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Modulation of Δ^9-tetrahydrocannabinol-induced hypothermia by fluoxetine in the rat

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1 It has been suggested that the dose of Δ^9-tetrahydrocannabinol (Δ^9-THC) that induces hypothermia in the rat increases the release of brain 5-hydroxytryptamine (5-HT). In light of this, we investigated the hypothermia produced by Δ^9-THC, and the effect the selective serotonin reuptake inhibitor fluoxetine has on this response.

2 A significant dose-dependent decrease in body temperature occurred after i.v. administration of 0.5 to 5 mg kg^{-1} Δ^9-THC; maximum decreases being 0.8 ± 0.2°C to 2.9 ± 0.3°C. This hypothermic response was attenuated by the cannabinoid CB_1 receptor antagonist SR 141716.

3 Fluoxetine (10 mg kg^{-1} i.p.) alone caused a decrease in body temperature of 0.6 ± 0.1°C ($n=32$, $P<0.05$) after 40 min. However, pretreatment with fluoxetine (10 mg kg^{-1} i.p.) 40 min before Δ^9-THC significantly reduced the Δ^9-THC-induced hypothermia ($n=7-8$, $P<0.05$). Contrary to this antagonist-like effect, fluoxetine administered 40 min after Δ^9-THC significantly potentiated the Δ^9-THC-induced hypothermia, producing a maximum decrease of 3.2 ± 0.3°C.

4 It is suggested that the effect of fluoxetine on the Δ^9-THC-induced hypothermic response is dependent on the time of its administration relative to that of Δ^9-THC. Pretreatment with fluoxetine increases extracellular 5-HT due to reuptake inhibition. Increased extracellular 5-HT can activate autoreceptors which may decrease serotonergic activity, thereby reducing the Δ^9-THC-induced hypothermia. Conversely, when fluoxetine is adminstered after Δ^9-THC, the reuptake block is thought to potentiate the already activated serotonegic system, hence potentiating the Δ^9-THC-induced hypothermia.

Keywords: Δ^9-THC; 5-HT; hypothermia; fluoxetine; SR 141716

Introduction

Cannabinoids have been reported to produce a vast array of pharmacological effects. One of these effects that provides a good quantitative measure of cannabinoid activity is the hypothermic response. Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of cannabis, has been shown to alter thermoregulation by acting centrally (Biegon *et al*., 1979; Fitton & Pertwee, 1982) and occurs irrespective of the route of administration or the animal species used (Holtzman *et al*., 1969; Hardman *et al*., 1971). The hypothermic response can be blocked by the cannabinoid CB_1 receptor antagonist SR 141716A (Compton *et al*., 1996), indicating a CB_1 receptor specific effect. Consequently, along with antinociception, hypoactivity and catalepsy, the cannabinoid-induced hypothermic response has been used to evaluate cannabimimetic activity in cannabinoid analogues (Compton *et al*., 1992) and endogenous substances such as the recently discovered endogenous cannabinoid anandamide (Lichtman *et al*., 1996).

It has long been recognized that 5-hydroxytryptamine (5-HT) is involved in the central regulation of temperature control (Feldberg & Myers, 1964; Feldberg & Lotti, 1967). Several lines of evidence support the view that 5-HT may be involved in the cannabinoid-induced hypothermic response. For example, pretreatment with the serotonin selective reuptake inhibitor (SSRI) clomipramine has been shown to modify the hypothermia induced by Δ^9-THC and alter Δ^9-THC-induced alterations in concentrations of brain 5-hydroxyindoleacetic acid (5-HIAA) in the rat (Fennessy & Taylor, 1978). In mice, pretreatment with drugs that alter serotonergic systems such as methysergide, *p*-chlorophenylalanine or clompiramine affected the hypothermic response of Δ^9-THC (Davies & Graham, 1980).

SSRIs such as citalopram (Chaput *et al*., 1986) and paroxetine (Gartside *et al*., 1995) have been shown to have an inhibitory effect on 5-HT neuronal firing. This effect appears to be the result of the local increase of 5-HT in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) produced by reuptake inhibition which activates somatodendritic autoreceptors (Artigas, 1993). This effect can be blocked by pretreatment with the 5-HT_{1A} antagonist WAY 100635, which suggests the somatodendritic autoreceptors are of the 5-HT_{1A} type (Gartside *et al*., 1995; Sharp *et al*., 1997). It has been reported that this effect on 5-HT neurones can occur in a matter of minutes following intravenous administration of the SSRI (Arborelius *et al*., 1995; Gartside *et al*., 1995). As mentioned earlier, 5-HT has been implicated in the Δ^9-THC-induced hypothermic response. Hence, the effect of SSRI's on the hypothermic response of cannabinoids may be dependent on the time of administration, as the rate of 5-HT neuronal firing would be expected to alter the Δ^9-THC-induced hypothermic effect.

In order to further investigate this hypothesis, the effects of pre Δ^9-THC and post Δ^9-THC treatment with the SSRI fluoxetine on the Δ^9-THC-induced hypothermic response have been examined. A preliminary account of these findings have been presented at the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) meeting (Malone & Taylor, 1997).

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Methods

Animals and housing

Female Glaxo-Wistar rats weighing between 200 and 300 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

For intravenous (i.v.) administration of drugs, rats were implanted with permanent polyethylene (PE 50) catheters into the external jugular vein under sodium methohexitone (18 mg kg^{-1} i.p.)/sodium amylobarbitone (30 mg kg^{-1} i.p.) anaesthesia. Following catheter insertion, rats were injected with ticarcillin 15 mg kg^{-1} i.v. During the recovery period, animals were kept in individual cages and food and water was readily available. Body temperature and behavioural studies were all undertaken in the afternoon. Body temperature was recorded by means of a thermister probe inserted 6–7 cm into the colon. Core body temperature was recorded before pretreatments and immediately before Δ^9-THC and at 30, 60, 90, 120 and 150 min after Δ^9-THC or vehicle administration. Fluoxetine was dissolved in water for injection to give a concentration of 5 mg ml^{-1}. Rats were given fluoxetine 10 mg kg^{-1} i.p. either 40 min before or 40 min after Δ^9-THC administration. Δ^9-THC was injected i.v. at a dose of 0.5, 2, or 5 mg kg^{-1}.

Δ^9-THC formulation

0.5 ml of Δ^9-THC 200 mg ml^{-1} in ethanol was transferred to a plastic centrifuge tube and the ethanol evaporated off under nitrogen gas. The resultant Δ^9-THC liquid was made up to 0.5 ml with triacetin and vortexed for 2 min. The required volume of the commercially available fat emulsion Intralipid® 10% w/v was kept on ice and the Δ^9-THC solution was added to the Intralipid® through a 26 gauge needle. An aliquot of 0.1 ml was added at a time and the emulsion was then sonicated after each addition using a Soniprep 150 Ultrasonic Disintegrator (MSE Scientific Instruments Sussex England) fitted with a probe assembly with a 9.0 mm diameter tip. The probe was operated at an amplitude of 14 μ for 30 s at a time. The emulsion was made up to the required volume with Intralipid®, sonicated and filtered using a 0.22 μm sterile filter to give the desired final concentration of Δ^9-THC. A concentration of 4 mg ml^{-1} Δ^9-THC in Intralipid® was used for 2 mg kg^{-1} 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} doses. SR 141716 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.

Drugs

The following drugs and solutions were used: amylobarbitone sodium (Amytal®, Ely Lilly), fluoxetine HCl (Prozac®, Eli Lilly), methohexitone sodium (Brietal®, Eli Lilly), Intralipid® (Baxter), SR 141716 (N-piperidino-5-(4-clorphenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide) (Sanofi Recherche) and (−)-trans-Δ^9-THC (National Institute on Drug Abuse, U.S.A.).

Analysis of data

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 ($P<0.05$), the Student-Newman-Kuels multi-comparison test was used to determine which treatment groups were different and the level of significance.

Results

Δ^9-THC caused a dose-dependent decrease in body temperature following intravenous administration. The maximum decrease for the 0.5 mg kg^{-1} dose was 0.8 ± 0.2°C and occurred 60 min after Δ^9-THC administration. The maximum decreases of 2.0 ± 0.2°C and 2.9 ± 0.3°C for doses of 2 and 5 mg kg^{-1} Δ^9-THC respectively, both occurred at 120 min after Δ^9-THC administration (Figure 1a). This hypothermia was significant when compared with vehicle alone during the first 90 min after the 0.5 mg kg^{-1} dose of Δ^9-THC ($n=6-10$, $P<0.05$). Following the 2 mg kg^{-1} and 5 mg kg^{-1} doses, the hypothermic response remained significantly different from vehicle alone, throughout the duration of the experiment ($n=6-11$, $P<0.01$). Following pretreatment with 2.5 mg kg^{-1} i.p. SR 141716, there was no significant change in body temperature produced by 0.5 mg kg^{-1}, 2 mg kg^{-1} or 5 mg kg^{-1} Δ^9-THC ($n=6-11$, $P>0.05$) (Figure 1b). SR 141716 alone had no signficant effect on body temperature when compared with vehicle.

Fluoxetine (10 mg kg^{-1} i.p.) over the 40 min pretreatment period caused a short lasting but significant decrease in body temperature of 0.6 ± 0.1°C ($n=32$, $P<0.05$). Pretreatment with fluoxetine (10 mg kg^{-1} i.p.) 40 min before Δ^9-THC significantly reduced the 2 and 5 mg kg^{-1} Δ^9-THC-induced hypothermia ($n=7-8$, $P<0.05$), but did not alter the effect of the 0.5 mg kg^{-1} dose of Δ^9-THC (Figure 2).

Contrary to this antagonist-like effect, fluoxetine administered 40 min after Δ^9-THC-significantly potentiated the 0.5 and 2 mg kg^{-1} Δ^9-THC-induced hypothermia, producing maximum decreases in body temperature of 1.7 ± 0.2°C and 3.2 ± 0.3°C respectively ($n=6-11$, $P<0.05$), but had no effect on the 5 mg kg^{-1} dose (Figure 3).

Discussion

The results of the present study agree with previous reports that showed a dose-dependent reduction in body temperature following Δ^9-THC administration in rats. Similar hypothermic responses have been shown in a number of studies using various cannabinoid CB_1 receptor agonists (Fennessy & Taylor, 1977; 1978; Lichtman & Martin, 1991; Compton *et al*., 1992; 1996). Δ^9-THC in the same doses and the same species of rat were used by Fennessy & Taylor (1978) as in the present study. Similar hypothermic responses were observed except at the 0.5 mg kg^{-1} dose of Δ^9-THC, when no decrease in body temperature was observed. This may be due to the sex differences between the two studies. It has been shown that female rats are more sensitive to the hypothermic response produced by pargyline (Biegon *et al*., 1979). It has also been suggested by Biegon *et al*. (1979) that the sex differences they observed may be due to a higher activity of 5-HT in the female brain. However, previous unpublished work in our laboratory comparing the hypothermic effect of 2 mg kg^{-1} Δ^9-THC in males with that of females showed no significant difference between the two groups ($P>0.1$, $n=5-6$).

In the present study SR 141716 antagonised the Δ^9-THC-induced hypothermic response. This is in agreement with Compton *et al*. (1996) who showed a dose dependent reduction

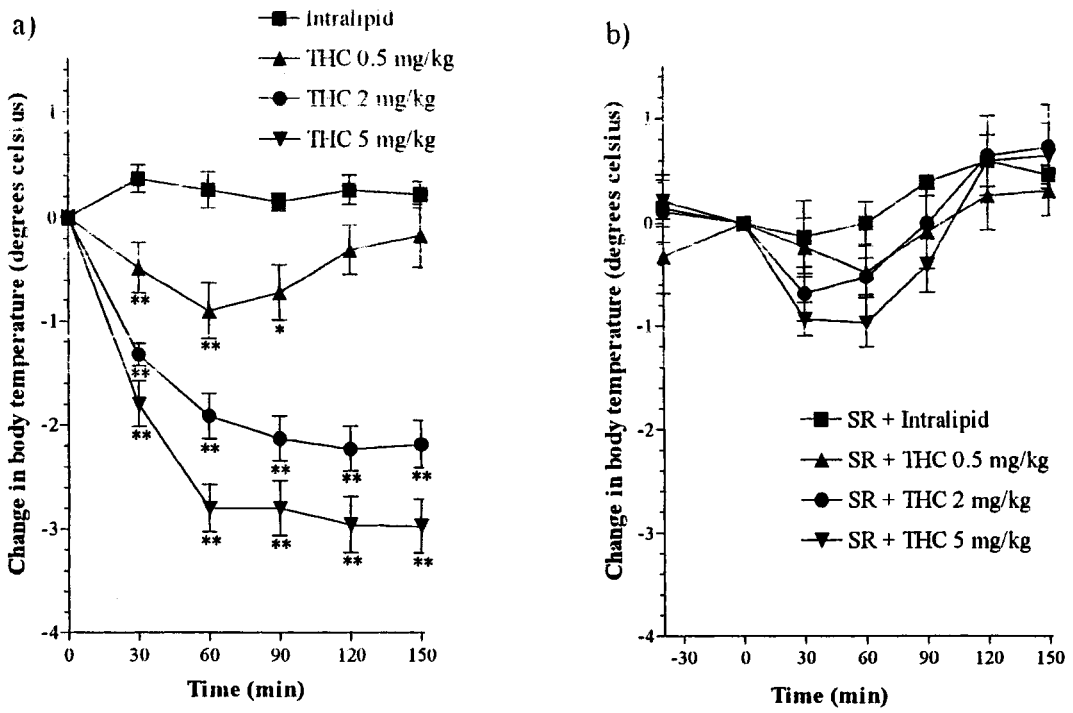

Figure 1 Effect of (a) Δ^9-THC i.v. and (b) SR 141716 2.5 mg kg^{-1} i.p. (administered at $t=-40$ min) + Δ^9-THC i.v. on body temperature (*$P<0.05$, **$P<0.01$ when compared with a) vehicle and b) SR 141716 + vehicle, $n=6-11$). Data is expressed as a change in temperature from that recorded immediately prior to Δ^9-THC or Intralipid (at $t=0$).

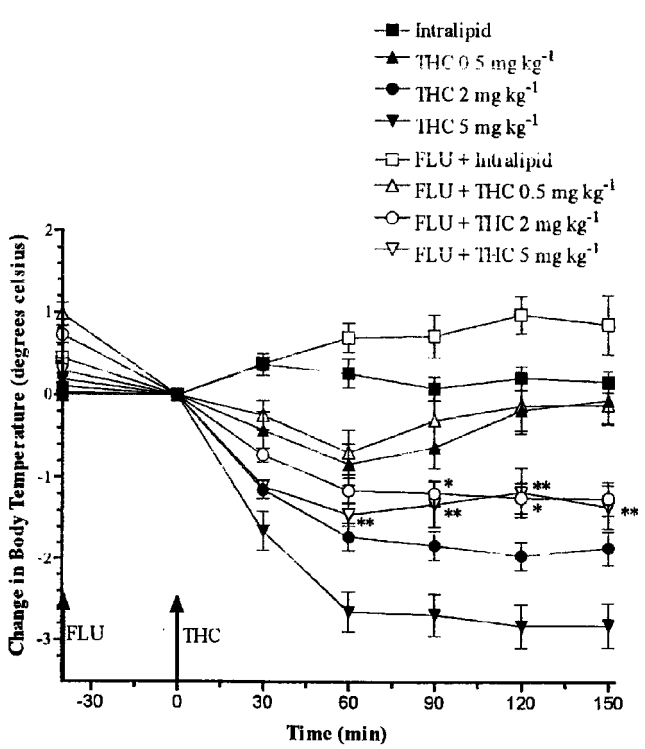

Figure 2 Comparison of fluoxetine 10 mg/kg i.p. pretreatment (at $t=-40$ min) +Δ^9-THC or Intralipid i.v. (open symbols) *versus* Δ^9-THC or Intralipid i.v. alone (filled symbols) on body temperature (*$P<0.05$, **$P<0.01$ when compared with no fluoxetine, $n=6-11$). Data is expressed as a change in temperature from that recorded immediately prior to Δ^9-THC or Intralipid (at $t=0$).

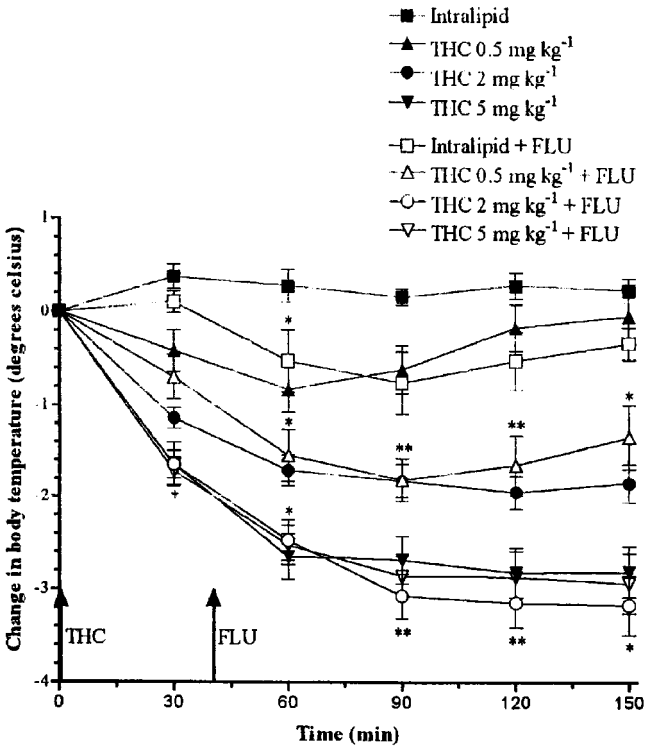

Figure 3 Comparison of fluoxetine 10 mg/kg i.p. post-treatment (at $t=40$ min) + Δ^9-THC or Intralipid i.v. (open symbols) *versus* Δ^9-THC or Intralipid i.v. alone (filled symbols) on body temperature (*$P<0.05$, **$P<0.01$ when compared with no fluoxetine, $n=6-11$). Data is expressed as a change in temperature from that recorded immediately prior to Δ^9-THC or Intralipid (at $t=0$).

of the Δ^9-THC-induced hypothermic response by SR 141716A, the hydrochloride salt of SR 141716.

The i.v. route of administration for Δ^9-THC was chosen as this route more accurately mimics the inhaled route of administration than the i.p. or oral routes. The inhaled route is the method that most recreational users opt for when using cannabis (Agurell *et al*., 1986), presumably because of the fast absorption rate achieved.

As Δ^9-THC is an extremely hydrophobic drug, a suitable formulation to enable Δ^9-THC to be administered i.v. was required. The Δ^9-THC in Intralipid® emulsion used in this study offers several advantages over previous methods of incorporating Δ^9-THC into a solution. The actions of Δ^9-THC *in vivo* have previously been investigated by suspending the Δ^9-THC in solution using a suspending agent such as polyvinylpyrrolidone (PVP) (Davies & Graham, 1980; Fennessy & Taylor, 1978). An emulsion has better dissolution characteristics than a suspension as it remains a homogeneous solution even upon standing for extended periods of time. Also, the Δ^9-THC in Intralipid® emulsion requires no ethanol as a co-solvent, which has also been used commonly (Compton *et al*., 1992; 1996; Aceto *et al*., 1995). Since ethanol is a centrally acting depressant drug, it may alter the neurochemical and behavioural effects of Δ^9-THC.

Fluoxetine 10 mg kg^{-1} i.p. produced a short lasting but significant hypothermia of 0.6 ± 0.1°C measured 40 min after fluoxetine injection. This result appears to be contrary to other reports of systemic administration of SSRI's that found no effect on body temperature (Maj & Moryl, 1993; Bartoszyk *et al*., 1997). However, Fennessy & Taylor (1978) reported a comparable decrease of 0.4 ± 0.1°C in body temperature 30 min after clomipramine administration.

The precise mechanisms responsible for the short lasting hypothermic response of fluoxetine observed remains to be elucidated, but may be due to increased extracellular 5-HT expected due to reuptake blockade by fluoxetine. Intracerebroventricular (i.c.v.) administration of 5-HT has been shown to produce hypothermia in the rat (Feldberg & Lotti, 1967). Also, several 5-HT agonists have been reported to produce hypothermia; in particular 5-HT$_{1A}$ agonists such as 8-hydroxy-(di-*n*-proplyamino) tetralin (8-OH-DPAT) (Patel & Hutson, 1996; Bagdy & To, 1997). Whether presynaptic or postsynaptic 5-HT$_{1A}$ receptors are involved in the hypothermic effect of fluoxetine remains questionable.

Fluoxetine administered 40 min before Δ^9-THC significantly attenuated the Δ^9-THC-induced hypothermia produced by 2 mg kg^{-1} and 5 mg kg^{-1} doses of Δ^9-THC (Figure 2). This observation is in agreement with Fennessy & Taylor (1978) who showed that clomipramine shifted the dose-response curves of Δ^9-THC-induced hypothermia and the Δ^9-THC-induced increase in 5-HIAA levels in whole brain to the right. Because of these observations, it was suggested that these doses of Δ^9-THC increased the release of 5-HT which was manifested as hypothermia. SSRI's have also been shown to have an inhibitory effect on 5-HT neuronal firing and neuronal release (Gartside *et al*., 1995). This appears to be due to the increase level of extracellular 5-HT (produced by reuptake inhibition) activating somatodendritic autoreceptors at cell bodies in areas of the raphe nuclei and presynaptic autoreceptors at 5-HT nerve terminals respectively (Sharp *et al*., 1997). Therefore, Δ^9-THC may cause the release of 5-HT in order to produce hypothermia (as was speculated by Fennessy & Taylor [1978]), and the resultant decreased activity of 5-HT neurones that may be expected following pretreatment with fluoxetine may cause a reduction in the Δ^9-THC-induced-hypothermia.

In contrast to this attenuation caused by fluoxetine pretreatment, fluoxetine administered 40 min after Δ^9-THC significantly potentiated the Δ^9-THC-induced hypothermic response (Figure 3). In keeping with the hypothesis that Δ^9-THC increases the release of 5-HT to produce hypothermia, the greater increase in extracellular 5-HT produced by fluoxetine appears to potentiate the Δ^9-THC-induced-hypothermia. This effect was only seen at 0.5 mg kg^{-1} and 2 mg kg^{-1} doses of Δ^9-THC and not at the higher dose of 5 mg kg^{-1}. It is possible that at this higher dose, the extracellular 5-HT and therefore the hypothermic effect had reached a maximum.

The Δ^9-THC-induced-hypothermia was shown to be a cannabinoid receptor mediated effect since it was blocked by the CB$_1$ receptor antagonist SR 141716. In autoradiographic studies using [^{3}H] CP 55,940 as a radiolabel, cannabinoid binding sites were found to be sparse in the hypothalamus compared with areas rich in cannabinoid binding such as the substantia nigra, globus pallidus, striatum and cerebellum (Herkenham *et al*., 1990). In contrast, another autoradiographic study using [^{3}H] WIN 55,212-2 as a radiolabel found the density of cannabinoid binding sites in the hypothalamus to be only marginally less than that in the cerebral cortex (Kuster *et al*., 1993), an area previously found to be quite dense in cannabinoid binding sites (Herkenham *et al*., 1990). It is possible that in the present study, Δ^9-THC is acting as an agonist at CB$_1$ receptors in the hypothalamus in order to produce hypothermia, although the activation of CB$_1$ receptors in other regions of the brain may also be involved.

The existence of cannabinoid receptors on presynaptic nerve endings has recently been suggested on noradrenergic neurones, as CB$_1$ receptor mRNA was found to be present in sympathetic ganglion (Ishac *et al*., 1996). Hence, the possibility exists that CB$_1$ receptors are located presynaptically on other types of neurones, for example, serotonergic neurones. Ishac *et al*. (1996) also showed that Δ^9-THC and anandamide inhibited electrically stimulated [^{3}H]-noradrenaline release from isolated atria and vas deferentia, indicating an inhibitory effect on neurotransmitter release following presynaptic CB$_1$ receptor activation. This is in keeping with other reports *in vitro* of cannabinoids showing an inhibitory effect on neuronal release. For example, Gifford & Ashby (1996) reported that electrically evoked acetylcholine release from hippocampal slices can be inhibited by a cannabinoid receptor agonist (WIN 55212-2). Cannabinoids have been shown to inhibit adenylate cyclase activity (Howlett & Fleming, 1984; Barg *et al*., 1995) and enhance voltage-sensitive potassium channels (Deadwyler *et al*., 1995); two cellular effects that would be expected to inhibit neurotransmitter release. If the predominant action of cannabinoids on presynaptic neurones is that of inhibition, and if as hypothesized in the present study, CB$_1$ receptor activation results in an increase in 5-HT release in order to produce hypothermia, then perhaps CB$_1$ receptor activation inhibits another neurotransmitter system which in turn affects the release of 5-HT. γ-Aminobutryric acid (GABA) is in abundance in the hypothalamus (Tappaz *et al*., 1977; Singewald *et al*., 1993) and it has been reported that GABA can exert an inhibitory effect on serotonergic neurones (Gallager & Aghajanian, 1976; Nishikawa & Scatton, 1983; Tao *et al*., 1996). Therefore it is possible that CB$_1$ receptor activation inhibits the release of GABA which causes a disinhibition of 5-HT neurones, and the resultant increase in basal 5-HT is responsible for the hypothermic response.

The prevalent theme that exists in the reports mentioned above that found an inhibitory effect of cannabinoids, is that in each case, the system being examined was stimulated; either

by forskolin in the case of adenylate cyclase activity (Howlett & Fleming, 1984) and potassium channel activity (Deadwyler *et al*., 1995), by prostaglandin E_1 (Barg *et al*., 1995) or by electrically evoked neurotransmitter release (Gifford & Ashby, 1996). Recent evidence suggests that CB_1 receptor activation can also produce opposing effects depending on the level of stimulation of the system being investigated. Maneuf & Brotchie (1997) showed that cannabinoids can inhibit forskolin stimulated cAMP accumulation, but paradoxically can increase basal cAMP accumulation in the absence of forskolin; that is in an unstimulated system. Hence, the speculative proposal that CB_1 receptors are located on serotonergic nerve endings, and when stimulated increase basal extracellular 5-HT levels which produces hypothermia cannot be ruled out.

Conclusion

It is suggested from the results of the present study that Δ^9-THC-induced hypothermia occurs via activation of CB_1 receptors. This may be a direct effect through activation of CB_1 receptors located presynaptically on 5-HT neurones, or an indirect effect through activation of CB_1 receptors located on neurones that modify the release of 5-HT in the hypothalamus. The activity of the 5-HT neurones involved may modify the body temperature response observed.

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