were sacrificed by anesthetic overdose, the brains removed, and standard histological analysis carried out on all brains to verify dialysis probe locations.

Results

Basal DA and metabolite levels (uncorrected for per cent recovery) in the baseline samples preceding drug or vehicle administration were 85.3 ± 3.2 fmol/20 min DA ($N = 23$), 24.3 ± 0.4 pmol/20 min DOPAC ($N = 23$), and 9.1 ± 0.3 pmol/20 min HVA ($N = 23$).

As compared to baseline levels, both 0.5 mg/kg Δ^9–THC and 1.0 mg/kg Δ^9–THC significantly augmented extracellular basal DA efflux in nucleus accumbens (simple main Δ^9–THC effect by analysis of variance for repeated measures: $F_{2,12} = 35.5$; $P < 0.0001$), with 1.0 mg/kg Δ^9–THC having an earlier peak effect than 0.5 mg/kg Δ^9–THC (time x Δ^9–THC interaction effect by analysis of variance for repeated measures: $F_{10,60} = 6.9$; $P < 0.0001$). These effects on basal DA efflux in nucleus accumbens are illustrated in Fig. 1. The lower dose of Δ^9–THC (0.5 mg/kg) produced maximum change at 60 min post-Δ^9–THC, subsiding to baseline by 120 min post-Δ^9–THC. The higher dose Δ^9–THC (1.0 mg/kg) produced maximum change at 40 min post-Δ^9–THC but with significantly enhanced extracellular DA efflux persisting for the duration of the dialysate collection periods

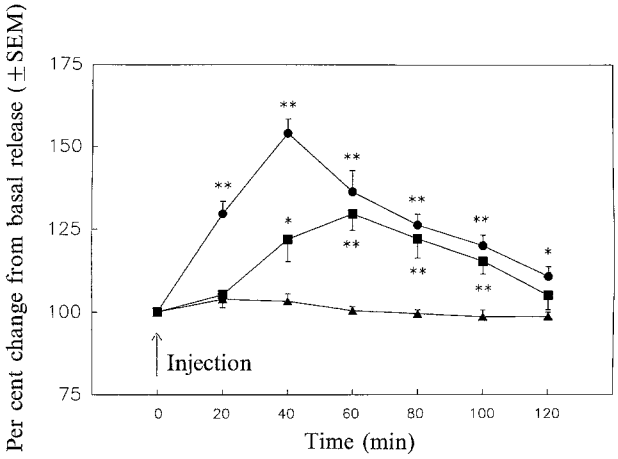

Fig. 1. Per cent enhancement (± SEM) of basal DA efflux in nucleus accumbens of conscious freely-moving rats produced by 20% polyvinylpyrrolidone vehicle. 0.5 mg/kg Δ^9-tetrahydrocannabinol (Δ^9–THC), and 1.0 mg/kg Δ^9–THC. *Asterisks* indicate degree of statistical significance of each Δ^9–THC data point as compared to the corresponding vehicle data point (* $P<0.05$; ** $P<0.01$), as tested using the Tukey-Kramer a posteriori test of individual comparisons. ●—● 1.0 mg/kg THC; ■—■ 0.5 mg/kg THC; ▲—▲ vehicle

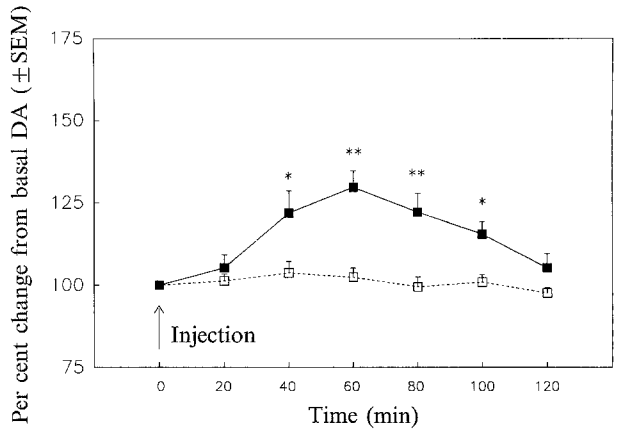

Fig. 2. Naloxone (5 mg/kg)-induced inhibition of the enhanced basal DA efflux in nucleus accumbens of conscious freely-moving rats produced by 0.5 mg/kg Δ^9-tetrahydrocannabinol (Δ^9–THC). *Asterisks* indicate degree of statistical significance of each Δ^9–THC versus Δ^9–THC-plus-naloxone comparison (* $P<0.05$; ** $P<0.01$), as tested using the Tukey-Kramer a posteriori test of individual comparisons. ■—■ 0.5 mg/kg THC; □---□ 0.5 mg/kg THC + 5.0 mg/kg NAL

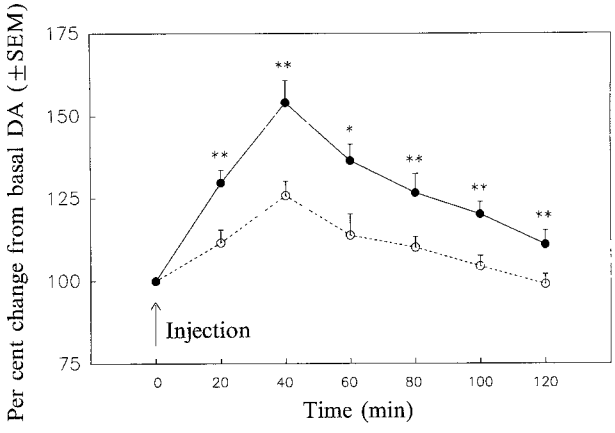

Fig. 3. Naloxone (5 mg/kg)-induced inhibition of the enhanced basal DA efflux in nucleus accumbens of conscious freely-moving rats produced by 1.0 mg/kg Δ^9-tetrahydrocannabinol (Δ^9–THC). *Asterisks* indicate degree of statistical significance of each Δ^9–THC versus Δ^9–THC-plus-naloxone comparison (* $P<0.05$; ** $P<0.01$), as tested using the Tukey-Kramer a posteriori test of individual comparisons. ●—● 1.0 mg/kg THC; ○---○ 1.0 mg/kg THC + 5.0 mg/kg NAL

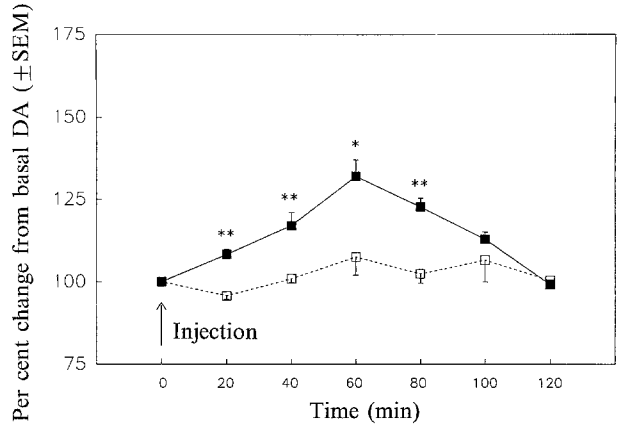

Fig. 4. Naloxone (0.1 mg/kg)-induced inhibition of the enhanced basal DA efflux in nucleus accumbens of conscious freely-moving rats produced by 0.5 mg/kg Δ^9-tetrahydrocannabinol (Δ^9–THC). *Asterisks* indicate degree of statistical significance of each Δ^9–THC versus Δ^9–THC-plus-naloxone comparison (* $P<0.05$; ** $P<0.01$), as tested using the Tukey-Kramer a posteriori test of individual comparisons. ■—■ 0.5 mg/kg THC; □---□ 0.5 mg/kg THC + 0.1 mg/kg NAL

(although subsiding from peak enhancement by roughly 75% at 120 min post-Δ^9–THC) (Fig. 1). Tests of individual comparisons revealed that the lower dose of Δ^9–THC (0.5 mg/kg) significantly enhanced basal DA efflux at 40, 60, 80, and 100 min post-Δ^9–THC (Fig. 1), while the higher dose (1.0 mg/kg) significantly enhanced basal DA efflux at all post-Δ^9–THC time periods tested (Fig. 1). Δ^9–THC at either dose failed to produce significant alterations in DOPAC or HVA. Vehicle injections of 15% PVP solution did not alter DA, DOPAC, or HVA levels.

The enhanced extracellular basal DA efflux produced by Δ^9–THC was significantly attenuated by naloxone. The enhanced basal DA efflux produced by 0.5 mg/kg Δ^9–THC was totally inhibited by naloxone at 5 mg/kg; this naloxone-induced inhibition was statistically significant ($F_{1,4}=28.5$; $P<0.01$) and is illustrated in Fig. 2. As shown in Fig. 2, tests of individual comparisons revealed that naloxone significantly blocked the DA-enhancing effect of 0.5 mg/kg Δ^9–THC at every post-Δ^9–THC time period at which Δ^9–THC had an enhancing effect on DA efflux (Fig. 1). The enhanced basal DA efflux produced by 1.0 mg/kg Δ^9–THC was inhibited approximately 50% by naloxone at 5 mg/kg; this naloxone-induced inhibition was statistically significant ($F_{1,4}=49.8$; $P<0.005$) and is illustrated in Fig. 3. As shown in Fig. 3, tests of

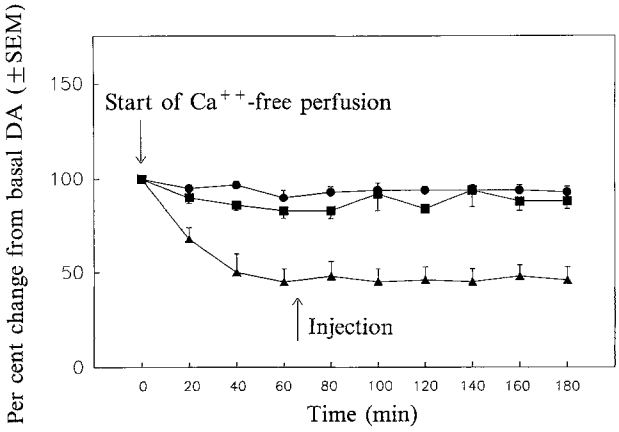

Fig. 5. Calcium-dependence of enhancing effect of Δ^9-tetrahydrocannabinol (Δ^9–THC) on extracellular DA efflux in nucleus accumbens. Extracellular DA efflux in nucleus accumbens was reduced approximately 50% by the Ca^{++}-free perfusion. Δ^9–THC 1.0 mg/kg administered 1 h after the start of Ca^{++}-free perfusion at the point marked "injection" had no effect on extracellular DA efflux. ●—● Dopac; ■—■ HVA; ▲—▲ dopamine

individual comparisons revealed that naloxone significantly attenuated the DA-enhancing effect of 1.0 mg/kg Δ^9–THC at every post-Δ^9–THC time period. The lower dose of naloxone (0.1 mg/kg) also inhibited the enhanced basal DA efflux produced by 0.5 mg/kg Δ^9–THC . This low dose naloxone-induced inhibition was statistically significant ($F_{1,6}=17.6$; $P<0.01$) and is illustrated in Fig. 4. Δ^9–THC's enhancing effect on basal DA efflux was Ca^{++}-dependent, as illustrated in Fig. 5. Basal DA efflux was decreased approximately 50% in the presence of Ca^{++}-free perfusate. Challenge with 1 mg/kg Δ^9–THC did not affect extracellular basal DA efflux in the Ca^{++}-free situation.

The animals were behaviorally unaffected by injections of the PVP vehicle or naloxone. They showed no evidence of stereotypy or psychomotor stimulation following injections of Δ^9–THC; to the contrary, all animals seemed slightly quiescent following Δ^9–THC, although still retaining full motoric capability.

Discussion

Loop-shaped dialysis probes were used in the present study. Even though this kind of probe can cause more tissue damage due to its size than smaller probes used both by us (Paredes et al. 1989) and by others (Di Chiara and Imperato 1986; Hernandez and Hoebel 1988), the basal DA efflux measured after 20–24 h recovery in the present experiments was tetrodotoxin (TTX) sensitive (TTX at 10^{-6} M in the perfusate reduced basal DA efflux to 59% of pre-TTX level; TTX at 10^{-5} M in the perfusate decreased basal DA efflux to 14% of its pre-TTX level), indicating the DA signal monitored in the present experiments was neuronal (Westerink et al. 1987).

The present findings with Δ^9–THC show that this compound, the psychoactive ingredient in marijuana and hashish, augments basal nucleus accumbens extracellular DA efflux. As such, the present findings are in fundamental agreement with the following: a) previous in vitro studies showing augmenting effects of Δ^9–THC on DA synthesis and release in synaptosomes (Poddar and Dewey 1980; Bloom 1982), b) previous in vitro studies showing an inhibitory effect of Δ^9–THC on DA uptake into synaptosomes (Banerjee et al. 1975; Johnson et al. 1976; Hershkowitz and Szechtman 1979), and c) previous in vivo work inferring a DA agonist-like effect for Δ^9–THC on the basis of Δ^9–THC's similarity to amphetamine in behavioral tests in animals with unilateral mesostriatal DA denervation (Sakurai et al. 1985). Also, the present findings are in close agreement with our own previous in vivo findings that Δ^9–THC augments K^+-evoked DA efflux in rat caudate-putamen, perhaps by inhibiting presynaptic DA reuptake mechanisms (Ng Cheong Ton et al. 1988).

Critically, however, the present findings show that Δ^9–THC's DA agonist-like effect is clearly manifest under the more physiologically and pharmacologically meaningful conditions used in the present experiments, i.e., in conscious freely-moving animals, and on basal rather than K^+-evoked DA efflux. Also, the present findings are the first to demonstrate a DA agonist-like effect of Δ^9–THC in portions of the mesocorticolimbic DA circuitry, known to be more critically involved in mediating or modulating the effects of drugs of abuse on brain reward mechanisms than the brain areas studied in previous synaptosomal and in vivo experiments. Also importantly, the present findings together with our previous findings in caudate-putamen (Ng Cheong Ton et al. 1988) constitute the first studies of Δ^9–THC's effect on brain DA biochemistry to use doses of Δ^9–THC that approximate those found in human use of marijuana. Rosenkrantz et al. (1975) have translated doses of Δ^9–THC in rats to inhalation doses in humans; taking their assumptions and calculations, the dose range of 0.5–1.0 mg/kg Δ^9–THC used in the present experiments translates into one to two marijuana cigarettes of moderate Δ^9–THC content. Thus, the presently observed robust effects of Δ^9–THC on DA brain systems implicated in mediating brain reward would appear to be pharmacologically and physiologically relevant to human marijuana use.

Psychostimulants such as amphetamine, cocaine, phencyclidine and nomifensine have been shown to increase extracellular DA concentration in nucleus accumbens of freely moving rats studied by in vivo microdialysis (Carboni et al. 1989). The DA-agonist effects of cocaine, phencyclidine and nomifensine were different from amphetamine, as shown by their dependence upon intact neuronal firing and on exocytosis, as indicated by the inhibiting action of γ-butyrolactone and the Ca^{++}-dependence of their effects (Carboni et al., 1989). When the Ca^{++} ions in the perfusion solution were replaced by 0.1 mM EGTA (a chelator of divalent ions) in the present study, the extracellular DA efflux was immediately reduced but not completely abolished. This could be explained by the availability of intracellular Ca^{++} ions, since it has been demonstrated that depolarization of terminals can release Ca^{++} ions from intracellular pools to the extracellular space (Illes 1986) and the EGTA

concentration (0.1 mM) used in the present study may have been too low to completely block all available Ca^{++} ions. However, the present study shows clearly that Δ^9–THC effect was Ca^{++}-dependent, indicating that Δ^9–THC has a cocaine-like and nomifensine-like effect at DA presynaptic terminals in nucleus accumbens, rather than an amphetamine-like effect.

That drugs of abuse act on the same brain reward mechanisms that are activated, perhaps trans-synaptically, by the intensely rewarding stimulation of electrical brain-stimulation reward, and derive their euphorigenic properties and abuse potential therefrom, is at present the most compelling hypothesis available on the neurobiology of drug abuse (Kornetsky et al. 1979; Wise 1980; Wise and Bozarth 1984; Kornetsky 1985; Wise and Rompre 1989). That the ascending DA neurons of the mesocorticolimbic DA system, originating in the ventral tegmental area and projecting to the nucleus accumbens, constitute a crucial "second-stage" anatomic convergence within this reward circuitry upon which drugs of abuse act to enhance brain reward is equally compelling (Wise 1980; Wise and Bozarth 1984; Wise and Rompre 1989). In support of this hypothesis, many lines of evidence can be adduced (see, e.g., Wise and Rompre 1989). First, virtually all drugs of abuse that have been adequately studied (including cocaine, amphetamines, ethanol, barbiturates, benzodiazepines, opiates, phencyclidine-like dissociative anesthetics, and marijuana) augment brain-stimulation reward or lower brain reward thresholds in brain loci closely associated with the mesocorticolimbic DA system (e.g., Esposito and Kornetsky 1978; Lorens and Sainati 1978; Kornetsky et al. 1979; Esposito et al. 1980; Nazzaro et al. 1980; Nazzaro et al. 1981; Kornetsky 1985; Gardner et al. 1988). Second, most drugs with DA agonist properties are euphorigenic (and have consequential abuse potential) while most DA antagonists are dysphorigenic (Wise 1982; Wise and Rompre 1989). Third, virtually all drugs of abuse that have been adequately studied have been found to augment either neuronal firing or DA release (or both) in the mesocorticolimbic DA system (e.g., Wise 1984; Di Chiara and Imperato 1986; Imperato and Di Chiara 1986; Westerink et al. 1987; Wise and Bozarth 1987; Hernandez and Hoebel 1988; Wise and Rompre 1989). Fourth, animals will work to receive microinjections of drugs of abuse into mesocorticolimbic DA loci, but for the most part not into other brain loci (e.g., Bozarth and Wise 1981; Phillips et al. 1981; Goeders and Smith 1983; Hoebel et al. 1983; Goeders et al. 1984; Wise and Rompre 1989). Fifth, lesions or pharmacological blockade of the mesocorticolimbic DA circuitry markedly inhibit the rewarding properties of systemically-administered drugs of abuse (e.g., Yokel and Wise 1975; Roberts et al. 1977; Lyness et al. 1979; Spyraki et al. 1983; Bozarth and Wise 1986). From all of this evidence, and other data, Wise and Bozarth (1987) have hypothesized that psychomotor stimulation and reward are homologous, and derive from neural activation of the mesocorticolimbic DA circuitry.

While not speaking directly to the correctness of Wise and Bozarth's (1987) derivative hypothesis that psychomotor stimulation and reward are homologous, the present data, together with our previous demonstrations that Δ^9–THC significantly augments direct electrical brain-stimulation reward of the medial forebrain bundle (Gardner et al. 1988), strongly suggest that marijuana is not as anomalous a drug of abuse as formerly thought, and may in fact resemble other drugs of abuse in producing, as a final common neuropharmacological action, acute facilitation of the mesocorticolimbic DA reward-relevant circuitry.

Within this conceptual context of the neuropharmacological substrates of brain-reward enhancement by drugs of abuse, the present fiding that naloxone significantly attenuates Δ^9–THC's augmenting effect on mesocorticolimbic DA release would appear congruent with our previous demonstration that naloxone significantly attenuates Δ^9–THC's augmenting effect on electrical brain-stimulation reward (Gardner et al. 1989). Similarly, these findings appear congruent with the fact that brain-reward facilitation induced by a wide variety of other drugs of abuse is also naloxone-reversible, at doses of naloxone which themselves have no effect on brain-stimulation reward (e.g., Esposito et al. 1980; Nazzaro et al. 1980; Wise 1980; Nazzaro et al. 1981; Wise and Bozarth 1984; Wise and Rompre 1989). Thus, the DA mesocorticolimbic mechanisms enhanced by marijuana to produce augmented brain-reward appear to be modulated by an endogenous opioid peptide system. We previously reported (Vaysse et al. 1987) that Δ^9–THC modulates brain δ and μ (but not κ) opioid receptors, probably at an allosteric site on the opioid receptor complex. However, the locus at which the functional interrelationship between the "second-stage" DA fibers of the reward system (Wise and Rompre 1989) and endogenous opioid peptide circuitry takes place, to modulate drug effects on brain reward, has remained unclear. Many possibilities suggest themselves (and are by no means mutually exclusive), including the nuclei and terminal projection areas of the mesocorticolimbic DA circuitry and transsynaptic modulation via afferents to the mesencephalic DA cell fields from the locus coeruleus (Wise 1980). Among these, an attractive possibility is that opioid receptors directly on mesocorticolimbic DA terminals (Gardner et al. 1980) may form axo-axonic connections with reward-relevant DA fibers to modulate reward-relevant neural signals by presynaptic excitation or inhibition. The present finding of inhibition of Δ^9–THC's effect by low dose naloxone (0.1 mg/kg) indicates an antagonistic effect by naloxone mediated by opiate receptors, and appears to be congruent with the work of Di Chiara and Imperato (1988), who found that pretreatment with 0.1 mg/kg naloxone partially inhibited enhanced presynaptic DA efflux induced by opiates in nucleus accumbens and caudate-putamen. A fuller explanation of the location and nature of this opioid modulation of Δ^9–THC's effects remains to be developed, an avenue of current investigation in our laboratory. From such future work may well come new insights into brain mechanisms subserving reward/euphoria and vulnerability to drug abuse, as well as novel therapeutic strategies for compulsive drug use.

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