



Modification of 5-HT₂ receptor mediated behaviour in the rat by oleamide and the role of cannabinoid receptors

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Abstract

Oleamide (*cis*-9,10-octadecenoamide) is an endogenous brain lipid which has been suggested to induce sleep in experimental animals. The mechanism of action is unclear but shares many of the characteristics of endogenous cannabinoids such as anandamide and has been shown to enhance *in vitro* responses to 5-HT and GABA. In the present study we investigated the effects of oleamide on two motor behaviours, back muscle contractions (BMC) and wet-dog shakes (WDS) induced in rats by treatment with the 5-HT₂ receptor agonist DOI ((+/-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride). We then examined the potential involvement of CB₁ cannabinoid receptors in the responses to oleamide and the mechanism of interaction between CB₁ and 5-HT₂ receptors. Oleamide and the cannabinoid receptor agonist HU210 (6aR)-*trans*-3-(1,1-dimethylheptyl)6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol produced a hypolocomotion which was prevented by the CB₁ antagonist SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride). Despite having no effect alone, oleamide and HU210 potentiated BMC induced by treatment with DOI. SR141716A alone did not affect the response to DOI but it blocked the potentiations caused by oleamide or HU210. WDS were unaffected by oleamide and slightly reduced by HU210. *In vitro*, oleamide and HU210 enhanced the high affinity binding of 5-HT to 5-HT₂ receptors on rat cerebral cortex membranes labelled with ³H-ketanserin. Neither agent, however, altered 5-HT-stimulated phosphoinositide hydrolysis in rat cerebral cortex slices. Oleamide occupied CB₁ cannabinoid receptors on rat brain membranes labelled with ³H-CP55940 with an IC₅₀ of 10 μM. The data presented are consistent with oleamide acting via a cannabinoid recognition site to enhance 5-HT₂ receptor function *in vivo*. The mechanism of the modulation is still unclear but it does not appear to involve a potentiation of 5-HT₂ receptor-stimulated phosphoinositide hydrolysis. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Oleamide (*cis*-9,10-octadecenoamide) is a lipid isolated from the cerebrospinal fluid of sleep-deprived cats and which, on injection into rats, caused a state resembling physiological sleep (Cravatt et al., 1995). On this basis, oleamide has been proposed to be an endogenous sleep-inducing agent.

Oleamide shares many of the characteristics of endogenous cannabinoid compounds typified by anandamide. For instance, both agents inhibit gap-junction-mediated Ca²⁺ fluxes (Cravatt et al.,

1996), lymphocyte proliferation (Langstein et al., 1996) and induce, in mice, a characteristic 'tetrad' of behaviours (Mechoulam et al., 1997).

Oleamide has been demonstrated to be synthesised from oleic acid and ammonia in rat brain microsomes (Sugiura et al., 1996) and mouse neuroblastoma cells (Bisogno et al., 1997), although other routes have been proposed for the formation of fatty acid primary amides (Merkler et al., 1996). An enzyme, fatty acid amide hydrolase (FAAH), that catalyses the hydrolysis of the amide bond of oleamide and anandamide has been cloned (Cravatt et al., 1996) and is widely distributed in rat, mouse and human tissues including liver and brain (Maurelli et al., 1995; Cravatt et al., 1996; Giang and Cravatt, 1997).

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The mechanism of action of oleamide is unclear. It is reported to be unable to stimulate GTP γ S binding and, therefore, not to be an agonist for the central (CB $_1$) cannabinoid receptor (Boring et al., 1996). However, oleamide is a substrate for FAAH and could potentiate the effects of other endocannabinoids by competing for the enzyme.

Alternatively, oleamide has been reported to act indirectly on serotonergic mechanisms. Huidobro-Toro and Harris (1996) reported that the Ca $^{2+}$ -dependent Cl $^-$ currents, generated in response to 5-HT in *Xenopus* oocytes expressing 5-HT $_{2A}$ and 5-HT $_{2C}$ receptors were potentiated selectively in the presence of low concentrations of oleamide. A similar effect of oleamide on second messenger responses to 5-HT was observed by Thomas et al. (1997) in mammalian cells expressing 5-HT $_{2A}$ or 5-HT $_7$ receptors. Very recently, Lees et al. (1998) reported that oleamide also enhances GABA $_A$ receptor-mediated responses in rat brain neurones in primary culture. It is, therefore possible that oleamide acts as an allosteric modulator of certain G protein linked and ion channel receptors.

The functional significance of the *in vitro* effects of oleamide on 5-HT responses in model cell systems is uncertain. Therefore, in this study, we have determined the effects of oleamide in a behavioural model of 5-HT $_2$ receptor activation. Furthermore we have addressed the possibility that oleamide might act via cannabinoid receptors by comparing its effects with those of the high affinity cannabinoid agonist HU-210 on 5-HT $_2$ receptor binding in rat brain membranes, 5-HT-mediated phosphoinositide hydrolysis in brain slices and in the behavioural model. We propose that the oleamide modulation of 5-HT $_2$ responses in rat brain is a cannabinoid receptor-mediated phenomenon, mediated via a modulation of the affinity of 5-HT for the 5-HT $_2$ receptor but which does not result in any change in phosphoinositide hydrolysis.

2. Methods

2.1. Behavioural measurements

2.1.1. Quantification of 5-HT $_2$ -receptor mediated behaviours

Experiments were performed on male Wistar rats (250–300 g Biomedical Services Unit, QMC, Nottingham, UK). Animals were housed in groups of five per cage, with a 12 h light–dark cycle (first light 07:00 h). Food and tap water were available *ad libitum*.

Animals were treated with the 5-HT $_2$ receptor agonist DOI ((+/-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride) (1 mg/kg *i.p.*) alone, or 5 min before administration of either saline, the cannabinoid receptor agonist HU-210 (dissolved in saline,

20 μ g/kg *i.p.*) or oleamide (ODA) which was dissolved in a ethanol/triacetin (50/50) solution and incorporated in the commercially available fat emulsion Intralipid. Briefly, the ethanol/triacetin/ODA solution was filtered into the required volume of Intralipid (10%) using a 26 gauge needle. Aliquots of 0.2 ml were added one at a time and the emulsion was sonicated after each addition using a Soniprep 150 Ultrasonic Disintegrator (MSE Scientific Instruments, Sussex, UK) fitted with a probe assembly with a 9.0 mm tip. The probe was operated at an amplitude of 14 μ for 30 s bursts. During sonication the emulsion was kept in ice to avoid inactivation of the compound due to subsequent rise in temperature. The emulsion was made up to the desired volume of Intralipid and injected at a dose of 2.0 mg/kg *i.p.*

Wet-dog shakes (WDS) and back muscle contractions (BMC) were counted immediately after the last injection. WDS were defined as ‘a paroxysmic shudder of the head, neck and trunk reminiscent of the purposeful movement seen in dogs’ according to Bedard and Pycocock (1977). BMC were counted only if a clear-cut powerful contraction sweeping from the back of the neck along the back to the tail was present (Fone et al., 1989). Behaviours were counted every 5 min for 50 min after the second injection by a trained observer ‘blind’ to the treatment protocol.

For the second set of experiments the rats were given the cannabinoid receptor antagonist SR141716A (in the same emulsion as ODA, 3.0 mg/kg *i.p.*) or saline 15 min before DOI (1.0 mg/kg) followed 5 min later by ODA (2.0 mg/kg) or HU-210 (20 μ g/kg).

2.1.2. Locomotor activity experiments

Rats were given SR141716A (3.0 mg/kg) followed 20 min later by HU-210 (100 μ g/kg) or ODA (10 mg/kg). Spontaneous locomotor activity was monitored using perspex observation cages. The animals had no prior experience with this type of environment. The movement of each rat from one side of the box to the other resulted in infra red (IR) beams being broken and an incremental count was recorded by a computer (Medical Physics, University of Nottingham). A set of five infra red beams 3 cm above the floor of the box measured locomotor activity whereas the five parallel beams situated 15 cm above the floor recorded rears. Twelve readings for each activity were taken every 5 min over a period of 1 h.

2.2. Binding of 3 H-CP55940 to cannabinoid receptors

The method employed to measure the binding of oleamide to cannabinoid receptors on rat cerebellar membranes was essentially that described by Devane et al. (1988).

2.3. Membrane preparation

Male Hooded Lister rats were decapitated, their brains removed and cerebella and cerebral cortices were dissected for membrane preparation. Cerebella were immersed in ice-cold sucrose solution (320 mM) containing 2 mM EDTA and 5 mM MgCl₂ and homogenized using a Potter–Elvehjem glass/teflon homogenizer. The homogenate was centrifuged at 1000 × *g* for 5 min, the pellet discarded, the supernatant diluted 4-fold with ice-cold Tris buffer (50 mM, pH7) and centrifuged at 38 000 × *g* for 20 min. The pellet was resuspended in Tris (50 mM)/EDTA (2 mM)/Mg²⁺ (5 mM) buffer using a Polytron disrupter, incubated at 37°C for 10 min and centrifuged at 38 000 × *g* for 10 min. The pellet was resuspended in the same buffer, incubated again (30°C for 40 min) and centrifuged at 38 000 × *g* for 10 min. The final washed pellet was resuspended in assay buffer (Tris (50 mM)/EDTA (2 mM)/MgCl₂ (5 mM) pH 7.4) to a protein content of about 4 mg/ml. The homogenate was stored at –30°C prior to use.

2.4. Binding assay

About 150 µg protein was added to incubation tubes containing 0.5 nM [³H]-CP55940, a cannabinoid receptor agonist, and competing drugs in a final vol. of 1 ml, made up with assay buffer containing 0.2 mg/ml bovine serum albumin (BSA). The radioligand and drug solutions were made up in assay buffer containing 5 mg/ml BSA. After incubation at 30°C for 90 min the incubations were terminated by rapid filtration (using a Brandel cell harvester) over glass fibre filters that had been presoaked for at least 1 h in assay buffer containing 5 mg/ml BSA. The filters were washed with ice cold buffer (Tris 50 mM pH 7 containing 0.5 mg/ml BSA) scintillation fluid added and trapped radioactivity measured by liquid scintillation spectrometry. Non-specific binding was measured in the presence of 1 µM HU-210. When appropriate, assays were conducted in the presence of 150 µM phenyl-methylsulphonyl fluoride (PMSF) an inhibitor of fatty acid amido hydrolase, the enzyme that degrades endogenous cannabinoids.

2.5. 5-HT₂ receptor binding

5-HT₂ receptors were labelled with [³H]ketanserin essentially as described by Kendall and Nahorski (1985). Briefly, rat cerebral cortical membranes (about 0.5 mg protein) were incubated for 15 min at 37°C in 1 ml of assay buffer (50 mM Tris, pH 7.7 plus ascorbic acid (5.7 mM), pargyline (10 µM) and CaCl₂ (4 mM)) containing 1 nM [³H]ketanserin, competing drugs and, when appropriate, oleamide or HU-210.

Incubations were terminated by rapid filtration through glass fibre filters followed by washing with ice cold buffer (Tris, 50 mM, pH 7). Non-specific binding was determined in the presence of 1 µM methysergide and represented about 50% of total binding.

2.6. Phosphoinositide hydrolysis

5-HT-stimulated phosphoinositide hydrolysis was determined by monitoring the accumulation of total [³H]-inositol phosphates ([³H]-InsP) in rat cerebral cortex slices in the presence of 5 mM LiCl, essentially as described by Brown et al. (1984). Cross-chopped slices (350 × 350 µm) were incubated at 37°C for 60 min in a Krebs Henseleit buffer (KHB) gassed with O₂/CO₂ (95/5%). Fifty microlitre aliquots of slices were then incubated with ³H-myoinositol (0.3 µCi) for 30 min. When appropriate, oleamide or HU-210 were added followed 10 min later by LiCl (5 mM) and increasing concentrations of 5-HT. After incubating at 37°C for 45 min the incubations were terminated by the addition of chloroform/methanol (1:2 v/v). After adding more chloroform and water (Brown et al., 1984) [³H]-InsPs were separated from the aqueous phase by ion exchange chromatography using Dowex-50 resin in the Cl⁻ form. [³H]-phospholipids ([³H]-PI) were separated from the chloroform phase by evaporation overnight at room temperature. Phosphoinositide hydrolysis was expressed as the ratio [³H]-InsPs(dpm): [³H]-PI (dpm) × 100.

3. Materials

Cis-oleamide was purchased from Tocris Cookson Chemicals (UK); the *trans* isomer was synthesized by Dr E.A. Boyd, School of Pharmaceutical Sciences, University of Nottingham. In some experiments oleamide was formulated as an oil in water emulsion by first dissolving in soya oil followed by sonication in water in the presence of an emulsifying agent. SR141716 was a generous gift from Sanofi. [³H]-myoinositol and [³H]-CP55940 was from Amersham International (UK). Intralipid was purchased from Pharmacia and Upjohn. All other drugs and chemicals were purchased from either Sigma (Dorset, UK) or Fisons (Fisher Scientific, UK).

4. Data analysis

Behavioural data were compared using the computer programme Prism 2 (Graph Pad, California, USA). Sigmoidal curves were fitted to concentration/response and binding data using the same software.

5. Results

5.1. Effects of oleamide on ^3H -CP55940 binding

The *cis* isomer of oleamide competed for [^3H]-CP55940 binding to rat cerebellar membranes in a concentration-dependent manner. For the emulsified preparation, (Fig. 1) IC_{50} values in the absence of PMSF ($11.1 \pm 1.3 \mu\text{M}$) were no different from those in the presence of $150 \mu\text{M}$ PMSF ($9.7 \pm 1.7 \mu\text{M}$). When oleamide was added as an ethanolic solution (final ethanol concentration 2.5%) the IC_{50} value in the absence of PMSF was $20.8 \mu\text{M}$ and in the presence of the protease inhibitor it was $10.8 \mu\text{M}$ ($n = 2$).

At a maximally effective concentration of $300 \mu\text{M}$, the *trans* isomer of oleamide displaced only $20.3 \pm 0.5\%$ of specific ^3H -CP55940 binding ($n = 3$).

5.2. Effects of oleamide on locomotor activity in the rat

Oleamide significantly decreased locomotor activity in a dose-dependent fashion (Fig. 2A). From this experiment a dose of 2.0 mg/kg was selected for use in the next experiment because, at this dose, ODA did not produce an obvious sedation. A similar decrease in locomotion was induced by the cannabinoid receptor agonist HU-210 (Fig. 2A). Despite having no effect alone on locomotor activity, the cannabinoid CB_1 receptor antagonist SR141716A (3.0 mg/kg) partially inhibited the decreases in activity induced by oleamide (Fig. 2B).

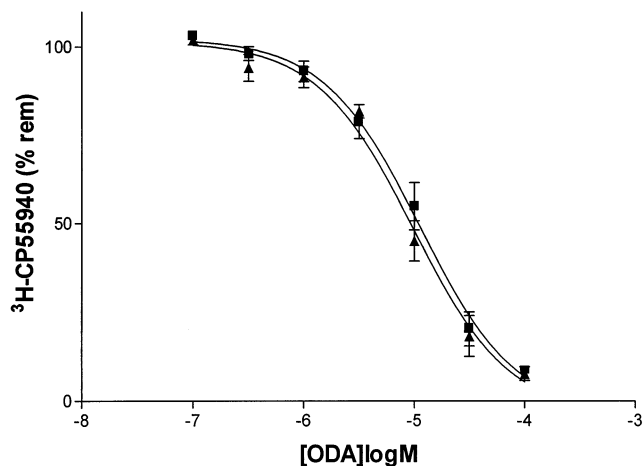


Fig. 1. Oleamide (ODA) competition for ^3H -CP55940 binding to rat cerebellar membranes. Data are presented as percentage specific ^3H -CP55940 binding remaining in the presence of increasing concentrations of ODA and are expressed as means \pm SEM of three independent experiments each conducted in triplicate. Experiments were conducted in the presence (s) or absence (n) of $150 \mu\text{M}$ PMSF. In the experiments shown ODA was prepared in an oil in water emulsion (Section 2).

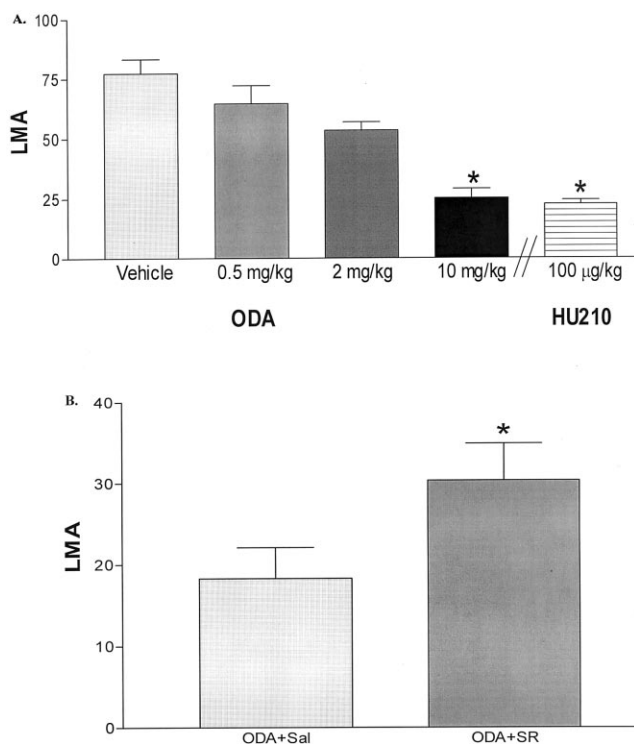


Fig. 2. (A) The effect of oleamide (ODA; 0.5 mg/kg , 2 mg/kg and 10 mg/kg , i.p.) and HU210 ($100 \mu\text{g/kg}$, i.p.) on rat locomotor activity over 60 min. Values are mean \pm SEM shown by vertical bars. * $P < 0.05$ Duncan New Multiple Range Test compared with vehicle-treated controls, $n = 10$. (B) The effect of the cannabinoid CB_1 receptor antagonist SR141716A (3 mg/kg , i.p.) injected 20 min before ODA (10 mg/kg)-induced hypoactivity. Data are shown as means \pm SEM. The vertical axes show total locomotor activity (LMA) and represent breaks of the IR beam in the middle of the activity box after injection of SR141716A and ODA (ODA + SR) or saline and ODA (ODA + Sal). * $P < 0.05$ Mann–Whitney Two Tailed U -test, $n = 12$ for each group.

5.3. Effect of oleamide on DOI-induced behaviours

Injections of saline (0.2 ml i.p.) or oleamide alone failed to provoke the appearance of either BMC or WDS. Injections of DOI (1.0 mg/kg i.p.), however, did induce both behaviours (Figs. 3 and 4). Animals treated with both oleamide and DOI exhibited a significantly greater number of BMC but the frequency of WDS was unchanged (Fig. 3A and B, respectively). HU-210 ($20 \mu\text{g/kg}$ i.p.) had a similar effect, potentiating DOI-induced BMC, but the frequency of WDS was reduced (Fig. 4A and B, respectively).

SR141716A (3.0 mg/kg i.p.) alone did not alter the number of DOI-induced BMC or WDS (data not shown) but it blocked the potentiation of the BMC due to oleamide without having an effect on WDS (Fig. 5A and B, respectively).

5.4. Effect of oleamide on the affinity of 5-HT for 5-HT₂ receptors in rat cerebral cortex membranes

5-HT competed for [³H]ketanserin-labelled binding sites in a concentration-dependent manner (Fig. 6A). The curves were best fitted to a two site model comprising a high affinity portion ($IC_{50} = 80 \pm 2$ nM) and a lower affinity portion ($IC_{50} = 13 \pm 0.5$ μ M). In the presence of 500 nM oleamide, the high affinity portion was shifted significantly, 5-fold, ($P < 0.05$, $n = 3$; Student's *t*-test) to the left ($IC_{50} = 16 \pm 0.6$ nM). The lower affinity portion was also shifted significantly ($P < 0.05$, $n = 3$, Student's *t*-test) but by a lesser degree (1.9-fold; $IC_{50} = 7 \pm 1$ μ M).

These effects are at least somewhat selective because oleamide (500 nM) had no effect on the affinity of the cholinergic agonist carbachol for the muscarinic receptor in rat cerebral cortex homogenates labelled with ³H-quinuclidinyl benzilate (data not shown).

5.5. Effect of HU-210 on the affinity of 5-HT for 5-HT₂ receptors in rat cerebral cortex membranes

In a manner similar to that of oleamide, the presence of HU-210 (500 nM) caused a shift in the IC_{50} of the higher affinity portion of the 5-HT competition curve, in these experiments, from 148 ± 50 to 43 ± 5 nM ($P < 0.05$, $n = 3$, Student's *t*-test) (Fig. 6B). The lower

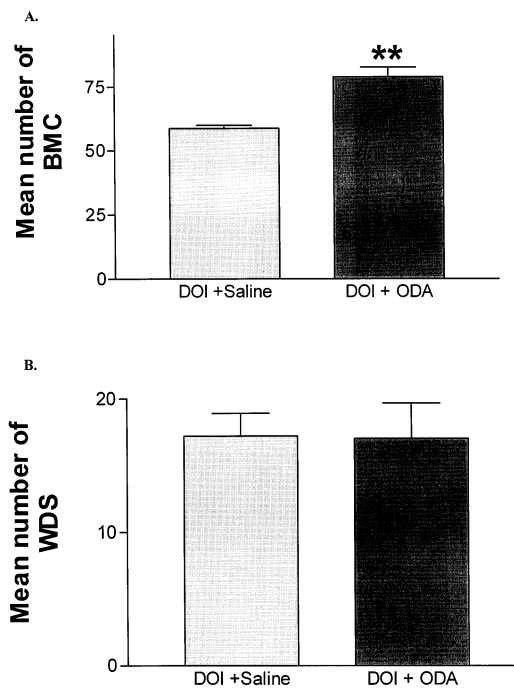


Fig. 3. The effect of oleamide (ODA, 2 mg/kg, i.p.) or saline injected 5 min after DOI (1 mg/kg) on the number of A) BMC or B) WDS observed over 50 min. ** $P < 0.01$, Mann-Whitney two tailed *U*-test, $n = 6$ for BMC. No significant effect was evident for WDS using a Mann-Whitney two tailed *U*-test, $n = 6$.

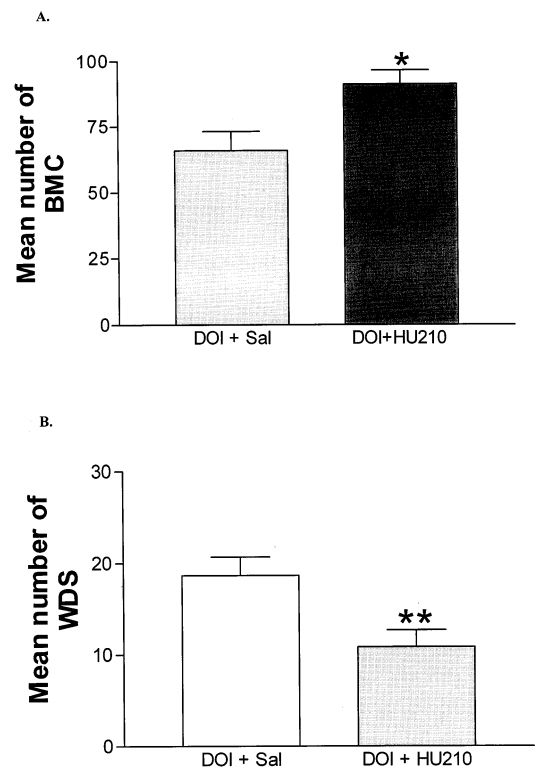


Fig. 4. The effects of the cannabinoid agonist HU210 (20 μ g/kg, i.p.) on the number of DOI (1 mg/kg)-induced: (A) BMC or (B) WDS observed over 50 min. HU210 or saline (Sal) were injected 5 min after DOI. Mean values \pm SEM are shown by vertical bars. * $P < 0.05$ and ** $P < 0.01$, Mann-Whitney two tailed *U*-test., $n = 6$ for BMC and WDS, respectively.

affinity portion was unchanged ($IC_{50} = 8 \pm 0.5$ μ M in the absence and 7 ± 0.4 μ M in the presence of HU-210).

5.6. Effect of oleamide on 5-HT-stimulated phosphoinositide hydrolysis in rat cerebral cortex slices

5-HT stimulated ³H-InsPs accumulation to a maximum of 6.8 ± 0.7 ratio units (Section 2) over basal with an EC_{50} of 9.8 ± 0.2 μ M ($n = 3$). This was unaltered by co-incubation with 500 nM oleamide ($E_{max} = 6.3 \pm 0.2$ μ M; $EC_{50} = 7.5 \pm 0.5$ μ M) (Fig. 7).

In a separate experiment HU-210 (500 nM) was similarly without effect on 5-HT-stimulated ³H-InsPs accumulation (data not shown).

6. Discussion

The results presented demonstrate that oleamide produces behavioural effects that appear to involve the activation of cannabinoid receptors. The oleamide-induced reduction in locomotor activity was reversed by the selective cannabinoid CB₁ receptor antagonist SR141716A (Rinaldi-Carmona et al., 1994) as was that

caused by the potent cannabinoid agonist HU-210 (Mechoulam et al., 1988). It has been suggested (Mechoulam et al., 1997) that the behavioural effects of oleamide resemble those of the endocannabinoid anandamide, not because of a common activation of CB₁ receptors, but indirectly, due to preservation of endocannabinoids via competitive inhibition of the enzyme FAAH for which oleamide is a substrate (Maurelli et al., 1995). It was also stated that oleamide is not a cannabinoid because it does not bind to the CB₁ receptor, even at a concentration of 10 μ M (Mechoulam et al., 1997). However, in the present study, this was clearly not the case. Oleamide inhibited the binding of [³H]-CP55940 to rat cerebellar membranes in an apparently stereospecific manner with an affinity in the micromolar range. The affinity was 2-fold higher in the presence of the serine protease inhibitor PMSF consistent with oleamide being a substrate for the PMSF-sensitive FAAH activity retained by brain-derived plasma membranes (Childers et al., 1994). Interestingly, the emulsified preparation of oleamide had a greater affinity than the ethanolic preparation and was unaf-

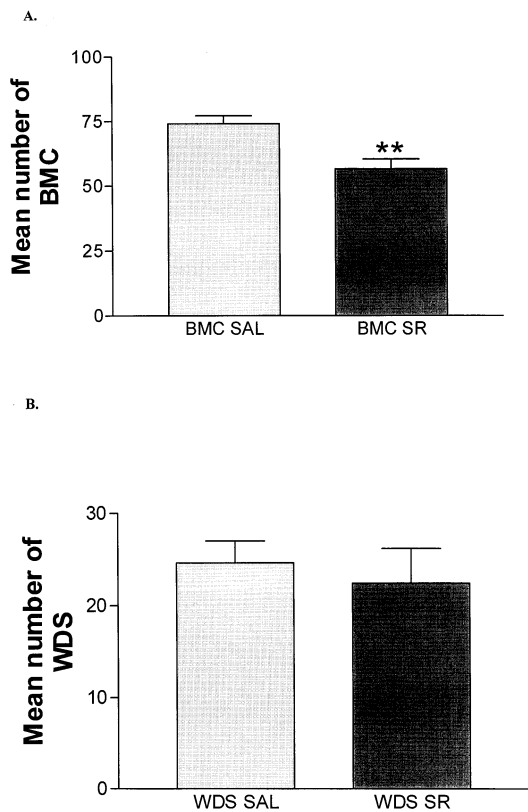


Fig. 5. The effects of SR141716A (3 mg/kg, i.p.) on the ODA-potentiated back muscle contraction and wet-dog shake responses to DOI. SR141716A or saline (SAL) was injected 15 min before DOI (1 mg/kg) followed 5 min later by ODA (2 mg/kg), indicated as (A) 'BMC SR' or (B) 'WDS SR'. Mean values \pm SEM are shown by vertical bars. A significant effect of SR141716A on BMC production is indicated by ** $P < 0.01$ Mann-Whitney two tailed U -test, $n = 10$. Again, no effect was observed on WDS production.

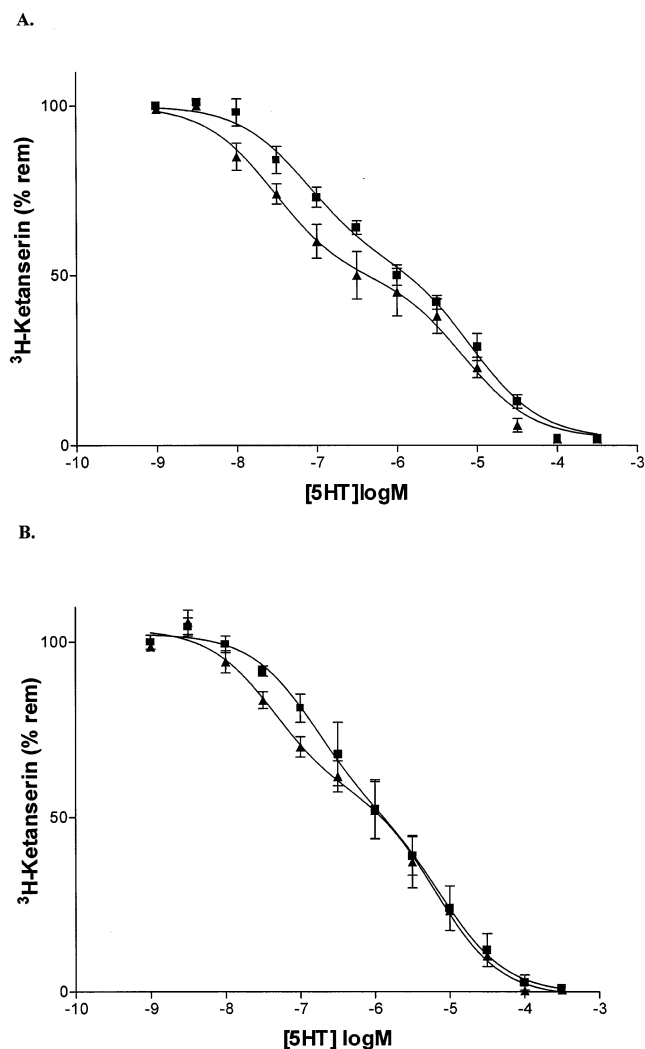


Fig. 6. The effects of oleamide (A) and HU210 (B) on 5-HT competition for ³H-ketanserin binding to rat cerebral cortex membranes. Membranes were incubated with 1 nM ³H-ketanserin and a range of concentrations of 5-HT in the presence (s) or absence (n) of 500 nM oleamide (A) and the presence (s) or absence (n) of 500 nM HU210 (B). The data represent the means \pm SEM of three separate experiments each conducted in triplicate. IC₅₀ values (see text) for each component of the curves were compared by Student's unpaired t -test.

ected by PMSF indicating that the emulsion form protects against FAAH catabolism. We have found a similar protective effect with emulsified preparations of anandamide (Walton et al., unpublished). Agonist activity cannot be inferred from the present radioligand binding studies and oleamide has been reported to be unable to enhance GTP γ S binding to brain preparations suggesting a lack of efficacy at CB receptors (Boring et al., 1996). However, other studies (Griffin et al., 1998) have reported that even well-recognised cannabinoid receptor agonists such as anandamide and tetrahydrocannabinol fail to enhance GTP γ S binding (although this is disputed, Petit et al., 1997) implying that this method may not reliably detect CB receptor

agonist activity. Oleamide does, however, appear to be a ligand at central CB₁ receptors and, in concert with its potential amplification of endocannabinoid responses via competition for FAAH, this could underlie some of its behavioural effects such as the hypolocomotion observed in the present study.

In vitro studies have indicated that another possible mechanism of action of oleamide is via indirect modulation of various 5-HT receptors including 5-HT_{2A}, 5-HT_{2C} and 5-HT_{1A} involving either potentiations or inhibitions of phosphoinositide hydrolysis and cyclic AMP formation (Huidobro-Toro and Harris, 1996; Thomas et al., 1997). Evidence from the present study shows that oleamide is also able to modify in vivo responses mediated by 5-HT receptors. Peripheral application of the 5-HT₂ agonist DOI provoked two readily identifiable and quantifiable motor behaviours, WDS and BMC. Oleamide enhanced BMC without affecting WDS. Both of these DOI-induced behaviours are thought to be mediated by 5-HT₂ receptors since they are blocked by the 5-HT₂ antagonists ketanserin, ritanserin and mianserin (Fone et al., 1991) although whether the same subtypes are involved is not known. It is likely that some of 5-HT receptors subserving WDS and BMC are spinal since the behaviours can be initiated by intrathecal administration of DOI, although supraspinal circuits are also involved in the WDS (Fone et al., 1991). It is unclear why BMC but not WDS were potentiated by oleamide. One possibility is that each is supported by a different 5-HT₂ receptor subtype only one of which is modulated by oleamide. Positive modulations of 5-HT_{2A} and 5-HT_{2C} responses by oleamide have been reported (Huidobro-Toro and Harris, 1996; Thomas et al., 1997) but its effects on 5-HT_{2B} are not known. There is, however, no direct

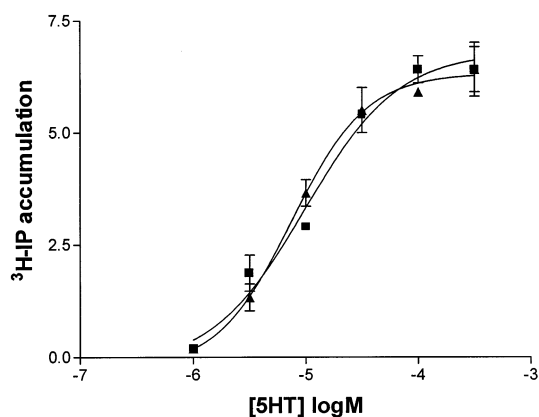


Fig. 7. No effect of oleamide on 5-HT-stimulated phosphoinositide hydrolysis in rat cerebral cortex slices. Slices were incubated with a range of concentrations of 5-HT in the presence (s) or absence (n) of oleamide (500 nM). The figure represents means \pm SEM from three separate experiments each conducted in triplicate. Maximum responses and EC₅₀ values (see text) were compared by Student's unpaired *t*-test.

evidence for the involvement of the 5-HT_{2B} receptor in motor behaviours and there is, even, doubt about the expression of 5-HT_{2B} receptors in the rat brain (Shmuck et al., 1994). Alternatively, oleamide might have differential effects on some other component of the polysynaptic pathways supporting WDS and BMC.

It is probable that, whatever the 5-HT receptor subtype involved, the potentiation of BMC by oleamide is mediated by a cannabinoid receptor. This is based on two findings. First, the potentiation was blocked by the CB₁ receptor antagonist SR141716A and, second, the potentiation was mimicked by the high affinity cannabinoid agonist HU210. The lack of effect of SR141716A alone on the response to DOI suggests that, at least under the prevailing experimental conditions, endogenous cannabinoids do not exert a tonic control over 5-HT₂-mediated responses.

A number of possibilities present themselves to explain the potentiated responses. First, there might be cell-cell interactions in which the cannabinoid receptors reside on different cells to those expressing the 5-HT₂ receptors affecting the latter by alterations in synaptic activity. However, potentiations of 5-HT receptor responses by oleamide have been observed in individual *Xenopus* oocytes (Huidobro-Toro and Harris, 1996) and in clonal cell cultures (Thomas et al., 1997) indicating that interactions within the same cell are possible. Whether such interactions are at the receptor or post-receptor level is yet to be clarified. In the present study, oleamide and HU210 modified the binding of 5-HT to 5-HT₂ receptors labelled with the antagonist ketanserin in a cerebral cortex membrane preparation. Both cannabinoids modestly, but significantly, enhanced high affinity 5-HT binding but this was not reflected by an increase in the second messenger response to 5-HT in cortical slices. Neither oleamide nor HU210 affected 5-HT-stimulated phosphoinositide hydrolysis. This contrasts with the enhancements in P11 cells reported by Thomas et al. (1997) but is in agreement with our previous study in which oleamide failed to affect 5-HT-stimulated phosphoinositide hydrolysis in SHSY-5Y cells transfected with the human 5-HT_{2A} receptor (Cadogan et al., 1998).

This failure to detect a correlation between oleamide-mediated increases in the affinity of 5-HT for the 5-HT₂ receptor and the second messenger response might indicate that there is no connection between the effects of oleamide at the 5-HT recognition site and the altered behavioural responses. However, it is also possible that the two effects are linked but via a different signal transduction system. Although all of the members of the 5-HT₂ receptor family can couple to phosphoinositide hydrolysis (Hoyer et al., 1994) the 5-HT_{2B} receptor in human pulmonary artery endothelial cells and in rat stomach fundus causes intracellular calcium release via

a mechanism independent of phosphoinositide hydrolysis (Cox and Cohen, 1996; Ullmer et al., 1996). Also, in the brain, changes in 5-HT₂ recognition sites and 5-HT₂-mediated behaviours do not necessarily result in parallel changes in phosphoinositide hydrolysis. For instance, chronic electroconvulsive shock increases the density of 5-HT₂ receptor binding sites and 5-HT₂-mediated head shakes without affecting 5-HT-stimulated phosphoinositide turnover in rat brain (Moorman et al., 1996).

It should also be kept in mind that the motor behaviours examined in the present study are probably mediated by spinal 5-HT₂ receptors whereas the second messenger responses were measured in cerebral cortex. It is possible that differences exist in 5-HT receptor transduction systems in the different regions of the central nervous system.

Modifications of receptor activity by oleamide are not restricted to the 5-HT receptor family or even to G-protein coupled receptors. Lees et al. (1998) reported recently that oleamide increases the affinity of GABA for the GABA_A receptor and enhances electrophysiological responses to the inhibitory amino acid. These authors suggested that oleamide is an endogenous ligand for allosteric modulatory sites on particular isoforms of the GABA_A receptor but they did not investigate the potential involvement of cannabinoid receptors using CB₁ agonists or antagonists.

In summary, oleamide increases the affinity of 5-HT for 5-HT₂ recognition sites in rat brain membranes and potentiates a behavioural response to a 5-HT₂ receptor agonist. The sensitivity of the potentiation to a cannabinoid CB₁ receptor antagonist and the similarity of the response to a CB₁ receptor agonist indicates that there is an interaction at the membrane level between the CB and 5-HT receptors. Alternatively there could be an allosteric modulatory site on the 5-HT₂ receptor that recognises cannabinoids. Whether endogenously produced oleamide or other endocannabinoids, play a similar modulatory role in animal behaviours is as yet unclear and should be the subject of future studies.

References

- Bedard, P., Pycock, C.J., 1977. 'Wet-Dog' shake behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. *Neuropharmacology* 16, 663–670.
- Bisogno, T., Sepe, N., De Petrocellis, L., Mechoulam, R., Di Marzo, V., 1997. The sleep-inducing factor oleamide is produced by mouse neuroblastoma cells. *Biochem. Biophys. Res. Commun.* 239, 473–479.
- Boring, D.L., Berglund, B.A., Howlett, A.C., 1996. Cerebrodiene, arachidonyl-ethanolamide and hybrid structures: potential for interaction with brain cannabinoid receptors. *Prostaglandins Leukot. Essent. Fatty Acids* 55, 207–210.
- Brown, E., Kendall, D.A., Nahorski, S.R., 1984. Inositol phospholipid hydrolysis in rat cerebral cortical slices: I. receptor characterisation. *J. Neurochem.* 42, 1379–1387.
- Cadogan, A.-K., Fletcher, C., Debank, P., Marsden, C.A., Kendall, D.A., 1998. Differences in the in vivo effects of the endogenous sleep-inducing agent oleamide on 5-HT₂ receptor function. *Br. J. Pharmacol.* 123, 247P.
- Childers, S.R., Sexton, T., Roy, M.B., 1994. Effects of anandamide on cannabinoid receptors in rat brain membranes. *Biochem. Pharmacol.* 47, 711–715.
- Cox, D.L., Cohen, M.L., 1996. 5-HT_{2B} receptor signalling in the rat stomach fundus: dependence on calcium influx, calcium release and protein kinase C. *Behav. Brain Res.* 73, 289–292.
- Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., Gilula, N.B., 1996. Molecular characterisation of an enzyme that degrades neuromodulatory fatty acid amides. *Nature* 384, 83–87.
- Cravatt, B.F., Prospero-Garcia, O., Siuzdak, G., Gilula, N.B., Henrikson, S.J., Boger, D.L., Lerner, R.A., 1995. Chemical characterisation of a family of brain lipids that induce sleep. *Science* 268, 1506–1509.
- Devane, W.A., Dysarz, F.A., Johnson, M.R., Melvin, L.S., Howlett, A.C., 1988. Determination and characterisation of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* 34, 605–613.
- Fone, K.C.F., Johnson, J.V., Bennett, G.W., Marsden, C.A., 1989. Involvement of 5-HT₂ receptors in the behaviours produced by intrathecal administration of selected 5-HT agonists and the TRH analogue (CG 3509) to rats. *Br. J. Pharmacol.* 96, 599–608.
- Fone, K.C.F., Robinson, A.J., Marsden, C.A., 1991. Characterisation of the 5-HT receptor subtypes involved in the motor behaviours produced by intrathecal administration of 5-HT agonists in rats. *Br. J. Pharmacol.* 103, 1547–1555.
- Giang, D.K., Cravatt, B.F., 1997. Molecular characterisation of human and mouse fatty acid amide hydrolases. *Proc. Natl. Acad. Sci. USA* 94, 2238–2242.
- Huidobro-Toro, J.P., Harris, R.A., 1996. Brain lipids that induce sleep are novel modulators of 5-hydroxytryptamine receptors. *Proc. Natl. Acad. Sci. USA* 93, 8078–8082.
- Kendall, D.A., Nahorski, S.R., 1985. 5-HT-stimulated inositol phospholipid hydrolysis in rat cerebral cortex slices: pharmacological characterisation and effects of antidepressants. *J. Pharmacol. Exp. Therap.* 233, 473–479.
- Griffin, G., Atkinson, P.J., Showalter, V.M., Martin, W.R., Abood, M.E., 1998. Evaluation of cannabinoid receptor agonists and antagonists using the guanosine-5'-O-(3-[³⁵S]thio)-triphosphate binding assay in rat cerebellar membranes. *J. Pharmacol. Exp. Therap.* 285, 553–560.
- Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P.A., 1994. VII International union of pharmacology classification of receptors for 5-hydroxytryptamine. *Pharmacol. Rev.* 46, 157–203.
- Langstein, J., Hofstadter, F., Schwarz, H., 1996. *Cis*-9,10-octadecenoamide an endogenous sleep-inducing CNS compound inhibits lymphocyte proliferation. *Res. Immunol.* 147, 389–396.
- Lees, G., Edwards, M.D., Hassoni, A.A., Ganellin, R., Galanakis, D., 1998. Modulation of GABA_A receptors and inhibitory synaptic currents by the endogenous CNS sleep regulator *cis*-9,10-octadecenoamide (cOA). *Br. J. Pharmacol.* 124, 873–882.
- Maurelli, S., Bisogno, T., De Petrocellis, L., Di Luccia, A., Marino, G., Di Marzo, V., 1995. Two novel classes of neuroactive fatty acid amides are substrates for mouse neuroblastoma anandamide amidohydrolase. *FEBS Lett.* 377, 82–86.
- Mechoulam, R., Frider, E., Hanus, L., Sheskin, T., Bisogno, T., Di Marzo, V., Bayewitch, M., Vogel, Z., 1997. Anandamide may mediate sleep induction. *Nature* 389, 25–26.
- Mechoulam, R., Feigenbaum, J.J., Lander, N., Segal, M., Jarbe, T.U.C., Hiltunen, A.J., Consroe, P., 1988. Enantiomeric cannabinoids: stereospecificity of psychotropic activity. *Experientia* 44, 762–764.

- Merkler, D.J., Merkler, K.A., Stern, W., Fleming, F.F., 1996. Fatty acid amide biosynthesis: a possible role for peptidylglycine α -amidating enzyme and acyl-coenzyme A: glycine *N*-acyltransferase. *Arch. Biochem. Biophys.* 330, 430–434.
- Moorman, J.M., Grahame-Smith, D.G., Smith, S.E., Leslie, R.A., 1996. Chronic electroconvulsive shock enhances 5-HT₂ receptor-mediated head shakes but not brain *c-fos* induction. *Neuropharmacology* 35, 303–313.
- Petitot, F., Jeantaud, B., Capet, M., Doble, A., 1997. Interaction of brain cannabinoid receptors with guanine nucleotide binding protein, a radioligand binding study. *Biochem. Pharmacol.* 54, 1267–1270.
- Rinaldi-Carmona, M., Barth, F., Heaulme, M., et al., 1994. SR141716A a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* 350, 240–244.
- Shmuck, K., Ullmer, C., Engels, P., Lubbert, H., 1994. Cloning and functional characterization of the human 5-HT_{2B} serotonin receptor. *FEBS Lett.* 342, 85–90.
- Sugiura, T., Kondo, S., Kodaka, T., Tonegawa, T., Nakane, S., Yamashita, A., Ishima, Y., Waku, K., 1996. Enzymatic synthesis of oleamide (*cis*-9,10-octadecenoamide), sleep inducing lipid, by rat brain microsomes. *Biochem. Molec. Biol. Int.* 40, 931–938.
- Thomas, E.A., Carson, M.J., Neal, M.J., Sutcliffe, J.G., 1997. Unique allosteric regulation of 5-hydroxytryptamine receptor-mediated signal transduction by oleamide. *Proc. Natl. Acad. Sci. USA* 94, 14115–14119.
- Ullmer, C., Boddeke, H.G.W.M., Shmuck, K., Lubbert, H., 1996. 5-HT_{2B} receptor mediated calcium release from ryanodine sensitive intracellular stores in human pulmonary artery endothelial cells. *Br. J. Pharmacol.* 117, 993–994.