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Marijuana's Interaction With Brain Reward Systems: Update 1991

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GARDNER, E. L. AND J. H. LOWINSON. *Marijuana's interaction with brain reward systems: Update 1991.* PHARMACOL BIOCHEM BEHAV **40**(3) 571–580, 1991.—The most pervasive commonality amongst noncannabinoid drugs of abuse is that they enhance electrical brain stimulation reward and act as direct or indirect dopamine agonists in the reward relevant dopaminergic projections of the medial forebrain bundle (MFB). These dopaminergic projections constitute a crucial drug sensitive link in the brain's reward circuitry, and abused drugs derive significant abuse liability from enhancing these circuits. Marijuana and other cannabinoids were long considered "anomalous" drugs of abuse, lacking pharmacological interaction with these brain reward substrates. It is now clear, however, that Δ^9-tetrahydrocannabinol (Δ^9-THC), marijuana's principal psychoactive constituent, acts on these brain reward substrates in strikingly similar fashion to noncannabinoid drugs of abuse. Specifically, Δ^9-THC enhances MFB electrical brain stimulation reward, and enhances both basal and stimulated dopamine release in reward relevant MFB projection loci. Furthermore, Δ^9-THC's actions on these mechanisms is naloxone blockable, and Δ^9-THC modulates brain μ and δ opioid receptors. This paper reviews these data, suggests that marijuana's interaction with brain reward systems is fundamentally similar to that of other abused drugs, and proposes a specific neural model of that interaction.

Marijuana Cannabis Cannabinoids Δ^9-Tetrahydrocannabinol Δ^9-THC THC Dopamine
Brain reward ICSS Self-stimulation Reward Reinforcement DA Release Presynaptic
Microdialysis Electrochemistry Voltammetry Drug abuse Drug addiction Opioids

SINCE the seminal discovery by Olds and Milner in 1954 of the reward circuits of the brain (77), a tremendous amount of research has been devoted to the phenomenon of brain stimulation reward and to its implications for neurobiology and psychobiology [e.g., (78, 85, 87, 112)]. These implications are wide ranging and profound, not the least of which being the implications for the neurobiology and psychobiology of substance abuse. Evidence that recreational and abuse prone drugs derive their rewarding properties by activating brain reward circuits either directly or indirectly was presented as early as 1957 by Killam and his colleagues (56). In the years since, many different lines of evidence have converged to confirm this hypothesis. First, virtually all adequately studied recreational and abuse prone drugs (including those in such disparate chemical and pharmacological classes as opiates, stimulants, sedative-hypnotics, ethanol, anxiolytics, and anesthetics) enhance brain stimulation reward or lower brain reward thresholds (103, 104, 106, 107, 110). Second, virtually all adequately studied recreational and abuse prone drugs enhance basal neuronal firing and/or basal neurotransmitter release in reward relevant brain circuits (6, 15, 38, 42, 46, 49, 50, 53, 54, 99, 109, 110). Third, laboratory animals will work for microinjections of abused drugs into brain reward loci, but not into other brain loci [e.g., (3, 35, 36, 45, 81)]. Fourth, lesions or pharmacological blockade of brain reward circuits markedly inhibit the rewarding properties of systemically administered drugs of abuse [e.g., (4,91)]. Thus acute enhancement of brain reward mechanisms appears to be the single essential commonality of abuse prone drugs, and the hypothesis that recreational and abused drugs act on these brain mechanisms to produce the subjective reward that constitutes the "high" or "rush" or "hit" sought by drug users is, at present, the most compelling hypothesis available on the neurobiology of recreational drug use and abuse (3, 57, 58, 104, 106, 108, 110).

NEUROANATOMY, NEUROPHYSIOLOGY AND NEUROCHEMISTRY OF BRAIN REWARD

As originally mapped by early workers in the field, the brain regions capable of supporting brain stimulation reward included a variety of brain stem, midbrain, and forebrain loci, with the vast majority of positive sites corresponding to the aggregate of ascending and descending tracts which constitute the medial forebrain bundle (MFB), together with the nuclei and terminal projection fields of the MFB [e.g., (79, 96, 101)]. With the development of histofluorescence mapping techniques and the resulting illumination of the monoaminergic anatomy of the brain, major portions of which are carried through the MFB [e.g., (68)], a striking correspondence was noted between sites positive for brain stimulation reward and the mesotelencephalic dopamine (DA) system [e.g., (11)]. This observation in turn spawned a host of neuropharmacological [e.g., (67,115)] and other [e.g., (10,18)] studies, all pointing to the importance of mesencephalic

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DA neurotransmission to brain stimulation reward. From such studies, and literally hundreds of other experiments [summarized in (17, 103, 105, 107, 110)], it is now abundantly clear that brain reward is, in fact, critically dependent on the functional integrity of DA neurotransmission within the mesotelencephalic DA systems.

However, the original conception of many researchers that electrical brain stimulation reward directly activates the DA fibers of the MFB was shown to be incorrect more than 10 years ago by the elegant electrophysiological studies of Yeomans, Gallistel, Shizgal, and their colleagues [e.g., (19, 20, 90, 111, 114)], which argue persuasively that the primary MFB substrate directly activated by electrical brain stimulation reward is a myelinated, caudally running fiber system whose neurons have absolute refractory periods of 0.5–1.2 ms and local potential decay time constants of approximately 0.1 ms. Since none of these neurophysiological properties agrees with those of the ascending mesotelencephalic DA neurons, Wise and his colleagues have argued that the DA neurons cannot be the "first-stage" reward neurons preferentially activated by electrical brain stimulation reward, but must instead constitute a crucial "second-stage" anatomic convergence within the reward circuitry of the brain, upon which the "first-stage" neurons synapse to form an "in series" reward relevant neural circuit (104, 108, 110). It is on this "second-stage" DA convergence that recreational and abuse prone drugs act to enhance brain reward (103, 104, 106, 107, 110). Although apparently preferentially activated by drugs, these DA substrates also appear capable of direct activation by electrical brain stimulation reward under the proper laboratory conditions (113). A current view of the reward circuitry of the brain, with the separate substrates differentially activated by electrical brain stimulation reward and by abuse prone drugs, is shown in Fig. 1. The synaptic interconnection of endogenous opioid peptide (enkephalinergic, endorphinergic) neurons with these brain reward mechanisms (Fig. 1) presumably constitutes the neural substrate for naloxone's modulation of brain reward enhancement by recreational and abuse prone drugs (see below).

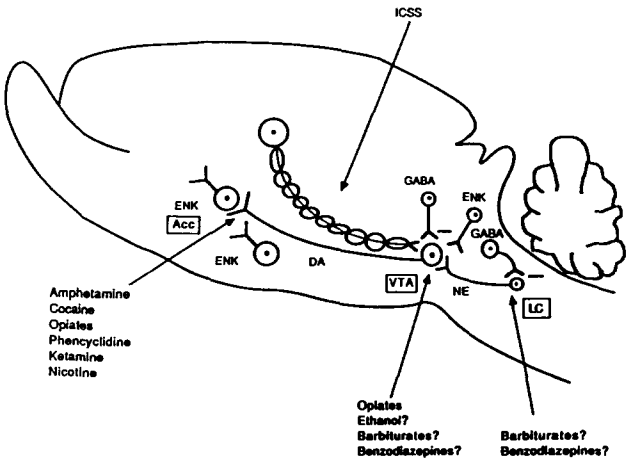

FIG. 1. Schematic diagram of the reward circuitry of the mammalian (rat) brain, with sites at which various drugs of abuse appear to enhance brain reward and thus induce the euphorigenic "high" sought by drug users. Abbreviations are as follows: ICSS: the descending, myelinated, moderately fast conducting component of the reward circuitry preferentially activated by electrical intracranial self-stimulation; DA: the ascending dopaminergic component preferentially activated by drugs of abuse; LC: locus coeruleus; VTA: ventral tegmental area; Acc: nucleus accumbens; NE: noradrenergic projections from locus coeruleus to ventral mesencephalon; GABA: GABAergic inhibitory systems synapsing on the LC NE system and on the VTA DA cell fields; ENK: endogenous opioid peptidergic (enkephalinergic or endorphinergic) neurons synapsing onto and within the reward circuitry at indicated sites. [Modified from (104), by permission of Pergamon Press.]

MARIJUANA ENHANCES ELECTRICAL BRAIN STIMULATION REWARD

Given that marijuana is the most widely used illicit recreational drug in North America (34, 59, 63, 71), the question obviously arises: Does marijuana act on the brain reward substrates outlined above in a manner similar to that of other recreational and abuse prone drugs? The answer is "yes." In 1988, my colleagues and I demonstrated that Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive constituent of marijuana, enhances electrical brain stimulation reward (i.e., lowers brain reward thresholds) in the MFB of laboratory rats. For these experiments (24), male Lewis rats were surgically implanted with chronic brain stimulation electrodes in the MFB and trained to self-deliver rewarding electrical brain stimulation by a titrating threshold stimulation procedure that we have extensively used in our studies of brain reward [e.g., (72,88)]. This paradigm allows the animal to indicate its threshold for brain reward on a minute-to-minute basis throughout the test session. The test chambers contain two response levers. Each response by an animal on the primary or "stimulation" lever delivered a 250 ms stimulus train of 60 Hz bipolar rectangular pulse pairs through the brain electrode. Initial current intensity for each animal was set at the lowest intensity supporting consistent stable responding of 1000 lever presses per 30-minute test session. The current decremented by 1/16 of this initial intensity at every third press of the primary lever. At any point during the ensuing self-administered decremental brain stimulation, the animal could reset the current back up to the initial maximum level by pressing the secondary or "reset" lever (which did not itself deliver brain stimulation). The current levels at which the animals reset were automatically recorded by microprocessors throughout each 30-minute test session, and the mean of the resulting frequency distribution of self-determined reset levels was operationally defined as the brain reward threshold. After training to stable performance, each animal was tested daily with intraperitoneal saline injections for a minimum of 6 weeks to ensure absolutely stable baselines of brain reward threshold before drug trials began. After this 6-week baseline period, each animal was injected intraperitoneally on the next day with the 20% polyvinylpyrrolidone (PVP) vehicle and tested on the brain reward paradigm. Two weeks of additional daily baseline testing then ensued, and then, on the next day, each animal was injected intraperitoneally with 1.5 mg/kg Δ^9-THC and tested on the brain reward paradigm. Two more weeks of daily testing then ensued, after which each animal was again given Δ^9-THC or vehicle and again tested on the brain reward paradigm. In contrast to the total ineffectiveness of both saline and the PVP vehicle, Δ^9-THC significantly lowered brain reward thresholds in the MFB (Fig. 2).

MARIJUANA ENHANCES PRESYNAPTIC DA EFFLUX IN BRAIN REWARD CIRCUITS

Given the enhancing action of all other well-characterized recreational and abuse prone drugs on presynaptic DA release in reward relevant brain circuits, and given that the DA link in the reward circuitry appears to be the crucial drug sensitive link (see review of these points above), the question arises: Does marijuana also enhance DA release in the reward circuitry? Again,

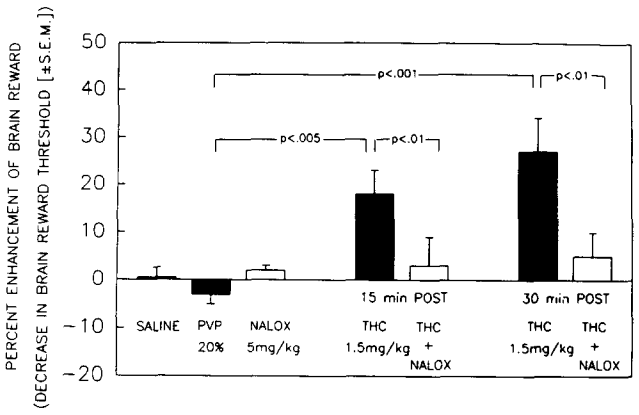

FIG. 2. Enhanced brain reward following acute administration of Δ^9-THC (1.5 mg/kg, IP), and attenuation of resulting enhanced brain reward by acute naloxone. Enhanced brain reward is experimentally equivalent to decreased electrical brain stimulation thresholds in the medial forebrain bundle (see text). "PVP 20%" is the 20% polyvinylpyrrolidone vehicle for the Δ^9-THC. Probability values shown are for the specific comparisons indicated. [Data redrawn from (24) and (28) by permission of Springer-Verlag Publishers and Pergamon Press.]

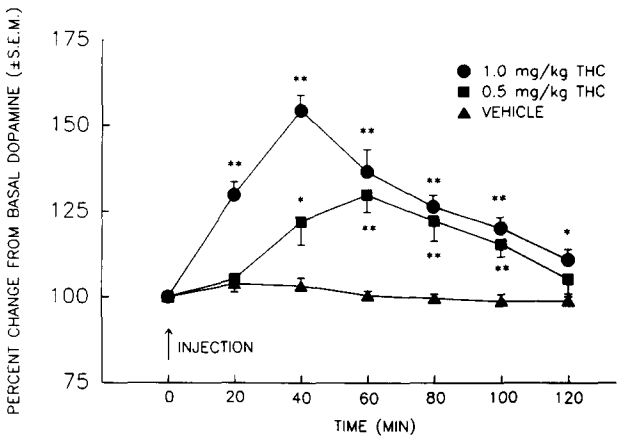

FIG. 3. Enhanced basal DA efflux in nucleus accumbens following acute administration of Δ^9-THC (0.5 and 1.0 mg/kg, IP) in conscious, freely moving rats as measured by in vivo brain microdialysis. Asterisks indicate degree of statistical significance of each post-Δ^9-THC data point as compared to the corresponding 20% PVP vehicle data point (*$p<0.05$; **$p<0.01$). [Reprinted from (8) by permission of Springer-Verlag Publishers.]

the answer is "yes" (7, 8, 75, 76). Explorations of this question have utilized two recently developed techniques for assessing real-time presynaptic neurotransmitter efflux in vivo in discrete brain loci of conscious freely moving animals. These techniques are in vivo brain microdialysis and in vivo brain voltammetric electro-chemistry (52, 66, 70). Using both techniques, we have shown that Δ^9-THC enhances both basal and potassium-stimulated presynaptic DA efflux in reward relevant brain loci, including striatum, nucleus accumbens, and medial prefrontal cortex (7, 8, 75, 76). This effect is illustrated, for the case of the nucleus accumbens, in Fig. 3. A similar DA-releasing effect of Δ^9-THC in neostriatum has been recently reported by Taylor and colleagues, also using in vivo brain microdialysis (92,93).

MARIJUANA'S ENHANCING EFFECTS ON BRAIN REWARD AND PRESYNAPTIC DA EFFLUX IN BRAIN REWARD CIRCUITS OCCUR AT PHYSIOLOGICALLY MEANINGFUL LOW DOSES

We have consistently seen the above-outlined effects of Δ^9-THC on brain reward and on presynaptic DA efflux in brain reward loci at doses ranging from 0.5 mg/kg to 2.0 mg/kg (7, 8, 24, 75, 76). These doses are very appreciably lower than those used in many previous studies of Δ^9-THC's effects on brain neurotransmitter mechanisms. In fact, Harris and Stokes (39) have criticized previous studies of Δ^9-THC's effects on neurochemical mechanisms as employing doses so high as to preclude physiological relevance to human recreational use of marijuana. In contrast, our studies appear to be the first that can conservatively be judged as within the range of relevance to human use and abuse. Rosenkrantz and colleagues (86) translated oral doses of Δ^9-THC in rats to inhalation doses in a 50 kg human by correcting for 7:1 differences in body surface area, 50% pyrolysis, and a 3:1 difference in oral:inhalation dose. Taking these assumptions, one marijuana cigarette per day weighing 1 g with 2% Δ^9-THC would result in absorption of 0.2 mg/kg Δ^9-THC. Recreational marijuana smoking of 1–3 cigarettes per day is well within the range of what is seen in human use. Making additional modest assumptions about the conversion of oral to intraperitoneal doses in laboratory rats, these calculations indicate that the robust effects we have consistently seen with Δ^9-THC in the 0.5–2.0 mg/kg range may well correspond to recreational human use of as little as one to two marijuana cigarettes of moderate Δ^9-THC content, and thus pharmacologically and physiologically relevant to human patterns of marijuana use.

MARIJUANA'S ENHANCING EFFECTS ON BRAIN REWARD AND PRESYNAPTIC DA EFFLUX IN BRAIN REWARD CIRCUITS IS STRAIN SPECIFIC IN LABORATORY RATS

For many years it has been well established that, for at least some drugs of abuse (e.g., ethanol), extensive genetically determined strain differences exist in both drug preference and propensity for drug self-administration (5, 31, 62, 84). We therefore studied whether similar differences exist between selected genetically inbred rat strains in the effects of Δ^9-THC on both electrical brain stimulation reward and presynaptic DA efflux in brain reward circuits. For these studies (25–27), all experimentation was carried out as outlined above, with the exception that, in addition to the inbred Lewis rat strain, rats of three additional inbred strains were also used: Sprague-Dawley, Long-Evans, and Fischer 344. We found that, for the Lewis rats, Δ^9-THC significantly facilitated MFB electrical brain stimulation reward (25,26), as we had seen in previous studies (24). However, in contrast to the robust and highly consistent facilitation of brain reward by Δ^9-THC in the Lewis strain rats, Δ^9-THC had no effect at any dose tested (0.37, 0.75, 1.5, 2.0, and 4.0 mg/kg) in the Sprague-Dawley, Long-Evans, and Fischer 344 strains. These strain differences in Δ^9-THC's effect on brain stimulation reward were striking; in the Lewis strain animals, Δ^9-THC facilitated brain stimulation reward in every animal and with every Δ^9-THC administration; in the Sprague-Dawley, Long-Evans, and Fischer 344 rats, Δ^9-THC never facilitated brain reward in any animal or at any dose. To see if these striking strain differences in susceptibility to Δ^9-THC's enhancement of electrical brain stimulation reward would be mirrored by similar strain differences in susceptibility to Δ^9-THC's enhancement of presynaptic DA efflux in brain reward loci, we studied Δ^9-THC's effects on basal DA efflux in the nucleus accumbens by in vivo brain microdial-

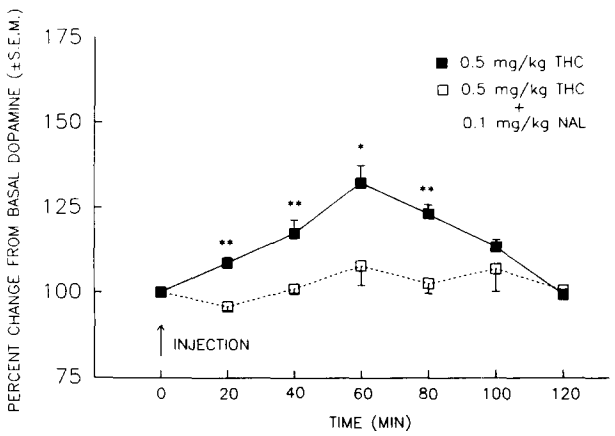

FIG. 4. Naloxone (0.1 mg/kg, IP)-induced attenuation of enhanced basal DA efflux in nucleus accumbens produced by Δ^9-THC (0.5 mg/kg, IP) in conscious, freely moving rats as measured by in vivo brain microdialysis. Asterisks indicate degree of statistical significance of each post-Δ^9-THC data point as compared to the corresponding Δ^9-THC-plus-naloxone point (*$p<0.05$; **$p<0.01$). [Reprinted from (8) by permission of Springer-Verlag Publishers.]

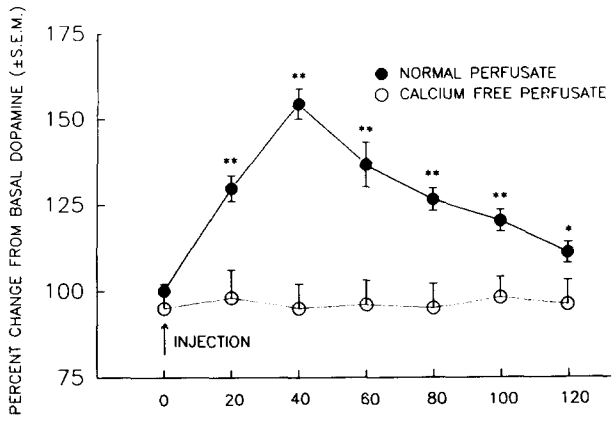

FIG. 5. Calcium-dependence of enhancing effect of Δ^9-THC (1.0 mg/kg, IP) on basal DA efflux in nucleus accumbens of conscious, freely moving rats as measured by in vivo brain microdialysis. Asterisks indicate degree of statistical significance of each post-Δ^9-THC data point with normal perfusate as compared to the corresponding post-Δ^9-THC data point with Ca^{++} free perfusate (*$p<0.05$; **$p<0.01$). [Data redrawn from (8) by permission of Springer-Verlag Publishers.]

ysis in awake behaving animals of the four inbred rat strains that we had previously used: Lewis, Sprague-Dawley, Long-Evans, and Fischer 344. We found that while basal (nondrugged) presynaptic DA release in nucleus accumbens was similar across all strains, clear strain differences emerged in vulnerability to Δ^9-THC's enhancement of presynaptic DA efflux (27). In the Lewis strain rats, Δ^9-THC at both 0.5 mg/kg and 1.0 mg/kg produced robust and long-lasting enhancement of basal presynaptic DA efflux; the other strains tested showed no enhancement of basal presynaptic DA efflux in nucleus accumbens at either dose (27). Provocatively, Lewis strain rats have also been shown to be more vulnerable to the effects of cocaine, and to have increased vulnerability for ethanol self-administration (31). On the basis of these data, we suggest that facilitation of brain reward, and of the neurotransmitter substrates mediating brain reward, by Δ^9-THC is strain specific, implicating significant genetic variation in vulnerability to the brain reward facilitating effects (and thus, presumably, to the euphorigenic effects) of marijuana.

MARIJUANA'S ENHANCING EFFECTS ON BRAIN REWARD AND PRESYNAPTIC DA EFFLUX IN BRAIN REWARD CIRCUITS IS NALOXONE REVERSIBLE

As noted at the beginning of this review, acute enhancement of brain reward mechanisms appears to be the single essential commonality of abuse prone drugs. Strikingly, this facilitation of brain stimulation reward is naloxone reversible for all well studied drugs of abuse (16, 64, 73, 74, 104, 108), implicating an endogenous opioid mechanism in mediating the euphorigenic action of such drugs. Given our findings that Δ^9-THC resembles other recreational and abuse prone drugs in enhancing electrical brain stimulation reward and enhancing presynaptic DA efflux in brain reward loci, the question obviously arises as to whether Δ^9-THC's effects on these mechanisms is also naloxone reversible. We addressed this question in a series of experiments using the marijuana sensitive Lewis strain rats and the electrical brain stimulation reward and in vivo brain microdialysis paradigms outlined above. As shown in Fig. 2, we found that Δ^9-THC's enhancing effects on electrical brain stimulation reward are indeed naloxone blockable, at doses of naloxone which themselves have no effect on brain reward (28). Furthermore, using in vivo brain microdialysis in the nucleus accumbens convergence of reward circuits, we found that naloxone, at doses as low as 0.1 mg/kg, completely blocked Δ^9-THC's enhancing effects on basal presynaptic DA efflux (8,29). This naloxone blockade of Δ^9-THC-induced enhancement of basal DA efflux is illustrated in Fig. 4.

MARIJUANA'S ENHANCING EFFECT ON PRESYNAPTIC DA EFFLUX IN BRAIN REWARD CIRCUITS RESEMBLES THAT OF A DOPAMINE REUPTAKE BLOCKER

As noted above, all adequately studied recreational and abuse prone drugs enhance basal DA neuronal firing and/or basal DA neurotransmitter release in brain reward circuits. However, the means by which each individual drug achieves these ends appear to differ widely. Thus, amphetamine appears to act as a presynaptic DA releaser, cocaine as a presynaptic reuptake blocker, opiates as transsynaptic enhancers of DA neuronal firing, and other abuse prone drugs by yet other means and mechanisms [e.g., (15, 33, 38, 42, 46, 49, 50, 53, 54, 99, 109, 110)]. The question arises: How does marijuana act? While not yet definitive, we believe that we have gathered sufficient data to strongly suggest that marijuana's action on DA reward neurons of the mesotelencephalic DA system resembles that of a DA reuptake blocker (8, 30, 76). First, we have shown that the DA efflux measured by our microdialysis probes (80) is tetrodotoxin sensitive (8), indicating that the DA signal monitored in our Δ^9-THC microdialysis experiments is neuronal (99). Second, we have shown that Δ^9-THC's enhancing effect on presynaptic DA efflux in brain reward loci is calcium dependent (8). Basal DA efflux in nucleus accumbens was decreased approximately 50% in the presence of calcium free perfusate. Challenge with 1.0 mg/kg Δ^9-THC did not enhance DA efflux at all in the calcium free paradigm, in contrast to the robust enhancement (to approximately 150% of pre-Δ^9-THC baseline) of DA efflux by the same dose of Δ^9-THC in the calcium-containing paradigm (8). This calcium dependence of Δ^9-THC's enhancing effect on pre-

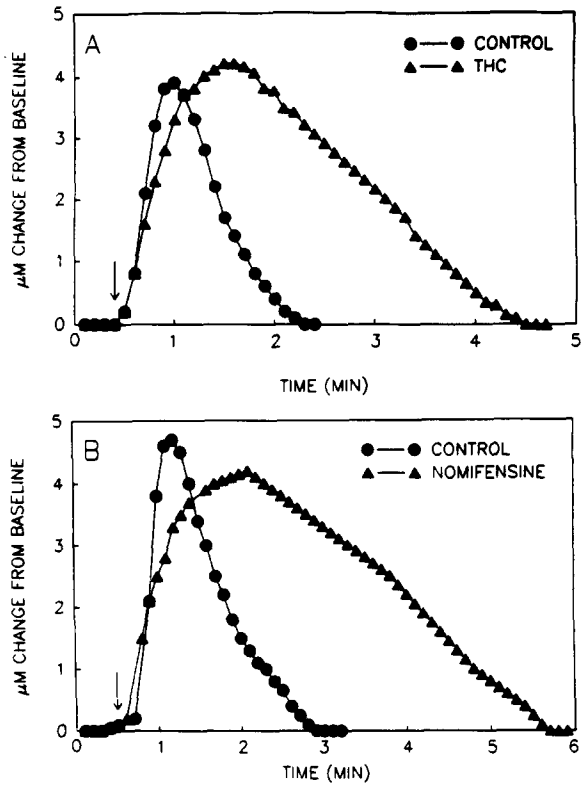

FIG. 6. Enhanced presynaptic DA efflux following acute administration of Δ^9-THC or nomifensine as measured by in vivo voltammetric brain electrochemistry. (A) Effects of Δ^9-THC (0.5 mg/kg, IP) on K^+-evoked voltammetric electrochemical signals corresponding to presynaptic DA efflux. (B) Effects of nomifensine (5.0 mg/kg, IP) on K^+-evoked voltammetric electrochemical signals corresponding to presynaptic DA efflux. The arrows indicate the timing of the localized intracerebral micropressure K^+ applications. [Data redrawn from (76) by permission of Elsevier Science Publishers B. V., Biomedical Division.]

synaptic DA efflux in brain reward loci is illustrated in Fig. 5. Third, we find that pretreatment (one hour prior to Δ^9-THC) with the DA receptor blocker haloperidol (0.1 mg/kg) has a synergistic effect on Δ^9-THC's enhancement of presynaptic DA efflux in the nucleus accumbens (30), and further that Δ^9-THC (1.0 mg/kg) pretreatment one hour before haloperidol has a similar synergistic effect on haloperidol's enhancement of presynaptic DA efflux in the nucleus accumbens (30). Tetrodotoxin perfused locally into the nucleus accumbens abolished the synergism between Δ^9-THC and haloperidol. Since impulse-induced facilitation of DA release underlies the synergistic effect between DA receptor blockers and DA reuptake inhibitors (100), these data are congruent with the conception that Δ^9-THC acts as a DA reuptake blocker. Finally, using high-speed in vivo voltammetric electrochemical techniques (32), we have shown that the high-speed time dynamics of Δ^9-THC's enhancement of the potassium-evoked electrochemical signal corresponding to released extracellular DA are identical to the high-speed time dynamics of nomifensine's enhancement of the same electrochemical signal (76). These findings are illustrated in Fig. 6. Inasmuch as nomifensine has been shown to be a relatively pure reuptake inhibitor (48), these findings are also congruent with the conception that Δ^9-THC augments presynaptic DA efflux by ultimately acting (perhaps indirectly) as a DA reuptake blocker (76).

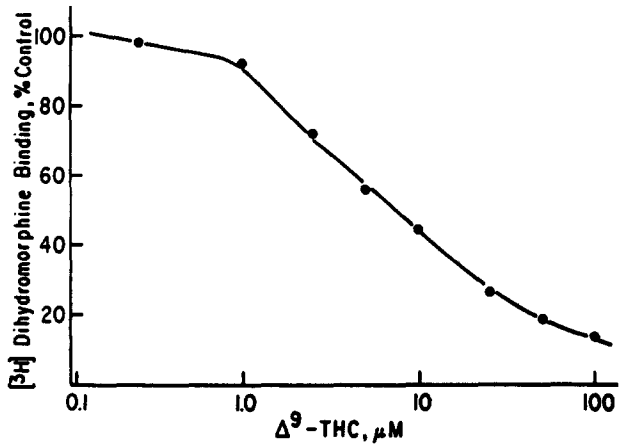

FIG. 7. Specific binding of [^{3}H]dihydromorphine to rat brain membranes as a function of Δ^9-THC concentration. Samples (1.0 ml, 0.8 mg of protein) were incubated in triplicate in 50 mM Tris HCl buffer pH 7.4, at 4°C for 45 min. Incubation samples included [^{3}H]dihydromorphine (0.5 nM) and Δ^9-THC at indicated concentrations in the presence or absence of levorphanol (5 μM). The mean Δ^9-THC concentration required to inhibit [^{3}H]dihydromorphine binding to brain by 50% was 7 ± 1 μM. [Reprinted from (98) by permission of the American Society for Pharmacology and Experimental Therapeutics.]

MARIJUANA MODULATES BRAIN OPIOID RECEPTORS

As noted above, the opiate antagonist naloxone antagonizes marijuana's effects on electrical brain stimulation reward and on presynaptic DA efflux in brain reward loci. On those grounds alone, one could entertain the suggestion that a functional and possibly anatomic interaction exists between endogenous brain opioid substrates and the neural substrates through which marijuana acts on brain reward mechanisms. Additionally, other findings support the concept that at least some of marijuana's effects on the brain are mediated by endogenous opioid systems. For example, pharmacological effects of Δ^9-THC additional to the above-noted ones on reward mechanisms are also blocked by opiate antagonists (95,102), and Δ^9-THC can serve to ameliorate naloxone-precipitated morphine abstinence syndromes (1,43). Also, the modulation by Δ^9-THC of luteinizing hormone releasing hormone and of prolactin is blocked by naloxone (60). For these reasons, we undertook to study the in vitro effects of Δ^9-THC and other cannabinoids, including the stereoisomers levonantradol and dextronantradol, on both membrane bound and solubilized, partially purified brain opioid receptors as well as cholinergic (muscarinic) and DA receptors (97, 98, 116). For these studies, rat brains were removed, dissected, and prepared for receptor binding assays as we had previously described (94). Binding assays for mu (μ), delta (δ), kappa (κ), and sigma (σ) opioid receptors, muscarinic receptors, and DA receptors were then carried out using standard receptor binding procedures (97, 98, 116). For preparation of the solubilized, partially purified opioid receptors and binding to them, previously published procedures were followed (9). We found that Δ^9-THC produced a dose dependent inhibition of μ and δ opioid receptor binding, but failed to alter κ, σ, DA, or muscarinic binding (98). Scatchard analyses of the μ receptor binding indicated that Δ^9-THC produced a significant decrease in receptor density with no significant change in receptor affinity, consistent with a noncompetitive mechanism for the inhibition of μ opioid receptors by Δ^9-THC. The effect of Δ^9-THC on solubilized, partially purified

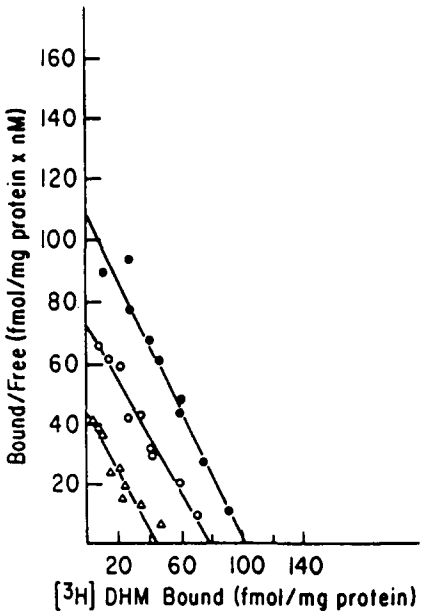

FIG. 8. Scatchard plot of [^{3}H]dihydromorphine binding to rat brain membranes in the absence (●) or presence of Δ^9-THC at indicated concentration (○, 5 μM; △, 10 μM). Aliquots of brain homogenate (1.0 ml) in 50 mM Tris HCl buffer, pH 7.4, were incubated with [^{3}H]dihydromorphine (0.05–10.0 nM) in the absence or presence of levorphanol (5 μM) and Δ^9-THC at the indicated concentration. Binding plots were analyzed by computer-assisted nonlinear regression analysis. [Reprinted from (98) by permission of the American Society for Pharmacology and Experimental Therapeutics.]

TABLE 1

POTENCIES OF Δ^9-THC IN COMPETING FOR RADIOLIGAND BINDING TO RAT BRAIN RECEPTORS

Receptor	Radioligand*	Δ^9-THC IC_{50} (μM)
Mu, delta and K opioid	[^{3}H]Etorphine	10 ± 1.2
		14† ± 3.0
	[^{3}H]Naloxone	
	100 mM NaCl	19 ± 1.0
	No NaCl	10 ± 1.5
Mu opioid	[^{3}H]Dihydromorphine	7 ± 1.0
Delta opioid	[^{3}H]D-Pen^2,D-Pen^5-enkephalin	16 ± 3.0
Kappa opioid		
(mu, delta blockers present)	[^{3}H]Ethylketocyclazocine	>100
Sigma/phencyclidine	[^{3}H]TCP	>100
Dopamine	[^{3}H]Spiroperidol	>100
Muscarinic	[^{3}H]Quinuclidinyl benzilate	>100

*Radioligand concentration was 0.5 nM in each assay, except in the case of [^{3}H]ethylketocyclazocine and [^{3}H]TCP, which were 2.5 nM, and [^{3}H]quinuclidinyl benzilate, which was 0.05 nM.

†IC_{50} value obtained for solubilized, partially purified opioid receptors.

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Whole rat brain was prepared and binding was carried out as described in (98). Aliquots (1.0 ml) of rat brain homogenate in either (for opioid and dopamine receptors assays) 50 mM Tris HCl, pH 7.4, or (for muscarinic receptor assays) 50 mM sodium potassium phosphate buffer, pH 7.4, with radioligand in the absence or presence of 5 μM levorphanol (mu receptor assay), D-Pen^2, D-Pen^5-enkephalin (delta receptor assay), ethylketocyclazocine (kappa receptor assay), 10 μM phencyclidine (sigma/phencyclidine receptor assay), naltrexone (opioid antagonist assay), 1 μM atropine (muscarinic receptor assay) or 10 μM (+)butaclamol (dopamine receptor assay) and Δ^9-THC (at eight concentrations). Incubation with [^{3}H]spiroperidol was carried out at 25 °C for 20 min; all other radioligand incubations were at 4 °C for 45 min. Log-probit plots were constructed, and the IC_{50} values were determined graphically. Each determination represents the mean ± S.E.M. of three independent experiments.

opioid receptors was qualitatively and quantitatively similar to the effect observed on membrane bound receptors, prompting us to suggest as early as 1985 (98,116) that allosteric modulation of the opioid receptor by Δ^9-THC results from a direct interaction with the receptor protein or with a specific protein-lipid complex and not merely from nonspecific perturbation of the lipid bilayer of the membrane. We also compared the potencies of a large series of cannabinoids and related compounds in their ability to inhibit μ receptor binding, and found a general correlation with psychoactive potencies in humans, with the exception of a lower-than-expected in vitro opioid receptor modulatory potency for 11-hydroxy-Δ^9-THC and a greater-than-expected in vitro opioid receptor modulatory potency for cannabidiol (98). These findings are illustrated in Figs. 7–8 and Tables 1–2.

POSSIBLE NEUROANATOMIC LOCATIONS FOR MARIJUANA'S INTERACTION WITH ENDOGENOUS BRAIN OPIOID MECHANISMS

From much of the above-cited evidence, it would appear that there exists an important functional and anatomic interrelationship between the crucial drug-sensitive "second-stage" DA fibers of the reward system (103, 104, 106, 107, 110) and endogenous opioid peptide circuitry, and furthermore that this interrelationship is important for the brain reward enhancement produced by marijuana and, hence, important for marijuana's abuse liability. Anatomically, there are many brain loci where such a functional interaction between reward relevant DA neurons and endogenous opioid peptide neurons could take place. Cell bodies, axons, and synaptic terminals of enkephalinergic and endorphinergic neurons are found in veritable profusion throughout the extent of the reward relevant mesotelencephalic DA circuitry [e.g., (55,65)]. We (23) and others (82,83) have shown that endogenous opioid peptide neurons synapse directly onto mesotelencephalic DA axon terminals, forming precisely the type of axo-axonic synapses one would expect of a system designed to modulate the flow of reward relevant neural signals through the DA circuitry. In addition to the DA axon terminal regions, other likely sites of enkephalinergic-DA functional interaction include the DA cell body region of the ventral mesencephalon (17) and transsynaptic modulation via afferents to the ventral mesencephalon from the region of the locus coeruleus (104). We also believe that some DA neurons of the reward system may synapse directly onto endogenous opioid peptide neurons located postsynaptically in the DA terminal regions, which may then carry the reward signal one synapse further. Our reasons for so believing are two-fold. First, we have demonstrated (89) that naloxone acutely attenuates the enhanced brain reward induced by chronic pharmacological up-regulation of DA receptors in the mesolimbic DA system, suggesting that a crucial naloxone blockable endogenous opioid peptide link lies efferent to the up-regulated DA receptors. Second, we have demonstrated (44) that naloxone significantly modulates behavioral responses induced by direct postsynaptic DA receptor ago-

TABLE 2

RELATIVE POTENCIES OF CANNABINOIDS IN INHIBITING [^{3}H]DHM BINDING TO MU OPIOID RECEPTORS OF RAT BRAIN MEMBRANES

Cannabinoid	$IC_{50}(K_i)$	Relative Potency*
Δ^9-THC	7 ± 1 μM†	1
(+)Δ^9-THC	45%‡	<0.07‡
11-Hydroxy-Δ^9-THC	20%‡	<0.07‡
Δ^8-THC	20 ± 5 μM	0.35
8α,11-Dihydroxy-Δ^9-THC	40%‡	<0.07‡
Equatorial hexahydrocannabinol	10 ± 1 μM	0.70
Cannabidiol	7 ± 1 μM	1.00
Cannabinol	35 ± 5 μM	0.20
Cannabinol acetate	20%‡	<0.07‡
1′-Oxocannabinol	48 ± 7 μM	0.15
1′,2′,3′,4′,5′-Pentanor-Δ^9-THC 3-carboxylic acid	20 ± 4 μM	0.35
Levonantradol	70 ± 10 μM	0.10
Dextronantradol	70 ± 10 μM	0.10

*Relative potencies are expressed relative to Δ^9-THC.

†IC_{50} value as reported is for a colloidal suspension of Δ^9-THC. IC_{50} value determined for a fully soluble Δ^9-THC solution is 3 ± 1 μM.

‡Because many of these cannabinoids were available in limited quantity, the approximate range of concentrations necessary to produce 50% inhibition was estimated by comparing the inhibition produced by each drug at 25 and 50 μM with that produced by Δ^9-THC.

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Whole rat brain was prepared and binding was carried out as described in (98). Samples (1.0 ml) (0.8 mg of protein) were incubated with [^{3}H]DHM (0.5 nM, 87.7 Ci/mmol) at 4 °C for 45 min in the absence or presence of levorphanol (5 μM) or other indicated drugs at eight concentrations. The incubation buffer was 50 mM Tris HCl, pH 7.4. The control binding value for 0.5 nM [^{3}H]DHM was 3000 cpm specifically bound per sample. The IC_{50} value is defined as the concentration of drug required to half-maximally displace [^{3}H]DHM (0.5 nM) binding to rat brain membranes. In the case of cannabinol acetate and 8α,11-dihydroxy-Δ^9-THC, the percentage of inhibition is reported at 0.1 mM.

nists in animals in whom the presynaptic DA fiber system has been destroyed by selective lesions of the DA mesotelencephalic system, again implicating a crucial naloxone sensitive endogenous opioid peptide link efferent to the ascending DA mesotelencephalic DA system. We believe, therefore, that on the basis of the best presently available data, the most likely site for marijuana's interaction with modulatory endogenous brain opioid mechanisms is in the DA terminal regions of the reward relevant ascending DA mesotelencephalic system, in reward relevant synapses containing both an opioid-DA axo-axonic link and a DA-opioid presynaptic-postsynaptic link.

MARIJUANA INTERACTS WITH SPECIFIC BRAIN Δ^9-THC BINDING SITES

Of course, one of the most seminal and important discoveries in the entire field of cannabinoid pharmacology in recent years has been the discovery that marijuana interacts with specific brain Δ^9-THC binding sites (2, 14, 40, 41, 47, 51). These sites, which appear to be allosterically regulated by a G protein, seem to fulfill standard criteria for high affinity, stereoselective, and pharmacologically distinct brain receptors (14). The relative potencies of natural and synthetic cannabinoids as competitors for these binding sites correlate closely with their relative potencies in biological and behavioral assays (14,41), suggesting that

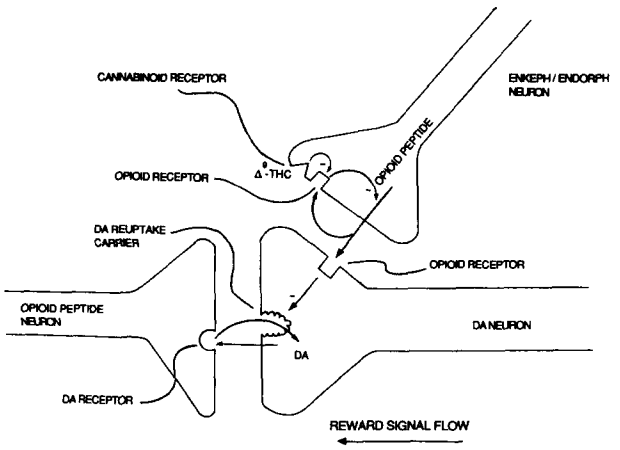

FIG. 9. Hypothetical model of marijuana's interaction with reward relevant synapses of the mesotelencephalic DA system (see text).

the specific brain Δ^9-THC binding sites identified in these experiments are the same brain receptors which mediate the behavioral and pharmacological effects of marijuana. The distribution in brain of these Δ^9-THC binding sites, as determined by autoradiography, is anatomically selective (41). Dense binding is observed in the striatum, cerebral cortex, substantia nigra pars reticulata, globus pallidus, hippocampus, and the molecular layers of the cerebellum. Sparse but significant binding is seen in the hypothalamus, basal amygdala, central gray, nucleus of the solitary tract, and certain spinal laminae. Very low and essentially nonspecific binding is seen in the thalamus, colliculi, and brain stem. It is provocative that very dense Δ^9-THC binding is seen in the ventral mesencephalic regions from which the DA reward relevant neurons arise, that dense binding is seen in the striatal regions to which these DA reward relevant neurons project, and that very dense binding is also seen in the globus pallidus, which receives an enkephalinergic projection from the reward relevant DA terminal projection fields of the mesotelencephalic DA system (13).

A HYPOTHETICAL MODEL OF MARIJUANA'S ACTIONS ON REWARD SYNAPSES

From all of the data reviewed above, it is possible to develop a preliminary hypothetical model of marijuana's interaction with reward relevant synapses of the mesotelencephalic DA system. Such a model is presented in Fig. 9. The model presumes that, for reasons outlined above, the principal marijuana sensitive link in the mesotelencephalic DA reward pathway is in the region of the DA terminal projections of the mesotelencephalic DA reward pathway, most likely within the nucleus accumbens and associated cell fields of the ventral striatum. It presumes that the reward relevant DA terminals of this system synapse upon endogenous opioid peptidergic neurons which carry the reward signal one synapse further, and that other modulatory endogenous opioid peptidergic neurons synapse, in axo-axonic fashion, onto the DA terminals of the reward relevant DA neurons. It presumes that this second class of opioid peptidergic neurons actively modulates the flow of reward relevant neural signals through the DA circuitry, possibly by classical presynaptic excitation and inhibition but also possibly by some kind of modulatory interaction between the opioid receptors located on the DA

terminals and the DA reuptake mechanism within those terminals. This last possibility is admittedly highly speculative, but finds support in previous work suggesting the existence within nerve endings of functional coupling between mechanisms of neurotransmitter uptake and presynaptic receptor activation [e.g., (12, 21, 22, 37, 61, 69)]. The model also speculates that there are opioid autoreceptors within this synaptic complex. It hypothesizes that specific Δ^9-THC binding sites are located on the axon terminals of the opioid peptidergic neurons which form axo-axonic connections with the DA terminals, and hypothesizes that these Δ^9-THC binding sites allosterically modulate opioid autoreceptors on these opioid peptidergic neurons. It appears to us to be the simplest model which fits the data summarized in the above review. It may be totally correct, partly correct, or totally incorrect. As with all preliminary neural models, the probability that it is totally incorrect or only partly correct far outweighs the probability that it is totally correct. Most importantly, as with all scientific models, it generates testable hypotheses. The pursuit of such hypotheses should produce additional data that will significantly advance our knowledge of marijuana's interactions with brain reward systems over the coming few years.

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