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THE CENTRAL NEUROPHARMACOLOGY OF PSYCHOTROPIC CANNABINOIDS

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1. INTRODUCTION

Since the identification in 1964 of delta-9-tetrahydrocannabinol (delta-9-THC) as the main psychically active constituent of cannabis (see Mechoulam 1973, for a review), most studies directed at establishing the mechanisms responsible for the psychotropic effect of cannabis have been carried out with delta-9-THC or other constituents of cannabis, rather than with the crude plant material itself. It is with these studies that this review is primarily concerned. Several important areas of research can be identified. One is the study of cannabinoids at the electrophysiological level, and another the study of the part played by putative central neurotransmitters in the psychopharmacology of cannabis. Other areas of importance are the study of interactions between constituents of cannabis and membranes and the study of effects of cannabis constituents on the activity of centrally located membrane-bound enzymes. All these areas are reviewed in this chapter. The chapter also contains a section which considers current thoughts about the molecular basis for the psychotropic activity of cannabis. Two sections in this chapter deal primarily with the human pharmacology of cannabis. One briefly outlines the therapeutic potential of cannabis constituents and related compounds and the other contains a short account of the central effects of cannabis in man and a more detailed discussion about the psychotropic activity of those constituents of cannabis whose neuropharmacology is discussed elsewhere in the chapter. Where possible, review articles have been cited in both these sections. There is also a section concerned with the solubility of delta-9-THC and related compounds. This is an important subject, the high lipid solubility and low water solubility of these substances markedly influencing their pharmacology and also giving rise to formidable practical and interpretational difficulties in the laboratory. The chapter ends with a general discussion and summary. It is important to note that apart from brief references to the existence of cannabis tolerance and dependence, discussions in this review have been limited to effects of single drug administration.

2. THE PSYCHOPHARMACOLOGICAL EFFECTS OF CANNABIS IN MAN

The effects of cannabis which make up a 'high' consist essentially of changes in perception, mood, emotion and cognition (Paton and Pertwee, 1973b; Hollister, 1986). Thus, after cannabis has been taken, there are reports that colors seem brighter and music more pleasant and that 'felt time' passes more slowly than 'clock time'. Effects on mood and emotion vary. Usually there is some euphoria. Sometimes, however, particularly in the inexperienced, mood may be unaffected or there may be dysphoria or anxiety. More serious adverse psychopharmacological responses can also occur, in particular, panic reactions and psychoses (Paton *et al.*, 1973; Hollister, 1986). Signs of changed cognitive function include difficulty in concentrating and thinking and impairment of memory (Miller, 1984). The 'high' is usually followed by a period of drowsiness. Associated with the 'high' are reductions in psychomotor coordination and performance and changes in autonomic processes. The most prominent autonomic changes are cardiovascular, in particular,

tachycardia, postural hypotension and supine hypertension (Jones, 1985; Hollister, 1986). There is also evidence, largely but not exclusively from animal studies, that cannabis can induce changes in thermoregulation (Pertwee, 1985a; Dewey, 1986) and in endocrine and reproductive function (Pertwee, 1985b; Rosenkrantz, 1985; Dewey, 1986; Hollister, 1986). On repeated administration, to animals or man, cannabis can give rise to tolerance and dependence (Pertwee, 1983; Agurell *et al.*, 1986; Beardsley *et al.*, 1986; Dewey, 1986; Hollister, 1986). The tolerance seems to be mainly pharmacodynamic in nature, resulting far more from adaptive changes within the brain than from changes in disposition or metabolism.

Like other natural cannabinoids, the main psychically active constituent of cannabis, (−) delta-9-6a,10a-*trans*-tetrahydrocannabinol (delta-9-THC), is a C_{21} compound, contains only carbon, hydrogen and oxygen and occurs naturally, exclusively in the plant, *Cannabis sativa*. The term 'cannabinoid' is also used to describe the transformation products and synthetic analogs of natural cannabinoids. The structure of delta-9-THC is shown in Fig. 1. Also shown in this figure are two numbering systems for tetrahydrocannabinol, both of which are still used. The dibenzopyran numbering system is the one that now appears most often in neuropharmacological papers and it is this system which has been adopted in this review. The structure of many of the other cannabinoids discussed in this article are shown in Figs 2 to 4.

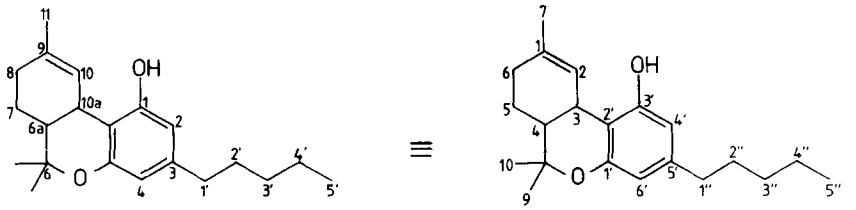

FIG. 1. The structure of the main psychically active constituent of cannabis showing the dibenzopyran numbering system on the left and the monoterpenoid system on the right.

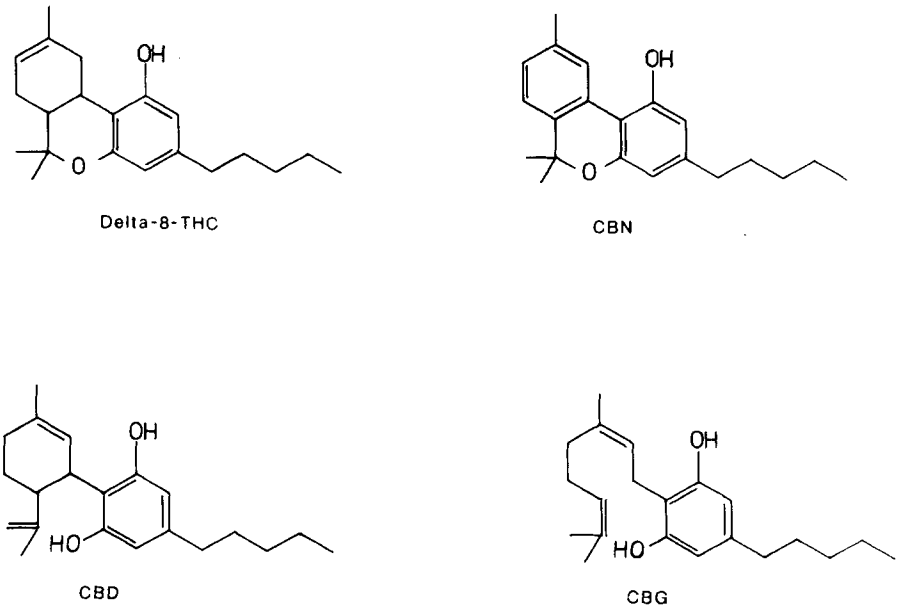

FIG. 2. The structure of four constituents of cannabis, delta-8-tetrahydrocannabinol (delta-8-THC), cannabinol (CBN), cannabidiol (CBD) and cannabigerol (CBG).

Present knowledge about the psychoactive potencies in man of cannabinoids mentioned later in this review is summarized in Table 1. More complete accounts of structure–activity relationships of cannabinoids are to be found in recent reviews by Razdan (1986) and by Martin (1986). As shown in Table 1, there is evidence that psychological changes in human subjects similar to those experienced recreationally with cannabis can be produced by delta-9-THC at doses of about 30 to 50 μg/kg given intravenously, at a dose of about 50 μg/kg inhaled in smoke and at a dose of about 120 μg/kg taken orally. The naturally occurring (−) delta-8-tetrahydrocannabinol (delta-8-THC) has been shown to be only slightly less potent (Hollister and Gillespie, 1973). However, it probably contributes little to cannabis-induced 'highs' since it is only present in the plant in relatively small amounts. Cannabidiol (CBD) has been found to be psychically inactive when administered orally or intravenously at doses well above those at which delta-9-THC is known to produce its psychic effects (Hollister, 1973; Perez Reyes *et al.*, 1973a). Experiments in which high doses of cannabinol (CBN) were administered orally yielded similar

TABLE 1. *The Psychoactivity of Cannabinoids in Human Subjects*

Cannabinoid	Dose (μg/kg)	*n*	Route	Presence of 'high'	Potency ratio	Reference
Δ^9-THC	50	10 to 40	i.h.	+*	1	1
	171†	6	i.h.	+	1	2
	120	10 to 40	p.o.	+*	1	1
	341 to 946	16	p.o.	+	1	3
	286†	6	p.o.	+	1	4
	29†	4	i.v.	+*	1	4
	14 to 16	3	i.v.	+	1	5
	13 to 14	6	i.v.	+	1	5
	50	15	i.v.	+	1	6
	41	6	i.v.	+*	1	7
	53	21	i.v.	+*	1	8, 9
Δ^8-THC	286†	6	p.o.	+	0.7	4
	571†	6	p.o.	+	0.7	4
	29†	3	i.v.	+	0.7	4
	43†	3	i.v.	+	0.7	4
$\Delta^{6a,10a}$-THC	214†	6	i.h.	+	0.2 to 0.3	2
	400	10 to 40	i.h.	0	<0.25	1
Synhexyl	214†	6	i.h.	+	0.2 to 0.3	2
	633 to 2666	13	p.o.	+	0.3	3
DMH-THC (R)	3	3	i.v.	0/+	?	10
(R)	0.5 to 8.3	33	i.v.	0	?	11
(2)	0.5 to 2.8	16	i.v.	0/+	?	11
Nabilone	43 to 71†	6	p.o.	+*	2 to 3	12
11-OH-Δ^9-THC	14 to 16	3	i.v.	+	>3.5	5, 13
	13 to 14	6	i.v.	+	>3.5	5
	32	6	i.v.	+*	1	7
	47	11	i.v.	+*	1	8
8α-OH-Δ^9-THC	>186	5	i.v.	0	<0.3	8
8β-OH-Δ^9-THC	183	9	i.v.	+	<0.3	8
CBN	286 to 5714†	6	p.o.	0	<0.02	14
	270	6	i.v.	+	<0.2	9
CBD	286 to 1429†	5	p.o.	0	<0.08	14
	71 to 429†	4	i.v.	0	<0.1	14
	>268	6	i.v.	0	<0.2	9

Psychoactive potency of each cannabinoid is expressed relative to that of delta-9-THC given by the same route and, where possible, in the same study. The values given in the Table are the ratios of doses producing approximately the same intensity of 'high' (delta-9-THC/other cannabinoid). The presence (+) or absence (0) of a 'high' is also shown. *Indicates that the 'high' (nature and intensity) was judged similar to 'highs' experienced previously with cannabis. (In most studies, the subjects used had had prior recreational experience of cannabis.) †Indicates that the dose shown has been calculated assuming a body weight of 70 kg. Inhalation (i.h.) indicates that the cannabinoid was inhaled in smoke. DMH-THC (delta-6a,10a-dimethylheptyl-THC) has 3 asymmetric carbon atoms and can exist as one or other of 8 optical isomers (numbered 1 to 8) or as a racemic mixture (R). References. 1, Isbell *et al.*, 1967; 2, Hollister, 1970; 3, Hollister *et al.*, 1968; 4, Hollister and Gillespie, 1973; 5, Lemberger *et al.*, 1973; 6, Hollister and Gillespie, 1975; 7, Perez Reyes *et al.*, 1972; 8, Perez Reyes *et al.*, 1973b; 9, Perez Reyes *et al.*, 1973a; 10, Lemberger *et al.*, 1974; 11, Sidell *et al.*, 1973; 12, Lemberger and Rowe, 1975; 13, Lemberger *et al.*, 1972; 14, Hollister, 1973.

results (Hollister, 1973). However, when CBN was injected intravenously, it was found to produce a cannabis-like 'high' (Perez Reyes *et al.*, 1973a). The maximum dose of CBN used, 270 μg/kg i.v., produced a 'high' less intense than that produced by delta-9-THC at a dose of 53 μg/kg i.v., suggesting that the i.v. delta-9-THC/CBN potency ratio exceeds five. 11-Hydroxy-delta-9-THC and 8-beta-hydroxy-delta-9-THC, both metabolites of delta-9-THC, have also been shown to possess cannabis-like psychoactive properties (Table 1.) The 11-hydroxy-compound has been found to be particularly potent, and indeed there is evidence that it can contribute significantly to the psychopharmacological effects of delta-9-THC. The extent of this is thought to depend on the route by which delta-9-THC is administered since this can affect the ratio of 11-hydroxy-delta-9-THC to its parent compound in the blood (Agurell *et al.*, 1986). The contribution made by the 11-hydroxy metabolite is probably far greater in man when delta-9-THC has been given orally than when it has been inhaled in smoke or injected intravenously (Agurell *et al.*, 1986).

The synthetic cannabinoid, delta-6a,10a-dimethylheptyl-THC, has so far been studied in man only at rather low doses, 3 to 6 times less than those at which delta-9-THC is known to produce a 'recreational high' (Sidell *et al.*, 1973; Lemberger *et al.*, 1974). These doses have been reported to produce euphoria or a mild 'high' in some subjects and to have no psychic effects in others. Other synthetic cannabinoids shown to have psychic activity in man are synhexyl which was 3 to 6 times less potent than delta-9-THC (Hollister, 1970; Hollister *et al.*, 1968) and nabilone which was somewhat more potent than

TABLE 2. *Delta-9-THC/Cannabinoid Potency Ratios in Three Behavioral Tests*

Cannabinoid	Rhesus monkey test (i.v.)	Squirrel monkey test (i.p.)	Dog ataxia test (i.v.)
(−)Δ^9-THC	1 (0.1 mg/kg)	1 (1.0 mg/kg)	1 (0.2 mg/kg)
11-OH-Δ^9-THC	ND	5	4
Δ^8-THC	0.1 to 0.2	ND	0.4
$\Delta^{6a,10a}$-THC	0.2	ND	ND
Δ^8-DMH-THC	2	ND	ND
8α-OH-Δ^9-THC	0.05	ND	ND
R-3′-OH-Δ^9-THC	ND	ND	<1
S-3′-OH-Δ^9-THC	ND	ND	>1
R/S-3′-OH-Δ^9-THC	ND	ND	>1
1′-OH-Δ^8-THC	<0.01	ND	ND
2′-OH-Δ^8-THC	0.05	ND	ND
3′-OH-Δ^8-THC	0.5	ND	ND
4′-OH-Δ^8-THC	0.2 to 0.4	ND	ND
5′-OH-Δ^8-THC	0.2	ND	ND
(+)Δ^9-THC	<0.1*	ND	<0.1*
(+)Δ^8-THC	<0.1*	ND	ca.0.1
CBN	Inactive	0.04	ca.0.2
CBD	Inactive	<0.01*	ND
CBG	<0.02*	ND	ND

In the squirrel monkey assay, cannabinoids were given to animals that had been trained to respond on a chain fixed-interval 4 min fixed-ratio 50 schedule of food presentation. The potency ratios shown have been calculated after inspection of dose–response curves (Carney *et al.*, 1979). In the other assays, the potency of 'test' cannabinoid relative to that of delta-9-THC was either the reported value (when given) or the value obtained by comparing doses required to produce a particular pattern of behavior. In the rhesus monkey assay (Grunfeld and Edery, 1969; Edery *et al.*, 1971; Mechoulam and Edery, 1973; Ohlsson *et al.*, 1979) the end point has been taken as behavior which the authors have scored + + ('stupor, ataxia, suppression of motor activity, full ptosis, typical crouched posture kept for up to three hours' and presence of reaction to external stimuli). In the dog assay (Martin *et al.*, 1981, 1984a, b) the end point has been taken as behavior which the authors have scored 3 ('tail is often tucked, some loss of tone in hind legs as evidenced by a semi-squatting position, static ataxia . . . seen after dog stands in one position for 1-2 min and nodding may be observed 30-60 min after injection'). CBG and DMH are abbreviations for cannabigerol and dimethylheptyl respectively. *Indicates inactive at the doses used. ND = not determined.

delta-9-THC (Lemberger and Rowe, 1975). Delta-6a,10a-THC was reported to be approximately equipotent with synhexyl in one study (Hollister, 1970) but to be psychically inactive in another (Isbell *et al.*, 1967).

The ability of several of the cannabinoids mentioned in this review to produce behavioral changes in rhesus monkeys, squirrel monkeys or dogs is summarized in Table 2. On the whole, the data in this Table are consistent with those obtained from human psychopharmacological studies (Table 1). Thus the animal data show that 11-hydroxy-delta-9-THC is more potent that delta-9-THC, that delta-8-THC, delta-6a,10a-THC and 8-alpha-hydroxy-delta-9-THC are all less potent than delta-9-THC and that CBD is inactive. CBN was reported to elicit no behavioral effects in rhesus monkeys (Mechoulam and Edery, 1973) but did produce delta-9-THC-like behavioral changes in squirrel monkeys and dogs (Carney *et al.*, 1979; Martin *et al.*, 1984a). As in the human studies of Perez Reyes *et al.*, 1973a) it was markedly less potent than delta-9-THC in both tests. The data in Table 2 also show that the unnatural (+) isomer of delta-9-THC was at least 10 times

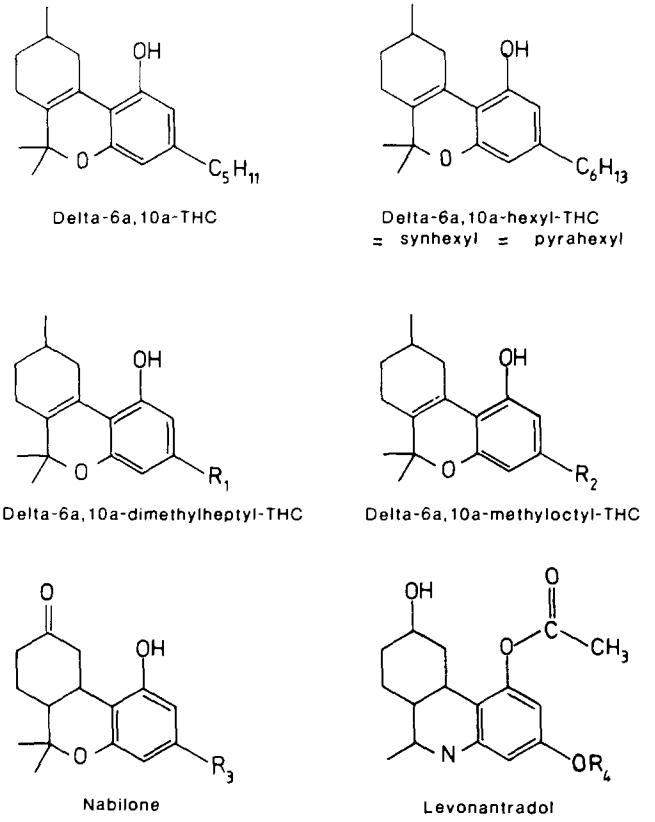

FIG. 3. The structure of several synthetic analogs of delta-9-tetrahydrocannabinol.
$R_1 = CH(CH_3)CH(CH_3)C_5H_{11}$; $R_2 = CH(CH_3)C_7H_{15}$; $R_3 = C(CH_3)_2C_6H_{13}$;
$R_4 = CH(CH_3)CH_2CH_2CH_2(C_6H_5)$.

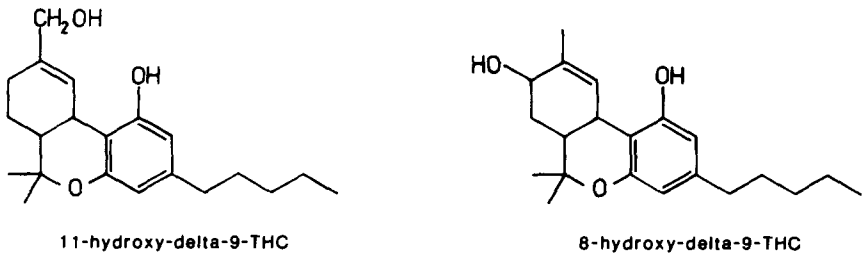

FIG. 4. The structure of two hydroxylated metabolites of delta-9-tetrahydrocannabinol.

less potent than its naturally occurring (−) isomer in both rhesus monkey and dog tests. The stereoselectivity of delta-9-THC has been demonstrated in numerous other animal experiments, both *in vivo* and *in vitro* (Pertwee, 1983; Martin, 1986), and it is likely that (−) delta-9-THC will also be much more potent than (+) delta-9-THC in producing cannabis-like 'highs' in human subjects. This has still to be confirmed. Also still to be confirmed in man is the evidence from animal data (Table 2) that delta-8-dimethylheptyl-THC and certain side chain hydroxylated metabolites of delta-8- and delta-9-THC possess marked psychic activity and that cannabigerol is psychically inactive.

One important synthetic analog of delta-9-THC not included in Tables 1 or 2 is levonantradol. Clinical trials directed at assessing the antiemetic activity of this drug in cancer patients undergoing chemotherapy have shown that it can produce affective, emotional, perceptual and cognitive changes and that it does so at oral doses (0.5 to 2 mg every 4 hr) well below those at which delta-9-THC is known to produce its psychopharmacological effects (Diasio *et al.*, 1981; Laszlo *et al.*, 1981; see also references cited by Pertwee, 1985b). There is also evidence that levonantradol (0.03 to 0.3 mg/kg i.v.) can produce delta-9-THC-like changes in the behavior of rhesus monkeys (Young *et al.*, 1981). The behavioral changes elicited by a levonantradol dose of 0.3 mg/kg i.v. were similar to those produced by delta-9-THC at a dose of 3 mg/kg i.v. These data suggest that levonantradol is a highly potent psychoactive cannabinoid. However, confirmatory controlled experiments with healthy human subjects are still required, there being a need for qualitative as well as quantitative comparisons to be made between the psychopharmacological properties of levonantradol and those of delta-9-THC or cannabis.

The results of experiments conducted with cannabis can be particularly difficult to interpret. There are three main reasons for this. First, cannabis contains a number of compounds, both cannabinoid and non-cannabinoid, having different pharmacological properties (Pertwee, 1983; 1985b). Second, the chemical composition of cannabis is not always the same (Paton, 1975; Pertwee, 1983). It is affected by heat when smoked and by storage and also depends on the geographical origin of the plant material. Third, there is evidence that the effects of delta-9-THC can be intensified or attenuated by CBN, CBD and other constituents of cannabis (Pertwee, 1983; Dewey, 1986; Martin, 1986). For these reasons, the remainder of this review follows the practice of many recent neuropharmacological studies and deals only with effects produced by individual cannabinoids.

Before proceeding to the next section it is important to note that like cannabis, delta-9-THC and many of the other cannabinoids referred to in this review have the ability to produce quite large falls in deep-body temperature (Pertwee, 1985a). A few neuropharmacological studies with whole animals have taken this into account, using ambient temperatures sufficiently high to prevent hypothermia. However, most have been conducted at normal room temperature and therefore presumably often with hypothermic animals. This must be borne in mind when results are being interpreted since brain function is markedly affected by hypothermia.

3. THE THERAPEUTIC POTENTIAL OF CANNABINOIDS

Cannabis has a long history not only as a recreational drug but also as a therapeutic agent. Although its traditional uses have been taken over by more effective and selective agents there has recently been an upsurge of interest in the possible clinical uses of individual cannabinoids. Experiments have been directed both at examining the therapeutic potential of existing cannabinoids and at producing new analogs with improved selectivity (Lemberger, 1980, 1985; Pertwee, 1985b; Hollister, 1986). The ability of delta-9-THC and of two of its synthetic analogs, nabilone and levonantradol, to suppress nausea and vomiting associated with cancer chemotherapy has attracted particular interest. Also of interest is the possible use of the psychically inactive cannabinoid, CBD, in the treatment of epilepsy. Other potential therapeutic applications which show some degree of promise include the use of delta-9-THC or its synthetic analogs as analgesics, as muscle relaxants and in the treatment of glaucoma.

4. THE SOLUBILITY OF CANNABINOIDS

Cannabinoids are very lipophilic. They have extremely low water solubilities, high lipid solubilities and high membrane/water and organic solvent/water partition coefficients. The values of the organic solvent/water coefficients vary somewhat from cannabinoid to cannabinoid. However, there is no correlation between these values and psychoactive potency. The octanol/water and benzene/water partition coefficients of delta-9-THC have both been reported to be about 6000 (Gill and Jones, 1972; Ho *et al.*, 1973). For delta-8-THC, the corresponding values are only slightly less, about 5000 (Ho *et al.*, 1973; Ohlsson *et al.*, 1980). Gill *et al.* (1973) found that the octanol/water partition coefficient of the more polar cannabinoid, 11-hydroxy-delta-9-THC, was about 3500. The 11-hydroxy metabolite of delta-8-THC was shown to have octanol/water and benzene/water partition coefficients of about 1500 and CBN had a benzene/water partition coefficient of 1175 (Ho *et al.*, 1973; Ohlsson *et al.*, 1980). The octanol/water partition coefficients of side chain hydroxylated derivatives of delta-8-THC were found to range from 1500 to 4000 (Ohlsson *et al.*, 1980).

Binder *et al.* (1984) studied the distribution of delta-9-THC in a system consisting of egg phosphatidylcholine, glycerol trioleate and phosphate buffer. The cannabinoid distributed almost equally between the two lipids and none of it was detectable in the aqueous phase. Seeman *et al.* (1972) measured the membrane/phosphate buffer partition coefficient of delta-9-THC using both guinea-pig brain synaptosomes and human erythrocyte ghosts. They reported that the partition coefficient varied inversely with the free concentration of delta-9-THC. It ranged from about 500 to 40 for the synaptosomal membranes and from about 800 to 35 for the erythrocyte membranes. More recently, Roth and Williams (1979) found the partition coefficient of delta-9-THC between rat brain synaptosomes and Krebs solution or phosphate buffer to be of the order of 12 000 to 14 000. They also reported that the value of the partition coefficient did not vary with the free concentration of delta-9-THC. These results are clearly quite different from those of Seeman *et al.* (1972). One possible explanation for this difference is that the latter group overestimated the concentration of delta-9-THC remaining in free solution. Whereas Roth and Williams (1979) used concentrations of delta-9-THC in the range 0.01 to 1 μM, Seeman *et al.* (1972) reported the use of concentrations of 4 to 40 μM, a range which extends well beyond the aqueous solubility of delta-9-THC (less than 10 μM; see below).

Because of their low solubility in water, cannabinoids are usually administered dissolved or dispersed in agents such as ethanol, dimethyl sulfoxide, Tween 80, Cremophor E. L. or polyvinylpyrrolidone. Most studies making use of vehicles of this kind have not included experiments to test for an effect of the vehicle (relative to an untreated group) or for the existence of synergistic or antagonistic interactions between cannabinoid and vehicle. (For further discussion on this point, see Burstein *et al.*, 1986a and the review by Dewey, 1986).

Although the administration of cannabinoids is facilitated by dissolving them in organic solvents, there is no guarantee that such solutions will not precipitate out immediately after administration. When Banerjee *et al.* (1975b) added 0.33 μmoles of delta-9-THC dissolved in dimethyl sulfoxide to 10 mls of Krebs–Henseleit buffer (final 'nominal' concentration = 33 μM), they found that only about 2% of the cannabinoid remained in solution (actual concentration = 0.63 μM). The same result was obtained when the buffer also contained rat brain synaptosomes. When delta-8-THC or CBD was added to the buffer (final 'nominal' concentrations = respectively 37 and 28 μM), 1% of delta-8-THC and 4.4% of CBD remained in solution. Only 0.3 to 0.5% of delta-9-THC remained in solution at 'nominal' concentrations in buffer ranging from 0.1 to 1 mM. Johnson *et al.* (1976a) added delta-9-THC dissolved in polyvinylpyrrolidone to Krebs–Henseleit buffer and found that 3.9% of the drug remained in solution (final 'nominal' concentration = 0.1 mM). The results obtained by Banerjee *et al.* (1975a), by Johnson *et al.* (1976a) and by Roth and Williams (1979) suggest that aqueous solutions of delta-9-THC become saturated at concentrations of about 1 to 4 μM. Consistent with this conclusion is the

observation by Bach *et al.* (1976a) that delta-9-THC and CBD precipitated out of 0.15 M aqueous NaCl and KCl solutions at a concentration of about 8 μM. and the report by Garrett and Hunt (1974) that the solubilities of delta-9-THC in water and in aqueous 0.15 M NaCl solutions are respectively 9 and 2.5 μM.

Some vehicles, for example Tween 80 and Cremophor E. L., combine with cannabinoids in aqueous media to form micelles, the vehicle–cannabinoid–water mixture existing in a dispersion rather than as a true solution. It is likely that after administration to animals or to *in vitro* preparations, such micelles remain intact so that the cannabinoids stay homogeneously dispersed. There is evidence that, at least in some media, the transfer of delta-9-THC from micelles to tissue is much slower and less complete than tissue absorption of delta-9-THC from free solution. Thus Roth and Williams (1979) found that Tween 80 markedly reduced the synaptosomal membrane/buffer partition coefficient of delta-9-THC, the size of the reduction increasing with the amount of Tween 80 present. Similar results were obtained with Cremophor E. L. Interestingly, ethanol also reduced the partition coefficient of delta-9-THC. However, its effect was much smaller than that of the other vehicles.

Reports about *in vitro* experiments with cannabinoids often give a value for the concentration used that implies uniform and complete dissolution in the aqueous bathing medium. For the want of more accurate values, such 'nominal' concentrations are also given in the Tables and text of this review. It is likely, however, that when, as is frequently the case, the vehicle used is an organic solvent, the final actual free concentration of cannabinoid in the medium will only equal the 'nominal' concentration when the latter is of the order of 1 μM or less (see above), an increasing proportion of added cannabinoid coming out of solution as the 'nominal' concentration is raised above 1 μM. Even when low concentrations of cannabinoids are used, the actual free concentration in the medium will probably be less than expected. This is because delta-9-THC and presumably other cannabinoids bind avidly to the surfaces of the vessels in which they are contained (Garrett and Hunt, 1974; Kujtan *et al.*, (1983). The extent to which precipitation of cannabinoids into aqueous media affects their ability to reach and interact with a responsive tissue is not certain. Complete loss of this ability is unlikely, however, and indeed Banerjee *et al.* (1975b) found that when delta-9-THC was dissolved in dimethyl sulfoxide and then added to an aqueous suspension of synaptosomes, it produced 'concentration dependent' inhibition of neurotransmitter uptake at 'nominal' concentrations ranging from 1 to 100 μM (Section 6).

Many of the experiments described in this review have involved addition of delta-9-THC directly to preparations of brain tissue suspended in aqueous media. In order to assess the importance of delta-9-THC-induced changes detected in such experiments, it would be helpful to know how the tissue concentrations achieved in these *in vitro* experiments compare with the concentrations of delta-9-THC reached in brain tissue after the drug has been administered *in vivo* at doses known to elicit pharmacological responses. This is an extremely complex task and indeed the data at present available are insufficient to make comparisons of this kind with any degree of accuracy. Gill and Jones (1972) measured whole brain levels of ^{3}H-delta-9-THC at various times after the drug had been injected intravenously into mice. The dose used, 2 mg/kg, produced detectable behavioral changes and is also known to lower body temperature (Pertwee and Tavendale, 1977). The maximum concentration of delta-9-THC found in the brain was 1.46 μmoles/kg. Ryrfeldt *et al.* (1973) showed that mouse brain concentrations of delta-9-THC rose to about 3.2 μmoles/kg following intravenous injection of this cannabinoid at a dose of 12 mg/kg. In rats, intravenous injections of delta-9-THC at doses of 4 and 5 mg/kg were reported to produce peak brain levels of 5.1 and 11.2 μmoles/kg, respectively (Klausner and Dingell, 1971; Ho *et al.*, 1973). These brain concentrations were all determined per unit wet weight of whole brain and, given the high lipid/water partition coefficient of delta-9-THC, it is likely that the drug reached higher concentrations in the lipid phase of neuronal membranes. [Roth and Williams (1979) estimated that a dose of delta-9-THC known to elicit behavioral changes in mice would give rise to a brain membrane concentration of about

20 μmoles/kg dry membrane.] Metabolites of delta-9-THC are thought to make a significant contribution towards the responses evoked by *in vivo* administration of delta-9-THC (Section 2) and this too must be taken into account when calculating the concentration of delta-9-THC required in brain tissue to bring about pharmacological changes.

Roth and Williams (1979) obtained values for the membrane concentration of delta-9-THC in various *in vitro* systems from the equation membrane concentration = free concentration × membrane/buffer partition coefficient. The equation is of limited value in interpreting the data described in this review since the amount of delta-9-THC added to *in vitro* systems has often exceeded its aqueous solubility. Another problem can be the selection of an appropriate value for the partition coefficient because, as already discussed, the partition coefficient of delta-9-THC can vary considerably not only with the vehicle used but also with vehicle concentration. For example, if the concentration of delta-9-THC in the aqueous medium of an *in vitro* preparation were 1 μM, then depending on the vehicle used, the membrane concentration of the drug might be as low as 0.1 or 0.5 mmoles/kg dry membrane or as high as 10 or 12 mmoles/kg dry membrane (Roth and Williams, 1979). Nonetheless, these calculations do suggest that, irrespective of the vehicle used, a 'nominal' delta-9-THC concentration in the medium of less than 1 μM will be sufficient to achieve a tissue concentration (20 μmoles/kg dry membrane) which will, when present in the brain, give rise to behavioral changes (see above). This conclusion is supported by evidence firstly that delta-9-THC can elicit responses in several *in vitro* preparations when

TABLE 3. *Effects of Cannabinoids on the Twitch Response Evoked in Isolated Preparations of the Guinea-pig Ileum by Repetitive Electrical Stimulation*

Procedure	Cannabinoid	Concentration	Twitch amplitude	Reference
Transmural stimulation of whole ileum	Δ^9-THC	3.18 μM upwards	–	1
	Δ^9-THC	0.16 μM ($=ED_{50}$)	–	2
	CBD	3.18μM	0	2
Supramaximal stimulation of whole ileum at 0.1 Hz (cannabinoid vehicle when named was ethanol)	(−)Δ^9-THC	c.a. 79.5 nM	–	3
	(−)Δ^8-THC	79.5 nM	–	3, 4
	(+)Δ^8-THC	318 nM	0	3
	(−) 11-OH-Δ^9-THC	c.a. 3 nM upwards	–	3
	(+) 11-OH-Δ^9-THC	76 nM	0	3
	(−) 11-OH-Δ^8-THC	3 nM upwards	–	3, 4
	(−) 1′-OH-Δ^8-THC	75.6 nM	−/0	5
	(−) 2′-OH-Δ^8-THC	75.6 nM	−/0	5
	(−) 3′-OH-Δ^8-THC	75.6 nM	–	5
	(−) 4′-OH-Δ^8-THC	75.6 nM	−/0	5
	(−) 5′-OH-Δ^8-THC	75.6 nM	–	5
	CBD	318 nM	0	3, 4
	CBN	322 nM	0	3, 4
Stimulation of whole ileum at 0.1 Hz (cannabinoid vehicle was ethanol)	(−)Δ^9-THC	100 nM ($=ED_{50}$)	–	6
	(+)Δ^9-THC	2 μM	0	6
	(−)Δ^8-THC	100 nM ($=ED_{50}$)	–	6
	11-OH-Δ^9-THC	15 nM ($=ED_{50}$)	–	6
	1′-OH-Δ^9-THC (A)	1.1 μM ($=ED_{50}$)	–	6
	1′-OH-Δ^9-THC (B)	2.3 μM	0	6
	2′-OH-Δ^9-THC	500 nM ($=ED_{50}$)	–	6
	3′-OH-Δ^9-THC	20 nM ($=ED_{50}$)	–	6
	4′-OH-Δ^9-THC	80 nM ($=ED_{50}$)	–	6
	5′-OH-Δ^9-THC	110 nM ($=ED_{50}$)	–	6
	Nabilone	100 nM ($=ED_{50}$)	–	6
	Levonantradol	10 nM ($=ED_{50}$)	–	6
	CBN	2 μM	0	6
	CBD	2 μM	0	6
	CBG	2 μM	0	6
Supramaximal field stimulation of longitudinal muscle strip at 0.2 Hz (cannabinoid vehicle was Cremophor E. L.)	(−)Δ^9-THC	6.4 nM to 0.64 μM*	–	7
	(+)Δ^9-THC	64 nM to 6.4 μM**	–	7

*Indicates $ED_{50}=0.125$ μM and ** indicates $ED_{50}=3.1$ μM. CBG is an abbreviation for cannabigerol. References: 1, Gill *et al.*, 1970; 2, Layman and Milton, 1971; 3, Rosell *et al.*, 1976; 4, Rosell and Agurell, 1975; 5, Rosell *et al.*, 1979; 6, Nye *et al.*, 1985; 7, Roth, 1978.

present in the medium at 'nominal' concentrations ranging from 10 pM to 300 nM and secondly that the ability of cannabinoids to produce changes at such concentrations does, in several preparations, correlate with psychoactive potency (see, for example, Sections 10.1.1. and 10.2.

5. ELECTROPHYSIOLOGICAL STUDIES

Delta-9-THC, 50 to 500 μM, has been shown to decrease the amplitude of the action potential in the desheathed cervical vagus nerve of the rabbit (Byck and Ritchie, 1973). In addition, at concentrations of 0.1 to 1.0 mM it has been found to reduce voltage-dependent sodium conductance in single myelinated fibers of frog sciatic nerve (Strichartz *et al.*, 1978). In the same preparation, the drug also reduced potassium conductance, albeit to a smaller extent. One preparation in which delta-9-THC has been reported to have no effect on impulse conduction is the giant squid axon (Brady and Carbone, 1973). Interestingly, its primary metabolite, 11-hydroxy-delta-9-THC, was found to be highly active in this preparation, concentrations as low as 5 nM reducing both the amplitude of the action potential and conduction velocity (Brady and Carbone, 1973).

There is evidence that as well as reducing the excitability of some isolated nerve preparations, delta-9-THC can depress the excitability of nerve cells in isolated buccal and parieto-visceral ganglia of *Aplysia californica* (Acosta-Urquidi and Chase, 1975) and decrease the activity of prejunctional neurons in isolated preparations of rat phrenic nerve-diaphragm (Kayaalp *et al.*, 1974; Hoekman *et al.*, 1976), frog sciatic nerve-sartorius muscle (Kumbaraci and Nastuk, 1980), crayfish neuromuscular junction (Aldridge and Pomeranz, 1977) and guinea-pig ileum (Table 3). Thus, for example, experiments with the rat phrenic nerve-diaphragm preparation showed that when just a portion of the phrenic nerve was exposed to delta-9-THC (95 μM), there was a marked reduction in the size of the twitch evoked by electrical stimulation of the nerve (Kayaalp *et al.*, 1974). In experiments with isolated preparations of the guinea-pig ileum, delta-9-THC was shown to decrease the size of the twitches induced by repetitive electrical stimulation (Table 3). The twitches observed in these experiments were produced by electrically stimulated prejunctional release of acetylcholine rather than by direct stimulation of ileal smooth muscle and it was found that delta-9-THC could reduce the size of these twitches at concentrations at which it had no effect on contractions induced by the addition of acetylcholine (Gill *et al.*, 1970; Layman and Milton, 1971; Roth, 1978). It was also found that concentrations of delta-9-THC known to reduce the twitch response, decreased the output of acetylcholine from resting strips of whole ileum (Layman and Milton, 1971) or of ileal longitudinal muscle (Paton *et al.*, 1972). Interestingly, the effect of delta-9-THC was greatest when the initial spontaneous release of acetylcholine had been high, suggesting that the drug acted to reduce acetylcholine output to a basal level (Paton *et al.*, 1972). It is noteworthy that CBD, which had no detectable effect on the twitch response, was reported by Layman and Milton (1971) to be more effective than delta-9-THC in reducing spontaneous acetylcholine output from lengths of whole ileum.

The ability of cannabinoids to decrease the size of electrically evoked twitches in the isolated guinea-pig ileum correlates well with psychoactive potency (see Table 3 for references). For example, (−) delta-8-THC and (−) delta-9-THC have been found to be approximately equipotent as inhibitors of the twitch response (Rosell *et al.*, 1976; Nye *et al.*, 1985). Both were less potent than their 11-hydroxy metabolites but markedly more potent than their (+) isomers (Rosell and Agurell, 1975; Rosell *et al.*, 1976; Roth, 1978; Nye *et al.*, 1985). CBN and CBD were reported not to attenuate the twitch response even at relatively high concentrations (Layman and Milton, 1971; Rosell and Agurell, 1975; Rosell *et al.*, 1976; Nye *et al.*, 1985).

Rosell and Agurell (1975) found that like delta-9-THC (see above), concentrations of delta-8-THC or 11-hydroxy-delta-8-THC known to decrease the size of the twitch response in the isolated guinea-pig ileum, did not reduce contractions elicited by the

addition of acetylcholine. They also found that the 11-hydroxy compound did not attenuate the response of guinea-pig ileum to histamine which acts directly on the smooth muscle but did reduce the response to 5-hydroxytryptamine which acts prejunctionally to increase acetylcholine release.

During their experiments with the isolated sciatic nerve-sartorius muscle preparation of the frog, Kumbaraci and Nastuk (1980) obtained evidence that delta-9-THC (30 μM) markedly decreased prejunctional release of acetylcholine per nerve impulse and suggested that the drug might have acted not only by diminishing prejunctional action potentials but also by reducing prejunctional Ca^{2+} influx. It is noteworthy, therefore, that Harris and Stokes (1982) have shown that *in vitro* addition of delta-9-THC can inhibit depolarization-dependent Ca^{2+} uptake into synaptosomes prepared from mouse whole brain or from rat cerebral cortex, striatum, cerebellum or brain stem. Effective concentrations ranged upwards from 10 nM and varied depending on the brain area from which the synaptosomes had been prepared. Delta-9-THC inhibited Ca^{2+} uptake at concentrations at which it had no apparent effect on Ca^{2+} efflux or on synaptosomal membrane potentials and it was concluded that it had altered Ca^{2+} uptake by interacting with voltage-dependent Ca^{2+} channels. The psychically inactive cannabinoid, CBD, was as potent as delta-9-THC as an inhibitor of Ca^{2+} uptake, albeit only in the experiments with mouse brain synaptosomes. 11-hydroxy-delta-9-THC was less potent than delta-9-THC, and CBN was inactive. Like delta-9-THC, CBD also inhibited Ca^{2+} uptake into rat brain stem synaptosomes. However, in contrast to delta-9-THC, it had no detectable effect on Ca^{2+} uptake into synaptosomes prepared from rat cerebral cortex or cerebellum. In more recent studies, Turkanis and Karler (1986a) found that psychically active cannabinoids can exert both depressant and excitant effects on transmission across the neuromuscular junction of the frog sartorius muscle. Using extracellular recording techniques, they obtained evidence that, at concentrations of 0.1 to 1.0 μM, delta-9-THC and its 11-hydroxy metabolite could act prejunctionally, initially to enhance transmitter release (an increase in the mean quantum content of the end plate potential) and subsequently to depress release. CBD, on the other hand, had only a depressant effect on release. All three cannabinoids also depressed the amplitude of miniature end-plate potentials, presumably a postjunctional effect. In addition, it was found that the frequency of the miniature end plate potentials was increased by delta-9-THC, unaffected by 11-hydroxy-delta-9-THC and decreased by CBD.

Niemi (1979) studied the effect of delta-9-THC on the electrical excitability of innervated electric eel electroplaques. At a concentration of 50 μM, the drug reduced the action potential elicited by direct stimulation of the electroplaque. Delta-9-THC also suppressed transmission across the nerve–electroplaque junction without altering the ability of carbamylcholine to depolarize the postjunctional membrane. It was concluded that, in this preparation, the drug can reduce the electrical excitability of the postjunctional membrane

TABLE 4. *Effects of Cannabinoids on Monosynaptic (Mono) and Polysynaptic (Poly) Spinal Reflexes*

Cannabinoid	Dose (mg/kg i.v.)	Preparation	Type of reflex	Effect	Reference
$\Delta^{6a,10a}$-DMH-THC	0.05	Anesthetized cat	Poly (Flexion)	0	1
	0.10	Anesthetized cat	Poly (Flexion)	−	1, 2
	0.05 and 0.10	Anesthetized cat	Poly (Linguomandibular)	−	1, 2
	0.20	Anesthetized cat	Mono (Patellar)	0	1
$\Delta^{6a,10a}$-MO-THC	0.20 to 0.40	Anesthetized cat	Poly (Flexion)	−	1
	0.20 to 0.40	Anesthetized cat	Poly (Linguomandibular)	−	1
Δ^{9}-THC	0.01 to 0.10	Anesthetized spinal cat	Mono	+	3
Δ^{9}-THC	0.05 to 0.15	Unanesthetized	Mono	+	4
	0.20 to 0.75	spinal cat	Mono	−	4
CBD	0.05 to 14.0	Unanesthetized spinal cat	Mono	−	4

DMH and MO are abbreviations for dimethylheptyl and methyloctyl, respectively. References: 1, Dagirmanjian and Boyd, 1962; 2, Boyd and Meritt, 1965; 3, Turkanis and Karler, 1983; 4, Tramposch *et al.*, 1981.

TABLE 5. *Effects of Cannabinoids on the Amplitude of Potentials Evoked at Sites in the Sensory Cortex, Thalamus, Midbrain and Hindbrain*

Cannabinoid	Dose (mg/kg)	Route	Preparation	Stimulation site	Recording site	Effect	Reference
Δ^8 and Δ^9-THC	0.25 to 1.5	i.v.	Unanesthetized	Somatosensory	Postarcuate	+	1
	0.50 to 1.5	i.v.	squirrel monkey (cerveau isolé)	cortex	polysensory cortex	−	1
Δ^9-THC	0.065 to 2.5	i.v.	Unanesthetized squirrel monkey (spinal or pontine)	Somatosensory cortex	Postarcuate polysensory cortex	+	2, 3
Δ^9-THC	0.032 to 1.0	i.v.	Unanesthetized squirrel monkey	Ear (stimulus = tone cue)	Postarcuate polysensory cortex	−	4
Δ^9-THC	0.1 to 1.0	i.p.	Unanesthetized	Parietal	Parietal	+	5
	1.2 to 6.0	i.p.	rat	cortex	cortex	−	
CBD	0.1 to 40.0	i.p.	Unanesthetized rat	Parietal cortex	(contralateral to stimulation site)	−	5
Δ^9-THC	0.1 to 0.3*	*	Anesthetized rat	Eye (stimulus = light)	Lateral geniculate nucleus	−	6
Δ^9-THC	0.1 to 0.3*	*	Anesthetized rat	Optic nerve	Lateral geniculate nucleus	−(5/8 rats)	6
THC	0.80 to 1.0†	i.v.	Anesthetized cat	Trigeminal afferent nerve	Superior sensory nucleus of trigeminal nerve	−	7
THC	0.80 to 1.0†	i.v.	Anesthetized cat	Trigeminal afferent nerve	Trigeminal afferent nerve	−	7
$\Delta^{6a,10a}$-DMH-THC	0.2	i.v.	Unanesthetized cat	Radial nerve	Mesencephalic medial lemniscus	0	8
$\Delta^{6a,10a}$-DMH-THC	0.2	i.v.	Unanesthetized cat	Radial nerve	Ventrobasal complex of thalamus	+(2/7 cats) −(1/7 cats)	8
$\Delta^{6a,10a}$-DMH-THC	0.2	i.v.	Unanesthetized cat	Radial nerve	Mesencephalic reticular formation	−(6/14 cats)	8

DMH is an abbreviation for dimethylheptyl. *Indicates administration by close arterial injection (catheter in internal carotid artery; dose in mg). †Indicates that these doses had no effect on tibialis nerve action potentials. References: 1, Boyd *et al.*, 1971; 2, Boyd *et al.*, 1974; 3, Boyd *et al.*, 1975 4, Boyd *et al.*, 1976 5, Turkanis and Karler, 1981b 6, Bieger and Hockman, 1973 7, Lapa *et al.*, 1968 8, Boyd and Meritt, 1966.

and that it can also act prejunctionally to reduce the size of the action potential or to impair excitation–secretion coupling.

Cannabinoids have been shown to alter transmission along central synaptic pathways in a variety of mammalian preparations (Tables 4 to 8). Their effects on spinal reflexes may well involve sites of action in the brain. Thus Dagirmanjian and Boyd (1962) found that although delta-6a, 10a-dimethylheptyl-THC could suppress the polysynaptic flexion and linguomandibular reflexes in unanesthetized cats sectioned at the level of the midbrain, it had no effect on the flexion reflex in animals with low spinal sections. Low spinal sections did not, however, abolish the effect of delta-6a, 10a-dimethylheptyl-THC on the linguomandibular reflex. In further experiments (Boyd and Meritt, 1965), delta-6a, 10a-dimethylheptyl-THC was shown in 6/10 cats to depress facilitation of the flexion reflex induced by electrical stimulation of the brain stem reticular formation. Similarly, facilitation of the knee jerk produced by brain stem stimulation was attenuated or abolished by the cannabinoid in 9/20 cats. In contrast to the earlier experiments, it was found that delta-6a, 10a-dimethylheptyl-THC was able to suppress the flexion reflex (5/8 cats) as well as the linguomandibular reflex in cats with low spinal sections. It would seem, therefore, that the drug can suppress spinal reflexes both by acting at the level of the brain stem to reduce motor facilitation and by interacting directly with the reflex system itself.

In several experiments, the psychically active cannabinoids delta-9-THC, delta-8-THC and delta-6a, 10a-dimethylheptyl-THC were found to have an excitatory effect on neuronal transmission at low doses and an inhibitory effect at higher doses. This was so for one or other of these drugs in experiments concerned with effects on spinal reflexes (Table 4), on the amplitude of potentials evoked in various parts of the brain by electrical stimulation (Tables 5 and 6) and on post-tetanic potentiation in cat spinal cord and rat hippocampus (Table 7).

Unlike psychically active cannabinoids, CBD has been found only to inhibit central transmission (Tables 4, 5 and 7). The effects of CBD and delta-9-THC on central transmission therefore parallel the changes produced by the two drugs in behavioral and electrophysiological models of epilepsy, CBD exerting only anticonvulsant actions and delta-

TABLE 6. *Effects of Cannabinoids on the Amplitude of Potentials Evoked at Sites in the Hippocampus*

Cannabinoid	Dose*	Preparation	Stimulation site	Recording site	Effect	Reference
In vivo experiments						
Δ^9-THC	2.5 to 10	Anesthetized	Fornix	Hippocampal	0	1
Δ^8-THC	2.5 to 10	rat		surface	0	
CBD	1.25 to 5	Anesthetized	Fornix	Hippocampal	0	1
CBN	2.5 to 10	rat		surface	0	
SP-111	10	Unanesthetized rat	Hippocampus	Hippocampus (contralateral to stimulation site)	–	2
Δ^9-THC	2 to 8	Unanesthetized	Afferents to	CA1 pyramidal	+	3, 4
	16	rat	CA1 pyramidal cells	cell body layer	–	4
In vitro experiments						
Δ^9-THC†	0.001 nM	Rat	Afferents to	CA1 pyramidal	0	5
	0.01 nM	hippocampal	CA1 pyramidal	cell body	+	
	0.1 to 10 nM	slices	cells	layer	–	
Δ^9-THC‡	0.01 μM	Guinea-pig	Afferents to	CA1 pyramidal	0	6
	0.1 μM	hippocampal	CA1 pyramidal	cell body	+	
	1.0 μM	slices	cells	layer	–	
Δ^9-THC‡	0.1 μM	Guinea-pig	Afferents to	Dentate	+	6
	1.0 μM	hippocampal slices	dentate granule cells	granule cells	–	

*Dose expressed as mg/kg i.p. (*in vivo*) or in molar terms (*in vitro*). †Indicates that the vehicle used was polyvinylpyrrolidone. ‡Indicates that no 'solubilizer' was used. SP-111 is a water soluble derivative of delta-9-THC. References: 1, Izquierdo *et al.*, 1973; 2, Segal, 1978; 3, Vardaris *et al.*, 1977; 4, Weisz *et al.*, 1982; 5, Foy *et al.*, 1982; 6, Kujtan *et al.*, 1983.

TABLE 7. *Effects of Cannabinoids on Post-tetanic Potentiation (PTP)*

Cannabinoid	Dose*	Preparation	Stimulation site	Recording site	Effect on PTP	Reference
In vivo experiments						
Δ^9-THC	0.05 to 0.15 i.v.	Unanesthetized	Dorsal root	Ventral root	+	1
	0.20 to 0.75 i.v.	spinal cat			−	
CBD	0.05 to 14 i.v.	Unanesthetized spinal cat	Dorsal root	Ventral root	−	1
Δ^9-THC	2 and 4 i.p.	Unanesthetized	Afferents to	CA1 pyramidal	+	2
	8 i.p.	rat	hippocampal	cell body layer	0	
	16 i.p.		CA1 pyramidal cells		−	
In vitro experiments						
Δ^9-THC†	1 to 25 μM	Isolated	Preganglionic	Ganglia	0	3 ‡
11-OH-Δ^9-THC	0.1 to 0.33 μM	paravertebral	neurons		−	
8α,11-diOH-Δ^9-THC	0.1 to 0.25 μM	ganglia X			−	
CBD	60 to 100 μM	of bull frog			−	

*Dose expressed as mg/kg (*in vivo*) or in molar terms (*in vitro*). †Indicates that cannabinoids were dispersed in Pluronic F68. ‡All the cannabinoids in this study reduced the amplitude of pretetanic potentials (delta-9-THC at 25 to 100 μM). References: 1, Tramposch *et al.*, 1981; 2, Weisz *et al.*, 1982; 3, Turkanis and Karler, 1975.

TABLE 8. *Effects of Cannabinoids on Paired Pulse Facilitation (f) and Inhibition (i)*

Cannabinoid	Dose*	Preparation	Stimulation site	Recording site	Effect	Reference
In vivo experiments						
Δ^8 and Δ^9-THC	0.5 to 1.5 i.v.	Unanesthetized squirrel monkey (cerveau isolé)	Somatosensory cortex	Postarcuate polysensory cortex	0/− (f)	1
CBD	3.5 i.p.	Anesthetized rat	Fornix or alveus of hippocampus	Alveus (contralateral to stimulated alveus)	− (f)	2
SP-111	10 i.p.	Unanesthetized rat	Hippocampus	Hippocampus (contralateral to stimulation site)	− (f) − (i)	3
Δ^9-THC	2 i.p.	Unanesthetized rat	Afferents to hippocampal CA1 pyramidal cells	CA1 pyramidal cell body layer	− (i)	4
Δ^9-THC	2 to 16 i.p.	Unanesthetized rat	Afferents to hippocampal CA1 pyramidal cells	CA1 pyramidal cell body layer	+ (i)	5
In vitro experiments						
Δ^9-THC†	0.1 and 1.0 μM	Guinea-pig hippocampal slices	Afferents to hippocampal CA1 pyramidal cells	CA1 pyramidal cell body layer	0 (f)	6
Δ^9-THC†	0.1 and 1.0 μM	Guinea-pig hippocampal slices	Afferents to hippocampal dentate granule cells	Dentate granule cells	0 (f)	6
Δ^9-THC†	0.1 μM 1.0 μM	Guinea-pig hippocampal slices	Alveus (conditioning pulse) and stratum radiatum (test pulse)	CA1 pyramidal cell body layer	− (i) 0 (i)	6

*Dose expressed as mg/kg (*in vivo*) or in molar terms (*in vitro*). †Indicates that no 'solubilizer' was used. SP-111 is a water soluble derivative of delta-9-THC. Stimuli were applied in pairs, the size of the response to the second stimulus (test pulse) being either greater (facilitation, f) or less (inhibition, i) than the response to the first stimulus (conditioning pulse). References: 1, Boyd *et al.*, 1971; 2, Izquierdo and Nasello, 1973; 3, Segal, 1978; 4, Vardaris *et al.*, 1977; 5, Weisz *et al.*, 1982; 6, Kujtan *et al.*, 1983.

9-THC a mixture of convulsant and anticonvulsant actions (Feeney, 1979; Karler and Turkanis, 1979; Turkanis and Karler, 1981a; Pertwee, 1985b).

The effect of delta-9-THC on paired pulse inhibition in rat hippocampal CA1 pyramidal cells (Table 8) was found by Weisz *et al.* (1982) to depend on the interval between the initial 'conditioning' pulse and the subsequent 'test' pulse. When this interval lay in the range 60 or 80 to 500 ms, delta-9-THC (2 to 16 mg/kg i.p.) produced dose-dependent increases in paired pulse inhibition. However, when the interval was above or below this range (20 to 60 or 750 to 2000 ms), the drug had no effect. These results are different from those of an earlier study in which delta-9-THC (2 mg/kg i.p.) was found to reduce paired pulse inhibition when the pulse interval was 40 ms (Vardaris *et al.*, 1977).

A number of mechanisms have been put forward to explain the effects of delta-9-THC on paired pulse inhibition in hippocampal CA1 pyramidal cells (Weisz *et al.*, 1982; Kujtan *et al.*, 1983). Among these is the hypothesis that delta-9-THC acts by altering feedback inhibition of these cells. When CA1 cells are stimulated, recurrent axon collaterals from CA1 cells are thought to activate gamma-aminobutyric acid-releasing interneurons. These in turn exert a feedback inhibition on the CA1 neurons. Paired pulse inhibition of CA1 cells probably arises because the first pulse triggers feedback inhibition so that the response to the second pulse is reduced. It is possible, therefore, that delta-9-THC alters paired pulse inhibition of these cells by interfering with feedback inhibition, perhaps by altering the release or fate of gamma-aminobutyric acid or perhaps by altering the sensitivity of CA1 neurons to this amino acid (Weisz *et al.*, 1982; Kujtan *et al.*, 1983; see also Section 6.6). Consistent with the latter possibility is an observation by Segal (1978) that reductions in the spontaneous activity of rat cerebellar cells induced by iontophoretic administration of gamma-aminobutyric acid could be enhanced by simultaneous iontophoretic administration of SP-111, a water soluble derivative of delta-9-THC.

There are two early reports that neither delta-9-THC nor delta-6a, 10a-dimethylheptyl-THC suppress transmission in the cat superior cervical ganglion (Dagirmanjian and Boyd, 1962; Dewey *et al.*, 1970). However, there is also some albeit rather weak, less direct evidence from other early electrophysiological experiments to suggest not only that cannabinoids may alter transmission across some synapses but also that these synapses are more susceptible than axons to the effects of cannabinoids. Thus (i) Dagirmanjian and Boyd (1962) found that polysynaptic reflexes of the cat were more sensitive than the

TABLE 9. *The Effect of Intraperitoneal Administration of Delta-9-THC on Rat Brain Levels of Norepinephrine*

Dose (mg/kg)	Time after injection (hr)	Brain area	Effect	Reference
20	1 to 1.5	Olfactory bulb	+	1
20	1 to 1.5	Diencephalon	+	1
100	0.75	Whole brain	+	2
5 and 50	0.5, 1 and 2	Hypothalamus	0	3
5	0.5, 1 and 2	Pons/medulla	0	3
10	2, 4 and 6	Brain stem	0	4
10	2, 4 and 6	Hypothalamus	0	4
10	1 to 2.5	Midbrain/diencephalon	0	5
20	0.5 to 1.5	Whole brain	0	6
25 and 30	2	Whole brain	0	7, 8
30	1 to 4	Whole brain	0	9
80	0.75	Whole brain	0	10
10	1	Pons/medulla	−	5
10	0.75 to 6	Whole brain	−	2
10	2	Hypothalamus	−	11
40	0.75	Whole brain	−	6
50	0.5	Pons/medulla	−	3

References: 1, Shiomi *et al.*, 1979; 2, Poddar and Ghosh, 1976a; 3, Yagiela *et al.*, 1974; 4, Hattendorf *et al.*, 1977; 5, Bhattacharyya *et al.*, 1980; 6, Bensemana and Gascon, 1974; 7, Maître *et al.*, 1970; 8, Maître *et al.*, 1972; 9, Filipovic and Trkovnik, 1980; 10, Schildkraut and Efron, 1971; 11, Steger *et al.*, 1983.

monosynaptic patellar reflex to the depressant effect of delta-6a, 10a-dimethylheptyl-THC and (ii) Boyd and Meritt (1966) found that delta-6a, 10a-dimethylheptyl-THC was particularly effective in reducing the amplitude of responses evoked by radial nerve stimulation in the mesencephalic reticular formation, a brain area rich in synapses. Consistent with these observations is the evidence that delta-9-THC can augment or suppress transmission along synaptic pathways in the hippocampus at concentrations far lower than those at which it has been shown to block axonal conduction along peripheral nerves (Byck and Ritchie, 1973; Tables 6 and 8).

Further evidence that cannabinoids can alter synaptic transmission comes from the more recent experiments of Turkanis and Karler (1983) in which it was found that delta-9-THC could increase the amplitude of excitatory postsynaptic potentials in cat spinal cord without producing detectable changes in afferent input to the cord. This evidence is not conclusive, however, since it was also found that the drug raised the membrane resistance of the postsynaptic spinal motoneurons, an effect which could have accounted for the enhancement of excitatory postsynaptic potentials. Interestingly, at the same dose levels, (0.01 to 0.1 mg/kg i.v.), delta-9-THC also had a depressant effect, raising the firing threshold for the motoneuron action potential. It also decreased the amplitude of inhibitory postsynaptic potentials demonstrating that its stimulatory properties may stem in part from an ability to reduce inhibition. In the same experimental model, CBD was found to have only a depressant effect (Turkanis and Karler, 1986b).

6. STUDIES CONCERNED WITH PUTATIVE CENTRAL NEUROTRANSMITTERS

This section contains a detailed account of the effects of cannabinoids on the concentration and turnover of putative transmitters in the central nervous system. Evidence that cannabinoids can alter the neuronal uptake and storage of certain transmitters is also presented. The section concludes with an account of the ability of cannabinoids to change the specific binding to brain tissue of various ligands, including benzodiazepines. The latter were included in this section because of the intimate functional relationship that is thought to exist between benzodiazepine receptors and receptors for the putative transmitter, gamma-aminobutyric acid. Effects of cannabinoids on monoamine oxidase activity and on prostaglandin production are discussed in Section 7.

6.1. Norepinephrine

6.1.1. *Brain Levels of Norepinephrine*

Delta-9-THC has been shown to alter brain levels of norepinephrine (NE) in rats, mice and squirrel monkeys. There is evidence that the direction as well as the degree of change

Table 10. *Rat Brain Levels of Norepinephrine One Hour After Intravenous Injection of Delta-9-THC*

Dose (mg/kg)	Ambient temperature (°C)	Brain area	Effect	Reference
0.05 to 0.5	21	Whole brain	0	1
1	21	Whole brain	+	1
2 and 5	21	Whole brain	0	1, 2
2	21	Whole brain	−	3
2	31	Whole brain	−	2
2	4	Whole brain	0	2
2	37	Whole brain	0	2
2	21	Hypothalamus	0	3
2	21	Medulla	0	3

References: 1, Taylor and Fennessy, 1977; 2, Fennessy and Taylor, 1977; 3, Taylor and Fennessy, 1979b.

in NE concentrations produced by delta-9-THC in rat or mouse brain may be dose dependent. Consider, for example, the effect of intraperitoneal administration of delta-9-THC on NE levels in rat whole brain (Table 9). The lowest dose studied, 10 mg/kg, depressed NE concentrations whereas the highest dose used, 100 mg/kg, had the opposite effect. Intermediate doses either lowered NE levels or had no effect. A similar biphasic effect of intraperitoneally administered delta-9-THC has been observed in experiments with mice (Holtzman *et al.*, 1969). Dose-dependent biphasic effects on NE levels in rat brain (Table 10) or mouse brain (Ho *et al.*, 1972) have also been detected when the drug was given intravenously. In these experiments, however, NE levels were elevated by low doses and depressed or unaffected by higher doses. In squirrel monkeys only decreases in NE concentrations have been observed after intravenous administration of delta-9-THC (Ho *et al.*, 1972). These decreases occurred in the hypothalamus with doses of 0.5, 2 and 10 mg/kg and in the cerebral cortex, midbrain and pons/medulla with a dose of 10 mg/kg. None of the doses used produced statistically significant changes in NE concentrations in squirrel monkey whole brain or cerebellum. In mice, an intraperitoneal dose of delta-9-THC, 10 mg/kg, reported to depress NE concentrations in whole brain (Holtzman *et al.*, 1969) had no detectable effect on NE levels in the telencephalon or brain stem (Welch *et al.*, 1971). Effects of intraperitoneal injection of delta-9-THC on NE levels in discrete areas of rat brain are summarized in Table 9.

Although delta-9-THC has been shown to alter brain levels of NE after intraperitoneal or intravenous administration, experiments in which the drug was given orally or subcutaneously have so far yielded only negative results. Maître *et al.*, (1970) failed to detect an effect on rat whole brain levels of NE after an oral dose of 75 mg/kg or after subcutaneous doses of 25 or 100 mg/kg. However, they also reported the absence of an effect when the drug was given intraperitoneally (Table 9). Bracs *et al.* (1975) reported that oral administration of 20 mg/kg of delta-9-THC to rats kept at 31°C to prevent hypothermia had no effect on NE levels measured 1 hr later in whole brain, striatum, cerebellum, pons/medulla or hypothalamus/midbrain/thalamus. It is noteworthy that Fennessy and Taylor (1977) found that when delta-9-THC was given intravenously to rats kept at 31°C, a dose of 2 mg/kg produced a small but significant fall in whole brain NE levels (Table 10).

Experiments with delta-8-THC have shown that it too can alter brain levels of NE at least in squirrel monkeys and mice. In squirrel monkeys, intravenous administration of the drug was found to lower NE levels in several brain areas although it did not have a significant effect on whole brain NE (Ho *et al.*, 1972). Intravenous injection of delta-8-THC was, however, found to alter NE levels in mouse whole brain (Ho *et al.*, 1972). In contrast to delta-9-THC (see above) it was found to decrease mouse brain NE levels at

TABLE 11. *Effects of Delta-9-THC on the Uptake of Norepinephrine into Mouse and Rat Brain Synaptosomes*

Species	Vehicle	Concentration of Δ^9-THC	Brain area	Effect	Reference
Mouse	Ethanol	1 and 5 μM	Whole brain	0	1
		10 μM		0/−	
		50 μM		−*	
Rat	PVP/saline	30 μM	Forebrain	−	2
Rat	PVP/saline	10 and 50 nM	Striatum	0	3
		0.1 μM		+	
		0.2 and 1 μM		0	
		10 and 100 μM		−	
Rat	PVP/saline	10 and 50 nM	Hypothalamus	0	3
		0.1 and 0.2 μM		+	
		1 μM		0	
		10 and 100 μM		−	
Rat	DMSO	1 μM	Hypothalamus	c.a.0	4
		50 and 100 μM		−**	

DMSO and PVP are abbreviations for dimethyl sulfoxide and polyvinylpyrrolidone respectively. *Indicates that synaptosomes were severely damaged by 50 μM delta-9-THC. **Indicates $K_i = 20$ μM. References: 1, Hershkowitz *et al.*, 1977; 2, Johnson *et al.*, 1976b; 3, Poddar and Dewey, 1980; 4, Banerjee *et al.*, 1975b.

low doses and to increase them at a higher dose. Rat experiments with delta-8-THC have produced only negative results, intraperitoneal doses of 10, 30 or 100 mg/kg having been reported to have no effect on NE levels in whole brain, striatum, or hypothalamus (Leonard, 1971; Maître *et al.*, 1972; Maclean and Littleton, 1977).

6.1.2. *Norepinephrine Turnover*

Delta-9-THC has been shown to increase the accumulation of ^{3}H-NE in the whole brains of rats (Maître *et al.*, 1970, 1972) and mice (Bloom *et al.*, 1978b, Bloom and Kiernan, 1980) injected intravenously with ^{3}H-tyrosine (after delta-9-THC). In rats, similar increases in ^{3}H-NE specific activity have been detected following pretreatment with delta-8-THC, 10 or 30 mg/kg i.p., both in whole brain and in hypothalamus, striatum and pons/medulla (Maître *et al.*, 1972; Maclean and Littleton, 1977). Increases in whole brain levels of ^{3}H-NE were also observed in rats injected with delta-6a, 10a-dimethylheptyl THC (Maître *et al.*, 1972). Interestingly, the effect of delta-8-THC on the conversion of tyrosine to NE in striatum and hypothalamus was no longer observed when the drug was administered to rats which had been stressed by isolation and food deprivation (Maclean and Littleton, 1977). In mice, increases in the accumulation of ^{3}H-NE have been observed after administration of delta-9-THC, 9-nor-9-beta-hydroxy-hexahydrocannabinol (beta-HHC) and 11-hydroxy-delta-9-THC (Bloom *et al.*, 1978b). CBN and CBD did not alter ^{3}H-NE levels and none of the cannabinoids studied affected brain levels of ^{3}H-tyrosine (Bloom *et al.*, 1978b). The doses of delta-9-THC, beta-HHC and 11-hydroxy-delta-9-THC used in the mouse studies were hypothermic. It is unlikely, however, that their effect on the accumulation of ^{3}H-NE in mouse brain was caused by a fall in body temperature since the ability of delta-9-THC to elevate brain ^{3}H-NE levels was not impaired when the drug was given to mice kept at an ambient temperature (31°C) sufficiently high to prevent hypothermia (Bloom and Kiernan, 1980). Moreover, Bloom (1982) found that delta-9-THC could increase the conversion of ^{3}H-tyrosine to ^{3}H-NE when it was added to a crude synaptosomal preparation obtained from mouse whole brain or brain stem.

The *in vitro* experiments of Bloom (1982) support the hypothesis that delta-9-THC can increase brain levels of ^{3}H-NE in ^{3}H-tyrosine-treated animals by interacting directly with NE-releasing neurons to stimulate NE synthesis. This it might do by reducing feedback inhibition of tyrosine hydroxylase (Bloom, 1982). There is little evidence in favor of alternative mechanisms such as inhibition of ^{3}H-NE metabolism (Bloom, 1982), suppression of the neuronal release of newly synthesized NE or facilitation of the neuronal uptake of released NE. Thus, concentrations of delta-9-THC effective in augmenting the conversion of ^{3}H-tyrosine to ^{3}H-NE in mouse brain synaptosomes (3 to 30 μM; Bloom, 1982) were found to fall within the delta-9-THC concentration range known to have an inhibitory effect on the synaptosomal uptake of both NE (Table 11) and tyrosine (Bloom, 1982). Consider also the effect of delta-9-THC on the ability of the tyrosine hydroxylase inhibitor, alpha-methyl-*para*-tyrosine, to lower rat brain levels of NE. Although delta-9-THC has been shown to reduce this effect of alpha-methyl-*para*-tyrosine in some experiments, a sign that the cannabinoid may indeed reduce NE release, in other experiments it has been shown to augment the effect of alpha-methyl-*para*-tyrosine on NE levels or to have no effect (Table 12). The absence of an effect on alpha-methyl-*para*-tyrosine-induced falls in rat brain levels of NE has also been noted in experiments with delta-8-THC (Leonard, 1971; Maclean and Littleton, 1977). Other evidence against the proposal that delta-9-THC can increase the rate at which ^{3}H-NE accumulates after ^{3}H-tyrosine injection by suppressing NE release comes from the experiments of Maître *et al.* (1972) in which it was found that delta-9-THC enhanced the disappearance rate of ^{3}H-NE from the brains of rats that had been preloaded with ^{3}H-tyrosine (before delta-9-THC administration).

TABLE 12. *Effects of Delta-9-THC on Norepinephrine Turnover in Mouse and Rat Brain*

Species	Dose and Route (mg/kg)	Brain area	Method of measuring NE turnover	Effect	Reference
Mouse	10 to 100 s.c.	Whole brain	Rise in ^{3}H-NE after ^{3}H-tyrosine (i.v.)	+	1
Mouse	2 to 16 i.v.	Whole brain*	Rise in ^{3}H-NE after ^{3}H-tyrosine (i.v.)	+	2
Rat	10 and 30 i.p.	Whole brain	Rise in ^{3}H-NE after ^{3}H-tyrosine (i.v.)	+	3
Rat	25 i.p.	Whole brain	Rise in ^{3}H-catecholamines after ^{3}H-tyrosine (i.v.)	+	4
	25 s.c.	Whole brain		+	
	75 p.o.	Whole brain		+	
Rat	c.a. 80 i.p.	Whole brain	Fall in ^{3}H-NE after intracisternal ^{3}H-NE	+	5
	c.a. 80 i.p.	Whole brain	Rise in ^{3}H-NM after intracisternal ^{3}H-NE	+	
Rat	10 i.p.	Whole brain	Fall in NE after α-methyl-*para*-tyrosine (i.v.)	+	6
	100 i.p.	Whole brain		−	
Rat†	20 and 60 p.o.	Whole brain	Fall in NE after α-methyl-*para*-tyrosine (i.p.)	0	7
Rat	10 i.p.	Hypothalamus	Fall in NE after α-methyl-*para*-tyrosine (i.p.)	−	8
Rat	5 and 50 i.p.	Hypothalamus	Fall in NE after α-methyl-*para*-tyrosine (i.v.)	0	9
Rat†	20 and 60 p.o.	Hypothalamus/ midbrain/ thalamus	Fall in NE after α-methyl-*para*-tyrosine (i.p.)	0	7
Rat	5 and 50 i.p.	Pons/medulla	Fall in NE after α-methyl-*para*-tyrosine (i.v.)	0	9
Rat†	20 and 60 p.o.	Pons/medulla	Fall in NE after α-methyl-*para*-tyrosine (i.p.)	0	7
Rat†	20 and 60 p.o.	Striatum	Fall in NE after α-methyl-*para*-tyrosine (i.p.)	0	7

*Indicates whole brain minus cerebellum. †Indicates experiments conducted at an ambient temperature of 31°C. NM is an abbreviation for normetanephrine. References: 1, Bloom *et al.*, 1978b; 2, Bloom and Kiernan, 1980; 3, Maître *et al.*, 1972; 4, Maître *et al.*, 1970; 5, Schildkraut and Efron, 1971; 6, Poddar and Ghosh 1976a; 7, Bracs *et al.*, 1975; 8, Steger *et al.*, 1983; 9, Yagiela *et al.*, 1974.

6.1.3. *Synaptosomal Uptake and Storage of Norepinephrine*

In vitro administration of delta-9-THC has been found to alter the uptake of ^{3}H-NE into synaptosomal preparations obtained from rat forebrain, striatum or hypothalamus or from mouse whole brain (Table 11). The effect on uptake was biphasic, increases in uptake being induced by delta-9-THC concentrations of 0.1 or 0.2 μM and decreases by concentrations of 10 μM or more. The cannabinoid exhibited a similar concentration-dependent biphasic effect on ^{3}H-NE release from preloaded rat brain synaptosomes. Release of ^{3}H-NE was decreased by a delta-9-THC concentration of 0.1 μM, unaffected by a concentration of 10 μM and increased by concentrations of 75 and 100 μM (Poddar and Dewey, 1980; Johnson *et al.*, 1976b). The changes in uptake and release produced by delta-9-THC at the higher concentrations used (50 μM or more) may well have been a reflection of its ability to damage synaptosomes (Hershkowitz *et al.*, 1977). Some of these *in vitro* findings are consistent with an earlier report that *in vivo* pretreatment with delta-9-THC, 10 mg/kg i.v., can increase NE uptake into mouse brain synaptosomes (Hershkowitz and Szechtman, 1979). The *in vitro* data are also consistent with the observation by Schildkraut and Efron (1971) that *in vivo* administration of delta-9-THC (ca. 80 mg/kg i.p.) increased the rate of disappearance of ^{3}H-NE from rat brains which had been preloaded with the tritiated catecholamine. It is noteworthy that at the same dose, delta-9-THC was reported to have no effect on uptake into rat brain of intracisternally administered ^{3}H-NE (Schildkraut and Efron, 1971).

The synaptosomal uptake of ^{3}H-NE has been reported to be inhibited by the *in vitro* administration not only of delta-9-THC but also of delta-8-THC and of the 11-hydroxy metabolites of delta-8- and delta-9-THC, all psychically active compounds (Banerjee *et al.*, 1975b; Poddar and Dewey, 1980). However, experiments with CBD (Banerjee *et al.*, 1975b; Hershkowitz *et al.*, 1977; Poddar and Dewey, 1980) and also some (Poddar and Dewey, 1980) but not all experiments (Banerjee *et al.*, 1975b) with CBN indicated that these cannabinoids can also decrease ^{3}H-NE uptake into rat or mouse brain synaptosomes. CBD has no psychic activity and CBN has been reported to be significantly less potent than delta-9-THC as a psychotropic agent or, like CBD, to be inactive (Section 2). Hence there is little support for a link between psychic activity and the ability to inhibit the neuronal uptake of NE. Similarly, there seems to be little correlation between psychic activity and the ability to enhance the release of ^{3}H-NE from preloaded synaptosomes (Poddar and Dewey, 1980). In contrast, the ability of delta-9-THC to increase synaptosomal ^{3}H-NE uptake and to decrease synaptosomal ^{3}H-NE release has been shown to be shared by delta-8-THC but not by CBN or CBD (Hershkowitz and Szechtman, 1979; Poddar and Dewey, 1980) supporting a link between these actions and the psychotropic properties of delta-8- and delta-9-THC.

TABLE 13. *The Effect of Intraperitoneal Administration of Delta-9-THC on Rat Brain Levels of Dopamine*

Dose (mg/kg)	Time after injection (hr)	Brain area	Effect	Reference
10	0.5 to 6	Whole brain	+	1
10	1.5 and 2	Caudate nucleus	+	2
10	1.5 and 2	Midbrain/diencephalon	+	2
100	0.5 to 6	Whole brain	+	1
10	2	Brain stem	+/0	3
10	2, 4 and 7	Hypothalamus	0	4
25	2	Whole brain	0	5
30	2	Whole brain	0	6
30	1 to 4	Whole brain	0	7
10	1	Caudate nucleus	−	2
10	1	Midbrain/diencephalon	−	2

References: 1, Poddar and Ghosh, 1976a; 2, Bhattacharyya *et al.*, 1980; 3, Hattendorf *et al.*, 1977; 4, Steger *et al.*, 1983; 5, Maître *et al.*, 1970; 6, Maître *et al.*, 1972; 7, Filipovic and Trkovnik, 1980.

TABLE 14. *Effects of Delta-9-THC on Dopamine Turnover in Mouse and Rat Brain*

Species	Dose and Route (mg/kg)	Brain area	Method of measuring DA turnover	Effect	Reference
Mouse	10 to 100 s.c.	Whole brain	Rise in ^{3}H-DA after ^{3}H-tyrosine (i.v.)	+	1
Mouse	2 to 16 i.v.	Whole brain*	Rise in ^{3}H-DA after ^{3}H-tyrosine (i.v.)	+	2
Rat	10 and 30 i.p.	Whole brain	Rise in ^{3}H-DA after ^{3}H-tyrosine (i.v.)	+	3
Rat	25 i.p.	Whole brain	Rise in ^{3}H-catecholamines after ^{3}H-tyrosine (i.v.)	+	4
	25 s.c.			+	
	75 p.o.			+	
Rat	10 and 30 i.p.	Whole brain	Rise in ^{3}H-DOPAC after ^{3}H-tyrosine (i.v.)	+	3
Rat	10 and 100 i.p.	Whole brain	Fall in DA after α-methyl-*para*-tyrosine (i.v.)	−	5
Rat †	20 and 60 p.o.	Whole brain	Fall in DA after α-methyl-*para*-tyrosine (i.p.)	0	6
Rat	10 i.p.	Anterior hypothalamus	Fall in DA after α-methyl-*para*-tyrosine (i.p.)	0	7
Rat	10 i.p.	Medial basal hypothalamus	Fall in DA after α-methyl-*para*-tyrosine (i.p.)	−	7
Rat†	20 and 60 p.o.	Striatum	Fall in DA after α-methyl-*para*-tyrosine (i.p.)	0	6
Rat	10 i.v.	Striatum	Rise in DOPA after haloperidol plus NSD-1024 or NSD-1015	−	8
Mouse	40 i.p.	Striatum	Concentrations of DOPAC and HVA	0	9
Rat	5 and 10 i.p.	Caudate nucleus	Concentration of HVA	0	10
	20 and 40 i.p.			+	
Rat	5 i.p.	Olfactory tubercle	Concentration of HVA	0	10
	10 to 40 i.p.			+	
Rat	5 i.p.	Prefrontal cortex	Concentration of HVA	0	10
	10 to 40 i.p.			+	

*Indicates whole brain minus cerebellum. †Indicates experiment conducted at an ambient temperature of 31°C. DOPA, DOPAC and HVA are abbreviations for dihydroxyphenylalanine, dihydroxyphenylacetic acid and homovanillic acid, respectively. NSD-1024 and NSD-1015 are aromatic amino acid decarboxylase inhibitors. References: 1, Bloom *et al.*, 1978b; 2, Bloom and Kiernan, 1980; 3, Maître *et al.*, 1972; 4, Maître *et al.*, 1970; 5, Poddar and Ghosh, 1976a; 6, Bracs *et al.*, 1975; 7, Steger *et al.*, 1983; 8, Koe, 1981; 9, Osgood and Howes, 1974; 10, Bowers and Hoffman, 1986.

6.2. Dopamine

6.2.1. *Brain Levels of Dopamine*

There are only a few reports that delta-9-THC can alter brain concentrations of dopamine (DA). Graham *et al.* (1974) found that oral administration of delta-9-THC (10 mg/kg) decreased DA concentrations both in rat whole brain and rat heart. Bhattacharyya *et al.* (1980) found that delta-9-THC, 10 mg/kg i.p., had a time-dependent biphasic effect on DA concentrations in rat caudate nucleus and diencephalon/midbrain (Table 13). In both brain areas, there was an initial fall in DA concentrations followed by a marked increase.

Poddar and Ghosh (1976a) found that DA concentrations in rat whole brain were increased by delta-9-THC at doses of both 10 and 100 mg/kg i.p., the effect being greater at the lower dose. A slight increase in DA concentration was also reported to occur in rat brain stem following delta-9-THC administration at a dose of 10 mg/kg i.p. (Hattendorf *et al.*, 1977). On the other hand, Maître *et al.* (1970, 1972) reported that at doses of 25 or 30 mg/kg i.p., delta-9-THC had no effect on DA concentration in rat whole brain. Similarly, intraperitoneal administration of delta-8-THC was reported to have no effect on rat striatal or hypothalamic DA concentrations at a dose of 10 mg/kg (Maclean and Littleton, 1977) and to have no effect on rat whole brain DA concentrations at doses of 30 or 100 mg/kg (Leonard, 1971; Maître *et al.*, 1972). The absence of an effect on DA concentrations was also noted (i) in rat whole brain after administration of delta-9-THC at doses of 0.5 to 5 mg/kg i.v. (Fennessy and Taylor, 1977; Taylor and Fennessy, 1977, 1979b), 25 mg/kg s.c. (Maître *et al.*, 1970) or 75 mg/kg p.o. (Maître *et al.*, 1970), (ii) in rat hypothalamus after administration of delta-9-THC at doses of 2 mg/kg i.v. (Taylor and Fennessy, 1979b) or 10 mg/kg i.p. (Steger *et al.*, 1983) (iii) in rat striatum after administration of delta-9-THC at doses of 10 or 100 mg/kg p.o. (Lew and Richardson, 1981) and (iv) in rat medulla oblongata after administration of delta-9-THC at a dose of 2 mg/kg i.v. (Taylor and Fennessy, 1979b). These experiments were all carried out at 'normal' room temperature. However, there are also reports that delta-9-THC had no effect on brain levels of DA when given orally or intravenously to rats kept at high or low ambient temperatures (Bracs *et al.*, 1975; Fennessy and Taylor, 1977).

In experiments with mice, delta-9-THC was found to have no effect on whole brain levels of DA when injected subcutaneously at doses of 1 to 100 mg/kg (Bloom *et al.*, 1978b). The drug was also found to have no effect on DA concentration in mouse telencephalon when injected intraperitoneally at a dose of 10 mg/kg (Welch *et al.*, 1971).

6.2.2. *Dopamine Turnover*

There are several reports that delta-9-THC can increase the accumulation of ^{3}H-DA in the brains of rats or mice injected intravenously with ^{3}H-tyrosine (Table 14). It is unlikely that this increase was caused by delta-9-THC-induced falls in body temperature, since it could be demonstrated in experiments with mice kept at an ambient temperature (31°C) sufficiently high to prevent hypothermia (Bloom and Kiernan, 1980). Increases in ^{3}H-DA levels in animals pretreated with ^{3}H-tyrosine have also been observed in response to other psychically active cannabinoids. In rats, for example, delta-6a, 10a-dimethylheptyl-THC (30 mg/kg i.p.) increased ^{3}H-DA levels in whole brain, hypothalamus and striatum although not in pons/medulla (Maître *et al.*, 1972). Rat whole brain, striatal and hypothalamic levels of ^{3}H-DA were also increased by delta-8-THC, again at a dose of 30 mg/kg i.p. (Maître *et al.*, 1972). A lower dose of delta-8-THC, 10 mg/kg i.p., was found to have no effect on striatal or hypothalamic ^{3}H-DA levels (Maclean and Littleton, 1977). In mice, whole brain levels of ^{3}H-DA were increased by 11-hydroxy-delta-9-THC and by 9-nor-9-beta-hydroxyhexahydrocannabinol but not by CBN or CBD, even at a dose of 200 mg/kg s.c. (Bloom *et al.*, 1978b).

Maître *et al.*, (1970) found that a dose of delta-9-THC increasing ^{3}H-catecholamine levels in the brains of rats injected intravenously with ^{3}H-tyrosine also decreased plasma

TABLE 15. *Effects of Delta-9-THC on the Uptake of Dopamine into Mouse and Rat Brain Synaptosomes*

Species	Vehicle	Concentration of Δ^9-THC	Brain area	Effect	Reference
Mouse	Ethanol	5 to 100 μM	Whole brain	−*	1
Mouse	Ethanol	0.1 to 100 μM	Striatum	−**	2
Rat	DMSO	100 μM	Striatum	−	3
Rat	PVP/saline	10 nM	Striatum	0	4
		0.05 to 0.2 μM		+	
		1 μM		0	
		10 and 100 μM		−	
Rat	PVP/saline	10 nM	Hypothalamus	0	4
		0.05 and 0.1 μM		+	
		0.2 and 1 μM		0	
		10 and 100 μM		−	

DMSO and PVP are abbreviations for dimethyl sulfoxide and polyvinylpyrrolidone respectively. *Indicates that synaptosomes were severely damaged by 50 μM delta-9-THC. **Indicates $IC_{50} = 5.4$ μM. References: 1, Hershkowitz *et al.*, 1977; 2, Howes and Osgood, 1974; 3, Banerjee *et al.*, 1975b; 4, Poddar and Dewey, 1980.

concentrations of endogenous tyrosine. This raises the possibility that the cannabinoid increased ^{3}H-catecholamine levels in the brain by reducing 'background' levels of unlabeled tyrosine so as to increase the specific activity of the intravenously injected ^{3}H-tyrosine. It is noteworthy, however, that delta-9-THC has been found not to affect brain concentrations of endogenous tyrosine in either rats (Maître *et al.*, 1970) or mice (Bloom *et al.*, 1978b). Furthermore, Bloom (1982) found that delta-9-THC could increase ^{3}H-DA accumulation in crude synaptosomal preparations obtained from mouse whole brain or striatum to which ^{3}H-tyrosine had been added. The cannabinoid was effective at concentrations known also to impair the ability of synaptosomes to retain DA (Section 6.2.3) and to inhibit the synaptosomal uptake of both DA (Table 15) and tyrosine (Bloom, 1982). It is unlikely, therefore, that delta-9-THC increased ^{3}H-DA accumulation in synaptosomes by attenuating DA efflux or facilitating reuptake. It is also unlikely that the drug acted by inhibiting DA metabolism (Bloom, 1982). Hence, there is good evidence that delta-9-THC can act directly to stimulate the synthesis of both DA and NE (Section 6.1.2.). Possible mechanisms are discussed in the paper by Bloom (1982) and also, briefly, in Section 6.1.2.

Results from *in vivo* experiments suggest that in addition to stimulating DA synthesis, delta-9-THC may also increase DA release. Maître *et al.* (1972) showed that delta-9-THC enhanced the disappearance rate of ^{3}H-DA from the brains of rats pretreated 2 hr earlier with intravenous injections of ^{3}H-tyrosine. They obtained similar results in experiments with delta-8-THC and delta-6a, 10a-dimethylheptyl-THC. Osgood and Howes (1974) reported that delta-9-THC (10 to 40 mg/kg i.p.) enhanced the ability of L-DOPA to increase mouse striatal concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). However, the cannabinoid had no effect on DOPAC or HVA levels in the absence of L-DOPA. On the other hand, in a more recent study with rats, Bowers and Hoffman (1986) found that delta-9-THC could increase HVA levels in several brain areas including the caudate nucleus (Table 14). Interestingly, the drug was more potent in its effects on HVA levels in the prefrontal cortex and olfactory tubercle than in its effect on caudate HVA. Against the hypothesis that delta-9-THC increases DA release are reports that falls in brain levels of DA induced by the tyrosine hydroxylase inhibitor, alpha-methyl-*para*-tyrosine, are either unaffected or else reduced by delta-9-THC (Table 14) and delta-8-THC (Maclean and Littleton, 1977).

6.2.3. *Synaptosomal Uptake and Storage of Dopamine*

The uptake of ^{14}C-DA or ^{3}H-DA into synaptosomal preparations obtained from rat hypothalamus, rat or mouse striatum or mouse whole brain has been shown in several studies to be markedly affected by delta-9-THC (Table 15). Drug concentrations of 5 μM

or more have been found to inhibit uptake. In one study (Howes and Osgood, 1974), a slight inhibitory effect was also noted at concentrations of 0.1 and 1 μM. In another study, however, concentrations ranging from 0.05 to 0.2 μM were found to stimulate ^{3}H-DA uptake (Poddar and Dewey, 1980).

Cannabinoids other than delta-9-THC that have been reported to inhibit ^{3}H-DA uptake into rat or mouse brain synaptosomal preparations are delta-8-THC, CBN and CBD (Hershkowitz *et al.*, 1977; Poddar and Dewey, 1980). Like delta-9-THC, delta-8-THC was found to exert a concentration-dependent biphasic effect on ^{3}H-DA uptake (Poddar and Dewey, 1980). It was stimulatory at a concentration of 10 nM and inhibitory at concentrations of 1 and 10 μM. CBN and CBD were at least as potent as delta-8- or delta-9-THC as inhibitors of ^{3}H-DA uptake (Hershkowitz *et al.*, 1977; Poddar and Dewey, 1980). However, there was no sign of a stimulatory effect when sub-inhibitory concentrations were used (Poddar and Dewey, 1980).

Effects of cannabinoids on ^{3}H-DA uptake have also been observed in experiments in which drug administration was made *in vivo*. Maclean and Littleton (1977) reported that there was a marked increase in ^{3}H-DA uptake into striatal homogenates prepared from isolated, food-deprived rats that had been injected with delta-8-THC (10 mg/kg i.p.). On the other hand, the drug had no effect on ^{3}H-DA uptake when given to animals kept under normal environmental conditions. Hershkowitz and Szechtman (1979) detected an enhancement of ^{3}H-DA uptake into synaptosomes prepared from the cerebral cortices or striata of mice which had been injected with delta-9-THC at doses of 5 or 10 mg/kg i.v. The effect of delta-9-THC on cortical uptake of ^{3}H-DA was greater than its effect on striatal uptake. At the same doses, CBD had a smaller but nonetheless significant stimulatory effect on ^{3}H-DA uptake. Intravenous injection of the psychically inactive isomer, (+) delta-8-THC, had no effect on ^{3}H-DA uptake.

There is evidence that as well as affecting the synaptosomal uptake of DA, delta-9-THC can alter tyrosine uptake. Delta-9-THC concentrations ranging from 3 to 100 μM were found to inhibit uptake of ^{3}H-tyrosine into crude synaptosomal preparations obtained from mouse whole brain, striatum and brain stem (Bloom, 1982). Several cannabinoids have also been shown to alter the release of radioactively labeled DA from preloaded synaptosomes. Howes and Osgood (1974) reported a significant increase in ^{14}C-DA release from mouse striatal synaptosomes in the presence of delta-9-THC (1 μM). Poddar and

TABLE 16. *The Effect of Intraperitoneal Administration of Delta-9-THC on Rat Brain Levels of 5-Hydroxytryptamine (5-HT) and 5-Hydroxyindoleacetic Acid (5-HIAA)*

Dose (mg/kg)	Time after injection (hr)	Brain area	Effect		Reference
			5-HT	5-HIAA	
6 to 80	0.5 to 4	Whole brain	+	ND	1, 2, 3
10	1	Whole brain*	+	+	4
20	0.5	Cerebellum	+	ND	1
20	0.5	Hypothalamus/midbrain	+	ND	1
20	1 to 1.5	Diencephalon	+	+	5
5 to 50	0.5 to 4	Hypothalamus	0	ND	6
5 to 50	0.5 to 4	Pons/medulla	0	ND	1, 6
5 to 5.5	0.5 to 4	Forebrain	0	0	7, 8
5.5	0.5 to 4	Brainstem	0	0	8
10	2 to 7	Hypothalamus	0	–	9
20	0.5	Cerebral cortex	0	ND	1
5 to 50	3 and 24	Brain stem	–	ND	10
10	1.5 and 2	Pons/medulla	–	ND	11
10	1.5 and 2	Midbrain/diencephalon	–	ND	11
20	1 to 1.5	Olfactory bulb	–	–	5
50	3	Cerebral cortex	–	ND	10

*Indicates whole brain minus cerebellum. ND = not determined. References: 1, Sofia *et al.*, 1971a; 2, Schildkraut and Efron, 1971; 3, Filipovic and Trkovnik, 1980; 4, Segawa *et al.*, 1976; 5, Shiomi *et al.*, 1979; 6, Yagiela *et al.*, 1974; 7, Gallager *et al.*, 1971; 8, Gallager *et al.*, 1972; 9, Steger *et al.*, 1983; 10, Ouellet *et al.*, 1973; 11, Bhattacharyya *et al.*, 1980.

Dewey (1980) detected decreased ^{3}H-DA release from a rat striatal synaptosomal preparation at a delta-9-THC concentration of 0.1 μM and increased release at 10 and 100 μM. At a concentration of 100 μM, delta-9-THC also increased ^{3}H-DA release from a hypothalamic preparation. However, it had no effect on DA release from this preparation at lower concentrations. In the same study, delta-8-THC increased ^{3}H-DA release from hypothalamic and striatal synaptosomal preparations at concentrations of 10 and 100 μM and decreased ^{3}H-DA release from the striatal preparation at lower concentrations (0.01 and 0.1 μM). It did not inhibit ^{3}H-DA release from the hypothalamic preparation at any of the concentrations used. CBN and CBD had a stimulatory effect on ^{3}H-DA release at concentrations of 10 and 100 μM but did not inhibit release at lower concentrations.

6.3. 5-HYDROXYTRYPTAMINE

6.3.1. *Brain Levels of 5-Hydroxytryptamine*

Delta-9-THC has been reported to increase, to decrease or to have no effect on the concentration of 5-hydroxytryptamine (5-HT) in the brain. These differences can to a large extent be attributed to the use of different routes of administration and species and to the study of different brain areas.

In rats, intracerebroventricular injection of delta-9-THC (50 μg) increased concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the forebrain (Gallager *et al.*, 1972). When given to rats by the intraperitoneal route, delta-9-THC at doses of 6 mg/kg or more increased the concentration of 5-HT and sometimes also of 5-HIAA in whole brain and cerebellum (Table 16). The drug either did not affect or else decreased concentrations of 5-HT or 5-HIAA in the rat forebrain, hypothalamus and brain stem (Table 16). In some areas, for example the diencephalon and midbrain, there were signs of an initial delta-9-THC-induced increase in 5-HT levels followed by a later depression. Intraperitoneal injection of delta-8-THC at a dose of 100 mg/kg had no effect on rat brain 5-HT or 5-HIAA (Leonard, 1971). Delta-9-THC given orally at a dose of 20 mg/kg to rats kept at an ambient temperature of 31°C to avoid hypothermia, was without effect on 5-HT levels in whole brain, striatum, hypothalamus/midbrain/thalamus or pons/medulla (Bracs *et al.*, 1975).

In mice, intraperitoneal or subcutaneous administration of delta-9-THC at doses ranging upwards from 10 mg/kg increased 5-HT concentrations in whole brain (Holtzman *et al.*, 1969), in whole brain minus the olfactory bulbs and cerebellum (Johnson and Dewey,

TABLE 17. *Rat Brain Levels of 5-Hydroxytryptamine (5-HT) and 5-Hydroxyindoleacetic Acid (5-HIAA) after Intravenous Injection of Delta-9-THC*

Dose of Δ^9-THC (mg/kg)	Ambient temperature (°C)	Time after injection (hr)	Brain area	Effect		Reference
				5-HT	5-HIAA	
2	4	1	Whole brain	+	0	1
2	37	1	Whole brain	+	+	1
2	21	1	Medulla	+	0	2
0.05	21	1	Whole brain	0	−	3
0.1 to 0.5	21	1	Whole brain	0	0	3
1	?	4, 7 and 19	Whole brain	0	ND	4
1	?	0.1 to 4	Brain stem	0	0	5
1	?	0.1 to 4	Forebrain	0	0	5, 6
2	21	1	Whole brain	0	0	2
2	21	1	Hypothalamus	0	+	2
2	31	1	Whole brain	0	+	1
1 to 5	21	1	Whole brain	0	+	1, 3
0.1 to 10	?	0.75, 3 and 24	Brain stem	0	0	7
0.1 to 1	?	0.75	Cerebral cortex	−	ND	7

ND = not determined. References: 1, Fennessy and Taylor, 1977; 2, Taylor and Fennessy, 1979b; 3, Taylor and Fennessy, 1977; 4, Englert *et al.*, 1973; 5, Gallager *et al.*, 1972; 6, Gallager *et al.*, 1971; 7, Ouellet *et al.*, 1973.

1978a) and in the telencephalon and brain stem (Welch *et al.*, 1971). CBN given intraperitoneally at a dose of 10 mg/kg also elevated 5-HT concentrations in mouse telencephalon although it had no effect on brain stem 5-HT levels (Welch *et al.*, 1971).

The effects of intravenous administration of delta-9-THC on rat brain levels of 5-HT and 5-HIAA are summarized in Table 17. It is noteworthy that the drug had a dose-related biphasic effect on 5-HIAA levels which were depressed by low doses and elevated by higher doses (Taylor and Fennessy, 1977). A similar biphasic effect was observed in experiments with mice injected intravenously with either delta-8- or delta-9-THC (Ho *et al.*, 1972). In contrast, intravenous administration of delta-8- or delta-9-THC to squirrel monkeys at doses of 0.5, 2 or 10 mg/kg produced only falls in brain 5-HT levels (Ho *et al.*, 1972).

There is evidence that the ability of delta-9-THC to alter rat brain levels of 5-HT and 5-HIAA depends on ambient temperature (Table 17). Fennessy and Taylor (1977) reported that at a dose of 2 mg/kg i.v., the cannabinoid had no effect on rat whole brain 5-HT levels in experiments carried out at 21° or 31°C but increased 5-HT levels at high or low ambient temperatures (37° and 4°C). They also detected delta-9-THC-induced increases in rat brain 5-HIAA levels at 21°, 31° and 37°C but not at 4°C.

6.3.2. *5-Hydroxytryptamine Turnover* (*Table 18*)

In experiments in which mice were injected intravenously with ^{3}H-tryptophan, Johnson and Dewey (1978a) found that pretreatment with delta-9-THC at doses known to augment endogenous brain levels of 5-HT increased the specific activity of ^{3}H-5-HT in the brain and concluded that the cannabinoid had increased the rate of 5-HT synthesis. In the same experiments, delta-9-THC also increased (i) the specific activity of ^{3}H-tryptophan in the brain, (ii) the brain to plasma ratio of ^{3}H-tryptophan and (iii) endogenous brain levels of tryptophan. It was therefore proposed that delta-9-THC can accelerate 5-HT synthesis by augmenting tryptophan uptake from blood to brain thereby increasing the quantity of brain tryptophan available for metabolism to 5-HT. Johnson and Dewey (1978a) found that delta-9-THC reduced total levels of endogenous plasma tryptophan without affecting free plasma levels of this amino acid. In experiments with rats, however, a dose of delta-9-THC elevating brain levels of 5-HT was shown to increase tryptophan concentrations in both brain and blood (Filipovic and Trkovnik, 1980). On the other hand, in another rat study, delta-8-THC was found to lower blood levels of tryptophan (Leonard, 1971). Effects of delta-8-THC on brain levels of tryptophan or 5-HT were not detected.

Although it would seem that delta-9-THC can increase the synthesis of 5-HT by increasing the brain concentration of its amino acid precursor, there is no evidence that the drug can also increase the proportion of brain tryptophan converted to 5-HT. Thus, delta-9-THC did not affect the ratio of ^{3}H-5-HT to ^{3}H-tryptophan in the brains of mice injected intravenously with ^{3}H-tryptophan (Johnson and Dewey, 1978a). Nor did it affect the rates of fall in the specific activities of ^{3}H-tryptophan and ^{3}H-5-HT in the brains of rats which had been injected intracerebroventricularly with ^{3}H-tryptophan (Taylor and Fennessy, 1979a). Moreover, *in vivo* administration of delta-9-THC has been reported to inhibit rat brain tryptophan hydroxylase, albeit only in the brain stem (Ouellet *et al.*, 1973). The activity of this enzyme in the cerebral cortex was not affected.

The mechanism by which delta-9-THC enhances the uptake of ^{3}H-tryptophan from blood to brain in mice remains to be elucidated since delta-9-THC has been found to inhibit rather than to facilitate uptake of ^{3}H-tryptophan into mouse brain synaptosomes (Johnson and Dewey, 1978b) and since *in vivo* pretreatment of mice with the cannabinoid has been found to have no effect on subsequent synaptosomal uptake of ^{3}H-tryptophan (Johnson and Dewey, 1978a,b). Interestingly, the effect of delta-9-THC on the uptake of ^{3}H-tryptophan from blood to brain has been shown to be abolished in mice by adrenalectomy (Johnson *et al.*, 1981). Now, delta-9-THC can elevate plasma corticosterone levels in rats and mice (Johnson *et al.*, 1981; Pertwee, 1985b) and since there is evidence that corticosteroids can facilitate the synaptosomal uptake of tryptophan, it is

TABLE 18. *Effects of Delta-9-THC on 5-Hydroxytryptamine Turnover in Mouse and Rat Brain*

Species	Dose and Route (mg/kg)	Brain area	Method of measuring 5-HT turnover	Effect	Reference
Mouse	10 to 100 s.c.	Whole brain*	Rise in ^{3}H-5-HT after ^{3}H-tryptophan (i.v.)	+	1
Mouse	3 to 100 s.c.	Whole brain	^{3}H-5-HT/^{3}H-tryptophan after ^{3}H-tryptophan (i.v.)	0	1
Mouse	10 s.c.	Whole brain	^{3}H-5-HIAA/^{3}H-tryptophan after ^{3}H-tryptophan (i.v.)	−	1
Rat	2 i.v.	Whole brain	Rise in ^{3}H-5-HT after ^{3}H-tryptophan (i.c.v.)	0	2
Rat	2 i.v.	Whole brain	Rise in ^{3}H-5-HIAA after ^{3}H-tryptophan (i.c.v.)	0	2
Rat	2 i.v.	Whole brain	Fall in 5-HIAA after pargyline	+	2
Rat	2 i.v.	Whole brain	Rise in 5-HT after pargyline	0	2
Rat	5 to 10 i.p.	Whole brain**	Rise in 5-HT after pheniprazine	−	3
Rat	20 i.p.	Whole brain	Rise in 5-HT after pargyline	−	4
Rat	5.5 i.p.	Forebrain	Rise in 5-HT after pargyline	0	5
Rat	5 and 50 i.p.	Pons/medulla	Rise in 5-HT after pargyline	0	6
Rat	5 and 50 i.p.	Hypothalamus	Rise in 5-HT after pargyline	0	6
Rat	2 i.v.	Whole brain	Rise in 5-HIAA after probenecid	0	2
Rat	20 i.p.	Whole brain**	Rise in 5-HIAA after probenecid	0	7
Rat	1 i.v.	Forebrain	Rise in 5-HIAA after probenecid	0	5
Rat	1 i.v.	Brain stem	Rise in 5-HIAA after probenecid	0	5
Rat	5.5 i.p.	Forebrain	Rise in 5-HIAA after probenecid	0	5
Rat	5.5 i.p.	Brain stem	Rise in 5-HIAA after probenecid	0	5

*Indicates whole brain minus cerebellum and olfactory bulbs. **Indicates whole brain minus cerebellum. 5-HIAA is an abbreviation for 5-hydroxyindoleacetic acid and i.c.v. indicates intracerebroventricular administration. References: 1, Johnson and Dewey, 1978a; 2, Taylor and Fennessy, 1979a; 3, Segawa *et al.*, 1977; 4, Sofia *et al.*, 1971a; 5, Gallager *et al.*, 1972; 6, Yagiela *et al.*, 1974; 7, Segawa *et al.*, 1976.

possible that delta-9-THC may enhance tryptophan uptake into the brain indirectly through its effect on corticosterone release (Johnson *et al.*, 1981).

At a dose (10 mg/kg s.c.) known to enhance the uptake of ^{3}H-tryptophan from blood to brain (see above), delta-9-THC has been shown to decrease the ratio in the brain of ^{3}H-5-HIAA to ^{3}H-tryptophan suggesting that it can either reduce the neuronal release of 5-HT or increase the transport of 5-HIAA from brain to blood (Johnson and Dewey, 1978a). Segawa *et al.* (1977) studied the effect of delta-9-THC on the accumulation of 5-HT in rat brain (whole brain minus cerebellum) induced by *in vivo* administration of the monoamine oxidase inhibitor, pheniprazine. The cannabinoid was found to reduce the ability of pheniprazine to elevate 5-HT levels in 'muricide positive' rats, an observation also consistent with the proposal that delta-9-THC can reduce the neuronal release of 5-HT. Similarly, delta-9-THC was found to reduce the rise in rat whole brain 5-HT concentrations produced by the monoamine oxidase inhibitor, pargyline (Sofia *et al.*, 1971a). In this case, however, the dose of delta-9-THC used, 20 mg/kg i.p., was reported to increase brain 5-HT levels when given by itself, making its effect on 5-HT levels in the presence of pargyline difficult to interpret.

Not all studies with rats have yielded results supporting an effect of delta-9-THC on the neuronal release of 5-HT. In experiments in which delta-9-THC was injected intravenously or in which 5-HT concentrations were measured in discrete brain areas (forebrain, hypothalamus and brain stem), the cannabinoid was found not to alter monoamine oxidase inhibitor-induced increases in central 5-HT levels (Gallager *et al.*, 1971, 1972; Yagiela *et al.*, 1974; Taylor and Fennessy, 1979a). In addition, the cannabinoid was reported not to enhance increases in brain 5-HIAA induced by probenecid, a drug known to inhibit the efflux of 5-HIAA from brain to blood (Gallager *et al.*, 1971, 1972; Segawa *et al.*, 1976; Taylor and Fennessy, 1979a). Taylor and Fennessy (1979a) found that delta-9-THC, 2 mg/kg i.v., enhanced pargyline-induced depletion of rat brain 5-HIAA without affecting pargyline-induced elevation of brain 5-HT, providing support for the hypothesis (see above) that delta-9-THC can accelerate the transport of 5-HIAA from brain to blood. It is also noteworthy that delta-8-THC, 100 mg/kg i.p., had no effect on the degree of rat brain 5-HT depletion induced by *para*-chlorophenylalanine, an inhibitor of 5-HT synthesis (Leonard, 1971).

6.3.3. *Synaptosomal Uptake and Storage of 5-Hydroxytryptamine*

There is good evidence from experiments with synaptosomal preparations obtained from rat or mouse brain that delta-9-THC can alter the neuronal uptake of 5-HT (Table 19). The drug has been found to inhibit ^{14}C-5-HT or ^{3}H-5-HT uptake into crude synaptosomal preparations derived from rat whole brain (Sofia *et al.*, 1971b), forebrain (Johnson *et al.*, 1976a, b) or hypothalamus (Banerjee *et al.*, 1975b). Kinetic analysis of

TABLE 19. *Effects of Delta-9-THC on the Uptake of 5-Hydroxytryptamine into Mouse and Rat Brain Synaptosomes*

Species	Vehicle	Concentration of Δ^9-THC	Brain area	Effect	Notes	Reference
Mouse	Ethanol	1 μM	Whole brain	+	Synaptosomes	1
		10 and 50 μM		0	damaged by	
		100 μM		−	Δ^9-THC (50 μM)	
Rat	PG	1 and 10 nM	Whole brain	0		2
		0.1 and 1 μM		−		
Rat	PVP/saline	10 μM	Forebrain	0		3
		30 and 100 μM		−	IC_{50} = 39 μM	
Rat	PVP/saline		Forebrain	−	IC_{50} = 29.3 μM	4
Rat	DMSO	1 μM	Hypothalamus	0/−		5
		50 and 100 μM		−	K_i = 25 μM	

PG, PVP and DMSO are abbreviations for propylene glycol, polyvinylpyrrolidone and dimethyl sulfoxide, respectively. References: 1, Hershkowitz *et al.*, 1977; 2, Sofia *et al.* 1971b; 3, Johnson *et al.*, 1976b; 4, Johnson *et al.*, 1976a; 5, Banerjee *et al.*, 1975b.

data derived from experiments with the hypothalamic tissue revealed that the inhibition was non-competitive (Banerjee *et al.*, 1975b).

Cannabinoids other than delta-9-THC have also been found to inhibit the synaptosomal uptake of 5-HT. The results of experiments with synaptosomal preparations derived from rat hypothalamus (Banerjee *et al.*, 1975b) or forebrain (Johnson *et al.*, 1976a) suggest that there is not a good correlation between psychic activity *in vivo* and the ability to inhibit 5-HT uptake *in vitro*. For example, even when solubility differences were taken into account, the psychically inactive CBD seemed to be no less potent than delta-9-THC as an inhibitor of 5-HT uptake (Banerjee *et al.*, 1975b; Johnson *et al.* (1976a).

Using a relatively pure synaptosomal preparation derived from mouse brain, Hershkowitz *et al.* (1977) detected a concentration related biphasic effect of delta-9-THC on 5-HT uptake. At a concentration of 1 μM, the drug increased 5-HT uptake. There was no effect at 10 or 50 μM and inhibition of uptake at 100 μM. The uptake of 5-HT was also inhibited but not enhanced by CBD. The synaptosomes showed signs of severe morphological damage in the presence of 50 μM delta-9-THC or CBD but no detectable damage when exposed to either cannabinoid at a concentration of 10 μM. Interestingly, the uptake of 5-HT into a crude synaptosomal preparation obtained from the cerebral cortices of mice was enhanced when the animals had been pretreated *in vivo* with delta-9-THC albeit with the rather high intravenous dose of 10 mg/kg (Hershkowitz and Szechtman, 1979). In contrast, *in vivo* pretreatment with CBD (10 mg/kg i.v.) did not affect synaptosomal uptake of 5-HT.

Johnson *et al.* (1976b) investigated the effect of delta-9-THC on synaptosomal retention of 5-HT. It was found that at concentrations greater than those required to inhibit 5-HT uptake, delta-9-THC reduced the ability of a rat forebrain crude synaptosomal preparation to retain preloaded ^{3}H-5-HT. Although the amount of ^{3}H-5-HT in the synaptosomes was reduced by delta-9-THC, there was no corresponding increase in the medium. An increase in ^{3}H-5-HIAA was detected in the medium, however, implying that the cannabinoid had increased the loss of ^{3}H-5-HT from synaptosomal storage vesicles to the surrounding cytosol, thereby (i) increasing the amount of free ^{3}H-5-HT available for metabolism to ^{3}H-5-HIAA within the cytosol and hence (ii) increasing the synaptosomal concentration of ^{3}H-5-HIAA and therefore its transfer to the medium by passive diffusion. Consistent with the hypothesis that delta-9-THC impairs vesicular storage of 5-HT is a report that 4 to 7 hr after intravenous administration of 5-HT the subcellular distribution of 5-HT is altered, there being a shift of 5-HT from the particulate or 'bound' fraction to the supernatant or 'free' fraction (Englert *et al.*, 1973). No such a change in the subcellular distribution of 5-HT was detected in rat brain 10 or 60 min after intravenous injection of delta-9-THC (Taylor and Fennessy, 1979b). However, at 10 min (but not at

TABLE 20. *The Effect of Intraperitoneal Administration of Delta-9-THC on Rat Brain Levels of Acetylcholine*

Dose (mg/kg)	Time after injection (hr)	Brain area	Effect	Reference
6	1	Amygdala	+	1
6	1	Striatum	+	1
32	0.5	Whole brain	+	2
3.2 and 10	0.5 and 1	Whole brain	0	2
6	1	Cerebral cortex	0	1
6	1	Diencephalon	0	1
6	1	Brain stem	0	1
10	1	Thalamus	0	2
10	1	Hippocampus	0	2
10	1	Hypothalamus	0	2
10	1	Caudate nucleus	0	2
10	1 to 2.5	Caudate nucleus	0	3

References: 1, Yoshimura *et al.*, 1974; 2, Domino, 1981; 3, Bhattacharyya *et al.*, 1980.

60 min) after its intravenous administration, the drug was found to shift 5-HIAA from the 'free' to the 'bound' fraction.

6.4. Histamine and Spermidine

6.4.1. *Histamine*

In experiments with rats, Fennessy *et al.* (1983) detected marked reductions in histamine (HA) concentrations in the cerebral cortex, hypothalamus and midbrain 30 min after intravenous injection of delta-9-THC (2 mg/kg) and in the pons/medulla 120 min after delta-9-THC. The drug had no effect on HA concentrations in the cerebellum. Further experiments are needed to determine whether the drug acted to reduce concentrations of HA in neurons and also to determine whether the drug acted to alter HA synthesis, release or metabolism. In more recent experiments, Oishi *et al.* (1985) found that intravenous administration of delta-9-THC at doses of 2 or 5 mg/kg had no detectable effect on HA concentrations in rat whole brain. The absence of an effect on whole brain concentrations of HA has also been noted in mice receiving delta-9-THC at doses of 1 to 50 mg/kg i.v. (Oishi *et al.*, 1985) or 20 mg/kg i.p. (Taylor and Snyder, 1972). At a dose of 5 mg/kg i.v., however, delta-9-THC has been found to reduce rat brain concentrations of the HA metabolite, tele-methylhistamine (Oishi *et al.*, 1985). Consistent with this observation, Oishi *et al.* (1985) also found that delta-9-THC, given intravenously at doses of 2, 5 or 10 mg/kg, could attenuate pargyline-induced increases in rat brain concentrations of tele-methylhistamine. Other signs of a delta-9-THC-induced decrease in the turnover of brain HA were observed in mice, albeit only at a very high intravenous dose (50 mg/kg) and in experiments with guinea pig hypothalamic slices in which the drug (30 and 100 μM) was found to produce a small but significant inhibition of K^+-induced HA release.

6.4.2. *Spermidine*

Lewis *et al.* (1986) have recently reported that 30 min after intravenous injection of delta-9-THC (2 mg/kg) concentrations of the aliphatic polyamine, spermidine, were reduced in rat cerebral cortex and midbrain although not in hypothalamus, cerebellum or pons/medulla. They proposed that some effects of delta-9-THC including its hypothermic

Table 21. *Effects of Delta-9-THC on Acetylcholine Turnover or Release in Mouse, Rat and Cat Brain*

Species	Dose and Route (mg/kg)	Brain area	Method of measuring ACh turnover	Effect	Reference
Mouse	30 i.p.	Cerebral cortex	Rise in ^{3}H-ACh relative to	0	1
		Hippocampus	^{3}H-choline and endogenous	–	
		Striatum	choline after ^{3}H-choline (i.v.)	0	
		Midbrain		0	
		Pons/medulla		0	
Rat	0.2 to 10 i.v.	Parietal cortex	Rise in ^{3}H-ACh relative to	0	2
	0.2 to 10 i.v.	Hippocampus	^{3}H-choline and endogenous	–	2, 3, 4
	0.2 and 0.5 i.v.	Striatum	choline after ^{3}H-choline (i.v.)	0	2
	5 and 10 i.v.	Striatum		–	
Mouse	10 s.c.	Whole brain	Fall in ACh after ASHC-3 (i.c.v.)	0	5
	32 and 56 s.c.	Whole brain		–	
Rat	10 and 30 i.p.	Whole brain	Fall in ACh after ASHC-3 (i.c.v.)	–	5
	1 i.p.	Hippocampus		0	
	3.2 to 32 i.p.	Hippocampus		–	
	1 to 32 i.p.	Thalamus		0	
		Caudate nucleus		0	
		Hypothalamus		0	
Cat*	1 to 11 i.v.	Somatosensory cortex	ACh release (collecting cups)	–	6

*Brain stem transected animals. ASHC-3 is an abbreviation for acetylsecohemicholinium-3 and i.c.v. for intracerebroventricular administration. References: 1, Tripathi *et al.*, 1979; 2, Revuelta *et al.*, 1978; 3, Revuelta *et al.*, 1979; 4, Revuelta *et al.*, 1980; 5, Domino, 1981; 6, Domino and Bartolini, 1972.

effect may result at least in part from delta-9-THC- induced spermidine release within the brain.

6.5. ACETYLCHOLINE

6.5.1. *Brain Levels of Acetylcholine*

Tripathi *et al.* (1979) reported that intraperitoneal injection of delta-9-THC at a dose of 30 mg/kg increased concentrations of acetylcholine (ACh) and choline in mouse cerebral cortex, hippocampus, striatum, midbrain and pons/medulla. When lower doses of delta-9-THC were used (1 or 10 mg/kg i.p.), or when CBD was injected instead (1 to 30 mg/kg i.p.), no significant changes in the concentrations of ACh or choline were detected. Domino (1981) found that delta-9-THC increased concentrations of ACh in rat whole brain when injected intraperitoneally at a dose of 32 mg/kg. Intraperitoneal administration of delta-9-THC at lower dose levels have also been reported to elevate ACh levels in rat amygdala and striatum, although not in other brain areas (Table 20). Friedman *et al.* (1976) found that neither delta-9-THC nor delta-8-THC altered ACh or choline concentrations in the striata of rats (10 mg/kg; route not given).

Askew *et al.* (1974) reported that after intravenous administration of delta-9-THC at a dose of 5 mg/kg, there was a decrease in ACh concentration in rat whole brain. A similar result was obtained in experiments with delta-8-THC but not with 11-hydroxy-delta-8-THC. Askew *et al.* (1974) also found that synaptosomes prepared from the brains of rats which had been pretreated with delta-8-THC (5 mg/kg i.v.) contained reduced amounts of ACh. In other studies, intravenous injection of delta-9-THC was found to have no effect on ACh concentrations either in rat whole brain (5 mg/kg; Domino, 1981) or in rat parietal cortex, hippocampus or striatum (0.2 to 10 mg/kg; Revuelta *et al.*, 1978, 1979, 1980). Similarly, delta-8-dimethylheptyl-THC had no effect on ACh or choline concentrations in rat parietal cortex or striatum when injected intravenously at doses of 0.12 to 1 mg/kg (Revuelta *et al.*, 1980). It did, however, elevate rat hippocampal concentrations of ACh at a dose of 1 mg/kg, although not at lower doses. In the same study, (+) and (−) delta-8-THC (1 to 20 mg/kg i.v.) had no effect on cortical, striatal or hippocampal levels of ACh. Nabilone too (0.1 to 1.0 mg/kg i.v.) was found to have no effect on ACh or choline concentrations in rat parietal cortex, striatum or hippocampus (Revuelta and Cheney, 1981). Similar experiments with levonantradol (0.3 and 3 mg/kg s.c.) also yielded negative results (Costa *et al.*, 1981).

TABLE 22. *Effects of Various Cannabinoids on Acetylcholine Turnover in Three Areas of Rat Brain*

Cannabinoid	Dose and Route (mg/kg)	Effect on ACh turnover*			Reference
		Parietal cortex	Striatum	Hippocampus	
(−)Δ^9-THC	0.2 i.v.	ND	0	–	1
	0.5 i.v.	0	0	–	
	5 and 10 i.v.	0	–	–	
(−)Δ^8-THC	1.0 i.v.	0	0	0	2
	5 to 20 i.v.	0	0	–	
(−)Δ^8-DMH-THC	0.025 i.v.	ND	ND	0	2
	0.05 i.v.	ND	ND	–	
	0.12 to 0.5 i.v.	0	0	–	
	1.0 i.v.	0	–	–	
Nabilone	0.1 i.v.	–	0	–	3
	1.0 i.v.	–	–	–	
Levonantradol	0.3 to 3.0 s.c.	0	–	–	4
(+)Δ^8-THC	1.0 to 20 i.v.	0	0	0	2
CBD	10 i.v.	0	0	0	1
	20 i.v.	0	–	0	

*ACh turnover measured using the method described by Revuelta *et al.*, 1979. ND = not determined. References: 1, Revuelta *et al.*, 1978 2, Revuelta *et al.*, 1980; 3, Revuelta and Cheney, 1981; 4, Costa *et al.*, 1981.

6.5.2. *Acetylcholine Turnover*

The results from several *in vivo* studies have provided evidence that delta-9-THC can reduce brain turnover of ACh (Table 21). In experiments with rats or mice, the drug was found to produce signs of reduced ACh turnover in whole brain, hippocampus and striatum. The drug was markedly more potent in reducing ACh turnover in rat hippocampus than in rat striatum and had no detectable effect on ACh turnover in any other brain area studied. Essentially similar results were obtained in experiments with delta-8-dimethylheptyl-THC and levonantradol (Table 22). The (−) isomer of delta-8-THC also reduced ACh turnover in rat hippocampus although not in the cerebral cortex or striatum whereas nabilone affected ACh turnover in rat hippocampus, striatum and cerebral cortex (Table 22). In contrast, the psychically inactive cannabinoids, CBD and (+) delta-8-THC, did not alter ACh turnover in rat hippocampus or cerebral cortex. CBD did alter ACh turnover in the striatum, however, albeit only at a rather high dose, whereas the (+) isomer of delta-8-THC had no effect on ACh turnover in this area (Table 22). In brain stem transected cats, delta-9-THC administered intravenously at doses of 1 to 11 mg/kg produced dose-related falls in ACh release from the sensorimotor cortex (Domino and Bartolini, 1972). In the same study, a dose of 0.5 mg/kg i.v. of delta-9-THC either had no effect on ACh release or caused a slight increase.

Signs of cannabinoid-induced alterations in the functioning of central ACh-releasing neurons have been observed not only with *in vivo* techniques but also in studies using brain slices or synaptosomes. Thus non-competitive inhibition of choline uptake has been reported to occur after *in vitro* addition of delta-9-THC in experiments with crude synaptosomal preparations obtained from rat hippocampus ($IC_{50} = 4.6\ \mu M$; Lindamood and Colasanti, 1980) or mouse forebrain ($IC_{50} = 16\ \mu M$; Johnson and Dewey, 1978b). *In vivo* pretreatment with delta-9-THC has also been found to reduce choline uptake into certain synaptosomal preparations. Inhibition was observed in rat hippocampal and hypothalamic preparations (Lindamood and Colasanti, 1980) but not in preparations obtained from rat cerebral cortex, striatum, midbrain or pons/medulla (Friedman *et al.*, 1976; Lindamood and Colasanti, 1980) or from mouse forebrain (Johnson and Dewey, 1978b).

Like delta-9-THC, CBD has been found to inhibit choline uptake when added directly to a rat hippocampal synaptosomal preparation ($IC_{50} = 16\ \mu M$; Lindamood and Colasanti, 1980). However, *in vivo* pretreatment with CBD had no effect on choline uptake into synaptosomal preparations obtained from rat cerebral cortex, striatum, hippocampus, hypothalamus, midbrain or pons/medulla (Lindamood and Colasanti, 1980).

Friedman *et al.* (1976) studied the effect of *in vivo* pretreatment with delta-8-THC, delta-9-THC (5 to 40 mg/kg) and CBD (20 and 40 mg/kg) on ^{3}H-ACh formation from ^{3}H-choline in slices of rat hypothalamus and striatum. Delta-8- and delta-9-THC were both inhibitory, the former cannabinoid producing the greater inhibition, whereas CBD had no detectable effect. *In vivo* pretreatment with delta-8- and delta-9-THC also reduced ^{3}H-ACh accumulation in rat cortical slices.

Observations that *in vivo* administration of delta-9-THC can produce decreases in central ACh turnover, in choline uptake into synaptosomes and in the synthesis of ACh in brain slices have been taken to indicate that the drug can reduce the firing rate of central ACh-releasing neurons (Friedman *et al.*, 1976; Revuelta *et al.*, 1978; Lindamood and Colasanti, 1980). The evidence for this hypothesis can be summarized as follows.

(i) Reductions in choline uptake, in ACh synthesis and in ACh turnover are all expected consequences of a reduction in impulse flow along cholinergic pathways.

(ii) Alternative hypotheses that delta-9-THC acts by inhibiting choline acetyltransferase or by increasing the activity of acetylcholinesterase are unlikely to be true since no such changes in enzyme activity have been detected in rat brain after *in vivo* administration of delta-8 or delta-9-THC (Askew *et al.*, 1974; Yoshimura *et al.*, 1974; Friedman *et al.*, 1976; Moss *et al.*, 1978). Moreover, reductions in ACh synthesis

provoked in rat brain slices by *in vivo* pretreatment with delta-8- or delta-9-THC occurred in the presence of physostigmine (Friedman *et al.*, 1976).

(iii) The effect of *in vivo* injection of delta-8-THC on ACh synthesis in rat brain slices could be prevented by using a K^+-rich incubation medium (Friedman *et al.*, 1976).

(iv) The effects of *in vivo* injection of delta-9-THC on hippocampal turnover of ACh and on ^{3}H-choline uptake by hippocampal synaptosomes could be mimicked by surgical or electrolytic procedures expected to reduce the neuronal activity of a septal–hippocampal cholinergic pathway (Revuelta *et al.*, 1979; Lindamood and Colasanti, 1981a). Conversely, electrical stimulation of the same pathway has been shown to increase the hippocampal turnover of ACh (Moroni *et al.*, 1978).

(v) The ability of delta-9-THC, administered *in vivo*, to reduce ACh turnover in rat hippocampus or to reduce uptake of ^{3}H-choline into hippocampal synaptosomes was abolished by surgical or electrolytic procedures expected to reduce impulse flow in septal–hippocampal cholinergic neurons (Revuelta *et al.*, 1979; Lindamood and Colasanti, 1981a).

Two hypotheses have been put forward to explain how delta-9-THC might act to reduce the firing rate of central ACh-releasing neurons. In one, it is proposed that delta-9-THC acts directly on cholinergic neurons to interfere with the propagation of action potentials or with the depolarization process (Friedman *et al.*, 1976). This hypothesis leaves unanswered the question of why delta-9-THC seems to alter ACh turnover in only a few brain areas. In the other hypothesis, put forward to account for the effect of delta-9-THC on ACh turnover in the hippocampus, it is proposed that delta-9-THC interacts with pathways that serve to regulate the activity of septal–hippocampal cholinergic neurons (Revuelta *et al.*, 1978). According to this hypothesis, possible sites of action include (i) septal GABA-releasing interneurons thought to exert an inhibitory influence on septal–hippocampal cholinergic neurons and (ii) dopamine and beta-endorphin-releasing neurons thought to decrease ACh release in the hippocampus by acting on septal GABAergic interneurons (Revuelta *et al.*, 1979). An involvement of GABA in the effect of delta-9-THC on hippocampal turnover of ACh is supported by the observation that this effect of delta-9-THC could be prevented by intraseptal injection of the GABA antagonist, bicuculline (Revuelta *et al.*, 1979). Also consistent with an involvement of GABA is the finding that delta-9-THC can increase GABA turnover in the septum (Revuelta *et al.*, 1979; see also Section 6.6). There is, however, no evidence for an involvement of DA or beta-endorphin releasing neurons in the effect of delta-9-THC on ACh turnover (Revuelta *et al.*, 1979; Lindamood and Colasanti, 1981b) or indeed for an involvement of NE or 5-HT (Lindamood and Colasanti, 1981b).

6.6. GAMMA-AMINOBUTYRIC ACID

Revuelta *et al.* (1979) studied the effects of delta-9-THC and CBD on central gamma-aminobutyric acid (GABA) turnover rate by simultaneous measurement of endogenous GABA and ^{13}C-GABA levels in the brains of rats which had been given an intravenous infusion of ^{13}C-glucose. At a dose of 5 mg/kg i.v., delta-9-THC was reported to double GABA turnover in the septum. At higher doses, respectively 10 and 20 mg/kg i.v., delta-9-THC and CBD increased GABA turnover in the substantia nigra. Neither drug affected the concentration of endogenous GABA. Nor did they alter GABA turnover in the caudate nucleus or nucleus accumbens.

In further studies with rats, Revuelta *et al.* (1982) found that (−) delta-8-dimethylheptyl-THC reduced GABA turnover both in the septum (1 mg/kg i.v.) and in the nucleus accumbens (0.25 and 1 mg/kg i.v.). Septal turnover of GABA was also reduced by nabilone at a dose of 1 mg/kg i.v. (Revuelta and Cheney, 1981). Neither cannabinoid altered endogenous brain concentrations of GABA. The dimethylheptyl compound had no detectable effect on GABA turnover in the caudate nucleus, globus pallidus or substantia nigra and the psychically inactive (+) isomer of delta-8-THC had

no effect on GABA turnover in the septum, even at a dose of 5 mg/kg i.v. (Revuelta *et al.*, 1982). Clearly additional experiments are required to determine why delta-9-THC had the opposite effect on GABA turnover to other psychically active cannabinoids. It would, for example, be interesting to test whether these differences reflect a dose-dependent biphasic effect, GABA turnover perhaps being reduced by low doses of delta-9-THC and increased by higher doses.

Edery and Gottesfeld (1975) reported that at a dose of 20 mg/kg i.p., delta-8-THC had no effect on GABA concentrations in the rat cerebellum, striatum or cerebral cortex. Delta-8-THC was also reported to have no effect on GABA concentrations in rat whole brain (minus cerebellum) when injected at a dose of 50 mg/kg i.p. (Leonard, 1971). In the same study, a long-lasting fall in GABA concentration was detected when the dose of delta-8-THC used was increased to 100 mg/kg i.p.

In vitro administration of delta-9-THC at concentrations of 10 to 100 μM was found to inhibit the uptake of ^{3}H-GABA into synaptosomal preparations obtained from rat cerebral cortex or mouse whole brain (Banerjee *et al.*, 1975b; Hershkowitz *et al.*, 1977). Other cannabinoids found to inhibit ^{3}H-GABA uptake include delta-8-THC, the 11-hydroxy metabolites of delta-8- and delta-9-THC, CBN, CBD and cannabigerol (Banerjee *et al.*, 1975b; Hershkowitz *et al.*, 1977). The inhibition of ^{3}H-GABA uptake by delta-9-THC, delta-8-THC and CBD was reported to be non-competitive (Banerjee *et al.*, 1975b). When administered *in vivo* at a dose of 10 mg/kg i.v., delta-9-THC was found to increase the uptake of ^{3}H-GABA into a mouse brain synaptosomal preparation (Hershkowitz and Szechtman, 1979). CBD injected at the same dose level, had no effect on ^{3}H-GABA uptake.

6.7. Binding Studies

6.7.1. *Neurotransmitters*

There is evidence from experiments with mouse brain preparations that when administered *in vitro,* delta-9-THC can alter binding to adrenoceptors, to dopamine receptors and to opioid receptors (Table 23).

Hillard and Bloom (1982, 1984) reported that delta-9-THC (3 and 10 μM) and its 11-hydroxy metabolite (10 μM) could facilitate specific binding of the beta-adrenoceptor antagonist, ^{3}H-dihydroalprenolol, to mouse brain tissue by increasing the affinity of the tritiated ligand for its binding sites. Both cannabinoids had a concentration-dependent biphasic effect, producing no significant changes in binding at concentrations below 3 μM or above 10 μM. Concentrations of CBD ranging from 1 to 100 μM had no effect on ^{3}H-dihydroalprenolol binding. It was also found that delta-9-THC could enhance the ability of several unlabeled beta-adrenoceptor antagonists to displace ^{3}H-dihydroalprenolol from its binding sites (Hillard and Bloom, 1984; Bloom and Hillard, 1985). In contrast, the ability of beta-adrenoceptor agonists to displace ^{3}H-dihydroalprenolol was unaffected or decreased by delta-9-THC. The experimental data suggest that delta-9-THC acted to induce a second population of binding sites with a particularly high affinity for beta-adrenoceptor antagonists (Hillard and Bloom, 1984; Bloom and Hillard, 1985). In other experiments with mouse brain tissue, Bloom and Hillard (1985) found that delta-9-THC decreased specific binding of ^{3}H-naloxone. The cannabinoid had no effect on ^{3}H-D-ala-D-leu-enkephalin binding suggesting that it had a selective effect on μ opioid binding sites.

In experiments with membrane preparations obtained from mouse corpus striatum, delta-9-THC, 11-hydroxy-delta-9-THC and CBD were all reported to decrease specific binding of ^{3}H-spiperone to D_2 DA sites and of ^{3}H-ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) to D_3 DA sites (Bloom, 1984; Bloom and Hillard, 1985). Interestingly, whereas the reductions in ^{3}H-spiperone binding were caused mainly by a decrease in the apparent affinity of D_2 sites for ^{3}H-spiperone, the reductions in ^{3}H-ADTN binding resulted largely from a decrease in the number of binding sites (a decrease in B_{max}). Neither delta-9-THC nor CBD altered the ability of haloperidol or (+)butaclamol to displace

TABLE 23. *Effects of Delta-9-THC on the Binding of Various Compounds to Mouse or Rat Brain Tissue*

Concentration (μM)	Vehicle	Experiment	Effect			Reference
			Displacement	Affinity constant	Number of binding sites	
1	Emulphor/	Specific binding of	0			1, 2
3 and 10	ethanol/	^{3}H-dihydroalprenolol	+	+	0	
30 and 100	buffer	(mouse cerebral cortex)	0			
10	Emulphor/ ethanol	Ability of (±) propranolol and other beta-adrenoceptor antagonists to displace ^{3}H-dihydroalprenolol (mouse cerebral cortex)	+			2, 3
10	Emulphor/ ethanol	Ability of (−) isoprenaline and other beta adrenoceptor agonists to displace ^{3}H-dihydroalprenolol (mouse cerebral cortex)	0/−			2, 3
30	Emulphor/ ethanol	Specific binding of ^{3}H-spiperone (mouse striatum)	−	−	0	2, 4
30	Emulphor/ ethanol	Ability of haloperidol and (+)butaclamol to displace ^{3}H-spiperone (mouse striatum)	0			2, 4
30	Emulphor/ ethanol	Ability of dopamine and apomorphine to displace ^{3}H-spiperone (mouse striatum)	+			2, 4
20 and 30	PVP/ saline	Specific binding of ^{3}H-ADTN* (mouse striatum)	−	0	−	4
0.6 to 3	Emulphor/	Specific binding of	0			2
5 to 40	ethanol	^{3}H-naloxone (mouse brain minus cerebellum)	−	−	0	

TABLE 23. *Effects of Delta-9-THC on the Binding of Various Compounds to Mouse or Rat Brain Tissue* (Continued)

Concentration (μM)	Vehicle	Experiment	Effect			Reference
			Displacement	Affinity constant	Number of binding sites	
0.6 to 40	Emulphor/ ethanol	Specific binding of ^{3}H-D-ala-D-leu enkephalin (mouse brain minus cerebellum)	0			2
400	Emulphor/ ethanol/ saline	Specific binding of ^{3}H-GABA (rat brain)	0			5

*2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene, a D_3 dopamine ligand. PVP is an abbreviation for polyvinylpyrrolidone. References: 1, Hillard and Bloom, 1982; 2, Bloom and Hillard, 1985; 3, Hillard and Bloom, 1984; 4, Bloom, 1984; 5, Leader *et al.*, 1981.

^{3}H-spiperone from its binding sites. However, delta-9-THC (unlike CBD) did enhance the ability of DA and apomorphine to displace ^{3}H-spiperone, raising the possibility that this cannabinoid can selectively increase the affinity of D_2 DA receptors for agonists. In view of these findings, it is noteworthy that Agrawal *et al.* (1985) have recently reported increased ^{3}H-spiperone binding in striatal tissue obtained from mice pretreated intraperitoneally 24 hr earlier with cannabis.

Leader *et al.* (1981) investigated the effects of *in vitro* administration of delta-9-THC and levonantradol on specific GABA binding to rat whole brain synaptic membranes. Levonantradol reduced GABA binding ($IC_{50}=4$ μM) whereas delta-9-THC had no effect at concentrations of up to 400 μM.

6.7.2. *Benzodiazepine Binding Sites*

In experiments with rat cerebral cortical membranes, levonantradol (50 and 100 μM) and dextronantradol (100 μM) were shown to enhance ^{3}H-diazepam binding (Koe and Weissman, 1981). Both cannabinoids also enhanced ^{3}H-diazepam binding to mouse brain tissue after *in vivo* administration, levonantradol at a dose of 0.47 mg/kg s.c. and dextronantradol at a dose of 1.5 mg/kg s.c. (Koe and Weissman, 1981). When ^{3}H-flunitrazepam was injected intravenously, increased benzodiazepine binding was only detected in brain tissue obtained from mice pretreated with levonantradol (0.05 to 1.5 mg/kg s.c.), dextronantradol (1.4 to 15 mg/kg s.c.) having no effect (Koe and Weissman, 1981; Koe *et al.*, 1985) In the same series of experiments, increased '*in vivo* binding' of ^{3}H-flunitrazepam was noted in mice pretreated subcutaneously with several other cannabinoids including delta-9-THC (32 mg/kg) and nabilone (1 to 12 mg/kg). CBD reduced ^{3}H-flunitrazepam binding at a dose of 10 mg/kg but had no effect on binding at higher doses (32 and 100 mg/kg).

7. STUDIES WITH MEMBRANE-BOUND ENZYMES

7.1. MONOAMINE OXIDASE

Both increases and decreases in monoamine oxidase activity have been detected following administration of delta-9-THC. The increases in activity have been observed after *in vivo* administration of the drug to rats and also in two *in vitro* studies, one with eye tissue in which very low (picomolar) concentrations of delta-9-THC were used and the other with hypothalamic tissue. Decreases in monoamine oxidase activity have been observed only after *in vitro* administration of delta-9-THC, the inhibitory concentrations of the drug lying in the micromolar range. The effect of delta-9-THC on monoamine oxidase activity has been shown to vary not only with dose but also with species and with the tissue and substrate used to measure the activity of the enzyme.

In rats, increases in the activity of whole brain monoamine oxidase activity have been detected 6 hr after intraperitoneal administration of delta-9-THC although not at other times after injection. (Table 24). Increases in monoamine oxidase activity have also been detected after intraperitoneal injection of delta-9-THC (10 and 50 mg/kg) in rat blood platelets and in mitochondrial preparations of rat hypothalamus and heart (Banerjee *et*

TABLE 24. *The Effect of Intraperitoneal Administration of Delta-9-THC on Rat Brain Monoamine Oxidase Activity*

Dose (mg/kg)	Time after injection (hr)	Brain area	Substrate	Effect	Reference
10 and 50	6	Whole brain	Tyramine	+	1
10 and 50	6	Hypothalamus	Tyramine	+	1, 2
20	0.5	Whole brain	Tryptamine	0	3
1 and 14	18 to 22	Whole brain	Tryptamine	0	4

References: 1, Banerjee *et al.*, 1975a; 2, Banerjee *et al.*, 1977; 3, Sofia *et al.*, 1971a; 4, Clarke and Jandhyala, 1977.

al., 1975a, 1977) and, 45 min after intravenous administration of the cannabinoid (10 mg/kg), in rat cerebral cortex although not in rat brain stem (Ouellet *et al.*, 1973). Banerjee *et al.* (1977) found that direct addition of delta-9-THC to their hypothalamic preparation also increased monoamine oxidase activity (Table 25). However, *in vitro* administration of delta-9-THC decreased monoamine oxidase activity in the rat heart preparation and had no effect on the activity of this enzyme in the rat platelet preparation (Banerjee *et al.*, 1977). Intraperitoneal administration of delta-9-THC at doses in the range 1 to 50 mg/kg had no detectable effect on monoamine oxidase activity in preparations obtained from rat liver (Sofia *et al.*, 1971a; Banerjee *et al.*, 1975a), rat kidney (Banerjee *et al.*, 1975a) or rat lung (Clarke and Jandhyala, 1977). In experiments with human blood platelets, delta-9-THC (3 to 30 μM) was reported to inhibit monoamine oxidase (Mazor *et al.*, 1982). The inhibition was non-competitive. In the same study, (−) delta-8-THC was shown to be a less potent inhibitor of monoamine oxidase than delta-9-THC. The psychically active (−) delta-8-dimethylheptyl-THC and the psychically inactive CBD and (+) delta-8-THC had no inhibitory effect.

In bovine eye preparations, delta-9-THC was reported to stimulate monoamine oxidase activity at concentrations ranging from 0.01 pM to 10 nM and to produce a slight inhibition at a concentration of 1 μM (Gawienowski *et al.*, 1982). On the other hand, in human brain and liver mitochondria, delta-9-THC (2.5 μM to 0.4 mM) was found to have no effect on monoamine oxidase activity (Schurr and Rigor, 1984, and Table 25). Similarly, the drug (0.1 mM) had no effect on the activity of the enzyme in porcine liver mitochondria (Schurr and Livne, 1976).

In porcine brain mitochondria, *in vitro* administration of delta-9-THC was found to inhibit monoamine oxidase (Table 25). The inhibition was non-competitive (Schurr *et al.*, 1978). Interestingly, the degree of inhibition observed varied not only with the dose of delta-9-THC added to the incubation medium but also with the substrate used in the enzyme assay (Schurr and Livne, 1976; Schurr *et al.*, 1978). Inhibition was greatest with

TABLE 25. *Effects of* In Vitro *Administration of Delta-9-THC on Brain Monoamine Oxidase Activity*

Species	Vehicle	Concentration of Δ^9-THC*	Brain area	Substrate	Effect	Reference
Man	Ethanol	1 to 100 μg/mg (2.5 to 398 μM)	Whole brain	2-phenyl-ethylamine	0	1
			Whole brain	5-hydroxy-tryptamine	0	1
Pig	Ethanol	3 μg/mg (3.2 μM)	Whole brain	Benzylamine	0/−	2
		10 to 300 μg/mg (10.6 to 318 μM)	Whole brain	Benzylamine	−	2
		3 and 10 μg/mg (3.2 and 10.6 μM)	Whole brain	Tyramine	0	2
		30 to 300 μg/mg (32 to 318 μM)	Whole brain	Tyramine	−	2
		158 μg/mg (84 to 168 μM)	Whole brain	Dopamine	−[1]	3
			Whole brain	Benzylamine	−[1]	3
			Whole brain	5-hydroxy-tryptamine	−[2]	3
			Whole brain	Tyramine	−[2]	3
			Whole brain	Dimethylamino-benzylamine	−[2]	3
			Whole brain	Tryptamine	−[3]	3
Rat	Propylene glycol	10 to 40 μg/ml (32 to 127 μM)	Whole brain	Tryptamine	0	4
	Tween/ saline	2 to 8 μg/mg	Hypothalamus	Tyramine	+	5

*μg/mg protein or μg/ml medium. The concentrations shown in round brackets have been calculated from the published values which were given in terms of μg/mg or μg/ml. The numbers in square brackets show how the degree of delta-9-THC-induced enzyme inhibition varied with substrate, [1] indicating the greatest degree of inhibition and [3] the least. References: 1, Schurr and Rigor, 1984; 2, Schurr and Livne, 1976; 3, Schurr *et al.*, 1978; 4, Sofia *et al.*, 1971a; 5, Banerjee *et al.*, 1977.

TABLE 26. *Effects of* In Vitro *Administration of Delta-9-THC on Brain ATPase Activity*

Species	Vehicle	Concentration of Δ^9-THC (μM)	Preparation	ATPase	Effect	Reference
Rat	Ethanol	1	Synaptic vesicles	Mg^{2+}-	+	1
		3 to 1000	from cerebral cortex		−	
Rat	Tween/ saline	3.2 to 12.7	Whole brain synaptosomes	Mg^{2+}-	−	2
			Whole brain microsomes		−	
Rat	Ethanol	3.2 to 95	Whole brain synaptosomes	Mg^{2+}-	−	3
		3.2 to 31.6	Cerebral cortex synaptosomes		−	
Mouse	Ethanol	5 to 50	Whole brain synaptosomes	Mg^{2+}-	−	4
Mouse	PVP	10 to 20	Whole brain synaptosomes	Mg^{2+}-	+	5
		5 to 20	Whole brain mitochondria		+	
		5 to 40	Whole brain microsomes		−(26 μM)	
		20 and 40	Whole brain myelin fraction		−(46 μM)	
Rat	Tween/ saline	3.2 to 12.7	Whole brain synaptosomes	Na^{+}-K^{+}-	−	2
			Whole brain microsomes		−	
Rat	Water/ acetone	3 and 30	'Lipoprotein fraction' of whole brain minus cerebellum	Na^{+}-K^{+}-	−	6
Rat	Ethanol	1.6 to 95	Whole brain synaptosomes	Na^{+}-K^{+}-	−	3
		1.6 to 31.6	Cerebral cortex synaptosomes		−	
Mouse	Ethanol	5 to 50	Whole brain synaptosomes	Na^{+}-K^{+}-	−	4
Mouse	PVP	1 to 40	Whole brain synaptosomes	Na^{+}-K^{+}-	−(8 μM)	5
		1 to 40	Whole brain mitochondria		−(7 μM)	
		2.5 to 40	Whole brain microsomes		−(11 μM)	
		10 to 40	Whole brain myelin fraction		−(23 μM)	
Mouse	PVP	20 and 40	Whole brain synaptosomes	Mg^{2+}-Ca^{2+}-	−(25 μM)	5
		40	Whole brain mitochondria		−(15 μM)	
		5 to 40	Whole brain microsomes		−(8 μM)	
		40	Whole brain myelin fraction		−(51 μM)	

PVP is an abbreviation for polyvinylpyrrolidone. The numbers in parentheses are IC_{50} values. References: 1, Gilbert *et al.*, 1977; 2, Poddar and Ghosh, 1976b; 3, Olmsted, 1976; 4, Hershkowitz *et al.*, 1977; 5, Bloom *et al.*, 1978a; 6, Toro-Goyco *et al.*, 1978.

benzylamine or DA as substrate and least with tryptamine. Intermediate degrees of inhibition were observed when the substrate was 5-HT, tyramine or dimethylaminobenzylamine. With benzylamine as substrate, signs of monoamine oxidase inhibition were detected at delta-9-THC concentrations of about 10 μM and more (Table 25). When the substrate was tryptamine, the drug inhibited porcine brain monoamine oxidase at concentrations of 84 to 170 μM (Table 25). In contrast, delta-9-THC concentrations of 32 to 127 μM had no effect on tryptamine deamination in rat brain or liver (Sofia *et al.*, 1971a, and Table 25).

The effect of CBD on monoamine oxidase activity has also been found to vary with the substrate used. With tyramine as substrate, CBD produced a slight inhibition of the enzyme, albeit only at a rather high concentration (Schurr and Livne, 1976). However, in marked contrast to delta-9-THC, CBD had no detectable effect on monoamine oxidase activity when the substrate was benzylamine. Interestingly, although lacking an effect when given by itself, CBD completely abolished the inhibitory effect of delta-9-THC on benzylamine deamination (Schurr and Livne, 1976).

In further experiments, Schurr *et al.* (1978) found that delta-9-THC could still markedly reduce the activity of porcine brain mitochondrial monoamine oxidase after the enzyme had been solubilized by sonication to separate it from the mitochondrial membrane, suggesting that the cannabinoid must interact either with the enzyme itself or with some phospholipid component bound to it. It was also found that the ability of delta-9-THC to inhibit monoamine oxidase was reduced after chloroform (in the presence of delta-9-THC or CBD) had been used to extract lipid material from the solubilized enzyme. The effect could be largely reversed by the addition of phosphatidylcholine and was not seen when the chloroformic extraction was performed in the absence of a cannabinoid. These findings led Schurr *et al.* (1978) to propose that delta-9-THC inhibits brain monoamine oxidase by interacting with phospholipids associated with the enzyme to induce a conformational change.

TABLE 27. *The Effect of Intraperitoneal Administration of Delta-9-THC on Rat or Mouse Brain Cyclic AMP Levels*

Species	Dose and route (mg/kg)	Time after injection (hr)	Brain area	Effect	Reference
Rat	10 i.v.	1	Cerebral cortex	0	1
			Hypothalamus	0	
			Midbrain	0	
			Cerebellum	0	
			Medulla	0	
Mouse	0.1 to 1 i.p.	0.5	Whole brain	+	2
	2 to 10 i.p.			−	
	50 i.p.			0	
Mouse	0.1 and 0.25 i.p.	0.5	Cerebral cortex	+	2
	0.5 i.p.			0	
	1 i.p.			+	
	2 to 10 i.p.			−	
	50 i.p.			0	
Mouse	0.1 and 0.25 i.p.	0.5	Hypothalamus	−	2
	0.5 and 1 i.p.			0	
	10 i.p.			+	
	2 and 50 i.p.			−	
Mouse	0.1, 0.5 and 1 i.p.	0.5	Cerebellum	+	2
	0.25 and 2 i.p.			0	
	10 and 50 i.p.			−	
Mouse	0.1 to 0.5 i.p.	0.5	Medulla	+	2
	1 and 10 i.p.			0	
	2 i.p.			−	
	50 i.p.			+	
Mouse	0.25 i.p.	0.5	Whole brain	ND*	3
	10 i.p.			ND**	

ND = not determined. *Indicates that adenylate cyclase activity was increased or unaffected. **Indicates that adenylate cyclase activity was decreased or unaffected. References: 1, Askew and Ho, 1974; 2, Dolby and Kleinsmith, 1974; 3, Dolby and Kleinsmith, 1977.

7.2. ATPases

In experiments with mice, intraperitoneal administration of delta-9-THC at a dose of 10 mg/kg has been reported to produce a 27% increase in whole brain ATPase activity (Jain *et al.*, 1974). In rats, doses of 10 and 100 mg/kg i.p. of delta-9-THC have been found to increase brain microsomal Mg^{2+}- and Na^+-K^+-ATPase activity, to decrease synaptosomal Na^+-K^+-ATPase activity and to have no effect on synaptosomal Mg^{2+}-ATPase activity (Poddar and Ghosh, 1976b). The drug has also been reported to inhibit synaptosomal Na^+-K^+-ATPase when given to rats at doses of 1 or 2 mg/kg i.p. (Lee and Olmsted, 1976).

When delta-9-THC was added directly to various preparations obtained from rat or mouse brains, concentrations in the low micromolar range were found to inhibit Na^+-K^+- and Mg^{2+}-Ca^{2+}-ATPases (Table 26). In rat brain synaptosomes and synaptic vesicles, delta-9-THC concentrations of 3 μM or more were also found to inhibit Mg^{2+}-ATPase (Table 26). However, a delta-9-THC concentration of 1 μM increased Mg^{2+}-ATPase activity in the vesicular preparation. *In vitro* administration of the drug also increased Mg^{2+}-ATPase activity in synaptosomal and mitochondrial fractions of mouse whole brain (Table 26).

In vitro administration of delta-9-THC has been shown to affect the activity of various ATPases not only in brain tissue but also in other types of preparation. Thus it has been found to increase ATPase activity in rat liver mitochondria in the presence and absence of Mg^{2+} (Chari-Bitron and Bino, 1971; Bino *et al.*, 1972) and to have an inhibitory effect on Na^+-K^+- and Mg^{2+}-ATPases in rat ileum microsomes (Laurent *et al.*, 1974; Laurent and Roy, 1975), on Ca^{2+}-ATPase activity in mouse heart microsomes (Collins and Haavik, 1979) and on Na^+-K^+-ATPase activity in an electric eel preparation and in Ehrlich ascites tumor cells (Toro-Goyco *et al.*, 1978).

There is no reason for believing that the ability of delta-9-THC to inhibit ATPases is linked with its ability to produce changes in mood and behavior. Thus CBN and CBD have been found to be as potent as delta-9-THC in inhibiting Na^+-K^+- and Mg^{2+}-ATPases in mouse or rat brain synaptosomes (Olmsted, 1976; Hershkowitz and Szechtman, 1979). Moreover, 11-hydroxy-delta-9-THC has been reported to be less effective than delta-9-THC as an inhibitor of Na^+-K^+- and Mg^{2+}-ATPases in rat brain synaptosomes (Olmsted, 1976) and CBD has been shown to be more potent than delta-9-THC as an inhibitor of Mg^{2+}-ATPase in rat brain synaptic vesicles (Gilbert *et al.*, 1977). Similarly, in experiments with mouse heart microsomes, the order of potency for inhibition of Ca^{2+}-ATPase has been found to be CBD > delta-9-THC > delta-6a,10a-dimethylheptyl-THC (Collins and Haavik, 1979).

7.3. Adenylate Cyclase and Guanylate Cyclase

In mice, *in vivo* administration of delta-9-THC has been shown to have a dose-dependent multi-phasic effect on brain levels of cyclic adenosine 3′,5′-monophosphate (cyclic AMP), the direction of the change produced in whole brain and in various brain areas varying with dose (Table 27). A dose-dependent effect of delta-9-THC on mouse brain adenylate cyclase activity has also been observed (Dolby and Kleinsmith, 1977). In contrast, CBN did not alter the activity of this enzyme. In rats, delta-9-THC injected intravenously at a dose of 10 mg/kg was reported to have no effect on brain concentrations of cyclic AMP (Table 27). At the same dose, delta-8-THC increased cyclic AMP levels in midbrain although not in cerebral cortex, hypothalamus, cerebellum or medulla (Askew and Ho, 1974). Delta-8-THC was also shown by Askew and Ho (1974) to decrease the activities of adenylate cyclase and cyclic nucleotide phosphodiesterase in the midbrain and to increase cyclic nucleotide phophodiesterase activity in the cerebellum.

Leader *et al.*, (1981) reported that delta-9-THC, at doses of 32 and 100 mg/kg i.p., attenuated increases in rat cerebellar cyclic guanosine 3′,5′-monophosphate (cyclic GMP) concentrations induced by isoniazid, an inhibitor of glutamic acid decarboxylase and

therefore of GABA synthesis. Levonantradol had the same effect at markedly lower doses whereas dextronantradol was inactive (Leader *et al.*, 1981; Koe *et al.*, 1985). Levonantradol was also shown to attenuate increases in cerebellar cyclic GMP levels induced by apomorphine and to depress basal cerebellar cyclic GMP levels (Leader *et al.*, 1981). The ability of delta-9-THC and levonantradol to oppose isoniazid-induced increases in cyclic GMP levels was taken as evidence in favor of the hypothesis that these cannabinoids can facilitate GABA function in the brain.

Several experiments have been carried out to test whether *in vitro* administration of delta-9-THC can alter either basal adenylate cyclase activity or the ability of a range of drugs to activate this enzyme. The experiments have yielded somewhat variable results, micromolar concentrations of delta-9-THC being without effect in some preparations and having stimulant or depressant effects in others (Table 28).

In experiments with mouse brain homogenates, Dolby and Kleinsmith (1977) reported that *in vitro* administration of delta-9-THC enhanced the ability of norepinephrine to stimulate adenylate cyclase. This effect of norepinephrine was also enhanced by CBN and 11-hydroxy-delta-9-THC, both of which were more potent than delta-9-THC. None of these cannabinoids changed the basal activity of adenylate cyclase or the activity of phosphodiesterase. Hillard and Bloom (1983) found that delta-9-THC could enhance the formation of cyclic AMP initiated in homogenates of mouse cerebral cortex by the addition of ATP and GTP. The delta-9-THC concentration–response curve was U shaped, concentrations above as well as below the effective concentration (30 μM) having no detectable effect on cyclic AMP synthesis. Similar results were obtained with CBD and levonantradol, which were both more potent than delta-9-THC and with CBN and 11-hydroxy-delta-9-THC. Dextronantradol, the (+) isomer of levonantradol, had no detectable effect on cyclic AMP formation. In further experiments with the same preparation, Bloom and Hillard (1985) found that delta-9-THC could also enhance the formation of cyclic AMP induced by isoprenaline. Again, the delta-9-THC concentration–response curve was U shaped. More recently, Hillard *et al.*, (1986) showed that delta-9-THC could enhance the ability of glucagon to increase cyclic AMP concentrations in rat liver plasma membranes and in isolated rat hepatocytes. Glucagon stimulation of cyclic AMP production in liver plasma membranes was also enhanced by 11-hydroxy-delta-9-THC (30 μM) but not by CBN or CBD. Indeed, CBD attenuated the effect of glucagon. None of the cannabinoids affected the basal production of cyclic AMP. Li and Ng (1984) reported that adenylate cyclase activity in ventricular tissue of rat heart could be reduced by delta-9-THC although not by delta-8-THC. As in the experiments of Hillard and Bloom (1983), the concentration–response curve was U shaped, the greatest degree of inhibition being produced by a delta-9-THC concentration of 145 μM.

Kelly and Butcher (1979) showed that delta-9-THC could increase the accumulation of cyclic AMP in human cultured diploid lung fibroblasts. In the same preparation, the drug was found to attenuate the ability of epinephrine or prostaglandin E_1 to increase cyclic AMP concentrations either in the incubation medium or within the cells (Kelly and Butcher, 1973, 1979). The response to prostaglandin E_1 was also reduced by CBD and CBN.

In experiments with intact or broken mouse neuroblastoma cells, delta-9-THC was found to reduce the ability of prostaglandin E_1, prostacyclin, secretin, vasoactive intestinal protein and forskolin to stimulate adenylate cyclase (Howlett, 1984; Howlett and Fleming, 1984). The cannabinoid had no detectable effect on basal adenylate cyclase activity in intact neuroblastoma cells but reduced basal enzyme activity in a broken cell preparation (Howlett, 1984). Forskolin-induced activation of neuroblastoma cell membrane adenylate cyclase was also attenuated by levonantradol and, to a lesser extent, by delta-8-THC, CBN and CBD (Howlett and Fleming, 1984). Secretin-induced activation of the enzyme was attenuated by levonantradol, delta-8-THC and CBN but slightly enhanced by CBD (Howlett and Fleming, 1984).

Receptor-mediated activation of adenylate cyclase is thought to take place as follows (for reviews see Helmreich and Pfeuffer, 1985; Taylor and Merritt, 1986): (i) the agonist

TABLE 28. *Effects of* In Vitro *Administration of Delta-9-THC on the Activities of Adenylate Cyclase and Phosphodiesterase*

Concentration of Δ^9-THC (μM)	Vehicle	Tissue	Measured effect	Change produced	Reference
0.02, 2 and 212	Ethanol	Mouse brain homogenate	Basal levels of cyclic AMP	0	1
0.02	Ethanol	Mouse brain	Norepinephrine-induced rise	0	1
2 and 212		homogenate	in cyclic AMP level	+	
1 to 10	Emulphor/	Mouse cerebral	Basal rise in	0	2
30	ethanol/	cortex	cyclic AMP	+	
100	water	homogenate	level (GTP present)	0	
1 and 3	Emulphor/	Mouse cerebral	Isoprenaline-	0	3
10	ethanol	cortex	induced rise in	+	
30 and 100		homogenate	cyclic AMP level	0	
1 to 50	Emulphor/ ethanol	Rat liver plasma membranes	Basal levels of cyclic AMP	0	4
1 and 3	Emulphor/	Rat liver	Glucagon-induced rise	0	4
10 and 30	ethanol	plasma membranes	in cyclic AMP level	+	
1 and 30	Emulphor/ ethanol	Rat liver plasma membranes	Rises in cyclic AMP levels induced by various drugs*	0	4
30	Emulphor/ ethanol	Rat isolated hepatocytes	Glucagon-induced rise in cyclic AMP level	+	4
29 and 72	PVP/	Ventricular homogenate	Basal cyclic AMP level	0	5
145 to 578	saline	of rat heart		−	
1160				0	
3.2	Ethanol	Cultured human lung fibroblasts (intact)	Epinephrine-induced rise in cyclic AMP level	−	6
1.1 and 5.5	Ethanol	Cultured human lung fibroblasts (intact)	Basal cyclic AMP level	+	7
0.32 to 32	Ethanol	Cultured human lung fibroblasts (intact)	Prostaglandin E_1-induced rise in cyclic AMP level	−	6, 7
1 and 30	Ethanol	Cultured mouse neuroblastoma cells (intact)	Basal cyclic AMP level	0	8

TABLE 28. *Effects of* In Vitro *Administration of Delta-9-THC on the Activities of Adenylate Cyclase and Phosphodiesterase* (Continued)

Concentration of Δ^9-THC (μM)	Vehicle	Tissue	Measured effect	Change produced	Reference
1 and 30	Ethanol	Cultured mouse neuroblastoma cells (intact)	Prostacyclin-induced rise in cyclic AMP level	–	8
10	Ethanol	Neuroblastoma cell membranes	Basal cyclic AMP level	–	8
0.1 to 10	Ethanol	Neuroblastoma cell membranes	Rises in cyclic AMP levels induced by various drugs†	–	8, 9
1	Ethanol	S49 lymphoma cell membranes	Forskolin, isoprenaline or Mn^{2+}-induced rise in cyclic AMP level	0	10
1	Ethanol	C6 glioma cell membranes	Prostaglandin E_1-induced rise in cyclic AMP level	0	10
1	Ethanol	Soluble adenylate cyclase from rat sperm	Mn^{2+}-induced rise in cyclic AMP level	0	10
9.6	Ethanol	Synchronously dividing Tetrahymena pyriformis	Levels of cyclic AMP and cyclic GMP	–	11
0.02 and 212	Ethanol	Mouse brain homogenate	Phosphodiesterase activity	0	1
5 and 30	Ethanol	Neuroblastoma cell membranes	Phosphodiesterase activity	0	8

*Drugs: Forskolin, guanosine triphosphate (GTP), guanosine 5′-(beta, gamma-imino)-triphosphate and sodium fluoride. †Drugs: prostacyclin, prostaglandin E_1, secretin, vasoactive intestinal peptide and forskolin. Delta-9-THC did not inhibit activation of adenylate cyclase by guanosine 5′-(beta,gamma-imino)-triphosphate or sodium fluoride (Howlett, 1984). PVP is an abbreviation for polyvinylpyrrolidone. References: 1, Dolby and Kleinsmith, 1977; 2, Hillard and Bloom, 1983; 3, Bloom and Hillard, 1985; 4, Hillard *et al.*, 1986; 5, Li and Ng, 1984; 6, Kelly and Butcher, 1973; 7, Kelly and Butcher, 1979; 8, Howlett, 1984; 9, Howlett and Fleming, 1984; 10, Howlett *et al.*, 1986; 11, Zimmerman *et al.*, 1981.

binds to its receptor, (ii) the receptor associates with a guanine nucleotide-dependent protein, G_s, (iii) GTP displaces GDP from the alpha subunit of the G_s protein, (iv) the G_s protein dissociates into its alpha and beta–gamma subunits and (v) the GTP-alpha subunit complex combines with adenylate cyclase to produce an active enzyme complex. According to the same hypothesis, GTP bound to the alpha subunit is later hydrolysed, causing deactivation of the adenylate cyclase and allowing reassociation of the alpha and beta–gamma subunits of the G_s protein. A similar GTP-dependent mechanism may be responsible for receptor-mediated inhibition of adenylate cyclase, the receptor combining with a G_i protein to trigger binding of GTP to G_i and subsequent enzyme inhibition.

It has been postulated that the ability of cannabinoids to attenuate drug-induced activation of neuroblastoma cell membrane adenylate cyclase is mediated by G_i protein (Howlett, 1984, 1985; Howlett and Fleming, 1984; Howlett *et al.*, 1986). The most direct evidence for this hypothesis is that pertussis toxin, which is thought to act by blocking the action of G_i protein, abolished the inhibitory effects of delta-9-THC and desacetyllevonantradol on secretin-induced activation of adenylate cyclase in neuroblastoma cells (Howlett *et al.*, 1986). Hillard and Bloom (1983) observed that increases in cyclic AMP levels induced by delta-9-THC in mouse brain homogenate took place only in the presence of exogenously added GTP. This too points to an involvement of G protein. They also observed that the effect of delta-9-THC on cyclic AMP levels in this preparation could be prevented by the phospholipase A_2 inhibitor, quinacrine, and by the cyclo-oxygenase inhibitors, acetyl salicylic acid and indomethacin. Since there is evidence that delta-9-THC can increase prostaglandin synthesis (Section 7.4) and that prostaglandins can stimulate the production of cyclic AMP, they proposed that delta-9-THC enhanced cyclic AMP formation in mouse brain by increasing prostaglandin levels, the prostaglandins binding to receptors and thereby activating G_s protein and adenylate cyclase. A hypothesis has also been put forward to explain the ability of delta-9-THC to enhance glucagon-stimulated cyclic AMP production in rat liver plasma membranes (Hillard *et al.*, 1986). In this case, no mention is made of a possible involvement of prostaglandins. The hypothesis proposes that the cannabinoid acts by increasing the efficiency with which glucagon-occupied receptors interact with G_s protein. Support for the hypothesis comes from data that rule out likely alternative mechanisms. In essence, the data suggest that the delta-9-THC-induced increases in cyclic AMP production (i) require the presence of glucagon and (ii) occur after glucagon receptor occupation but before G_s protein activation (Hillard *et al.*, 1986).

7.4. PHOSPHOLIPASES A_2 AND C, CYCLO-OXYGENASE AND THROMBOXANE SYNTHETASE

Results from experiments with a variety of *in vitro* preparations (Table 29) suggest that delta-9-THC can raise the concentration of free arachidonic acid thereby increasing the availability of this acid for conversion to a number of products including prostaglandins, prostacyclins, thromboxanes and leukotrienes. The cannabinoid is thought to act by stimulating phospholipase A_2 which catalyses the hydrolysis of acylester bonds of phospholipids in the 2-position and hence brings about the release of phospholipid-bound fatty acids including arachidonic acid (Burstein and Hunter, 1984; Martin, 1986). Results from recent experiments with a crude synaptosomal preparation derived from mouse brain suggest that delta-9-THC may also stimulate phospholipase C (Hunter *et al.* 1986). This enzyme catalyses the conversion of phosphoinositides to diacylglycerol and inositol triphosphate. Diacylglycerol is thought to be hydrolysed subsequently to its constituent fatty acids (usually stearic acid and arachidonic acid) and glycerol by the action of diacylglycerol lipase and it is noteworthy that delta-9-THC has been reported to be an inhibitor of this lipase (Hunter *et al.*, 1986). There is evidence that inositol triphosphate and diacylglycerol function as second messengers (see Abdel-Latif, 1986, for a recent review). Thus it is thought that interactions between agonists and certain types of receptor, for example the $alpha_1$-adrenoceptor, can lead to the activation of phospholipase C and hence to an

increase in the production of inositol triphosphate and diacylglycerol, the former subsequently triggering intracellular Ca^{2+} mobilization and the latter activation of Ca^{2+}/phospholipid-dependent protein kinase. It is too early, however, to comment on the possible consequences of any interactions between cannabinoids and this putative second messenger system.

Although delta-9-THC has been shown to increase the synthesis of prostaglandins in some *in vitro* preparations, in others it has been reported to inhibit prostaglandin synthesis (Table 29). This inhibitory effect probably reflects an ability to inhibit cyclo-oxygenase since, in some experiments, delta-9-THC has been found to reduce prostaglandin synthesis from added free arachidonic acid. There is also evidence from experiments with lysed human platelets that delta-9-THC can inhibit thromboxane synthetase although not arachidonate lipoxygenase (White and Tansik, 1980). Hence delta-9-THC seems to have two opposing effects on the synthesis of prostaglandins, prostacyclins and thromboxanes. In some systems its stimulatory effect on the activity of phospholipases appears to predominate whereas in other systems, the drug may act primarily to inhibit cyclo-oxygenase and (at least in some systems) thromboxane synthetase and diacylglycerol lipase.

There is evidence that cannabinoids other than delta-9-THC, including CBN, CBD, cannabichromene, cannabicyclol and hydroxylated metabolites of delta-9-THC, share the ability of delta-9-THC to stimulate phospholipase A_2 (Burstein and Hunter, 1978, 1981; White and Tansik, 1980; Burstein *et al.*, 1982a, 1983, 1984, 1985a, b, 1986a; Hunter *et al.*, 1986) and to inhibit cyclo-oxygenase (Burstein *et al.*, 1973; Spronck *et al.*, 1978; Dalterio *et al.*, 1978; White and Tansik, 1980; Reich *et al.*, 1982) and thromboxane synthetase (White and Tansik, 1980). The ability to interact with each of these enzymes has been shown to vary from cannabinoid to cannabinoid. Thus, for example, White and Tansik (1980) reported that CBD was almost 4 times less potent than delta-9-THC as an inhibitor of thromboxane synthetase in lysed human platelets. With regard to the other two enzymes, there is little sign of any correlation between the ability of cannabinoids to alter enzyme activity and psychoactive potency.

In a recent study, Burstein *et al.* (1986b) showed that although the delta-9-THC metabolite, delta-9-THC-11-oic acid, had no effect on prostaglandin E_2 synthesis in human lung fibroblast cultures when given by itself, it could reduce the stimulant effect of delta-9-THC on prostaglandin synthesis. Results from additional experiments suggested that the two cannabinoids were interacting with different enzymes, delta-9-THC acting primarily to stimulate phospholipase A_2 and its metabolite to inhibit cyclo-oxygenase. In other experiments with human lung fibroblasts, Burstein *et al.* (1986a) found that the (−) isomers of delta-9-THC, delta-8-THC and delta-8-dimethylheptyl-THC were more potent than their (+) isomers in stimulating prostaglandin E_2 production. Interestingly, these cannabinoids did not show significant stereoselectivity in their stimulant effect on release of phospholipid-bound arachidonic acid. Burstein *et al.* (1985b) have also reported that repeated exposure of human lung fibroblast cultures to cannabinoids (delta-9-THC, CBD or cannabicyclol) rapidly rendered the cells tolerant to cannabinoid-induced stimulation of prostaglandin E_2 synthesis. Tolerance also developed to cannabinoid-induced stimulation of release of phospholipid-bound arachidonic acid, suggesting that the cells had become tolerant to the stimulant effect of the cannabinoids on phospholipase activity.

In line with the results from *in vitro* experiments, there are a number of reports concerning changes in prostaglandin production induced by delta-9-THC *in vivo*. Thus a dose of delta-9-THC (2 mg i.p.) which prevented the proestrous rise in rat plasma levels of luteinizing hormone, follicle-stimulating hormone and prolactin and which delayed rat ovulation was found to prevent rises in rat ovarian prostaglandin E content normally occurring on the evening of proestrus (Ayalon *et al.*, 1977). In the same study, delta-9-THC was shown to have no effect on hypothalamic concentrations of prostaglandin E. However, in other rat experiments, Coupar and Taylor (1982) found that a dose of delta-9-THC producing marked hypothermia and catatonia (2 mg/kg i.v.) reduced the concentration of

TABLE 29. *Effects of* In Vitro *Administration of Delta-9-THC on the Production of Free Arachidonic Acid and Prostaglandins*

Concentration of Δ^9-THC (μM)	Vehicle	Preparation	Measured effect	Change produced	Reference
16 and 160	Ethanol	HeLa cells	Release of phospholipid-bound ^{14}C-arachidonic acid	+	1, 2
3.2 to 32	Ethanol	Human lung WI-38 fibroblasts	Release of phospholipid-bound ^{14}C-arachidonic acid	+	3, 4, 5
8 to 32	Ethanol	Mouse peritoneal macrophages	Release of phospholipid-bound ^{14}C-arachidonic acid	+	6
80	Ethanol	Intact platelets from human blood	Release of phospholipid-bound ^{14}C-arachidonic acid	+	7
64	Ethanol	Mouse C1300 neuroblastoma cells (NBA_2)	Release of phospholipid-bound ^{14}C-arachidonic acid	+	7
3.2. to 32	Ethanol	Rat testis Leydig cells	Release of phospholipid-bound ^{14}C-arachidonic acid	+	2
32	Ethanol	Neuroblastoma cells (N2a)	Release of phospholipid-bound ^{14}C-arachidonic acid	+	8
32	Ethanol	C-6 glioma cells	Release of phospholipid-bound ^{14}C-arachidonic acid	+	8
32	Ethanol	Mouse brain synaptosomal fraction	Release of phospholipid-bound ^{14}C-arachidonic acid	+	9
1.6, 8 and 16	Ethanol	Mouse brain synaptosomal fraction	Production of ^{14}C-arachidonic acid from ^{14}C-arachidonyl-phosphatidylcholine	+	9
1.6 to 16	Ethanol	Mouse brain myelin fraction	Production of ^{14}C-arachidonic acid from ^{14}C-arachidonyl-phosphatidylcholine	+	9
1.6 to 16	Ethanol	Mouse brain mitochondrial fraction	Production of ^{14}C-arachidonic acid from ^{14}C-arachidonyl-phosphatidylcholine	+	9
8	Ethanol	Human lung WI-38 fibroblasts	Production of ^{14}C-PGE_2 from phospholipid-bound ^{14}C-arachidonic acid	+	10
0.8 to 320	Ethanol	Human lung WI-38 fibroblasts	Production of PGE or PGE_2	+	3, 5, 10
c.a. 0.16 to 1.6	Ethanol	Human lung WI-38 fibroblasts	Production of PGE_2	+	4

TABLE 29. *Effects of* In Vitro *Administration of Delta-9-THC on the Production of Free Arachidonic Acid and Prostaglandins* (Continued)

Concentration of Δ^9-THC (μM)	Vehicle	Preparation	Measured effect	Change produced	Reference
32	Ethanol	Neuroblastoma (N2a) cells	Production of PGE	+	8
32	Ethanol	C-6 glioma cells	Production of PGE	+	8
40	Ethanol	Lysed platelets	Production of labeled PGE_2 and	+	7
100 ($=IC_{50}$)		from human blood	$PGF_{2\alpha}$from ^{14}C-arachidonic acid	−	
9.6	Propylene glycol/ethanol	Ovine seminal vesicle microsomal fraction	Production of ^{14}C-PGE_2 from ^{14}C-arachidonic acid	−	11
318 ($=IC_{50}$)	Ethanol	Bovine seminal vesicle microsomal fraction	Production of ^{14}C-PGE_1 from ^{14}C-8,11,14-eicosatrienoic acid	−	12
110 ($=IC_{50}$)	?	Ovine seminal vesicle microsomal fraction	Rate of metabolism of 8,11,14-eicosatrienoic acid	−	13
39.8	Ethanol	Decapsulated mouse testis	Production of PGE and PGF in the presence of human chorionic gonadotropin	−	14
50 to 200	Ethanol	Rat ovary cultured Graafian follicles	Production of PGE in the presence of luteinizing hormone	−	15
750 ($=IC_{50}$)	?	Rat brain crude synaptosomal fraction	Release of $PGF_{2\alpha}$	−	16

References: 1, Burstein and Hunter, 1978; 2, Burstein and Hunter, 1981; 3, Burstein *et al.*, 1982a; 4, Burstein *et al.*, 1985b; 5, Burstein *et al.*, 1986b; 6, Burstein *et al.*, 1984; 7, White and Tansik, 1980; 8, Burstein *et al.*, 1985a; 9, Hunter *et al.*, 1986; 10, Burstein *et al.*, 1983; 11, Burstein and Raz, 1972; 12, Burstein *et al.*, 1973 13, Spronck *et al.*, 1978; 14, Dalterio *et al.*, 1978; 15, Reich *et al.*, 1982; 16, Raffel *et al.*, 1976.

'prostaglandin E_2-like material' in the hypothalamus. Concentrations in whole brain, cerebral cortex, midbrain, cerebellum and pons/medulla were not altered. More recently, Bhattacharya (1986) found that at a dose of 2 mg/kg i.p., delta-9-THC increased concentrations of prostaglandin E_2 and $F_{2\alpha}$ in rat whole brain. Signs of delta-9-THC-induced increases in prostaglandin synthesis have also been detected in isolated perfused guinea pig lung (Kaymakcalan *et al.*, 1975) and, *in vivo*, in the uteri of progesterone pretreated ovariectomized rats injected simultaneously with estradiol (Jordan and Castracane, 1976). In addition, they have been detected in mouse peritoneal macrophages taken from animals given delta-9-THC before removal of the cells (Burstein *et al.*, 1985a). It is noteworthy that in this study *in vivo* pretreatment with the psychically inactive cannabinoid CBD did not stimulate prostaglandin synthesis. This observation complicates the interpretation of structure–activity data derived from *in vitro* experiments with cannabinoids since CBD appears to be more potent than delta-9-THC as a stimulator of prostaglandin synthesis or phospholipase A_2 when added directly to *in vitro* preparations (Burstein and Hunter, 1978; White and Tansik, 1980; Burstein *et al.*, 1983).

Fairbairn and Pickens (1979, 1980) proposed that the cataleptic effect of delta-9-THC in mice may depend on the presence of prostaglandin E_2. They found that delta-9-THC-induced catalepsy could be abolished by inhibitors of cyclo-oxygenase and that the effect of these inhibitors on this response to delta-9-THC could be reversed by administration of prostaglandin E_2. They also found that the cataleptic response to delta-9-THC was reduced in mice that had been maintained on a diet deficient in arachidonic acid. Arachidonic acid administration restored the response. In a more recent study, Burstein *et al.* (1982b) found that the hypotensive effect of delta-9-THC in anesthetized dogs could be reduced by aspirin pretreatment and suggested that delta-9-THC decreases dog blood pressure by stimulating the production of a vasoactive metabolite of arachidonic acid, possibly prostacyclin.

TABLE 30. *The Effect of Delta-9-THC on the 'Fluidity' of Phospholipids and Phospholipid-containing Membranes*

Phospholipid or membrane preparation	Technique	Fluidity change	Reference
Egg yolk phosphatidylcholine/cholesterol spin-labeled vesicles	Electron spin resonance	+	1
Dipalmitoylphosphatidylcholine	Differential scanning calorimetry	+	2
Dipalmitoylphosphatidylcholine bilayers	Differential scanning calorimetry	+	3
Dioleoylphosphatidylcholine bilayers	Differential scanning calorimetry	+	3
Dipalmitoylphosphatidylcholine/ distearoylphosphatidylcholine bilayers	Differential scanning calorimetry	+	3
Dioleoylphosphatidylcholine/ distearoylphosphatidylcholine bilayers	Differential scanning calorimetry	+	3
Dipalmitoylphosphatidylcholine/ cholesterol bilayers	Differential scanning calorimetry	+	3
Egg yolk phosphatidylcholine vesicles	Nuclear magnetic resonance	0	4
Egg yolk phosphatidylcholine/ cholesterol vesicles	Nuclear magnetic resonance	+	4
Egg yolk phosphatidylcholine vesicles	Fluorescence polarization	−	5
Egg yolk phosphatidylcholine/ cholesterol vesicles	Fluorescence polarization	+	5
Dimyristoylphosphatidylcholine vesicles	Fluorescence polarization	+	5
Dimyristoylphosphatidylcholine/ cholesterol vesicles	Fluorescence polarization	+	5
Synaptic plasma membranes	Fluorescence polarization	+/−*	5

*'Fluidity' was increased by delta-9-THC concentrations of 1 to 10 μM but decreased by a concentration of 30 μM. Delta-9-THC was either premixed with phospholipids (Refs 1, 2 and 3) or added to vesicular preparations in which case the drug was dissolved in dimethyl sulfoxide (Refs 4 and 5). References: 1, Lawrence and Gill, 1975; 2, Bach *et al.*, 1976b; 3, Bruggemann and Melchior, 1983; 4, Tamir and Lichtenberg, 1983; 5, Hillard *et al.*, 1985.

8. MEMBRANE STUDIES

Experiments using a variety of physical methods have yielded results which suggest that delta-9-THC can produce small but significant changes in the 'fluidity' of phospholipid-containing membranes (Table 30). In some experiments (Lawrence and Gill, 1975; Bach *et al.*, 1976b; Bruggemann and Melchior, 1983), delta-9-THC was incorporated into the membranes while they were being prepared and the results obtained from these studies suggested that the drug could increase membrane fluidity. In other experiments (Tamir and Lichtenberg, 1983; Hillard *et al.*, 1985), delta-9-THC was added after the membranes had been prepared. In these studies the effect on membrane fluidity was shown to vary both with the concentration of delta-9-THC used and with membrane composition. For example, when added to rat brain synaptic plasma membranes, delta-9-THC had a concentration-dependent biphasic effect on polarization of the fluorescence emission of 1,6-diphenyl-1,3,5-hexatriene (DPH; Hillard *et al.*, 1985). At delta-9-THC concentrations of 1 to 10 μM, polarization decreased (a sign of increased fluidity) whereas at a concentration of 30 μM, polarization increased. In contrast, only decreases in fluorescence polarization of DPH were detected when delta-9-THC was added to vesicles prepared from cholesterol plus egg yolk phosphatidylcholine or dimyristoylphosphatidylcholine or from dimyristoylphosphatidylcholine alone. These decreases occurred at delta-9-THC concentrations ranging from about 1 μM to 30 μM.

In experiments with mice, Leuschner *et al.* (1984) studied the effect of *in vivo* administration of delta-9-THC on the fluidity of erythrocyte membranes. Intravenous doses of 3.2, 10 and 16 mg/kg all increased membrane fluidity as measured by electron spin resonance. When the dose used was 10 mg/kg, the plasma concentration of delta-9-THC at the time of blood sampling (+60 min) was about 1 μM. The membrane concentrations of delta-9-THC ranged from 1 to 10 μmoles per kg dry membrane and it was calculated that for each delta-9-THC molecule in the membrane there were about 20 000 phospholipid molecules.

In addition to delta-9-THC, several other cannabinoids have been shown to alter membrane fluidity. Bach *et al.* (1976b) showed that like delta-9-THC, CBD affected the transition of dipalmitoylphosphatidylcholine from the gel to the liquid crystalline state by decreasing both the melting temperature and the enthalpy of melting. Similarly, in fluorescence experiments with vesicles prepared from cholesterol plus egg yolk phosphatidylcholine or dimyristoylphosphatidylcholine or from dimyristoylphosphatidylcholine alone, Hillard *et al.* (1985) obtained evidence that CBD acted like delta-9-THC to increase membrane fluidity. In both of these studies, CBD was no less potent or effective than delta-9-THC as a fluidizing agent. In other experiments, however, important differences between CBD and delta-9-THC have been detected, the results obtained not only with CBD and delta-9-THC but also with other cannabinoids demonstrating a correlation between the ability to increase the fluidity of phospholipid-containing membranes and psychoactive potency. For example, using an electron spin resonance technique, Lawrence and Gill (1975) found that the ability to fluidize egg yolk phosphatidylcholine/cholesterol vesicles decreased in the order (−) delta-9-dimethylheptyl-THC, 11-hydroxy-delta-9-THC, (−) delta-9-THC and (+) delta-9-THC. CBN and CBD both reduced membrane fluidity. It was suggested by Lawrence and Gill (1975) that the stereoselectivity observed in their experiments was due to the presence of cholesterol which, being optically active, imposed asymmetry on the lipid bilayer of the vesicles. In experiments with the fluorescent probe, DPH, Hillard *et al.* (1985) showed that 11-hydroxy-delta-9-THC was more effective although not more potent than delta-9-THC in fluidizing rat brain synaptic plasma membranes, that CBN was less potent than delta-9-THC and that CBD did not increase membrane fluidity at all. Interestingly, at a concentration of 30 μM, CBD and CBN, like delta-9-THC at the same concentration (see above), decreased fluidity in this membrane preparation. Tamir and Lichtenberg (1983), using nuclear magnetic resonance spectrometry, found that (−) delta-8-dimethylheptyl-THC was slightly more effective than (−) delta-9-THC in fluidizing egg yolk phosphatidylcholine/cholesterol vesicles. (−) Delta-8-

THC was less potent than (−) delta-9-THC whereas (+) delta-9-dimethylheptyl-THC and CBD reduced fluidity. The correlation between ability to increase membrane fluidity and psychoactive potency was not perfect in this study, however, since the psychically active cannabinoid, nabilone, was found to reduce membrane fluidity.

Lawrence and Gill (1975) observed that the fluidizing effect of delta-9-THC in vesicles prepared from egg yolk phosphatidylcholine and cholesterol rapidly leveled off as the drug concentration in the vesicular membrane was increased. A similar leveling off of effect was detected in the same study both with other cannabinoids and with the 'partial' anesthetics, hexadecanol and tetradecane. It was also detected in studies concerning the effects of cannabinoids on rat brain synaptic plasma membrane fluidity (Hillard *et al.*, 1985).

To investigate the role of membrane proteins in the effect of cannabinoids on the fluidity of synaptic membranes, Hillard *et al.* (1985) studied the ability of 11-hydroxy-delta-9-THC and CBD to alter fluorescence polarization of DPH in total lipid extracts of mouse brain synaptic plasma membranes. As in intact synaptic membranes, DPH polarization was increased by CBD and decreased by the 11-hydroxy compound, demonstrating that cannabinoids can produce their effects on synaptic membrane fluidity in the absence of membrane proteins.

The effect of cannabinoids on lipid membrane fluidity has been shown to be markedly influenced by cholesterol content. In experiments with vesicles containing egg yolk phosphatidylcholine, phosphatidic acid and cholesterol, Pang and Miller (1978) found that CBN decreased membrane fluidity when the cholesterol content of the vesicles was 20 mole% or less. However, when the cholesterol content was raised to 25 mole% or more, CBN had a fluidizing effect. In agreement with these findings, CBN decreased fluidity in rat liver mitochondrial membranes (cholesterol content 6 mole%) and increased fluidity in human erythrocyte ghost membranes (cholesterol content 40 mole%). In more recent studies, the addition of cholesterol to dipalmitoylphosphatidylcholine bilayers was found to enhance the ability of delta-9-THC to increase membrane fluidity (Bruggemann and Melchior, 1983; Tamir and Lichtenberg, 1983). Hillard *et al.*, (1985) found that several cannabinoids, including delta-9-THC, increased fluidity in egg yolk phosphatidylcholine vesicles containing cholesterol but decreased fluidity in vesicles consisting entirely of phosphatidylcholine. Egg yolk phosphatidylcholine forms relatively fluid bilayers that are far less rigid than those formed by phosphatidylcholine mixed with cholesterol (Pang and Miller, 1978) and indeed there is evidence to suggest that cannabinoids tend to decrease the ordering of molecules in rigid phospholipid bilayers and to increase ordering in bilayers that are more fluid (Pang and Miller, 1978; Hillard *et al.*, 1985).

Other evidence that cannabinoids can alter the physical properties of membranes derives from reports concerning the effects of cannabinoids on the stability or morphology of individual cells, subcellular organelles and model membranes. As shown in Table 31, there are signs from such experiments that delta-9-THC can stabilize membranes. Several of the studies referred to in Table 31 included experiments with CBN (Sofia *et al.*, 1974) or CBD (Raz *et al.*, 1972, 1973; Sofia *et al.*, 1974; Raz and Goldman, 1976; Hershkowitz *et al.*, 1977) and both of these were found to behave much like delta-9-THC. In some experiments, a concentration-dependent biphasic effect of cannabinoids on membrane stability has been observed. Thus, in their experiments with rat erythrocytes, Sofia *et al.* (1974) found that delta-9-THC protected against hemolysis at concentrations of 20 to 50 μM and promoted hemolysis at a concentration of 75 μM. Essentially similar results were obtained with CBN and CBD. Other erythrocyte studies with delta-9-THC or CBD (0.1 to 100 μM) revealed only an antihemolytic effect (Chari-Bitron, 1971; Raz *et al.*, 1972). It is noteworthy, however, that in one of these studies (Raz *et al.*, 1972) the antihemolytic effect of CBD diminished when the concentration was raised above 20 μM. Raz *et al.* (1973) found that delta-9-THC and CBD reduced leakage of acid phosphatase from rat liver lysosomes at a concentration of 2 μM but enhanced the leakage at concentrations of 50 and 100 μM. These results are supported by those of Britton and Mellors (1974) who also detected increases in acid phosphatase release from rat liver lysosomes in the presence

TABLE 31. *Effects of* In Vitro *Administration of Delta-9-THC on Membrane Stability and Organelle Morphology*

Concentration of Δ^9-THC (μM)	Vehicle	Preparation or organelle	Measured effect	Response	Reference
c.a. 1 to 80	Ethanol	Rat erythrocytes	Hemolysis	Protection	1
c.a. 0.5 to 100	?	Human erythrocytes	Hemolysis	Protection	2
c.a. 10 to 50	Phosphate buffer	Rat erythrocytes	Hemolysis	Protection	3
c.a. 6 to 12	Ethanol	Egg yolk phosphatidyl-choline vesicles	Osmotic lysis	Slight protection	4
c.a. 2 to 80	Ethanol	Erythrocyte lipid vesicles	Osmotic lysis	Protection	4
c.a. 2 to 40	Ethanol	Lamb brain lipid vesicles	Osmotic lysis	Protection	4
2	Methanol	Rat liver lysosomes	Release of acid phosphatase	Reduction	5
50 and 100				Enhancement	
250 to 1000	Ethanol +DMSO	Rat liver lysosomes	Release of acid phosphatase	Enhancement	6
10 to 16	Ethanol	Rat liver mitochondria	Release of malate dehydrogenase	Enhancement	7
10 to 16	Ethanol	Rat liver mitochondria	Morphology	Swelling	7
17 to 150	Ethanol	Rat liver mitochondria	Morphology	Swelling	8
200 to 300	Ethanol	Bull sperm mitochondria	Morphology	Swelling	9
100	Ethanol	Mouse peritoneal macrophage mitochondria	Morphology	Some swelling	10
100	Ethanol	Mouse peritoneal macrophages	Morphology	Extensive vacuolization	10
10	Ethanol	Mouse brain synaptosomes	Morphology	No detectable change	11
50	Ethanol	Mouse brain synaptosomes	Morphology	Disruption	11
10	Ethanol	Rat erythrocytes	Morphology	No detectable change	12
15 to 35	Ethanol	Rat erythrocytes	Morphology	Invagination of cells	12

DMSO is an abbreviation for dimethyl sulfoxide. References: 1, Chari-Bitron, 1971; 2, Raz *et al.*, 1972; 3, Sofia *et al.*, 1974; 4, Alhanaty and Livne, 1974; 5, Raz *et al.*, 1973; 6, Britton and Mellors, 1974; 7, Mahoney and Harris, 1972; 8, Bino *et al.*, 1972; 9, Shahar and Bino, 1974; 10, Raz and Goldman, 1976; 11 Hershkowitz *et al.*, 1977; 12, Chari-Bitron and Shahar, 1979.

of high concentrations of delta-9-THC (Table 31). Bino *et al.* (1972) showed that the degree of mitochondrial swelling induced by delta-9-THC increased with concentration in the range 17 to 56 μM but decreased in response to higher concentrations. Similarly, Alhanaty and Livne (1974) found that egg yolk phosphatidylcholine vesicles were protected against osmotic lysis by delta-9-THC concentrations of about 6 to 12 μM but were not protected by concentrations of 20, 40 or 60 μM. Hershkowitz *et al.* (1977) found that at a concentration of 50 μM both delta-9-THC and CBD severely disrupted mouse brain synaptosomes. This observation must be borne in mind when interpreting neuropharmacological data from experiments with synaptosomes.

In view of the evidence that cannabinoids can alter the physical properties of membranes it is noteworthy that there is also evidence that delta-9-THC can alter membrane composition within the brain and affect the biosynthesis of membrane lipids.

Six hours after intraperitoneal injection of delta-9-THC at a dose of 10 mg/kg, Sarkar and Ghosh (1975) detected changes per unit weight of protein in rat brain levels of total lipid, total phospholipid and total cholesterol. There were increases in the amounts of these lipid components in a microsomal fraction and decreases in mitochondrial, synaptosomal and myelin fractions of the brain. The changes in phospholipid content were attributed to changes in phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, the amount of sphingomyelin present remaining unaltered. In some fractions, changes in ganglioside and sialoglycoprotein content were also detected (Sarkar and Ghosh, 1976). None of the fractions studied showed any change in total cerebroside content.

In experiments with mice, delta-9-THC given intraperitoneally 30 min before an intravenous injection of [methyl-^{14}C]-choline was found to alter the amount of radioactive label incorporated into the brain (Wing and Paton, 1979). At an ambient temperature of 22°C, the incorporation of radioactivity into a brain lipid fraction was reduced by a dose of 15 mg/kg although unaffected by a dose of 3.75 mg/kg. Incorporation of radioactivity into an aqueous fraction of the brain was not reduced by either dose. The effect of the larger dose was attenuated but not abolished when the experiment was repeated at a higher ambient temperature (33.5°C) to prevent hypothermia. Under these conditions, 3.75 mg/kg of delta-9-THC increased incorporation of label into both the brain lipid fraction and the aqueous fraction.

In experiments with mouse spleen lymphocytes, Greenberg and Mellors (1978) showed that delta-9-THC, at a concentration of 10 μM, significantly inhibited ^{14}C-acetate incorporation into the membrane lipids phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and triglyceride. In the same preparation, delta-9-THC was shown to inhibit lysophosphatidylcholine acyltransferase, an enzyme catalysing the formation of phosphatidylcholine from lysophosphatidylcholine and coenzyme A-activated fatty acids (Greenberg *et al.*, 1977; Greenberg and Mellors, 1978; Mellors, 1979). It also inhibited lysophosphatidate acyltransferase which catalyses the conversion of lysophosphatidic acid to phosphatidic acid, a precursor of phospholipids and triglycerides (Mellors, 1979). Lysophosphatidylcholine acyltransferase was inhibited by concentrations of delta-9-THC ranging from 0.2 to 10 μM, the concentration for half maximal inhibition (K_i) being 0.35 μM. The K_i for the other enzyme was 6 μM. Inhibition of lysophosphatidylcholine acyltransferase was also detected in mouse brain synaptosomes after *in vitro* administration of delta-9-THC ($K_i = 0.3$ μM) and in mouse spleen lymphocytes and mouse brain synaptosomes 2 hr after *in vivo* administration of delta-9-THC, albeit with doses (20 to 70 mg/kg i.v.) far higher than are required to produce behavioral changes in mice (Huszar *et al.*, 1977; Greenberg *et al.*, 1978; Mellors, 1979). In addition to delta-9-THC, a number of other cannabinoids were found to inhibit lysophosphatidylcholine acyltransferase when added directly to lymphocytes or synaptosomes. The most potent inhibitors were psychically active, the ability to inhibit the enzyme decreasing in the order delta-9-THC, delta-8-THC, 11-hydroxy-delta-9-THC, synhexyl and CBN (Greenberg and Mellors, 1978; Greenberg *et al.*, 1978; Mellors, 1979). The psychically inactive cannabinoid, cannabigerol, was equipotent with synhexyl.

At concentrations ranging upwards from about 3 μM, cannabinoids have been reported to inhibit cholesterol esterase activity in preparations obtained from brain, adrenal gland, liver, pancreas, ovary and testis and in cultures of human dermal fibroblasts and of human aortic medial cells (Burstein *et al.*, 1978, 1979a,b, 1980; Shoupe *et al.*, 1980; Cornicelli *et al.*, 1981). In a microsomal preparation obtained from rat brain, the ability of cannabinoids to inhibit the enzyme decreased in the order delta-9-THC, CBN and 11-hydroxy-delta-9-THC (Burstein *et al.*, 1980). In cultured human aortic medial cells, however, CBN and CBD were reported to be more potent than delta-9-THC as inhibitors of the enzyme (Cornicelli *et al.*, 1981). From studies with a purified cholesterol esterase preparation, Shoupe *et al.* (1980) concluded that delta-9-THC is a competitive inhibitor of this enzyme and that it can compete with cholesterol esters for a position on the active site of the enzyme because its structure is somewhat similar to that of cholesterol. Cornicelli *et al.* (1981) found that in cultured human fibroblasts, although delta-9-THC, CBN and CBD reduced the incorporation of ^{14}C-oleate into cholesterol esters, they did not alter the incorporation of label into cellular phospholipid or triglyceride.

Finally, there is evidence that as well as inhibiting the production of membrane phospholipids, cannabinoids can also hasten their metabolism by stimulating phospholipases A_2 and C (Section 7.4).

9. MOLECULAR MECHANISMS

There is strong evidence that the primary sites of action of delta-9-THC reside in neuronal cellular and subcellular membranes. Thus, as discussed elsewhere in this review, the drug has been shown to alter the transport of transmitters and ions across membranes, to change the activities of important membrane-bound enzymes and to affect the physical properties of membranes.

The ability of cannabinoids to interact with neuronal membranes, like their ability to produce changes in mood and behavior is markedly influenced by their chemical shape and structure. This raises the possibility that there might be a specific cannabinoid receptor (Binder and Franke, 1982; Binder *et al.*, 1979, 1984; Harris *et al.*, 1978; Nye *et al.*, 1985) and has led to a search for binding sites in the brain. In studies with rat brain tissue, Harris *et al.* (1978) were unable to detect saturable, high affinity binding sites for ^{3}H-delta-8-THC. They did, however, detect such sites in intact hepatoma cells. These contained two populations of binding site, one of which had a very high affinity for the tritiated ligand.

The cannabinoids are particularly difficult substances with which to carry out binding studies. Their low water solubility introduces the need for a special vehicle (Section 4) and their high lipid solubility gives rise to a considerable amount of non-specific binding (Harris *et al.*, 1978). In addition, delta-9-THC has been shown to bind avidly to glass, plastic, rubber, stainless steel and filter paper (Garrett and Hunt, 1974; Kujtan *et al.*, 1983). In order to circumvent some of these difficulties, Nye *et al.* (1985) carried out binding studies with ^{3}H-5′-trimethylammonium delta-8-THC (TMA), a positively charged, hydrophilic analogue of delta-8-THC. Their experiments with TMA demonstrated the presence of single populations of saturable, high affinity binding sites both in various regions of rat brain (affinity constant = 0.01 litres/nmole in crude cerebral cortical membrane homogenates) and in a number of peripheral organs. They also showed that like delta-8- and delta-9-THC, TMA could reduce the size of the twitch response induced in the isolated guinea-pig ileum by electrical stimulation. Its ED_{50} was 10 times greater than that of delta-9-THC. The correlation between the ability of cannabinoids to displace TMA from its binding sites and psychoactive potency was poor, however, suggesting that TMA binding sites do not mediate effects of cannabinoids on mood or behavior. An alternative interpretation, yet to be fully explored, is that psychically inactive cannabinoids with a high affinity for TMA binding sites are delta-9-THC antagonists. It is also possible that the TMA binding sites mediate actions of the cannabinoids other than those which are responsible for psychoactivity (Nye *et al.*, 1985).

The finding that there is a correlation between psychoactive potency and the ability to produce changes in the physical properties of artificial membranes containing only cholesterol and phospholipid (Section 8) weakens the main argument for the existence of a specific cannabinoid receptor. Indeed, Lawrence and Gill (1975) have proposed that psychoactive cannabinoids do not interact with a specific receptor but rather with the lipid phase of the cell membrane and that the pharmacological activity of these substances results from a structure-dependent ability to disorder membrane lipids. According to this hypothesis, pharmacological activity depends on 'awkwardness of fit into the hydrocarbon matrix' rather than on goodness of fit between drug and site of action (Gill and Lawrence, 1976). Differences in the abilities of cannabinoids to alter membrane fluidity can be attributed to the presence of cholesterol, which being chiral, would be expected to impose asymmetry on the lipid phase of the membrane (Lawrence and Gill, 1975; Gill and Lawrence, 1976). Burstein and Hunter (1981) have pointed out that delta-9-THC is a competitive inhibitor of cholesterol esterase (Section 8) and have speculated that the cannabinoid may be able to 'mimic' membrane cholesterol. More recently, Bruggemann and Melchior (1983) proposed that delta-9-THC acts by forming a complex with some specific membrane lipid to produce changes in membrane function these changes possibly resulting from an alteration in membrane organization. In addition, they suggested that membrane cholesterol could influence the interaction of delta-9-THC with the membrane lipid, the cholesterol having either an inhibitory or a facilitatory effect depending on its concentration in the membrane. It has also been suggested that cannabinoids may induce conformational changes in membrane proteins or modify interactions between membrane proteins and lipids (Leuschner *et al.*, 1984).

Further support for the notion that the psychoactivity of delta-9-THC is mediated by mechanisms which require a particular chemical structure comes from evidence that nonspecific molecular interactions of the sort that probably underlie anesthesia do not contribute significantly towards delta-9-THC's psychoactive properties. Thus, unlike general anesthetics, there is no correlation among the cannabinoids between psychoactive potency and lipid solubility (Section 4). Moreover, the cannabis 'high' is quite different from the collection of signs and symptoms experienced after the taking of alcohol or other general anesthetics at subanesthetic doses (Paton and Pertwee, 1973a, 1973b). There is also evidence from several studies (Seeman *et al.*, 1972; Roth and Williams, 1979; Leuschner *et al.*, 1984) to suggest that delta-9-THC can elicit pharmacological responses when its concentration in cell membranes is markedly less than that which is needed, according to the Meyer–Overton rule, to produce either local anesthesia (about 40 mmoles/kg dry membrane) or general anesthesia (about 3 mmoles/kg dry membrane; Seeman, 1972). For example, Roth and Williams (1979) using data produced by Gill and Lawrence (1974), calculated that behaviorally effective doses of delta-9-THC would give rise to membrane concentrations in mouse brains of about 20 μmoles/kg dry membrane. Similarly, Leuschner *et al.* (1984) calculated that the concentration of delta-9-THC in erythrocyte membranes taken from mice pretreated with behaviorally effective doses of the drug ranged from 1 to 10 μmoles/kg dry membrane. It is also noteworthy that some psychically active cannabinoids have been reported to be far more potent in inhibiting lymphocytic or synaptosomal lysophosphatidylcholine acyltransferase than was to be expected from their anesthetic activity as measured by their molar volume or their ability to protect erythrocytes against hemolysis (Greenberg and Mellors, 1978; Greenberg *et al.*, 1978). This is of interest because the ability of cannabinoids to inhibit lysophosphatidylcholine acyltransferase has been found to correlate with psychoactive potency (Section 8).

Since cannabinoids are highly lipid soluble compounds, they should, according to the Meyer–Overton rule, have anesthetic properties (Seeman, 1972). Indeed, delta-9-THC has been shown to decrease the amplitude of action potentials in isolated preparations of rabbit vagus nerve and rat phrenic nerve (Section 5). However, the action potentials were by no means abolished. Moreover, in whole animals, delta-9-THC has never been shown to produce general anesthesia. It has been proposed, therefore, that delta-9-THC is a 'partial anesthetic', unable in spite of its high lipid solubility to reach a concentration in

nerve membranes sufficiently large to produce complete anesthesia (Paton *et al.*, 1972). This may be because it has such a low solubility in water, the maximum concentration that any substance can attain in membrane lipid being a function not only of its membrane/extracellular fluid partition coefficient but also of its solubility in extracellular fluid (Seeman, 1972).

Even though delta-9-THC cannot itself produce general anesthesia, there is evidence that it can, at relatively low doses, decrease the concentrations of conventional general anesthetics or barbiturates required to produce antinociception or loss of consciousness. Vitez *et al.* (1973) showed that delta-9-THC could reduce the minimum alveolar concentration (MAC) of cyclopropane required to abolish the motor response of rats to clamping of the tail. MAC was reduced about 15% by 1 mg/kg i.p. of delta-9-THC and about 25% by 2 mg/kg i.p. of the cannabinoid. No experiments were carried out with these doses of delta-9-THC in the absence of cyclopropane and it is therefore not clear whether the cannabinoid was acting additively, sub-additively or synergistically. Using the rat tail flick test, Novelli *et al.* (1983) found that delta-9-THC (10 mg/kg i.p.) could potentiate the antinociceptive effect of nitrous oxide. Both drugs were active in the test when given by themselves. When given together they had a super-additive effect. In experiments with mice, Morrison and Pertwee (1985) found that delta-9-THC (20 mg/kg i.p.) brought about a reduction (about 40%) in the dose of sodium barbitone needed to abolish the righting reflex, the dose required to abolish this reflex in 50% of a group of mice (ED_{50}) decreasing from approximately 230 mg/kg s.c. to 140 mg/kg s.c. In an earlier study with mice, Pertwee (unpublished) found that doses of delta-9-THC ranging from 2 to 20 mg/kg i.v. reduced the loss of righting reflex ED_{50} of diethyl ether (inhaled) by 10% (see also Paton, 1974). The mechanism by which delta-9-THC can augment the antinociceptive or hypnotic effects of general anesthetics and barbiturates remains to be elucidated. It is possible that these interactions reflect the ability of delta-9-THC to act as a 'partial anesthetic' and it would be of interest, therefore, to determine if other cannabinoids can also augment the effects of anesthetics and, if they do, whether this ability correlates best with chemical structure or with lipid solubility.

Reports that the ability of cannabinoids to increase the 'fluidity' of membranes correlates with psychoactive potency (Section 8) raise the possibility that such fluidity changes may underlie important cannabinoid-induced changes in membrane function. It is noteworthy, therefore, that delta-9-THC has been shown to increase membrane fluidity only at concentrations well above those at which it can produce functional changes in membranes, in particular, facilitation of the synaptosomal uptake of NE and DA (Sections 6.1.3 and 6.2.3). Either such changes in membrane function do not arise from delta-9-THC-induced increases in membrane fluidity or they are produced by fluidity changes that are too subtle to detect.

Several of the neuropharmacological changes produced by delta-9-THC, *in vivo* or *in vitro*, seem to be biphasic in nature, the direction of change depending on the dose used. Often, there is evidence that the ability of low doses of cannabinoids to produce a change in one direction correlates with psychoactive potency whereas the ability of higher doses to produce the opposite effect is independent of cannabinoid structure. Examples include effects on the amplitude of potentials evoked in the cerebral cortex (Section 5), on the synaptosomal uptake of NE and DA (Sections 6.1.3 and 6.2.3) and on the fluidity of synaptic plasma membranes (Section 8). The existence of such biphasic effects implies that, at least at some sites, cannabinoids have two types of action, one that is structure-dependent and detectable at low doses, and the other that is non-specific and detectable only at higher doses. It is noteworthy that the ability of cannabinoids to produce changes at low doses does not always parallel psychic activity. For example, both delta-9-THC and CBD have been reported to be highly potent as inhibitors of depolarization-dependent Ca^{2+} uptake into brainstem synaptosomes (Section 5). Indeed, this action has been postulated to account for the anticonvulsant properties of delta-9-THC and CBD (Harris and

Stokes, 1982). It is also noteworthy that not all 'high dose' effects of cannabinoids are independent of chemical structure, an example in this case being the hypothermic effect of cannabinoids (Pertwee, 1985a).

In conclusion, in spite of its high lipid solubility, the psychic activity of delta-9-THC probably depends on an ability to undergo structure-dependent interactions with neuronal membranes rather than on non-specific membrane interactions of the sort which are thought to underlie general anesthesia. There is still no detailed information either about the molecular basis of these interactions or about the way in which they alter membrane function. Thus, it is not yet known whether delta-9-THC interacts with a specific delta-9-THC receptor or undergoes some other kind of structure-dependent interaction with membrane phospholipids or proteins. The receptor hypothesis has little experimental support at present since no one has yet developed a satisfactory selective delta-9-THC antagonist (Martin, 1986) or demonstrated the presence of specific delta-9-THC binding sites in the brain. The failure to detect such binding sites may of course simply reflect the extra practical difficulties associated with delta-9-THC binding studies rather than an absence of receptors.

10. GENERAL DISCUSSION AND SUMMARY

10.1. The Role of Transmitters in the Psychopharmacology of Cannabinoids

10.1.1. *Norepinephrine, Dopamine and 5-Hydroxytryptamine*

In vivo experiments directed at examining the ability of delta-9-THC to alter turnover of NE, DA or 5-HT by measuring its effects on changes in the brain levels of these amines induced by inhibition of their synthesis or metabolism have yielded variable results (Sections 6.1, 6.2 and 6.3). In some experiments, delta-9-THC had no apparent effect on turnover whereas in others either increases or decreases in turnover were detected. Equally variable results were obtained when effects of delta-9-THC were measured on levels of NE, DA or 5-HT in the brains of animals that had not received a second drug treatment. Brain levels rose or fell in some experiments and remained unchanged in others. This variability is no doubt due in part to the study of different brain areas and to the use of different species, different ambient temperatures and different doses of delta-9-THC.

In vivo and *in vitro* experiments designed to detect delta-9-THC-induced changes in the rates of synthesis of NE, DA or 5-HT in the brain have yielded more consistent data (Sections 6.1, 6.2 and 6.3). The results suggest that delta-9-THC can accelerate the rate of formation of NE and DA from tyrosine and that it can increase 5-HT synthesis by raising brain levels of the 5-HT precursor, tryptophan. There is also evidence from *in vitro* experiments with brain tissue that delta-9-THC can alter the neuronal uptake or storage of NE, DA, 5-HT and tyrosine (Sections 6.1.3, 6.2.3 and 6.3.3), inhibit monoamine oxidase (Section 7.1), increase the affinity of DA D_2 receptors for DA agonists (Section 6.7) and enhance the coupling of central beta-adrenoceptors to adenylate cyclase (Section 7.3).

Effects most likely to contribute to the psychoactivity of delta-9-THC are those which can be produced by concentrations of 1 μM or less (Section 4). These include (i) facilitation of synaptosomal uptake of ^{3}H-NE and ^{3}H-DA, (ii) increased retention of ^{3}H-NE and ^{3}H-DA by preloaded synaptosomes and (iii) inhibition of synaptosomal uptake of ^{3}H-5-HT (Table 32). The first of these effects is of special interest since *in vivo* administration of delta-9-THC has also been shown to facilitate *in vitro* uptake of NE and DA into brain synaptosomes (Sections 6.1.3 and 6.2.3). The doses used were rather high and further *in vivo* experiments are required to test whether delta-9-THC can produce similar changes in synaptosomal uptake when given at lower doses. Also noteworthy is evidence from *in vitro* studies with synaptosomes, albeit with only a limited number of cannabinoids, that the ability to facilitate ^{3}H-NE and ^{3}H-DA uptake and to increase ^{3}H-NE and ^{3}H-DA retention correlates with psychoactive potency. Correlations with psychoactive potency have also been observed for some effects so far detected *in vitro* only at concentrations of 1 μM or above (Table 32).

The extent to which interactions of delta-9-THC with central NE, DA or 5-HT releasing neurons can account for its effects in man, for example the production of mood changes or psychoses (Section 2) or the suppression of nausea and vomiting induced by anticancer drugs (Section 3), remains to be established. There is evidence, however, for an involvement of DA in certain behavioral effects produced by delta-9-THC in animals. For example, Gough and Olley (1977, 1978) have obtained results from experiments with rats which suggest that delta-9-THC can produce catalepsy by interacting directly with the striatum to reduce dopaminergic transmission. In line with the evidence that delta-9-THC can produce biphasic changes in central neuronal activity there are also signs that the drug can produce behavioral changes by augmenting transmission along central dopaminergic pathways. Thus results obtained by Conti and Musty (1984) from experiments with rats suggest that delta-9-THC may increase dopaminergic transmission in the nucleus accumbens, this in turn giving rise to increases in locomotor activity. In more recent studies, Sakurai *et al.* (1985) found that delta-9-THC shared the ability of methamphetamine to elicit ipsilateral circling behavior in rats with unilateral lesions in the substantia nigra. This circling behavior could be prevented by haloperidol, supporting the hypothesis that delta-9-THC had acted by increasing dopaminergic transmission in the surviving nigrostriatal pathway.

10.1.2. *Acetylcholine*

Delta-9-THC has been shown to reduce ACh release both in cat somatosensory cortex (Section 6.5) and in peripheral nerve-containing preparations (Section 5). It has also been shown to reduce ACh turnover in rat and mouse hippocampus and in rat striatum. ACh turnover in several other brain areas was not affected by delta-9-THC. Reductions in ACh turnover in rat brain were produced by quite a low dose of delta-9-THC. Moreover, the ability to reduce hippocampal turnover of ACh in rats correlated well with psychoactive potency. It is likely that the psychic activity of delta-9-THC may depend at least in part on an ability to reduce the firing rate of ACh neurons in certain brain areas, particularly the hippocampus. Indeed, a delta-9-THC induced suppression of cholinergic transmission in the hippocampus could well account for the ability of delta-9-THC and cannabis to impair memory (Miller and Branconnier, 1983).

10.1.3. *Gamma-aminobutyric Acid*

GABA turnover has been shown to be increased by delta-9-THC in rat septum and substantia nigra but not in rat caudate nucleus or nucleus accumbens (Section 6.6). So far only rather high doses of delta-9-THC have been used to study its effect on GABA turnover and there is some reason for expecting that at lower doses, the drug may reduce turnover (Section 6.6). Delta-9-THC is known to produce a mixture of excitant and depressant effects, both behavioral and electrophysiological, a good example being its ability to act both as a convulsant and as an anticonvulsant (Section 5). It would therefore be of interest to know whether low doses of delta-9-THC can indeed reduce GABA turnover in the brain since this might well reflect an ability to reduce GABA release, an effect which could account for at least some of the excitant properties of this cannabinoid. Similarly, the finding that delta-9-THC can increase GABA turnover could reflect an ability to enhance GABA release. Such an effect might account for the anticonvulsant action of delta-9-THC and could possibly also explain reports that the drug has anxiolytic, hypnotic and muscle-relaxant properties (Musty, 1984; Hollister, 1986). Interestingly, CBD, which shares the ability of delta-9-THC to increase GABA turnover in the substantia nigra, has also been reported to be anxiolytic (Musty, 1984) and anticonvulsant (Section 5). Indeed, Consroe *et al.* (1982) have found that CBD could prevent tonic convulsions produced in mice by drugs thought to act by reducing GABAergic transmission but did not inhibit tonic convulsions induced by the glycine antagonist, strychnine. The hypothesis that delta-9-THC has a dose-dependent biphasic effect on transmission

TABLE 32. *The Lowest Concentrations at which Delta-9-THC has been Shown to be Active in* In Vitro *Studies with Brain Tissue or with Peripheral Nerve Preparations*

Concentration	Preparation or tissue	Measured effect and direction of change (±)	Correlation with psychoactivity*	Section
Delta-9-THC concentrations below 1 μM				
0.01 nM	Rat hippocampal cells	Amplitude of evoked potential (+)	ND	5
0.1 nM	Rat hippocampal cells	Amplitude of evoked potential (−)	ND	5
6.4 nM	Guinea-pig ileum	Electrically evoked twitch amplitude (−)	Yes	5
10 nM	Rat brain stem synaptosomes	Depolarization-dependent Ca^{2+} uptake (−)	No	5
50 nM	Rat brain synaptosomes	^{3}H-DA uptake (+)	Yes	6.2.3
100 nM	Rat brain synaptosomes	^{3}H-DA retention (+)	Yes	6.2.3
	Rat brain synaptosomes	^{3}H-NE uptake (+)	Yes	6.1.3
	Rat brain synaptosomes	^{3}H-NE retention (+)	Yes	6.1.3
	Rat brain synaptosomes	^{3}H-5-HT uptake (−)	No	6.3.3
	Rat striatal synaptosomes	^{14}C-DA uptake (−)	ND	6.2.3
	Guinea-pig hippocampal cells	Amplitude of evoked potential (+)	ND	5
	Guinea-pig hippocampal cells	Paired pulse inhibition (−)	ND	5
300 nM	Mouse brain synaptosomes	Lysophosphatidylcholine acyltransferase activity (−)	Yes	8
Delta-9-THC concentrations of 1 to 10 μM				
1 μM	Mouse brain synaptosomes	^{3}H-5-HT uptake (+)	Yes	6.3.3
	Rat brain synaptosomes	^{3}H-DA retention (−)	No	6.2.3
	Rat brain synaptic vesicles	Mg^{2+}-ATPase activity (+)	ND	7.2
	Rat/mouse brain synaptosomes	Na^+-K^+-ATPase activity (−)	No	7.2
	Guinea-pig hippocampal cells	Amplitude of evoked potential (−)	ND	5
	Rat brain synaptic plasma membranes	Membrane fluidity (+)	Yes	8
2 μM	Mouse brain homogenate	Activation of adenylate cyclase by NE (+)	No	7.3
	Mouse brain synaptosomes	Activity of phospholipases (+)	ND	7.4
3 μM	Mouse brain synaptosomes	^{3}H-Tyrosine uptake (−)	ND	6.2.2
	Mouse brain synaptosomes	Conversion of ^{3}H-tyrosine to ^{3}H-NE (+)	ND	6.1.2
	Mouse striatal synaptosomes	Conversion of ^{3}H-tyrosine to ^{3}H-DA (+)	ND	6.2.2
	Mouse cerebral cortex	^{3}H-dihydroalprenolol binding (+)	Yes	6.7
	Rat/mouse brain synaptosomes	Mg^{2+}-ATPase activity (−)	No	7.2
	Rat brain microsomes	Cholesterol esterase activity (−)	No	8
5 μM	Rat/mouse brain synaptosomes	Choline uptake (−)	No	6.5.2
	Mouse brain	^{3}H-naloxone binding (−)	ND	6.7

TABLE 32. *The Lowest Concentrations at which Delta-9-THC has been Shown to be Active in* In Vitro *Studies with Brain Tissue or with Peripheral Nerve Preparations* (Continued)

Concentration	Preparation or tissue	Measured effect and direction of change (±)	Correlation with psychoactivity*	Section
10 μM	Rabbit vagus nerve	Amplitude of action potential (−)	ND	5
	Rat/mouse brain synaptosomes	^{3}H-NE uptake (−)	No	6.1.3
	Rat/mouse brain synaptosomes	^{3}H-DA uptake (−)	No	6.2.3
	Rat/mouse brain synaptosomes	^{3}H-GABA uptake (−)	No	6.6
	Rat striatal synaptosomes	^{3}H-DA retention (−)	No	6.2.3
	Pig brain	Monoamine oxidase activity (−)	Yes	7.1
	Mouse brain synaptosomes	Mg^{2+}-ATPase activity (+)	ND	7.2
	Mouse cerebral cortex	Activation of adenylate cyclase by isoprenaline (+)	ND	7.3
	Mouse cerebral cortex	Attenuation of ^{3}H-dihydroalprenolol binding by beta-adrenoceptor antagonists (+)	ND	6.7
Delta-9-THC concentrations above 10 μM				
20 μM	Mouse brain synaptosomes	Mg^{2+}-Ca^{2+}-ATPase activity (−)	ND	7.2
30 μM	Mouse brain synaptosomes	^{3}H-ADTN (D_3) binding (−)	No	6.7
	Mouse brain homogenate	Basal activity of adenylate cyclase (+)	No	7.3
	Mouse striatum	^{3}H-spiperone (D_2) binding (−)	No	6.7
	Mouse striatum	DA-induced attenuation of ^{3}H-spiperone binding (+)	Yes	6.7
	Rat brain synaptic plasma membranes	Membrane fluidity (−)	No	8
50 μM	Mouse brain synaptosomes	Morphological integrity (−)	No	8
100 μM	Rat brain synaptosomes	^{3}H-NE retention (−)	No	6.1.3
	Rat brain synaptosomes	^{3}H-5-HT retention (−)	ND	6.3.3
	Mouse brain synaptosomes	^{3}H-5-HT uptake (−)	No	6.3.3
	Frog sciatic nerve	Voltage-dependent Na^+ conductance (−)	ND	5
750 μM	Mouse brain synaptosomes (crude preparation)	Release of prostaglandin $F_{2\alpha}$(−)	ND	7.4

*The evidence is usually based on studies with very few cannabinoids and must therefore be interpreted with caution. ND = not determined.

along certain GABA-releasing pathways is supported by the evidence that at low doses, it can oppose recurrent inhibition in the hippocampus, whereas at higher doses it has the opposite effect (Section 5).

In view of the evidence that delta-9-THC can facilitate GABA transmission in the brain, it is noteworthy that the drug has also been reported to undergo synergistic interactions with benzodiazepines, drugs which are thought to produce many of their effects by enhancing the response to neuronally released GABA. Thus delta-9-THC (100 mg/kg s.c.) has been shown in experiments with mice to enhance the ability of diazepam to suppress pentylenetetrazol-induced convulsions (Koe *et al.*, 1985). In addition, at a much lower dose (20 mg/kg i.p.), delta-9-THC has been found to abolish the righting reflex of mice pretreated with doses of flurazepam or chlordiazepoxide well below those required to abolish this reflex in the absence of the cannabinoid (Morrison and Pertwee, 1985). There is also evidence that delta-9-THC and benzodiazepines have a super-additive hypothermic effect in rats and mice (Pryor *et al.*, 1977; Morrison and Pertwee, 1985). It is worth noting, therefore, that delta-9-THC retained the ability to abolish the righting reflex in mice pretreated with sub-hypnotic doses of chlordiazepoxide when the ambient temperature was kept at 34°C to prevent hypothermia (Morrison and Pertwee, 1985). Interestingly, the synergism between delta-9-THC and benzodiazepines seems to be far greater than that between delta-9-THC and barbiturates or general anesthetics (Morrison and Pertwee, 1985; see also Section 9).

Although synergistic interactions between delta-9-THC and benzodiazepines could be the result of a delta-9-THC-induced enhancement of GABA release, there are other equally plausible explanations. It is possible, for example, that the cannabinoid acts to increase the affinity of benzodiazepines for their receptors and indeed a dose of delta-9-THC which potentiates the anticonvulsant effect of diazepam has been shown also to enhance the '*in vivo*' binding of ^{3}H-flunitrazepam to mouse brain (Section 6.7.2; Koe *et al.*, 1985). Similar results have been obtained with levonantradol. In these experiments, all the drugs were administered *in vivo* so that it is possible that delta-9-THC and levonantradol could have acted by increasing the entry of ^{3}H-flunitrazepam into the brain rather than by augmenting ^{3}H-flunitrazepam binding. Although levonantradol has also been shown to enhance benzodiazepine binding in experiments in which the benzodiazepine was added directly to brain tissue (Section 6.7.2), such experiments have not yet been carried out with delta-9-THC. Other possible mechanisms for a delta-9-THC-benzodiazepine synergism include delta-9-THC-induced changes in the metabolism or disposition of benzodiazepines, an area which has yet to be explored, and delta-9-THC-induced increases in the synaptic concentration of GABA achieved through inhibition of GABA uptake into nerve terminals (Section 6.6). There is at present no evidence that delta-9-THC interacts with benzodiazepines by enhancing the affinity of GABA for its receptors. Indeed, levonantradol has been shown to reduce GABA binding to brain tissue (Section 6.7.1).

10.2. THE SYNAPSE—A PRIMARY SITE OF ACTION?

There are several reasons for believing that the synapse is an important site of action for delta-9-THC. These may be summarized as follows.

(i) After entry into the brain there is evidence that delta-9-THC and its metabolites accumulate in the grey matter (Kennedy and Waddell, 1972; Shannon and Fried, 1972) and may be particularly tightly bound to nerve endings (Hattori and McGeer, 1977).

(ii) There is some indirect evidence from electrophysiological experiments that delta-9-THC can alter synaptic transmission in the central nervous system and that it does so at doses lower than those required to impair impulse conduction along peripheral axons (Section 5).

(iii) Results from *in vitro* experiments suggest that delta-9-THC can produce a number of important changes at nerve terminals. Thus it has been shown to affect the synthesis, storage and/or fate of NE, DA, 5-HT and GABA in synaptosomes (Section 6), the depolarization-dependent uptake of Ca^{2+} into synaptosomes (Section 5), the activities of ATPases present in synaptosomal or synaptic vesicular membranes (Section 7.2), the activity of synaptosomal lysophosphatidylcholine acyltransferase (Section 8) and the 'fluidity' of brain synaptic plasma membranes (Section 8). Of particular interest are effects which can be produced by delta-9-THC at concentrations of 1 μM or less (Section 4), especially if in addition they can only be produced by psychically active cannabinoids. Among such effects are certain delta-9-THC-induced changes in the synaptosomal uptake and storage of NE and DA and delta-9-THC-induced inhibition of lysophosphatidylcholine acyltransferase (Table 32). Delta-9-THC has also been shown to be highly potent in its inhibitory effect on depolarization-dependent synaptosomal uptake of Ca^{2+}. Results from experiments with CBN, CBD and 11-hydroxy-delta-9-THC (Section 5) have suggested that the ability of cannabinoids to produce this effect does not correlate with psychoactive potency.

(iv) There is evidence that as well as interacting with nerve terminals, delta-9-THC may act postsynaptically. For example, the results of binding studies (Section 6.7) suggest that delta-9-THC can increase the affinity of DA D_2 receptors for DA. However, the concentration of delta-9-THC used in these studies, 30 μM, was rather high. CBD did not alter DA binding. There is also evidence that delta-9-THC can enhance the coupling of central beta-adrenoceptors to adenylate cyclase (Section 7.3).

10.3. Final Comments

It is clear from the material presented in this review that the central effects of delta-9-THC are mediated by more than one transmitter and that it can produce neuropharmacological changes in many areas of the brain (Section 10.1). There is good evidence for an involvement of NE, DA, 5-HT, ACh and GABA. Further studies are required to investigate the role of HA and spermidine and experiments are also needed to test whether peptides or amino acids other than GABA have a part to play in the psychopharmacology of delta-9-THC. The synergism between delta-9-THC and benzodiazepines is intriguing. Not only could it throw light on the mode of action of delta-9-THC but it could also come to be exploited clinically.

The synapse seems to be a particularly important target for delta-9-THC and there are signs that it can act presynaptically to alter neurotransmitter synthesis, storage, release and fate and postsynaptically to alter neurotransmitter receptor-mediated events both at the level of the recognition site and at the level of second messenger systems (Sections 7.4 and 10.2). The extent to which each of these changes contributes towards the psychological, behavioral, physiological and neuropharmacological effects of delta-9-THC in the whole organism remains to be determined.

It is likely that the neuropharmacological basis for each of the numerous effects produced by delta-9-THC *in vivo* is not always the same and that the part played by particular transmitters and brain areas varies from effect to effect. The possible roles of hippocampal ACh-releasing neurons in the effect of delta-9-THC on memory, of DA-releasing neurons of the nucleus accumbens in the effect of the drug on locomotor activity and of DA-releasing neurons of the striatum in the cataleptic effect of delta-9-THC have already been mentioned (Sections 10.1.1 and 10.1.2). There is also evidence that delta-9-THC produces its effects on heart rate, blood pressure and respiration by interacting with the brain stem (Hosko *et al.*, 1984) and that its effects on thermoregulation involve interactions with both brain stem and hypothalamus (Pertwee, 1985a).

Little is known about the nature of the molecular events underlying the effects of delta-9-THC other than that many, including psychic activity, probably depend on the ability of the drug to undergo structure-dependent interactions with neuronal membranes rather

than on its high lipid solubility (Section 9). In particular, it is not yet known whether there is a specific delta-9-THC receptor. Also unknown is the extent to which membrane fluidity changes produced by delta-9-THC account for its psychopharmacological properties. The dose-dependent biphasic nature of many of the responses to delta-9-THC points to the existence of more than one mode of action. Consistent with this conclusion is the evidence that the relationship between cannabinoid structure and pharmacological activity is not the same for all responses.

The observation that the effect of cannabinoids on membrane fluidity can be altered by changing the chemical composition of the membrane (Section 8) raises the possibility that slight differences in the composition of neuronal membranes within the brain could impose some degree of selectivity on cannabinoids by causing them to interact more readily with some neuronal pathways than with others. Since there is evidence that delta-9-THC can change the lipid composition of brain membranes (Section 8) the question also arises as to whether changes in membrane composition could lead to delta-9-THC tolerance. Indeed, it has been suggested that changes in membrane lipid composition could have accounted for the development of tolerance to the stimulatory effect of cannabinoids on arachidonic acid release and prostaglandin production in lung fibroblasts (Burstein *et al.*, 1985b and Section 7.4). A similar 'membrane lipid adaptation' mechanism has been proposed to account for ethanol tolerance (Littleton, 1978; Little and Wing, 1983).

The extent to which some of the actions of delta-9-THC, for example those which affect the rate of removal of neurotransmitter from the synapse, alter transmission along a particular pathway probably depends on the pathway's 'ongoing activity'. The observations that changes in ambient temperature can alter the effect of delta-9-THC on brain levels of 5-HT (Section 6.3.1) and that stress can influence the effect of *in vivo* administration of delta-8-THC on the conversion in the brain of tyrosine to NE (Section 6.1.2) support this view since ambient temperature and stress are both expected to influence central neuronal activity. Because ongoing neuronal activity in the brain is highly variable, dependence on it could be one reason why the effect of delta-9-THC on central transmitter turnover appears to vary from brain area to brain area. Another reason for this could be that some of the changes in transmitter turnover produced by delta-9-THC are initiated indirectly, 'upstream' of the sites at which they are detected.

In conclusion, research into the central neuropharmacology of psychically active cannabinoids has led to advances in our understanding of how cannabinoids act at the electrophysiological level and has provided knowledge about the effects of these drugs on the synthesis, storage, release, actions and fate of central neurochemicals. It has also provided convincing evidence that the cannabinoids exert their central effects through interactions with targets located in cellular and/or subcellular membranes. There is still much to be learnt about the central effects of cannabinoids especially with regard to the underlying molecular mechanisms and with regard to the part played by individual neurotransmitters. Cannabinoid research would be greatly facilitated if a selective delta-9-THC antagonist were to be developed and it is therefore important to establish the basis of the stereoselectivity shown by cannabinoids and, in particular, to resolve the question of whether or not there is a specific delta-9-THC receptor. The wide-ranging neuropharmacological properties of psychically active cannabinoids form a unique pharmacological spectrum that distinguishes the cannabinoids from all other centrally active drugs and, consequently, the notion that cannabinoids could be the prototypes of important novel therapeutic agents, is well worth pursuing. Although some modest advances have already been made in the search for roles for the cannabinoids in modern medicine, further basic preclinical research with these drugs is required if their therapeutic potential is to be fully explored.

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