



Modulation by fluoxetine of striatal dopamine release following Δ^9 -tetrahydrocannabinol: a microdialysis study in conscious rats

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1 The present study was undertaken to investigate the effect of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and possible serotonergic involvement on the extracellular level of dopamine (DA) in the striatum using microdialysis in conscious, freely-moving rats.

2 A dose-dependent increase in striatal DA release occurred after i.v. administration of 0.5–5 mg kg⁻¹ Δ^9 -THC when compared with vehicle ($n=5-8$, $P<0.05$). Maximum increases, ranging from 42.1 ± 5.4% to 97.4 ± 5.9% (means ± s.e.mean) of basal levels occurred 20 min after Δ^9 -THC. This effect was abolished by pretreatment with the cannabinoid CB₁ receptor antagonist, SR 141716 (2.5 mg kg⁻¹ i.p.).

3 Pretreatment with fluoxetine (10 mg kg⁻¹ i.p.) abolished the Δ^9 -THC-induced DA release. Fluoxetine 10 mg kg⁻¹ i.p. administered 40 min after Δ^9 -THC had no significant effect on Δ^9 -THC-induced DA release. However, fluoxetine perfused locally into the striatum by adding it to the microdialysis perfusion fluid (10 μ M) 40 min after Δ^9 -THC significantly potentiated the Δ^9 -THC-induced DA release ($n=6-8$, $P<0.05$).

4 These results suggest that DA release induced by Δ^9 -THC is modulated by serotonergic changes induced by fluoxetine, the effect of which depends on the time of its administration relative to that of Δ^9 -THC. Fluoxetine induces an acute increase in extracellular 5-HT through reuptake inhibition, which can activate autoreceptors which may decrease serotonergic neuronal activity. This may be the reason fluoxetine pretreatment abolished the Δ^9 -THC-induced DA release. The potentiation of Δ^9 -THC-induced DA release by fluoxetine perfusion added 40 min after Δ^9 -THC may be due to an acute increase in 5-HT produced by reuptake inhibition.

Keywords: Δ^9 -THC; 5-HT; dopamine; microdialysis; fluoxetine

Abbreviations: DA (dopamine); FLU (fluoxetine); 5-HT (5-hydroxytryptamine); SR (SR 141716); Δ^9 -THC (Δ^9 -tetrahydrocannabinol)

Introduction

Δ^9 -Tetrahydrocannabinol (Δ^9 -THC) is the major psychoactive constituent of the plant *Cannabis sativa*, one of the most commonly used drugs of abuse in the world (Grinspoon & Bakalar, 1993). Δ^9 -THC produces stereotypical behaviours and has a pronounced depressive effect on locomotor activity. It has been suggested that the nigrostriatal dopaminergic system plays an important role in the manifestation of these behaviours (Navarro *et al.*, 1994). In support of the role of dopamine (DA) in these actions, the results from a number of studies using *in vivo* microdialysis have reported an increase in extracellular levels of DA following Δ^9 -THC administration in various regions of the nigrostriatal and mesolimbic systems (Chen *et al.*, 1990a,b; Ng Cheong Ton *et al.*, 1998; Tanda *et al.*, 1997). In addition, *in vitro* experiments using rat striatal slices have shown that Δ^9 -THC can facilitate the release of DA (Sakurai Yamashita *et al.*, 1989).

There is also considerable evidence to suggest that various effects of Δ^9 -THC are mediated by serotonergic mechanisms. A number of studies using rat forebrain (Johnson *et al.*, 1976) or whole brain homogenates (Sofia *et al.*, 1971) have reported increased levels of extracellular 5-hydroxytryptamine (5-HT) following Δ^9 -THC administration. However, another study using rat striatal slices showed no significant effect (Navarro *et al.*, 1994). Nevertheless, further evidence of Δ^9 -THC affecting

serotonergic mechanisms is apparent through the alteration of the behavioural effects of Δ^9 -THC, using drugs affecting 5-HT systems (Davies & Graham, 1980; Taylor & Fennessy, 1978; Verberne *et al.*, 1980). More recently, we published a study that showed that the CB₁ receptor mediated hypothermia can be augmented or antagonized by the addition of the selective serotonin reuptake inhibitor (SSRI), fluoxetine (Malone & Taylor, 1998b). The direction of the observed modulation was dependent on the time of administration of fluoxetine relative to that of Δ^9 -THC. From these results, we hypothesized that the amount of extracellular 5-HT influenced the postjunctional effects observed by altering the activity of the 5-HT neurones by acting prejunctionally. Furthermore, we speculated that CB₁ receptor activation by Δ^9 -THC results in an increase in extracellular 5-HT in order to produce hypothermia.

Several lines of evidence suggest that the serotonergic fibres projecting from the dorsal raphe nucleus (DRN) can alter dopaminergic activity in the mesolimbic and nigrostriatal systems (Benloucif & Galloway, 1991; Blandina *et al.*, 1989; Jacocks & Cox, 1992; Nedergaard *et al.*, 1988; Schmidt *et al.*, 1992; Sinton & Fallon, 1988; Yadid *et al.*, 1994). Through neuroanatomical studies, the projection of serotonergic neurones from the rostral DRN to DA cell bodies in the ventral tegmental area (VTA) and the substantia nigra (SN) as well as their respective terminal projections in the NAcc and the striatum have been identified (Benloucif & Galloway, 1991). Furthermore, administration of 5-HT or the SSRI,

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alaproclate, perfused into the striatum has been reported to dose-dependently facilitate striatal DA release (Benloucif & Galloway, 1991; Yadid *et al.*, 1994). In light of this, and the neuroanatomical evidence for serotonergic modulation of DA release in the striatum discussed previously, the present study was undertaken to examine the effect of fluoxetine on the Δ^9 -THC-induced DA release. This study was undertaken using microdialysis in the striatum of conscious, freely-moving rats. A preliminary account of these findings has been presented at the Fourth IUPHAR Satellite Meeting on Serotonin (Malone & Taylor, 1998a).

Methods

Animals and housing

Male Glaxo-Wistar rats weighing between 260 and 380 g were used. Prior to experiments, they were housed in group cages and kept at 22°C with a 12 h light–dark cycle. Food and water were available *ad libitum*.

Surgery

Two days prior to experimentation, rats were anaesthetized with a sodium methohexitone (18 mg kg⁻¹ i.p.)/sodium amylobarbitone (30 mg kg⁻¹ i.p.) mixture. A polyethylene (PE 50) catheter was surgically inserted into the external jugular vein for i.v. administration. The skull was exposed and a hole (2 mm i.d.) was drilled for the implantation of a 21 gauge microdialysis guide cannula above the left striatum (coordinates: L +20 mm, R +2.3 mm, V -5.0 mm). The coordinates were chosen according to the stereotaxic atlas of Paxinos & Watson (1986). The guide cannula was held in place using dental cement anchored to two stainless steel screws implanted into the skull. A stainless steel 25-gauge needle, cut to size, was inserted into the cannula as an obturator. Body temperature was maintained at 37°C with a heating pad used throughout the course of the operation. Following the operation, rats were injected with ticarcillin 15 mg kg⁻¹ i.v. to prevent infection.

Microdialysis

Prior to implantation, each rat was lightly anaesthetized with halothane and a 25-gauge microdialysis probe (4.5 mm longer than the guide cannula) with a 3 mm dialysis membrane at the tip was inserted. The probe was then connected *via* Teflon tubing to a 1.0 ml Exmire micro syringe (Ito Corporation, Fuji, Japan), filled with an artificial cerebrospinal fluid (composition in mM): NaCl, 125; KCl, 2.5; CaCl₂·2H₂O, 1.5; NaHCO₃, 27; NaH₂PO₄·H₂O, 0.5; Na₂HPO₄, 1.2; Na₂SO₄, 0.5; MgCl₂·6H₂O, 1.0; pH 6.2. The probe was continuously perfused at a rate of 2.0 μ l min⁻¹ by means of a CMA/100 microinjection pump (Carnegie Medecin, Stockholm, Sweden). The perfusate fractions (40 μ l) were collected every 20 min in Eppendorf microcentrifuge tubes containing 10 μ l 1 M perchloric acid to prevent the oxidation of the monoamines. The mean of the two perfusate collections immediately prior to Δ^9 -THC or vehicle injection was used to calculate the basal level of DA (100%).

At the end of the experiment, the rat was killed with an overdose of pentobarbitone. The brain was quickly removed, frozen and coronal sections were cut to verify the correct position of the probe. Only results obtained from rats with correctly positioned guide cannulae are presented.

Drugs

Δ^9 -THC (National Institute on Drug Abuse, U.S.A.) or vehicle was injected i.v. ($t=0$). Δ^9 -THC was incorporated into the triglyceride/phospholipid emulsion vehicle Intralipid[®] (Baxter) as described previously (Malone & Taylor, 1998b). A concentration of 4 mg ml⁻¹ Δ^9 -THC in Intralipid[®] was utilized for the 2 and 5 mg kg⁻¹ doses and 0.5 mg ml⁻¹ Δ^9 -THC in Intralipid[®] was used for 0.5 mg kg⁻¹ dose. SR 141716 (N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; Sanofi Recherche) was incorporated in a similar manner into Intralipid[®] and, when used, was injected 40 min before Δ^9 -THC at a dose of 2.5 mg kg⁻¹ i.p..

For intraperitoneal (i.p.) injection, fluoxetine HCl (Prozac[®], Eli Lilly) was dissolved in water for injection to give a concentration of 5 mg ml⁻¹. Rats were administered fluoxetine HCl (10 mg kg⁻¹ i.p.) either 40 min before or 40 min after Δ^9 -THC administration. When fluoxetine was administered *via* the microdialysis probe, a 10 μ M concentration of fluoxetine was added to the microdialysis perfusion fluid 40 min after Δ^9 -THC administration and remained in the perfusion fluid for the duration of the experiment, i.e. 200 min after Δ^9 -THC administration. Other drugs used were amylobarbitone sodium (Amytal[®], Ely Lilly) and methohexitone sodium (Brietal, Eli Lilly). All other reagents used were of analytical grade.

Chromatography

The striatal perfusates were assayed for DA using reverse-phase ion-pair high performance liquid chromatography (HPLC) coupled with electrochemical (EC) detection (Hurd & Ungerstedt, 1989). Perfusate samples (40 μ l) were collected over 20 min fractions into microcentrifuge tubes containing 10 μ l 1 M perchloric acid, and the resultant 50 μ l samples were injected directly onto the HPLC column. The HPLC system consisted of a BAS PM-60 pump operating at a pressure of about 2200 p.s.i., a Rheodyne BH 7125 injector, a reverse-phase Spherisorb 5 ODS column (250 mm \times 4.6 mm i.d. Phase Sep. Ltd., Deeside, U.K.) which was encapsulated in a temperature-controlled oven set at 28°C (Waters, Division of Millipore, MA, U.S.A.) and a LC-4B dual electrode EC detector equipped with a TL-5A glassy carbon working electrode (Bioanalytical Systems Inc., IN, U.S.A.). The analysis was carried out at a flow rate of 1.3 ml min⁻¹ and a detector potential of +750 mV (vs Ag/AgCl/3M NaCl) with a sensitivity of either 10.0 or 20.0 nA FS. Quantification was performed using a BBC Goerz Metrawatt SE 120 dual channel pen recorder.

The mobile phase consisted of methanol: 0.15 M sodium phosphate buffer containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 0.5 mM sodium 1-octanesulphonic acid (14:86 v v⁻¹). The 0.15 M sodium phosphate buffer was prepared by dilution of a stock solution of 1.0 M disodium hydrogen orthophosphate/phosphoric acid buffer pH 2.9. The final solution was adjusted to pH 3.7 with 10.0 M NaOH and was filtered through a 0.45 μ m vacuum filter apparatus (Waters, Division of Millipore, MA, U.S.A.). An external standard (0.05 μ M DA) was used for the identification of sample peaks.

Statistics

When comparing pretreatments to vehicle only, a Student's *t*-test was used. When comparing pretreatment or post treatment values plus Δ^9 -THC or vehicle, a one-way analysis of variance

was used. When values were found to be significant, the Student-Newman-Keuls multi-comparison test was used to determine which treatment groups were different and the level of significance ($P < 0.05$ or $P < 0.01$).

Results

Intralipid[®] administration had no effect on the extracellular levels of DA. Δ^9 -THC caused a dose-dependent increase in basal DA levels following i.v. administration. Maximum increases were to 142.1 ± 5.4 , 178.7 ± 9.4 and $197.4 \pm 5.9\%$ of basal levels following the 0.5, 2 and 5 mg kg⁻¹ doses of Δ^9 -THC respectively and occurred 20 min after Δ^9 -THC administration (Figure 1). The increase in basal DA levels was significant at all doses 20 min after Δ^9 -THC administration ($n = 5-7$, $P < 0.05$). Forty minutes after Δ^9 -THC administration DA levels remained significantly different from vehicle following the 5 mg kg⁻¹ dose of Δ^9 -THC.

SR 141716 (2.5 mg kg⁻¹) given 40 min before vehicle injection decreased DA levels when compared with vehicle. This decrease reached significance at 120 and 140 min after SR 141716 administration ($n = 5$, $P < 0.05$) (Figure 2). Following pretreatment with i.p. SR 141716, there was no significant

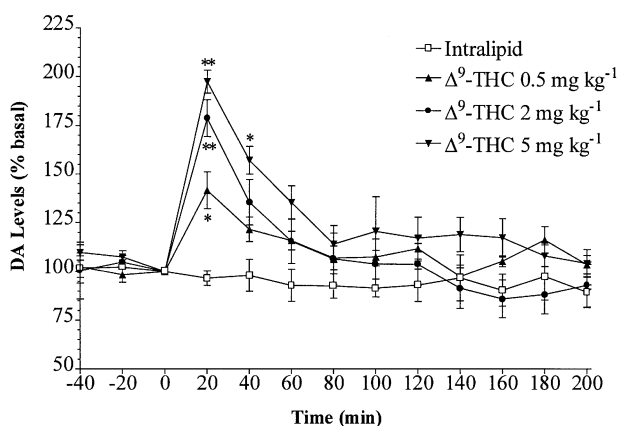


Figure 1 Effect of 0.5, 2 and 5 mg kg⁻¹ Δ^9 -THC i.v. or vehicle on the level of extracellular DA in the rat striatum. Results are expressed as mean \pm s.e. mean of 5-7 experiments. (* $P < 0.05$, ** $P < 0.01$ when compared with vehicle alone.)

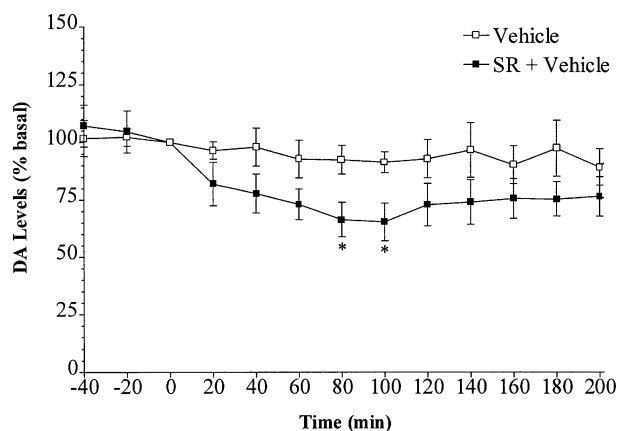


Figure 2 Effect of SR 141716 or vehicle on the level of extracellular DA in the rat striatum. Results are expressed as mean \pm s.e. mean of five experiments. (* $P < 0.05$ when compared with vehicle alone.)

change in DA levels produced by 0.5, 2 or 5 mg kg⁻¹ Δ^9 -THC ($n = 5-7$, $P > 0.05$) (Figure 3).

When fluoxetine was added to the microdialysis perfusion fluid at a concentration of 10 μ M, a highly significant increase in basal DA levels occurred ($n = 5-6$, $P < 0.01$) compared with vehicle alone 20 min after the fluoxetine perfusion commenced (Figure 4). Also, a significant increase in basal DA levels occurred 40 min after the perfusion commenced ($n = 5-6$, $P < 0.05$).

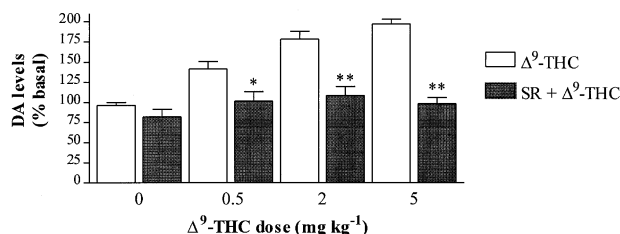


Figure 3 Effect of SR 141716 (SR) 2.5 mg kg⁻¹ i.p. injected at time = -40 min on the extracellular DA levels in the rat striatum 20 min after Δ^9 -THC or vehicle injection. Results are expressed as mean \pm s.e. mean of 5-7 experiments. (* $P < 0.05$, ** $P < 0.01$ when compared with Δ^9 -THC or vehicle alone.)

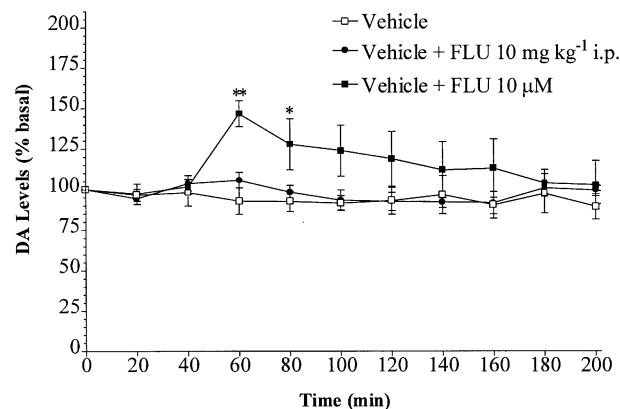


Figure 4 Effect of fluoxetine perfusion 10 μ M and fluoxetine 10 mg kg⁻¹ i.p., commencing at $t = 40$ min, on the level of extracellular DA in the rat striatum. Results are expressed as mean \pm s.e. mean of 5-6 experiments. (* $P < 0.05$, ** $P < 0.01$ when compared with vehicle alone.)

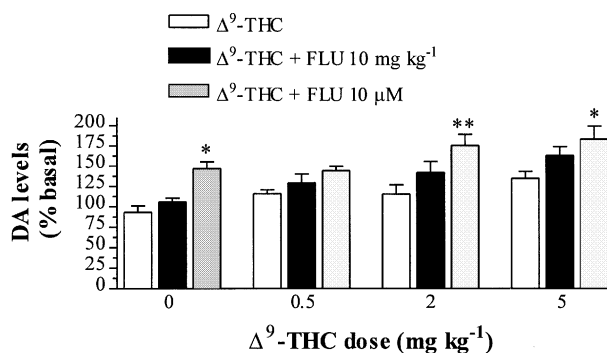


Figure 5 Effect of fluoxetine perfusion 10 μ M and fluoxetine 10 mg kg⁻¹ i.p., commencing at $t = 40$ min on Δ^9 -THC-induced DA levels in the rat striatum 60 min after Δ^9 -THC or vehicle injection. Results are expressed as mean \pm s.e. mean of 5-10 experiments (* $P < 0.05$, ** $P < 0.01$ when compared with Δ^9 -THC or vehicle alone.)

Fluoxetine perfusion significantly potentiated the increase in DA levels produced by the 2 mg kg⁻¹ ($n=5-6$, $P<0.01$) and 5 mg kg⁻¹ ($n=5-6$, $P<0.05$) doses of Δ^9 -THC (Figure 5). Fluoxetine perfusion had no significant effect on DA levels following the 0.5 mg kg⁻¹ dose of THC ($n=5-6$, $P>0.05$) although a slight increase was observed.

Fluoxetine (10 mg kg⁻¹ i.p.) given 40 min after Δ^9 -THC administration caused a slight but non-significant increase in DA levels produced by 0.5, 2 or 5 mg kg⁻¹ Δ^9 -THC ($n=5-10$, $P>0.05$) (Figure 5).

Fluoxetine (10 mg kg⁻¹ i.p.) given 40 min before vehicle injection had no significant effect on DA levels (Figure 6). Fluoxetine (10 mg kg⁻¹ i.p.) given 40 min before Δ^9 -THC administration abolished the change in basal DA levels produced by 0.5, 2 or 5 mg kg⁻¹ Δ^9 -THC ($n=5-7$, $P>0.05$) (Figure 6).

Discussion

The release of DA observed following Δ^9 -THC administration is comparable with previous studies using microdialysis. Chen *et al.* (1990a) observed an increase of approximately 125 and 160% from basal levels of DA in the NAcc following 0.5 and 1 mg kg⁻¹ Δ^9 -THC respectively. An increase to 160% of basal levels was reported in the medial prefrontal cortex following 2 mg kg⁻¹ Δ^9 -THC i.p. (Chen *et al.*, 1990b). In the present study, increases to approximately 142, 179, and 197% following 0.5, 2 and 5 mg kg⁻¹ doses of Δ^9 -THC occurred, respectively (Figure 1). Although the magnitude of increase in DA levels was comparable, one difference in the results of previous studies and the present study was the time taken for the increase in DA levels to reach a maximum. In the present study, the maximum effect was determined in the first sample collected, ie within 20 min after Δ^9 -THC administration whereas 40–60 min elapsed before a maximum effect was observed in previous studies. The studies described above used the i.p. route of administration but in the present experiment, the i.v. route was chosen, which provides a faster absorption to Δ^9 -THC's sites of action in the brain (Aguirell *et al.*, 1986). In other experiments mentioned, DA release peaked 40 min after Δ^9 -THC i.p.. This may account for the more rapid onset of action in the present study. The i.v. route of administration was chosen as this route more accurately mimics the inhaled route of administration, which is the method that most recreational users choose when using cannabis (Aguirell *et al.*, 1986), presumably because of the fast absorption rate produced.

The antagonism of the Δ^9 -THC-induced DA release by SR 141716 indicates that the Δ^9 -THC-induced DA release is CB₁ receptor-mediated (Figure 3). This is in agreement with Tanda

et al. (1997) who showed the increase in DA in the NAcc was prevented by SR 141716A, the hydrochloride salt of SR 141716. The dose of SR 141716 used in this study was shown to antagonize the catalepsy produced by the CB₁ agonist, CP 55940 (Anderson *et al.*, 1996).

Interestingly, SR 141716 administration 40 min before vehicle decreased basal DA to 66% of basal levels (Figure 2). This effect reached a maximum decrease at 120 to 140 min after SR 141716. The reason for this decrease is unclear, but may be due to either SR 141716 antagonising an endogenous cannabinoid system in the brain, or that it is an inverse agonist. *In vitro*, SR 141716A has been shown to display properties opposite to a CB₁ receptor agonist (Pertwee & Fernando, 1996) whilst *in vivo*, SR 141716A causes hyperalgesia in a rodent model of thermal pain (Richardson *et al.*, 1997). In cell lines expressing human recombinant CB₁ receptors, SR 141716A reduced basal incorporation of [³⁵S]-GTP γ S (Landsman *et al.*, 1997; MacLennan *et al.*, 1998) whereas cannabimol had no effect, ruling out the possibility of SR 141716A blocking the effect of an endogenous agonist (MacLennan *et al.*, 1998). This raises the possibility that SR 141716A (and indeed the base form SR 141716) is an inverse agonist. However, the physiological relevance of these results remains to be determined.

Previous studies show that 10 μ M fluoxetine added to microdialysis perfusion fluid causes substantial 5-HT reuptake inhibition (Matsumoto *et al.*, 1995; Pullar & Findlay, 1992). Based on these studies, a concentration of 10 μ M fluoxetine was added to the perfusion fluid 40 min after Δ^9 -THC or vehicle injection, and remained in the perfusion fluid until the end of the experiment. Fluoxetine 10 μ M perfused into the striatum *via* the microdialysis probe facilitated DA release (Figure 4). This effect of increasing basal DA levels has been reported previously with alaproclate perfusion, another SSRI (Yadid *et al.*, 1994). An increase in extracellular 5-HT in the striatum resulting in an increase in extracellular DA is in agreement with a study that showed that direct perfusion of 5-HT into the striatum dose-dependently facilitates DA release (Benloucif & Galloway, 1991). Also, it has been reported that an increase in local serotonergic activity results in an increase in DA release (Jacocks & Cox, 1992; Yadid *et al.*, 1994) whereas a decrease in serotonergic activity can reduce striatal DA release (Blandina *et al.*, 1989; Carboni *et al.*, 1989). As well as fluoxetine increasing extracellular 5-HT acutely by blocking 5-HT reuptake (Perry & Fuller, 1992), there is also evidence to suggest that selective 5-HT reuptake inhibitors may decrease 5-HT release (Adell & Artigas, 1991; Gartside *et al.*, 1995; Invernizzi *et al.*, 1997; Rutter & Auerbach, 1993). This is thought to occur following the increased extracellular 5-HT (produced by reuptake inhibition) stimulating autoreceptors on cell bodies and dendrites in the DRN to inhibit firing of 5-HT neurones, and stimulating terminal autoreceptors to inhibit neuronal release of 5-HT (Adell & Artigas, 1991; Invernizzi *et al.*, 1997; Rutter & Auerbach, 1993). The DA release reported by Yadid *et al.* (1994) with alaproclate was only apparent in the first hour after the perfusion commenced. Similarly, in the present study, the DA release induced by fluoxetine was only significant in the first hour. It is therefore possible that the increased DA levels produced by these 5-HT reuptake inhibitors were not sustained because of the elevated 5-HT activating inhibitory autoreceptors on the 5-HT nerve terminal and hence decreasing 5-HT release.

The increase in extracellular DA observed following local perfusion of fluoxetine was not observed following systemic fluoxetine (Figure 4). A possible explanation for this difference is that, when injected peripherally, fluoxetine would be

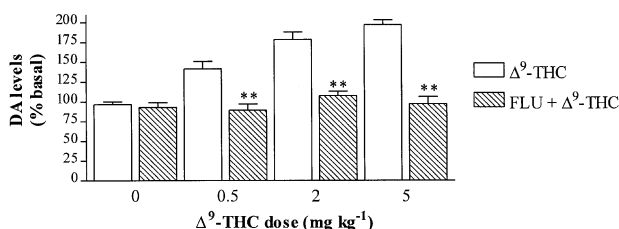


Figure 6 Effect of 10 mg kg⁻¹ fluoxetine (FLU) i.p. injected 40 min before Δ^9 -THC or vehicle on the extracellular DA levels in the rat striatum 20 min after Δ^9 -THC or vehicle injection. Results are expressed as mean \pm s.e. mean of 5–8 experiments. (* $P<0.05$, ** $P<0.01$ when compared with Δ^9 -THC or vehicle alone.)

expected to inhibit 5-HT reuptake in the striatum, as with local fluoxetine perfusion, but also at the 5-HT cell bodies in the DRN. Peripheral administration of 5-HT reuptake inhibitors has been shown to inhibit the firing of 5-HT neurones through activation of somatodendritic autoreceptors in the DRN in less than 5 min (Gartside *et al.*, 1995). This effect could be long-lasting since the increase in extracellular 5-HT levels in the DRN following 5-HT reuptake inhibition has been reported to persist for hours (Adell & Artigas, 1991; Gartside *et al.*, 1995). In contrast, when fluoxetine is administered 40 min before Δ^9 -THC, the Δ^9 -THC-induced DA release was abolished (Figure 4). As SSRIs can inhibit the firing of 5-HT neurones in minutes (Gartside *et al.*, 1995), when Δ^9 -THC is administered 40 min later, the firing rate of raphe-striatal 5-HT neurones would be minimal. Hence, Δ^9 -THC-induced DA release may be dependent on a normal firing rate of serotonergic neurones.

Although there is considerable evidence of the involvement of 5-HT and DA in the actions of Δ^9 -THC, the exact nature of how Δ^9 -THC produces its pharmacological effects remains to be elucidated. In the present study, it appears that increasing the amount of extracellular 5-HT in the striatum (as seen when fluoxetine was perfused into the striatum after Δ^9 -THC) potentiates Δ^9 -THC-induced DA release. Conversely, Δ^9 -THC-induced DA release is attenuated when the activity of raphe-striatal serotonergic neurones is decreased (as seen with fluoxetine pretreatment). Previously, we have hypothesized that Δ^9 -THC increases extracellular 5-HT in the hypothalamus in order to produce hypothermia (Malone & Taylor, 1998b). It is possible that Δ^9 -THC also produces an increase in 5-HT, in the striatum, in order to increase extracellular DA.

In autoradiographic studies using [3 H]-CP 55,940 as a radiolabel, the distribution of CB₁ receptors in the brain has been shown to correlate well with some of the pharmacological effects of Δ^9 -THC (Herkenham *et al.*, 1990). For example, the high density of CB₁ receptors in the forebrain and the cerebellum correlate with the altered cognition and movement produced by cannabinoids (Dewey, 1986). Fewer receptors were found in the areas controlling cardiovascular and respiratory functions in the lower brainstem, which may explain why high doses of Δ^9 -THC, unlike other drugs of abuse such as morphine are not lethal (Dewey, 1986).

The existence of CB₁ receptors in the striatum suggests an association with the same DA neurones activated by other

drugs of abuse. However, when these neurones were lesioned with 6-OHDA, DA but not CB₁ receptors were lost (Herkenham *et al.*, 1991). This suggests that the CB₁ receptors found in the striatum are not localized on DA neurones. The existence of cannabinoid receptors on presynaptic nerve endings has been suggested on noradrenergic neurones, as CB₁ receptor mRNA was found to be present in sympathetic ganglion (Ishac *et al.*, 1996). Hence, the possibility exists that CB₁ receptors are located presynaptically on other types for neurones. Several reports have suggested that striatal CB₁ receptors are located on opioid (Gardner & Lowinson, 1991) or GABA (Herkenham *et al.*, 1991) neurones that are thought to modulate striatal DA release. In the present study, it was found that altering striatal 5-HT output could modulate the Δ^9 -THC-induced DA release. In light of this discovery, it may be hypothesized that CB₁ receptors are located on striatal serotonergic neurones and the stimulation of these receptors by Δ^9 -THC may modulate striatal 5-HT output in order to evoke DA release. In addition, CB₁ receptors are quite dense in the SN and the DRN (Herkenham *et al.*, 1990) and local application of 5-HT agonists and antagonists to the SN modulates the firing of DA neurones (Benloucif & Galloway, 1991; Sinton & Fallon, 1988). Hence, the SN and the DRN are other potential sites for Δ^9 -THC to act in order to increase striatal DA levels. Further studies are required to test the hypothesis that Δ^9 -THC increases extracellular 5-HT in order to increase striatal DA.

Conclusion

It is suggested from the results of the present study that Δ^9 -THC administration results in a dose-dependent increase in extracellular levels of DA which occurs *via* activation of CB₁ receptors. Since an apparent increase in serotonergic activity in the striatum resulted in an increase in DA release induced by Δ^9 -THC, whereas an apparent decrease in serotonergic activity attenuated the Δ^9 -THC-induced DA release, it is hypothesized that Δ^9 -THC increases extracellular 5-HT in the striatum in order to increase DA release.

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