British Journal of Pharmacology (1996) 119, 107-114

Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat

[1,*,†]Elliot A. Stein, *Scott A. Fuller, †William S. Edgemond & †William B. Campbell

*Departments of Psychiatry and †Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, U.S.A.

1 Arachidonylethanolamide (AEA; anandamide) has been isolated from mammalian brain and found to bind to, and is thought to be, an endogenous ligand for the cannabinoid receptor. In order to understand better its behavioural and physiological properties, we have examined its acute effects in unanaesthetized freely behaving rats.

2 Intravenous AEA caused dose-related decreases in locomotor behaviour, a pronounced hyperreflexia, and a moderate antinociceptive state. At doses between 3 and 30 mg kg^{-1}, a dose-dependent hypothermia and profound, time-dependent cardiovascular changes were also observed.

3 An immediate bradycardia exceeding 50% was seen within 10-15 s of administration and lasted up to 11 min following the highest dose of the drug. In contrast, the change in mean arterial pressure was biphasic: an immediate 20% decrease in mean arterial pressure followed by a significant increase in blood pressure that lasted about 13 min after the highest dose.

4 These data demonstrate that AEA in the unanaesthetized rat exerts behavioural and physiological effects generally similar to those seen following natural cannabinoids and synthetic cannabimimetic agents and suggests a role for AEA in regulation of various physiological processes.

Keywords: Anandamide; heart rate; blood pressure; endogenous cannabinoid; temperature

Introduction

Several recent observations have collectively enhanced our understanding of the mechanism of action of cannabinoids in the brain. These include the recent discovery and characterization of a specific brain receptor that binds with high affinity to a number of synthetic cannabimimetic agents (Devane *et al.*, 1988; Howlett *et al.*, 1990), the heterogeneous localization of the cannabinoid receptor (Herkenham *et al.*, 1991a,b; Mailleux & Vanderhaeghen, 1992; Matsuda *et al.*, 1993), the cloning of the receptor (Matsuda *et al.*, 1990), and the observation that cannabinoid binding can be inhibited by a substance released from brain slices (Evans *et al.*, 1992). This latter observation enhanced speculation concerning the existence of an endogenous cannabinoid ligand. The structure of one putative ligand, arachidonylethanolamide (AEA; anandamide), has recently been identified (Devane *et al.*, 1992) and appears to bind to and activate the cloned human cannabinoid receptor (Felder *et al.*, 1993).

Several reports suggest that AEA possesses biological activity. It has been shown to inhibit the electrically evoked contractions of mouse vas deferens (Devane *et al.*, 1992), to bind to rat brain membranes (Hillard *et al.*, 1995), inhibit calcium currents in neuroblastoma cells (Mackie & Hille, 1992), and inhibit forskolin-stimulated adenylate cyclase in COS cells (Vogel *et al.*, 1993) and rat brain membranes (Childers *et al.*, 1994). Enzymes for its synthesis and degradation also appear to be present in brain (Deutsch & Chin, 1993). Finally, several reports have indicated that AEA possesses behavioural activity commensurate with that of the principal psychoactive constituent of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC) (Fride & Mechoulam, 1993; Crawley *et al.*, 1993; Smith *et al.*, 1994; Varga *et al.*, 1995). While the pharmacological and behavioural profile of Δ^9-THC is well known (Dewey, 1986), our understanding of the behavioural and physiological effects of AEA are considerably more limited. As such, we have chosen to examine the dose-response profile of AEA in the freely behaving, unanaesthetized rat while monitoring several behavioural and physiological parameters known to be altered by Δ^9-THC. The results described here are consistent with the hypothesis that AEA possesses biological activity similar to Δ^9-THC, leading to the speculation that AEA may subserve one or more normal physiological functions.

Methods

Forty two male, Sprague-Dawley rats (Sasco, Madison, WI, U.S.A.) weighing between 250-350 g, were individually housed in plastic tubs and maintained on food and water *ad lib* in a temperature (22°C) and light controlled facility (lights on between 20 h 30 min-08 h 30 min) for at least 1 week prior to surgery. Rats to be used in the physiological experiments had chronic indwelling femoral arterial and venous catheters surgically implanted under pentobarbitone anaesthesia (60 mg kg^{-1}, i.p.) The arterial catheter was constructed from PVC and Tygon tubing, while the venous catheter was constructed from silicone rubber and polyethylene. They were passed subcutaneously to exit on the animals' back immediately adjacent to a Nylon screw that had been passed through a small piece of Nylon screen and sutured subcutaneously to serve as an anchor during subsequent physiological monitoring. Arterial catheters were flushed daily with a 1% heparinized saline solution and filled at the end of each daily experiment with a 5% solution of fibrinolysin (Sigma Chemical, St. Louis, MO, U.S.A.) in 500 units of heparin. A second series of rats was used to test for the effects of AEA on antinociception and had chronic i.v. catheters implanted into the right jugular vein. At least 3 days elapsed for surgical recovery prior to any experimental manipulations.

Procedure

Rats in group 1 were used to measure the effects of AEA or its principal metabolite, arachidonic acid (AA) on heart rate (HR), mean arterial pressure (MAP), core body temperature and general behaviour. On the day of the experiment, rats were placed in a Plexiglas chamber (30 × 21 × 21 cm) and allowed at

[1] Author for correspondence at Department of Psychiatry.

least 30 min to habituate to the environment. Arterial and venous lines were connected through a protective spring attached to the Nylon screw on the animal's back into a double fluid swivel (Kent Scientific, Litchfield, CT, U.S.A.) permitting relative freedom of movement. Arterial blood pressure was measured with a disposable pressure transducer (Cobe Instruments, Arvada, CO, U.S.A.) connected to a computerized polygraph (Dataq Instruments, Columbus OH, U.S.A.). Body temperature was continuously monitored with a Telethermometer (YSI Instruments, Yellow Springs, OH, U.S.A.) and a rectal thermocouple inserted 6 cm into the anus. General locomotor behaviour and sensory responsivity were monitored by two independent observers for up to 60 min after each i.v. drug injection. Doses of AEA were 3.0, 10.0 and 30.0 mg kg^{-1} while the AA group received 1.0, 3.0 and 10.0 mg kg^{-1}. Rats in each group received each dose (plus vehicle) in a Latin Square design twice/week. A final group of animals received doses of 3.0 and 10.0 mg kg^{-1} AEA in the presence of 5.0 mg kg^{-1} indomethacin (Indo) in 5 mg ml^{-1} olive oil s.c. 40 min prior to i.v. AEA.

The antinociceptive effects of AEA were tested in a separate group of animals by the hot plate method (O'Callaghan & Holtzman, 1975). Briefly, rats were placed on a copper surface through which hot water was passed to maintain surface temperature at 52.4°C. Rats were restricted to the plate by a 27 cm high by 28 cm diameter Plexiglas cylinder placed over the chamber. Rats were pre-exposed to the testing apparatus at room temperature 3 days prior to experimentation. Each rat in this series was tested 3 times with an inter-trial interval of 3 days. On the day of testing, rats were given 1 of 3 doses of AEA (0.3, 3.0 or 10.0 mg kg^{-1}) or vehicle. Rats received either all 3 doses in a Latin Square design to control for order effect or repeated vehicle administration to control for any effects of conditioning. Drug or vehicle was administered i.v. and 1 or 5 min later, rats were placed on the hot surface and the time to lick a hind paw determined. A maximum period of 45 s was established to prevent tissue damage.

Drug preparation

AEA was synthesized from arachidonyl chloride following the method of Devane *et al.* (1992) and stored under N_2 at −20°C until used. Briefly, arachidonic acid (AA) was added to dry dichloromethane with 1.2 equivalents of oxalyl chloride in the presence of 1 equivalent of dimethylformamide at 0°C to form the acid chloride of AA. After 15 min, the acid chloride was added to a 10 fold excess of ethanolamine and incubated for 60 min at 0°C. The reaction mixture was washed with water followed by removal of the solvent under N_2. AEA was separated from arachidonic acid by thin layer chromatography on silica gel HL plates developed with hexane/ethyl acetate/methanol (60/40/5) and stored under N_2 at −20°C until used. Purity and identity of AEA was established by n.m.r. and mass spectrometry. The drug was prepared fresh each day in an emulphor-ethanol vehicle (1:1) and diluted with saline to maintain a constant injection volume of 0.5 ml.

Statistics

Heart rate and blood pressure were normalized to percentage of predrug baseline and, along with core body temperature and hot plate response, were analyzed by separate 2 way analyses of variance (ANOVA) of time × dose. Due to the very rapid cardiovascular effects of AEA, separate HR and MAP analyses were performed for the first min post drug (5 s time resolution) and for the entire 30 min observation period (1 min time bins). Post hoc tests (Tukey's or paired *t* tests) were performed as appropriate. Differences in locomotor and sensory responsivity were determined via paired *t* tests. An *P* value of 0.05 was considered significant throughout.

Results

Anandamide (AEA)

Intravenous AEA administration exerted profound, brief duration, dose-related behavioural and physiological effects in the unanaesthetized rat. Administration of either 10 or 30 mg kg^{-1} AEA produced an immediate waxy catatonia; rats receiving the high dose assumed a prone position, abdomen flat on the chamber floor with all 4 limbs hanging through the cage bars, their legs unable to support their weight. At high (10 and 30 mg kg^{-1}) AEA doses, rats became ataxic and often lost

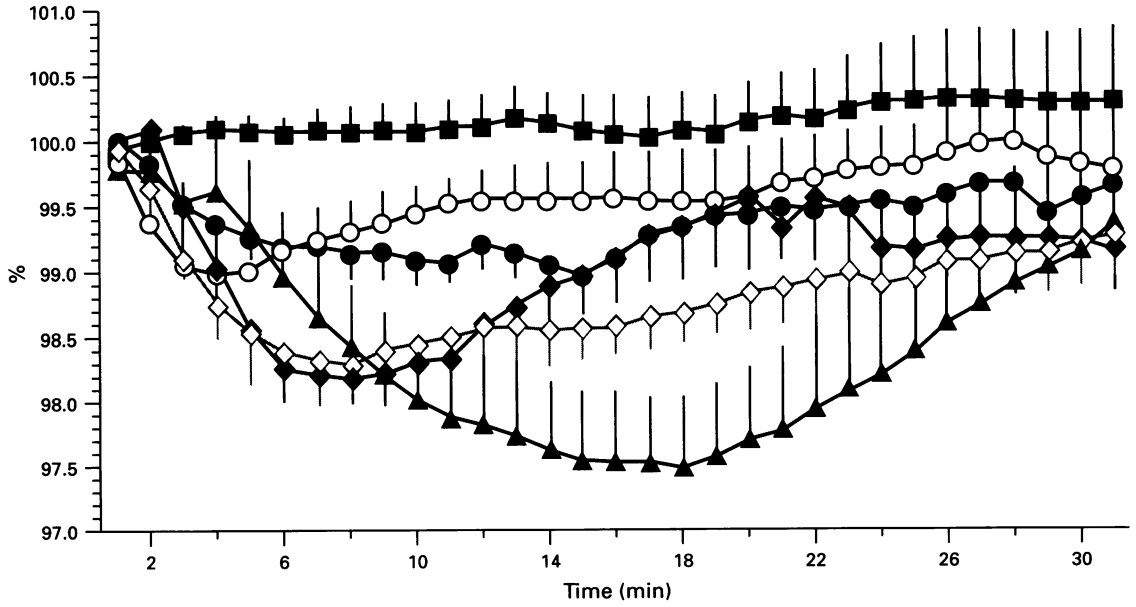

Figure 1 Dose-response effects of intravenous AEA on core body temperature during the 30 min test period. In contrast to the lack of effect of vehicle, all doses of AEA significantly ($P \leq 0.05$) reduced body temperature with an onset and duration of action of about 3 to 15 min for the low and medium dose and 8 to 16 min for the high dose. (■) Vehicle ($n=8$); (●) 3.0 mg kg^{-1} ($n=5$); (○) 3.0 mg kg^{-1} AEA + 5.0 mg kg^{-1} Indo ($n=5$); (◆) 10.0 mg kg^{-1} ($n=6$); (◇) 10.0 mg kg^{-1} AEA + 5.0 mg kg^{-1} Indo ($n=6$); (▲) 30.0 mg kg^{-1} ($n=6$).

their righting reflex. This behavioural state lasted for 13±3.9 min following 30 mg kg^{-1} and 4.2±1.9 min for the 10.0 mg kg^{-1} dose group (t=4.02; d.f.=6; P≤.007). In contrast, the 3 mg kg^{-1} dose produced virtually no locomotor depression, and it was difficult to identify this group from vehicle controls on the basis of visual inspection.

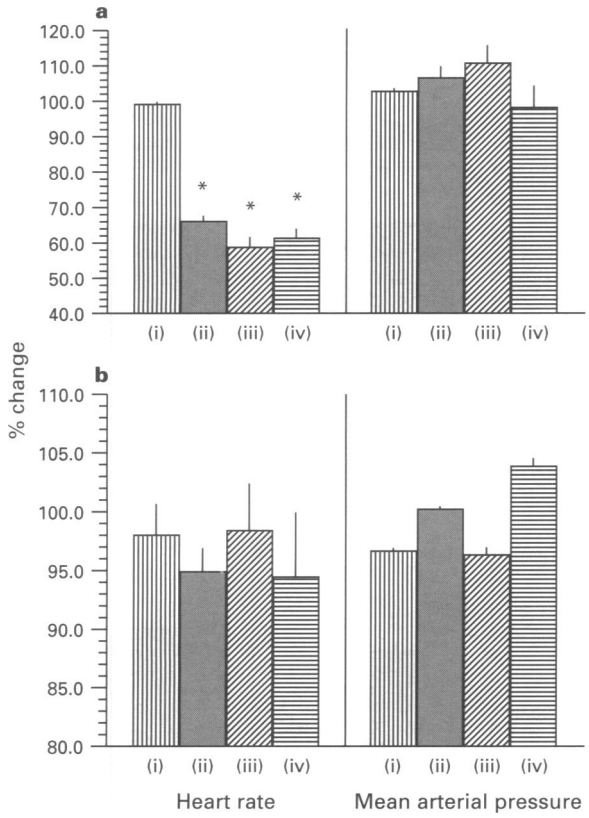

Figure 2 Averaged effects of AEA on heart rate and mean arterial blood pressure during the first min (a) and for 30 min (b) post drug administration. *Different from vehicle (P≤.05). (i) Vehicle (n=8); (ii) 3.0 mg kg^{-1} (n=5); (iii) 10.0 mg kg^{-1} (n=6); (iv) 30.0 mg kg^{-1} (n=6).

Although catatonic, rats displayed a hypersensitivity to many environmental stimuli including auditory and somatosensory (air puff) stimulation. While rats would not initiate spontaneous locomotor behaviour at this time, they were not sedated as they exhibited a powerful hyper-responsiveness to normal sensory stimuli. Brief, loud sounds (a hand clap) or somatosensory stimuli (air puff to the face) induced strong reflexive, escape-like responses. Rats would immediately orient to the stimulus, only to return to the cataleptic-like state in the absence of any external stimuli. This hyper-reflexia was similar to the behaviour seen in rodents after THC administration (Dewey, 1986). However, in contrast to the immediate onset of catatonia, the hyper-reflexia had a more delayed onset; for the high dose, a delay of between 5–13 min ensued with a duration of effect that averaged 22.3 min (range 14–43 min). At 10 mg kg^{-1}, onset varied from 2–5 min and lasted for a mean of 18.4 min (range 8–35 min), while the hyper-reflexia following 3.0 mg kg^{-1} AEA was quite mild and seen occasionally between 1–10 min. Although not specifically measured, no long term or residual effects of AEA were noted in any animal. Pretreatment with indomethacin did not appear to alter the AEA-induced behavioural effects. Arachidonic acid exerted little behavioural effect at the doses tested.

Two way analyses of variance revealed a significant dose- and time-dependent effect of AEA on core temperature (Figure 1). Compared to vehicle, which by itself did not alter core temperature, all doses of AEA significantly depressed temperature and did so in a dose-dependent manner [F(3,30)=54.9; P≤.001]. The 3.0 and 10.0 mg kg^{-1} doses decreased body temperature from control levels of 39.0±0.9°C between 3 and 15 min post injection, while the 30.0 mg kg^{-1} AEA caused a significant hypothermia between 8 and 26 min post injection.

AEA administration also profoundly altered several cardiovascular parameters. Due to the very rapid onset of effect, data were analyzed every 5 s during the first min post drug administration and in 1 min time bins thereafter for the next 30–40 min. Data from each time epoch (i.e. 1 and 30 min) were analyzed independently. A rapid, transient bradycardia was seen immediately following AEA administration. During the first min post drug, all 3 doses were equi-effective, de-

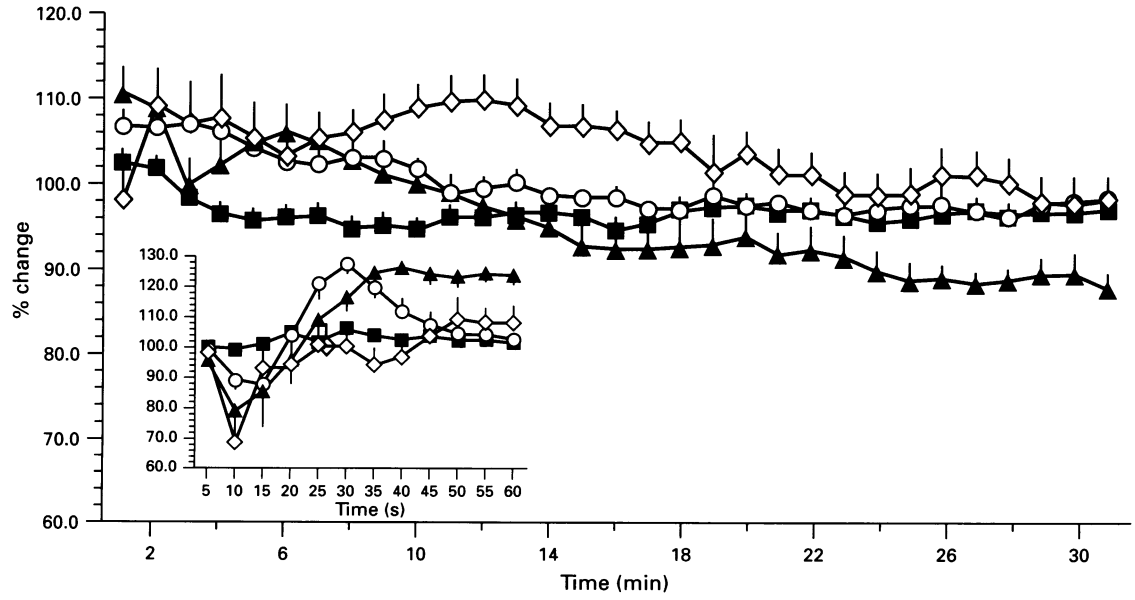

Figure 3 Effects of AEA on MAP as a function of dose and time. A significant (P≤.05) increase in blood pressure occurred for up to 5 min after 3.0 mg kg^{-1}, 2 min after 10.0 mg kg^{-1} and 13 min after 30.0 mg kg^{-1} AEA. Note also the delayed hypotensive effect with the middle dose between about 15 to 30 min. Inset illustrates the rapid biphasic depressor/pressor effect of AEA during the first minute post drug administration. (■) Vehicle (n=8); (○) 3.0 mg kg^{-1} (n=5); (▲) 10.0 mg kg^{-1} (n=6); (◇) 30.0 mg kg^{-1} (n=6).

creasing HR from a mean baseline rate of 445.8±61.6 b.p.m. by about 50–60% within 5 s [$F(3,30)=90.5$; $P\leqslant.001$], while vehicle was without effect on heart rate at any time point (Figure 2a). The maximal depression lasted about 20 s, with HR returning to about 70% of baseline by the end of the first min. However, for the 30 min observation period, the effects of the three doses did not differ significantly from each other (Figure 2b). Analysis of the time courses for the 3 doses, however, revealed that HR remained significantly below baseline for about 12, 5 and 6 min after the 30.0, 10.0 and 3.0 mg kg^{-1} doses, respectively (Figure 9).

A dose- and time-dependent effect of AEA was also seen on MAP (Figures 2 and 3). Following an immediate, brief, dose-related decrease in blood pressure after drug administration, a prolonged hypertensive response was seen from about 30 s and lasting for up to 5 min after the low, 2 min after the middle and 13 min after the high dose of AEA. Finally, a significant hypotension was evident from about 15–30 min following the 10 mg kg^{-1} injection (Figure 3). Vehicle administration alone caused a small (2–4%) significant decrease in MAP between 4 and 30 min.

AEA also produced antinociception as measured by the hot plate assay. Analysis of variance revealed a significant main effect of treatment (AEA vs. vehicle) [$F(1,39)=4.71$; $P\leqslant.036$], with AEA increasing hot plate latency from a mean response time of 7.3±0.5 s to 18.1±1.9 s. This antinociceptive effect was significant at the 10 mg kg^{-1} dose at 1 min compared to controls (treatment × dose × time) and was significantly reduced when tested again at 5 min (Figure 4). In view of the profound catatonic-like effect observed following 30 mg kg^{-1} AEA, this dose was not tested on the hot plate assay.

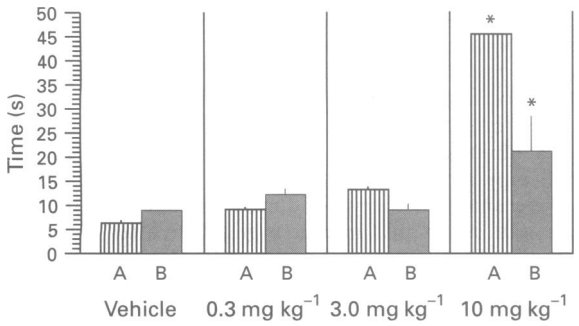

Figure 4 Effect of AEA on hot plate response. Only the 10 mg kg^{-1} dose caused a significant ($P\leqslant.05$) antinociceptive response at both 1 (A) and 5 (B) min post drug administration. The 5 min response was also significantly less than that after 1 min. Group sizes were: vehicle ($n=4$); 0.3 mg kg^{-1} ($n=5$); 3.0 mg kg^{-1} ($n=7$); 10.0 mg kg^{-1} ($n=5$).

Arachidonic acid (AA)

The effects of the AEA metabolite, AA, were determined on the same behavioural and physiological parameters in separate groups of animals. Unlike AEA, AA had no effect on core body temperature at any dose (data not shown). Minimal be-

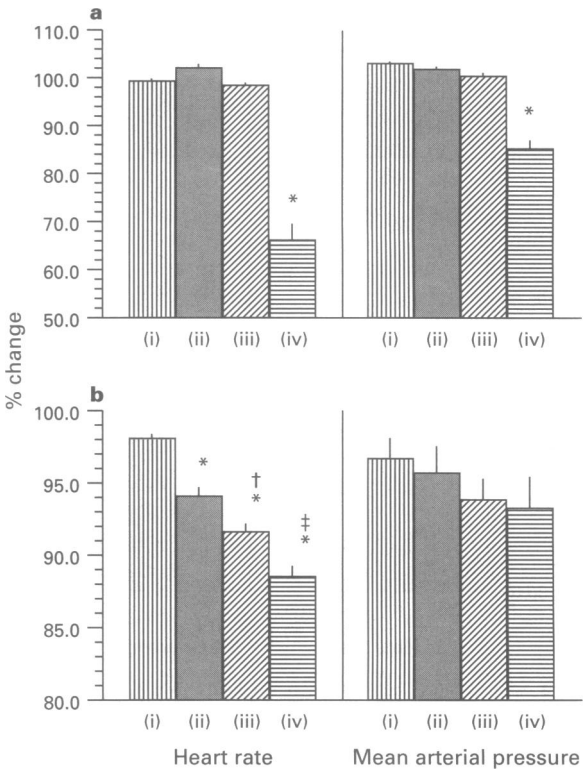

Figure 5 Effects of arachidonic acid (AA) on heart rate and blood pressure during the first min (a) and for 30 min (b) post drug administration. *Different from vehicle ($P\leqslant.05$); †different from 1.0 mg kg^{-1}; ‡different from 3.0 mg kg^{-1}. (i) Vehicle ($n=8$); (ii) 1.0 mg kg^{-1} ($n=5$); (iii) 3.0 mg kg^{-1} ($n=4$); (iv) 10.0 mg kg^{-1} ($n=6$).

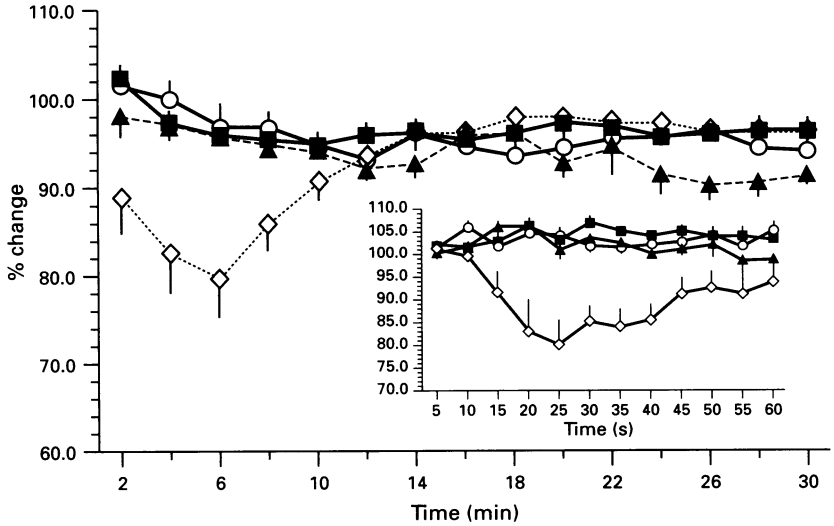

Figure 6 Effects of arachidonic acid (AA) on mean arterial pressure as a function of dose and time. Only the 10.0 mg kg^{-1} dose significantly depressed arterial blood pressure between 2 and 8 min post administration. (■) Vehicle ($n=8$); (○) 1.0 mg kg^{-1} ($n=5$); (▲) 3.0 mg kg^{-1} ($n=4$); (◇) 10.0 mg kg^{-1} ($n=6$).

havioural and antinociceptive properties were seen only following the highest dose tested (10.0 mg kg^{-1}), with brief (less than 2 min) hyper-reflexia and antinociception evident. However, a significant dose-dependent bradycardia was seen following all doses (1.0, 3.0 and 10.0 mg kg^{-1}) of AA [$F(3,30)=58.9$; $P\leqslant.001$] with the effects of each dose significantly greater than the next lower dose (Figure 5). Bradycardia developed slowly over time; during the first min post drug only the highest dose significantly depressed HR.

The actions of AA on MAP were likewise both dose- and time-related. While the effect of dose was significant [$F(3,30)=62.2$; $P\leqslant.001$], only the 10.0 mg kg^{-1} dose significantly depressed MAP during the first min post injection (Figure 5). Compared to vehicle, this moderate hypotension (~20%), with a latency of about 20 s, persisted for about 8 min (Figure 6). However, no significant main effects were observed for the entire 30 min period.

Indomethacin (Indo)

In a separate group of rats ($n=8$), Indo 5.0 mg kg^{-1}, a cyclooxygenase inhibitor, was administered s.c. 40 min prior to the i.v. administration of 3.0 or 10.0 mg kg^{-1} of AEA. Once again, both doses of AEA decreased core temperature compared to vehicle [$F(4,30)=78.34$; $P\leqslant.001$]. Indo pretreatment increased the duration of the hypothermic effects of the high (10.0 mg kg^{-1}) AEA dose from 14 to 21 min. In addition, when compared to baseline, Indo pretreatment prevented the hypothermic effect of 3.0 mg kg^{-1} AEA for up to 15 min (Figure 1). Indo was also able to block completely the rapid (few s) effects of 3.0 mg kg^{-1} AEA on HR. However, the same dose of Indo was only able to block partially (though significantly) the bradycardia following 10.0 mg kg^{-1} AEA from 58.6±3% to 76.6±3.4% (Figure 7). Finally, Indo plus 3.0 mg kg^{-1} AEA led to significantly higher HR for the first 9 min compared to the vehicle or AEA alone group (Figure 9). In contrast, Indo pretreatment prevented the hypotensive effect of 10.0 mg kg^{-1} AEA and led to a mild (10%) hypertension for most of the first 15 min (Figure 8).

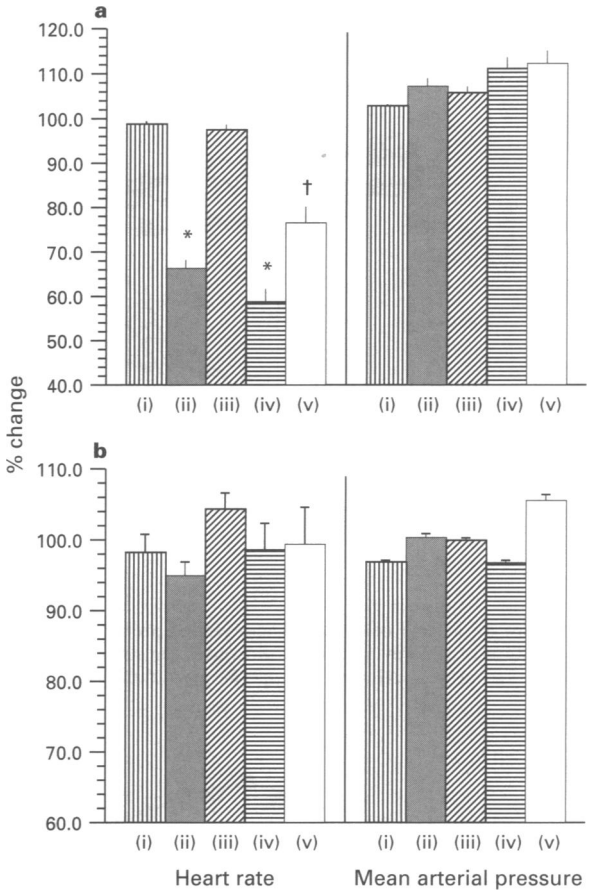

Figure 7 Effects of indomethacin (Indo) pretreatment (5 mg kg^{-1} s.c.) on AEA-induced blood pressure and heart rate alterations during the first (a) and 30 (b) min after AEA administration. When administered prior to 3.0 mg kg^{-1} AEA, Indo completely blocked the rapid (1 min) bradycardic effects of 3.0 mg kg^{-1} but only partially blocked the 10.0 mg kg^{-1} AEA effect. *Different from vehicle ($P\leqslant.05$) †different from AEA alone group. (i) Vehicle ($n=8$); (ii) 3.0 mg kg^{-1} AEA ($n=5$); (iii) 3.0 mg kg^{-1} AEA + Indo ($n=4$); (iv) 10.0 mg kg^{-1} AEA ($n=6$); (v) 10.0 mg kg^{-1} AEA + Indo ($n=4$).

Discussion

Data from the current experiment demonstrate that the putative endogenous cannabinoid ligand, AEA, can exert potent but relatively short lived behavioural and physiological effects when administered intravenously to freely behaving rats. These effects are generally similar, although of much shorter duration, to those reported after Δ^9-THC (Dewey, 1986). Most of the disparate time effects may be attributed to differences in drug pharmacokinetics; e.g. cannabinoids have been shown to be eliminated from the body relatively slowly via metabolism by cytochrome P450 (Agurell *et al.*, 1986), while AEA is metabolized much more rapidly to arachidonate, with an *in vitro* $t_{1/2}$ of approximately 80 min at 37°C (Hillard *et al.*, 1995). Alternatively, it is possible that in view of their very different chemical structures, each agent may act either somewhat differently at a common receptor or via different mechanisms or sites.

A dose- and time-dependent cataleptic-like waxy rigidity, hypomobility, and hyper-reflexia were seen following a single acute i.v. injection of AEA. This behavioural profile closely resembles the 'popcorn-like' hyper-reactive behaviour often reported in rodents when exposed to cannabinoids (Dewey, 1986; Ferri *et al.*, 1981). The onset of these behavioural effects following an i.v. injection varied from essentially no latency for catatonia, to between 2–5 min for the sensory hyper-responsiveness to develop; the duration of the hyper-reflexia somewhat paralleled the locomotor depression. The depressed locomotor behaviour is similar both in duration and extent to that reported previously for cannabinoids (Tulunay *et al.*, 1982) and for i.p. and i.v. AEA administration in mice (Fride & Mechoulam, 1993; Crawley *et al.*, 1993; Smith *et al.*, 1994). Direct comparisons of AEA studies using i.p. and i.v. drug administration in mice have revealed considerable attenuation of effects using the i.p. route. Thus route of administration appears to be a critical variable when determining the strength and duration of action of both the cannabinoids (Martin, 1985) and AEA (Smith *et al.*, 1994).

The antinociceptive properties of AEA were assessed by the hot plate method. A short duration (5 min), rapid onset (1 min) antinociception was seen at the highest dose tested (10 mg kg^{-1}). These data are consistent with the well known antinociceptive properties of the cannabinoids in rodents as assessed by tail flick, hot plate, writhing and pinch tests (Buxbaum, 1972; Martin, 1985). While more profound effects of AEA have been reported using the tail-flick (Smith *et al.*, 1994) and hot plate assay (Fride & Mechoulam, 1993) in mice, higher doses and/or different routes of administration were required. In view of the profound locomotor depression induced in our rats by high doses of AEA, doses comparable to those used previously were not tested in the present study so as to avoid a behavioural confound. In addition to these dose differences, different CNS sites of action or species differences could account for the milder results reported here. The cannabinoids have been reported to block pain at the level of the spinal cord (Lichtman & Martin, 1991). However, the hot plate assay employed in this study is more sensitive to supraspinal mechanisms whereas the tail flick assay engages mostly spinal

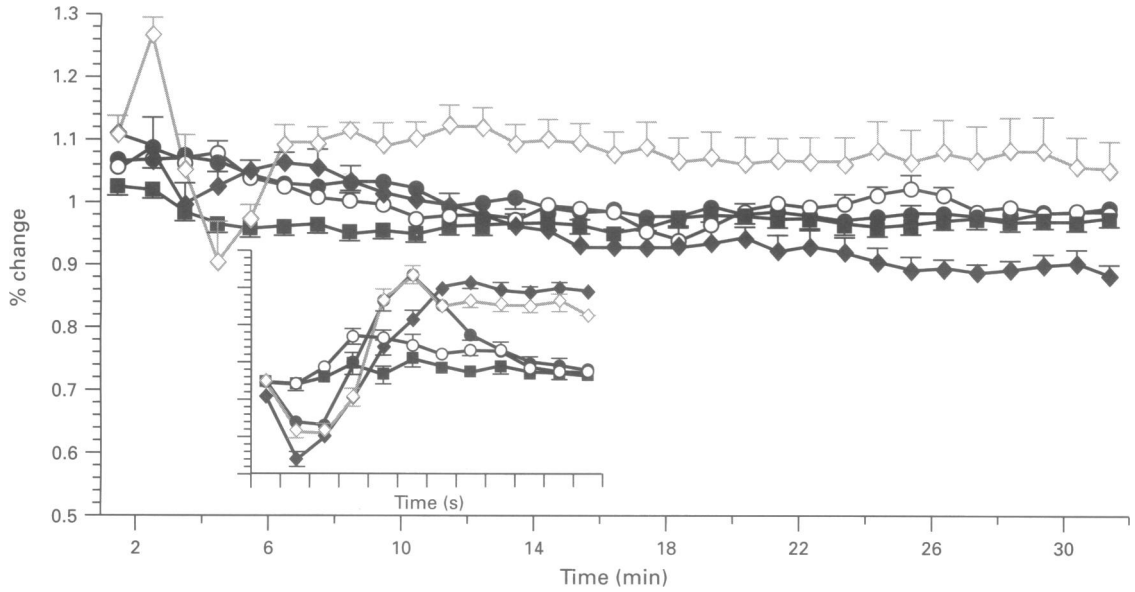

Figure 8 Effects of indomethacin (Indo) pretreatment (5 mg kg^{-1} s.c.) on AEA-induced blood pressure alterations plotted as a function of time in min. Inset illustrates the rapid (60 s) effects of these agents. (■) Vehicle ($n=8$); (○) 3.0 mg kg^{-1} AEA ($n=5$); (●) 3.0 mg kg^{-1} AEA + Indo ($n=4$); (◇) 10.0 mg kg^{-1} AEA ($n=6$); (◆) 10.0 mg kg^{-1} AEA + Indo ($n=4$).

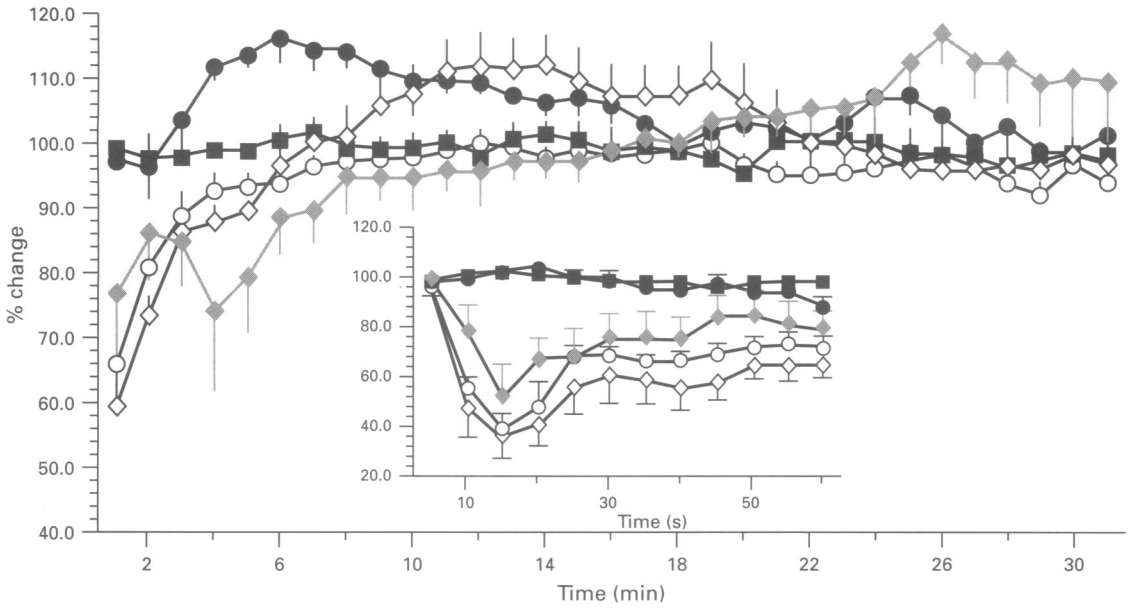

Figure 9 Effects of indomethacin (Indo) pretreatment (5 mg kg^{-1} s.c.) on AEA-induced heart rate alterations plotted as a function of time in min. Inset illustrates the rapid (60 s) effects of these agents. (■) Vehicle ($n=8$); (○) 3.0 mg kg^{-1} AEA ($n=5$); (●) 3.0 mg kg^{-1} AEA + Indo ($n=4$); (◇) 10.0 mg kg^{-1} AEA ($n=6$); (◆) 10.0 mg kg^{-1} AEA + Indo ($n=4$).

mechanisms (Irwin *et al.*, 1951; Schmauss & Yaksh, 1984). Thus, similar to the cannabinoids, AEA-induced antinociception may be predominantly spinally mediated.

The ability of the cannabinoids to induce hypothermia in rodents is well known (Pertwee, 1985; Taylor & Fennessey, 1977). Likewise, a dose-dependent depression in core body temperature of up to 1.4°C after 20 mg kg^{-1} AEA and lasting at least 30 min has been reported in rats (Crawley *et al.*, 1993), while Fride & Mechoulam (1993) and Smith *et al.* (1994) found somewhat greater hypothermia of up to 2.6°C after 20–50 mg kg^{-1} in mice. In the present study, a somewhat smaller, but statistically significant dose- and time-dependent hypothermia of about 1.1°C was seen following i.v. AEA with the middle and high doses leading to an 11 and 26 min hypothermia respectively. Interestingly, the magnitude of effect was about equal for both effective doses. Arachidonic acid in doses as high as 10 mg kg^{-1} had little effect on core temperature, nor much effect on locomotion or pain responsivity. When compared to vehicle, 5.0 mg kg^{-1} indomethacin, a cyclo-oxygenase inhibitor, enhanced the duration of the hypothermic effects of 10.0 but not 3.0 mg kg^{-1} AEA. Arachidonic acid, the main metabolite of AEA, exerted little behavioural or hypothermic effects when administered systemically. While only the high dose AEA effects were enhanced by Indo, when taken together, these data suggest that the parent compound, AEA, and/or a metabolite other than AA, is probably the active hypothermic, antinociceptive and behavioural depressant causative agent.

Coincident with the profound behavioural effects, equally pronounced cardiovascular alterations occurred. An extremely

short-lived depression of both heart rate and mean arterial pressure were observed following acute AEA administration. Both actions were immediate, with the bradycardia initially exceeding 60% of resting heart rate. However, within about 30 s, heart rate stabilized to about 30% above baseline. After the first min, both the duration and intensity of drug action was dose-dependent. While HR in the 3 mg kg^{-1} group returned to predrug baseline within 6 min, the 10 mg kg^{-1} group demonstrated a rebound tachycardia between about 9 and 14 min. Finally, the high AEA dose depressed heart rate for about 11 min, with only a brief (2 min), delayed (24–25 min) tachycardia evident. Likewise, AA depressed HR in a dose-dependent fashion, while Indo pretreatment blocked the AEA-induced bradycardia. Together, these data suggest that the profound HR effects of AEA may have been secondary to active metabolite formation.

Finally, the effect of AEA on MAP was somewhat less predictable. While the initial (first 10–15 s) hypotension was brief and directly dose-dependent, the secondary hypertensive effect although not continuous, lasted about 11, 5 and 2 min for the 30, 3 and 10 mg kg^{-1} doses respectively. Only the 10 mg kg^{-1} group demonstrated a delayed hypotension with a latency of about 15 min. There have been no previously published reports on the cardiovascular effects of exogenous AEA administration in the unanaesthetized rat. However, Varga *et al.* (1995) using urethane-anaesthetized rats, recently showed triphasic pressure alterations following 4 mg kg^{-1} AEA i.v., with MAP decreasing, increasing and finally decreasing again by about 40%. The initial depressor effect was believed secondary to a decreased cardiac output as a result of a profound bradycardia. Based on several pharmacological manipulations, they concluded that the long lasting depressor effects of AEA were likely to be mediated by the CB_1 receptor localized in brain. Our responses were similar, with the initial phases leading to dose-dependent changes between 10–30% (Figure 3). However, in the unanaesthetized state, the late hypotension was only seen after the 10 mg kg^{-1} dose. In contrast, AA caused only an initial depressor effect after the highest dose tested (10 mg kg^{-1}) lasting about 8 min. Finally, Indo pretreatment blocked the early phase depressor effect of 10 mg kg^{-1} AEA and caused a 10% increase in pressure for about 15 min. These data suggest that some of the depressor effects of AEA may be due to active metabolite actions as administration of AA also decreased blood pressure while pretreatment with Indo was able to block the AEA-induced hypotensive response. Likewise, Lukacsko *et al.* (1980) also showed a dose-dependent decrease in MAP after intra-arterial AA administration. In contrast, the increase in MAP following AEA administration may be independent of prostaglandins and AA formation as Indo was not able to prevent this effect.

While Δ^9-THC does not induce the transient bradycardia and depressor effect of AEA, it does lead to a delayed depressor response similar to AEA. Adams *et al.* (1976) found that Δ^9-THC elicited a dose-dependent biphasic effect on blood pressure with an initial transient hypertension peaking by about 1 min, followed by a prolonged decrease in pressure peaking at about 5 min and returning to baseline in 30 min. While no heart rate changes were seen during the initial pressor epoch, a delayed bradycardia occurred that reached a peak between 5–10 min.

In conclusion, it appears that acute administration of AEA produces a behavioural and physiological profile very similar to that seen following cannabinoid administration in rats and, while some of the cardiovascular effects seen may have been secondary to AEA metabolites (a conclusion also put forth by Smith *et al.*, 1994), other behavioural and physiological effects are likely to be dependent upon the parent compound. Since enzymes for the synthesis and degradation of AEA are known to exist in brain, these data are consistent with the hypothesis that AEA subserves a role in normal physiological and behavioural regulation.

Supported in part by grants from the Peters Foundation, National Heart Lung and Blood Institute (HL37981) and National Institutes on Drug Abuse (DA09465).

References

ADAMS, M.D., EARNHARDT, J.T., DEWEY, W.L. & HARRIS, L.S. (1976). Vasoconstrictor actions of Δ8 and Δ9-tetrahydrocannabinol in the rat. *J. Pharmacol. Exp. Ther.*, **196,** 649–656.

AGURELL, S., HALLDIN, M., LINDGREN, J., OHLSSON, A., WIDMAN, M., GILLESPIE, H. & HOLLISTER, L. (1986). Pharmacokinetics and metabolism of Δ^1tetrahydrocannabinoids with emphasis on man. *Pharmacol. Rev.*, **38,** 21–43.

BUXBAUM, D.M. (1972). Analgesic activity of Δ^9-tetrahydrocannabinol in the rat and mouse. *Psychopharmacologia*, **25,** 275–280.

CHILDERS, S.R., SEXTON, T. & ROY, M.B. (1994). Effects of anandamide on cannabinoid receptors in rat brain membranes. *Biochem. Pharmacol.*, **47,** 711–715.

CRAWLEY, J.N., CORWIN, R.L., ROBINSON, J.K., FELDER, C.C., DEVANE, W.A. & AXELROD, J. (1993). Anandamide, an endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia in vivo in rodents. *Pharmacol. Biochem. Behav.*, **46,** 967–972.

DEUTSCH, D.G. & CHIN, S.A. (1993). Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem. Pharmacol.*, **46,** 791–796.

DEVANE, W.A., DYSARZ, F.A. III., JOHNSON, M.R., MELVIN, L.S. & HOWLETT, A.C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.*, **34,** 605–613.

DEVANE, W.A., HANUS, L., BREUER, A., PERTWEE, R.G., STEVENSON, L.A., GRIFFIN, G., GIBSON, D., MANDELBAUM, A., ETINGER, A. & MECHOULAM, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, **258,** 1946–1949.

DEWEY, W.L. (1986). Cannabinoid pharmacology. *Pharmacol. Rev.* **38,** 151–178.

EVANS, D.M., JOHNSON, M.R. & HOWLETT, A.C. (1992). Calcium-dependent release from rat brain of a cannabinoid binding activity. *J. Neurochem.*, **58,** 780–782.

FELDER, C.C., BRILEY, E.M., AXELROD, J., SIMPSON, J.T., MACKIE, K. & DEVANE, W.A. (1993). Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 7656–7660.

FERRI, S., COSTA, G., MURARI, G., PANICO, A.M., RAPISAARDA, E., SPERONI, E. & ARRIGO-REINA, R. (1981). Investigations on behavioral effects of an extract of Cannibis sativa L in the rat. *Psychopharmacology*, **75,** 144.

FRIDE, E. & MECHOULAM, R. (1993). Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. *Eur. J. Pharmacol.*, **231,** 313–314.

HERKENHAM, M., GROEN, B.G.S., LYNN, A.B., DECOSTA, B.R. & RICHFIELD, E.K. (1991a). Neuronal localization of cannabinoid receptors and second messengers in mutant mouse cerebellum. *Brain Res.*, **552,** 301–310.

HERKENHAM, M., LYNN, A.B., JOHNSON, M.R., MELVIN, L.S., DECOSTA, B.R. & RICE, K.C. (1991b). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J. Neurosci.*, **11,** 563–583.

HERKENHAM, M., LYNN, A.B., LITTLE, M.D., JOHNSON, M.R., MELVIN, L.S., DECOSTA, B.R. & RICE, K.C. (1990). Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. U.S.A.*, **87,** 1932–1936.

HILLARD, C.J., EDGEMOND, W.S. & CAMPBELL, W.B. (1995). Characterization of ligand binding to the cannabinoid receptor of rat brain membranes using a novel method: application to anandamide. *J. Neurochem.*, **64,** 677–683.

HOWLETT, A.C., BIDAUT-RUSSELL, M., DEVANE, W.A., MELVIN, L.S., JOHNSON, M.R. & HERKENHAM, M. (1990). The cannabinoid receptor: biochemical, anatomical and behavioral characterization. *Trends Neurosci.*, **13,** 420–423.

IRWIN, S., HAUDE, R.W., BENNERR, D.R., HENDERSHOT, L.C. & SEEVERS, M.H. (1951). The effects of morphine, methadone and meperidine on some reflexive responses of spinal animals to nociceptive stimulation. *J. Pharmacol. Exp. Ther.*, **101,** 132–143.

LICHTMAN, A.H. & MARTIN, B.R. (1991). Spiral and supraspiral mechanisms of cannabinoid-induced antinociception. *J. Pharmacol. Exp. Ther.*, **258,** 517–523.

LUKACSKO, P., MESSINA, E.J. & KALEY, G. (1980). Reduced hypotensive action of arachidonic acid in the spontaneously hypertensive rat. *Hypertension*, **2,** 657–663.

MACKIE, K. & HILLE, B. (1992). Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 3825–3829.

MAILLEUX, P. & VANDERHAEGHEN, J.J. (1992). Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. *Neuroscience*, **48,** 655–668.

MARTIN, B.R. (1985). Structural requirements for cannabinoid-induced antinociceptive activity in mice. *Life Sci.*, **36,** 1523–1530.

MATSUDA, L.A., BONNER, T.I., LOLAIT, S.J. (1993). Localization of cannabinoid receptor mRNA in rat brain. *J. Comp. Neurol.*, **327,** 535–550.

MATSUDA, L.A., LOLAIT, S.J., BROWNSTEIN, M.J., YOUNG, A.C. & BONNER, T.I. (1990). Structure of a cannabinoid receptor: functional expression of the cloned cDNA. *Nature*, **346,** 561–563.

O'CALLAGHAN, J.P. & HOLTZMAN, S.G. (1975). Quantification of the analgesic activity of narcotic antagonists by a modified hot plate procedure. *J. Pharmacol. Exp. Ther.*, **192,** 497–505.

PERTWEE, R.G. (1985). Effects of cannabinoids on thermoregulation: a brief review. In *Marijuana '84*, ed. Harvey, D.J., pp 263–277. Oxford: IRL Press.

SCHMAUSS, C. & YAKSH, T.L. (1984). In vivo studies on spinal opiate receptor systems mediating antinociception II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. *J. Pharmacol. Exp. Ther.*, **228,** 1–12.

SMITH, P.B., COMPTON, D.R., WELCH, S.P., RAZDAN, R.K., MECHOULAM, R. & MARTIN, B.R. (1994). The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. *J. Pharmacol. Exp. Ther.*, **270,** 219–227.

TAYLOR, D.A. & FENNESSEY, M.R. (1977). Biphasic nature of the effects of Δ^9-tetrahydrocannabinol on body temperature and brain amines of the rat. *Eur. J. Pharmacol.*, **46,** 93–99.

TULUNAY, F.C., AYHAN, I.H. & SPARBER, S.B. (1982). The effects of morphine and delta-9 tetrahydrocannabinol on motor activity in the rat. *Psychopharmacology*, **78,** 358–360.

VARGA, K., LAKE, K., MARTIN, B.R. & KUNOS, G. (1995). Novel antagonist implicates the CB1 cannabinoid receptor in the hypotensive action of anandamide. *Eur. J. Pharmacol.*, **278,** 279–283.

VOGEL, Z., BARG, J., LEVY, R., SAYA, D., HELDMAN, E. & MECHOULAM, R. (1993). Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. *J. Neurochem.*, **61,** 352–355.

(Received April 16, 1996
Revised May 28, 1996
Accepted June 3, 1996)