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European Journal of Pharmacology 491 (2004) 181–189
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Cannabidiol lacks the vanilloid VR1-mediated vasorespiratory effects of capsaicin and anandamide in anaesthetised rats

Daniel S. McQueen[a,*], Susan M. Bond[a], Paula J.W. Smith[a,1], Kia Balali-Mood[a], Darren Smart[b]

[a] Division of Neuroscience, College of Medicine and Veterinary Medicine, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, Scotland, UK
[b] Neurology and GI CEDD, GlaxoSmithKline, New Frontiers Science Park, 3rd Avenue, Harlow, Essex CM19 5AW, UK

Received 11 March 2004; accepted 23 March 2004

Abstract

The results of vasorespiratory studies in rats anaesthetised with pentobarbital show that (±) cannabidiol, a cannabinoid that lacks psychotropic actions and is inactive at cannabinoid (CB) receptors, does not affect respiration or blood pressure when injected (1–2000 μg; 3.2–6360 nmol i.a.). Cannabidiol in doses up to 2 mg (6360 nmol) i.a. or i.v. did not affect the fall in mean blood pressure or the increase in ventilation (respiratory minute volume) caused by capsaicin and high doses of anandamide, responses that are mediated by activation of vanilloid VR1 (TRPV1) receptors in this species. Similar results were obtained with (−) cannabidiol (30–100 μg i.a.; 95–318 nmol). It has previously been shown using human embryonic kidney (HEK) cells over-expressing vanilloid human VR1 (hVR1) receptors that cannabidiol is a full agonist at vanilloid VR1 receptors in vitro. However, in the intact rat cannabidiol lacked vanilloid VR1 receptor agonist effects. We conclude that there are substantial functional differences between human and rat vanilloid VR1 receptors with respect to the actions of cannabidiol as an agonist at vanilloid VR1 receptors. Studies in vivo show that cannabidiol lacks any significant effect on mean blood pressure or respiratory minute volume when injected i.a. or i.v., and that this cannabinoid does not modulate the vanilloid VR1 receptor-mediated cardiovascular and ventilatory changes reflexly evoked by capsaicin or anandamide in rats anaesthetised with pentobarbital.

Keywords: Cannabidiol; Anandamide; Vanilloid VR1 receptors; Blood pressure; Ventilation

1. Introduction

Cannabidiol is a major constituent of *Cannabis sativa* (Adams et al., 1977) that appears to be devoid of psychotropic actions and lacks efficacy at cannabinoid (CB) receptors (Pertwee, 1997). It has recently been reported from experiments on human embryonic kidney (HEK) cells over-expressing human vanilloid VR1 (TRPV1, capsaicin-sensitive) receptors that cannabidiol can activate vanilloid VR1 receptors. The EC_{50} for cannabidiol stimulation was 3 μM, and its maximal effect was similar to that of the vanilloid VR1 receptor agonist, capsaicin (Bisogno et al., 2001). Vanilloid VR1 receptors have been implicated in nociceptive cardiovascular-respiratory reflexes in anaesthetised rats (Smith and McQueen, 2001); anandamide, an endogenous cannabinoid, has also been reported to activate VR1 receptors in vitro and in vivo (Gauldie et al., 2001; Malinowska et al., 2001; Smart et al., 2000; Smart and Jerman, 2000; Tucker et al., 2001).

The aim of the study was to test the hypothesis that cannabidiol is an agonist at rat vanilloid VR1 receptors in vivo, which can be predicted to be the case from findings in vitro (Bisogno et al., 2001). We measured changes in blood pressure and ventilation evoked by injecting cannabidiol and compared these with the effects of capsaicin and anandamide to determine whether cannabidiol activates vanilloid VR1 receptors in anaesthetised rats. We also investigated whether cannabidiol modulates the vasorespiratory actions of capsaicin and anandamide. A preliminary communication of some of the results has been presented (McQueen et al., 2002).

* Corresponding author. Tel.: +44-131-650-3525; fax: +44-131-650-6530.
E-mail address: D.S.McQueen@ed.ac.uk (D.S. McQueen).
[1] Present address: School of Life Sciences, Napier University, 10 Colinton Road, Edinburgh, EH10 5DT, Scotland, UK.

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doi:10.1016/j.ejphar.2004.03.045

2. Materials and methods

The experimental protocol was in accord with European Community guidelines, approved by the institutional ethics committee and licensed under UK Home Office regulations.

2.1. Animals

Adult male Wistar rats were supplied by Charles River (Margate, England) and housed with food and water ad lib for 7–14 days prior to use. Their mean body weight was 350 g, range 214–455 g, $n = 37$.

2.2. Procedures

The rat was anaesthetised with pentobarbital 60 mg kg^{-1} i.p.; anaesthesia was maintained by continuously infusing 6 mg pentobarbital hourly (0.2 ml h^{-1} 30 mg ml^{-1} solution i.v., jugular vein, adjusted as required to maintain a steady level of anaesthesia); rectal temperature was maintained at 36–37 °C by a heating blanket connected to a thermistor probe in the rectum. A tracheal cannula was inserted and airflow, tidal volume and respiratory frequency were measured using an electrospirometer (MacLab). The right carotid and femoral arteries were cannulated for measuring mean arterial blood pressure (MacLab) and drug administration, respectively, and an external jugular vein was cannulated for administration of anaesthetic solution. Arterial blood samples were taken at intervals in some experiments to monitor arterial blood gas tensions and pH, which were measured using a blood gas analyser (Rapidlab 248, Bayer).

2.3. Drug administration

Agonists were injected in a volume of 0.1 ml, washed in with 0.2 ml of saline (0.9% w/v aqueous sodium chloride), the i.a. and i.v. injection being completed within 2 s. The dead space in the catheter was 0.1 ml, and it took approximately 2–3 s from commencement of the injection for the drug to reach the hind limb following an i.a. injection. Antagonists were injected i.a. over 10 s (in a volume of 0.1 ml 100 g^{-1} body weight). The intra-arterial route was selected because vanilloid VR1 receptors in the hind limb vasculature rapidly (within 5 s of commencing an injection) evoke potent reflex changes in blood pressure and respiration when activated (Smith and McQueen, 2001). We administered cannabidiol as near to the vanilloid VR1 receptors as possible in order to achieve the maximum possible local concentration (we estimated from dye dilution that the amount injected will be transiently present in about 1 ml of blood on bolus injection) and reduce the metabolism that occurs on administration (Alozie et al., 1980). In two separate experiments cannabidiol and capsaicin were injected intravenously (i.v.) for comparison with response to cannabidiol given i.a.

2.4. Data analysis

Systolic and diastolic blood pressures were measured from the chart record (see Fig. 1), as was respiratory frequency and tidal volume. Values for mean blood pressure were computed mean blood pressure = diastolic blood pressure + 0.33 (systolic blood pressure − diastolic blood pressure), and respiratory frequency, tidal volume and respiratory minute volume were calculated from 5 s ramps of respiratory volume, each step in the ramp representing an individual breath (respiratory minute volume = height of 5 s ramp × 12).

Measurements were made during the 15 s period preceding an injection of drug, and at the point of maximum change during the 120-s period immediately following drug administration. The effects of drugs were determined by comparing responses to agonists alone and following administration of an antagonist. Where there was no obvious response, measurements were made 30 s post-injection. Data are expressed as the average change in mean blood pressure or respiratory minute volume, ± S.E.M. or for ED_{50} values, the 95% confidence interval (CI) for n values, where n is the number of individual animals studied.

"Apparent" ED_{50} values (dose causing half the maximum response) were determined from individual dose–response plots before and after cannabidiol, as illustrated in Fig. 4. Log dose–response curves were generated from data using a sigmoidal function (GraphPad Prism v4.00; GraphPad Software, USA).

Group means were compared statistically (GraphPad Prism 4.0, GraphPad Software) using either a one- or two-way analysis of variance (ANOVA), the Kruskal–Wallis test, a paired or unpaired t-test, or the Mann–Whitney test—as appropriate and indicated in the text. The null hypothesis that there was no difference between means or medians was rejected at the 0.05 level of probability.

2.5. Drugs used

The drugs used (and the supplier) were: cannabidiol and capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) (Sigma), anandamide (Tocris), pentobarbital (Rhône Mérieux) and (−) cannabidiol (kindly gifted by Prof. R. Mechoulam, Hebrew University, Jerusalem, Israel). Capsaicin was prepared as a 10 mg ml^{-1} stock solution in Tween 80 (10% v/v), ethanol (10% v/v) and PBS (phosphate buffered saline) and diluted prior to use with saline; the stock solution of anandamide was in soya oil/water (1:4) emulsion, and dilutions were made with saline; cannabidiol stock solution was in 100% methanol, and dilutions were made with saline. Methanol (Fisher Scientific) diluted with saline was used as a control for cannabidiol.

In order to study higher doses of cannabidiol without the complications of methanol, the solvent in commercial solutions, a 1 mg ml^{-1} solution of (±) cannabidiol in 100% ethanol was mixed with a 2 mg ml^{-1} solution of Tween 80 in ethanol. The ethanol was then evaporated off. Saline was

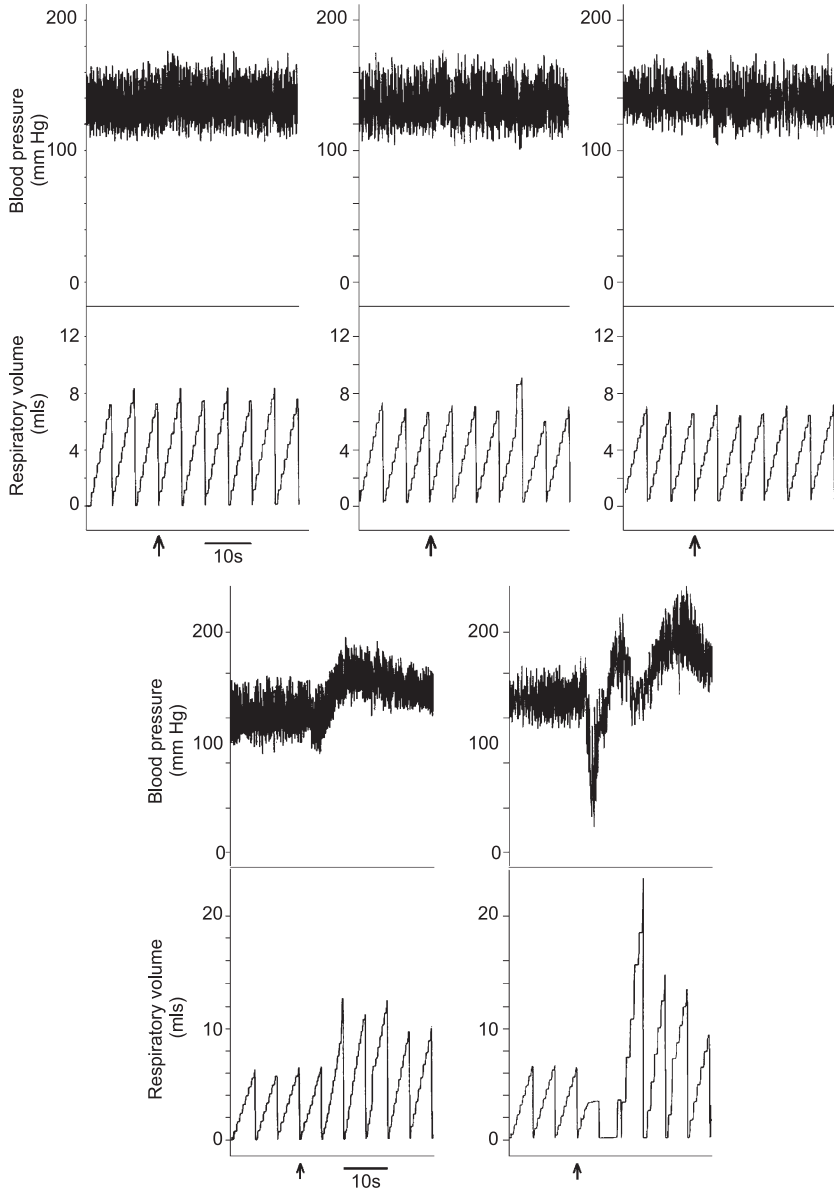

Fig. 1. Results from an individual experiment on a male Wistar rat (445 g; pentobarbital anaesthesia) showing the effect of injecting at time shown by arrow: (a) Tween 80/saline solvent used for cannabidiol 1000 μg i.a.; (b) ± cannabidiol 1000 μg (3.2 μmol) i.a.; (c) ± cannabidiol 1000 μg (3.2 μmol) i.v.; (d) capsaicin (3 μg; 10 nmol) i.a. after cannabidiol 1000 μg (3.2 μmol) i.a.; (e) capsaicin (3 μg; 10 nmol) i.v. after cannabidiol 1000 μg (3.2 μmol) i.v. High doses of cannabidiol injected as a bolus either i.v. or i.a. had no significant effect on either blood pressure or ventilation (P>0.05 versus vehicle control). Blood pressure and ventilatory responses to capsaicin injected either i.a. or i.v. 2 min after cannabidiol were similar to those obtained in experiments without cannabidiol pretreatment, indicating that cannabidiol did not affect the actions of capsaicin on vanilloid VR1 receptors.

added in small aliquots and the solution vortexed after each addition to give a final cannabidiol concentration of 10 mg ml^{-1}.The vehicle was prepared from Tween 80 dissolved in saline at equivalent concentrations.

3. Results

3.1. Cannabidiol

Experiments were performed using racemic (±) cannabidiol, and (−) cannabidiol was also studied in three animals. Injection of cannabidiol (3.2–32 nmol; 1–10 μg i.a.) had no appreciable effect on either mean blood pressure or respiratory minute volume (see Fig. 1) in comparison with the minimal changes evoked by saline or 10% methanol in saline, the vehicle for 10 μg cannabidiol (see left-hand panels in Fig. 2). The slight change in mean blood pressure following saline did not differ significantly from the hypotension obtained following injection of cannabidiol 1, 3 and 10 μg, (−) cannabidiol 10 μg, or 10% methanol (P=0.26; Kruskal–Wallis test). There was also no significant difference in ventilatory responses between these

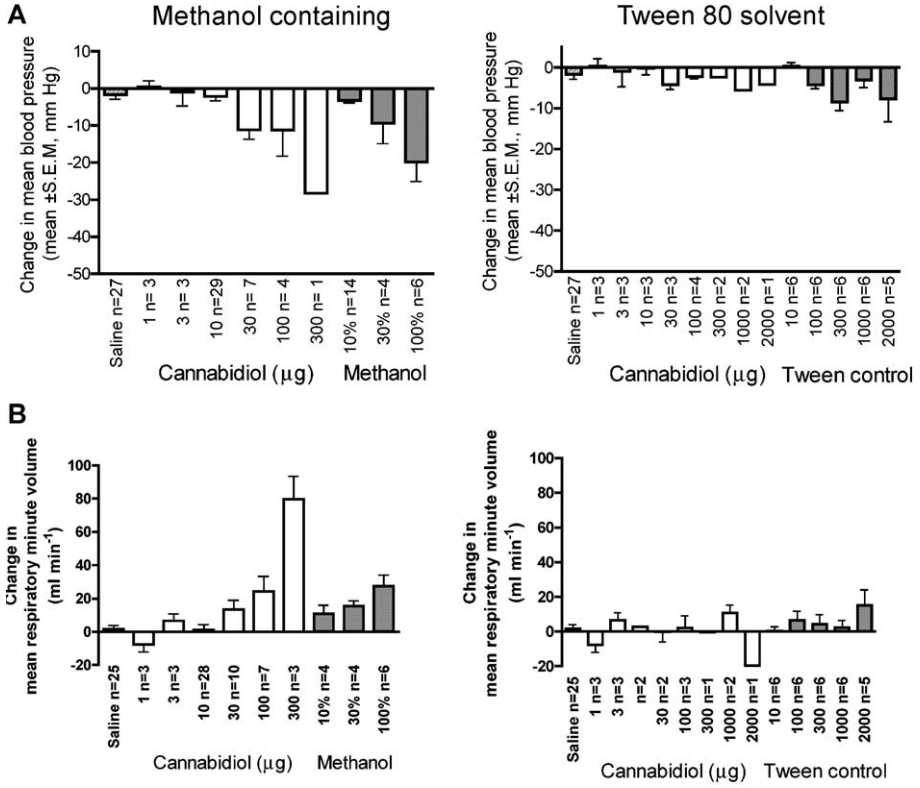

Fig. 2. Left panels: pooled data showing lack of effect of low doses of cannabidiol (1–10 μg) on blood pressure or ventilation (respiratory minute volume), relative to the drug vehicle (10% methanol) or saline, in anaesthetised rats ($P=0.12$; Kruskal–Wallis test). Basal mean blood pressure before cannabidiol (10 μg i.a) averaged 124 ± 4 mm Hg, and basal respiratory minute volume 162 ± 10 ml min^{-1}, $n=26$. Overall, cannabidiol (1–10 μg) had no effect on mean blood pressure or respiratory minute volume. Higher doses of cannabidiol (30–300 μg, 95–954 nmol i.a.) reduced mean blood pressure and increased respiratory minute volume. Statistical analysis showed no significant difference between the vehicle (30% methanol in saline to 100% methanol—stock solution for 100 μg cannabidiol 30–100 μg). The cardiovascular and respiratory changes associated with injecting higher doses of cannabidiol were attributable to the drug vehicle. Right panels: pooled data showing the response to cannabidiol (1–2000 μg; 3–6360 nmol i.a.) or the drug solvent (appropriate Tween 80/saline mixtures i.a.). There was no significant difference between the responses to vehicle or any of the doses of cannabidiol, either in terms of changes in mean blood pressure ($P=0.07$, Kruskal–Wallis for 13 groups where $n \geq 2$) or in respiratory minute volume ($P=0.53$, Kruskal–Wallis for 12 groups where $n \geq 2$).

groups ($P=0.12$). Arterial blood gas tensions and pH were unaffected by cannabidiol (Table 1), which provided further evidence that this cannabinoid did not affect ventilation.

Higher doses of cannabidiol (95–318 nmol; 30–100 μg) caused slight hypotension and hyperventilation (see Fig. 3). Comparison of the mean hypotensive and hyperventilatory responses to the drug vehicle (0.1 ml 100% methanol), (±) cannabidiol 30 and 100 μg, and (−) cannabidiol 30 and 100 μg showed there was no statistically significant difference between the effects of cannabidiol and the drug solvent, either in terms of mean blood pressure ($P=0.15$) or respiratory minute volume ($P=0.17$; Kruskal–Wallis test). Marked hypotension and hyperventilation was observed after the highest dose of cannabidiol studied (954 nmol, 300 μg i.a., as either the racemic (±) or the (−) isomer, $n=1$ of each). This effect was obtained using the stock solution of commercially supplied cannabidiol, and was clearly very similar to that of the drug vehicle (0.3 ml 100% methanol).

Table 1
Arterial blood gas tensions and pH values

Procedure	n	pH	$PaCO_2$ (mm Hg)	PaO_2 (mm Hg)
Control, pre-drug	11	7.41 ± 0.02	37.61 ± 1.4	82.1 ± 6.9
After cannabidiol (10 μg)	13	7.42 ± 0.01	36.4 ± 1.6	75.1 ± 2.5
After 10% methanol	5	7.41 ± 0.01	37.2 ± 2.4	75.9 ± 5.0

Mean values ± S.E.M from n experiments in which blood samples were taken. Kruskal–Wallis test for the three groups showed no statistically significant difference between the group mean values for any of the individual variables: pH values $P=0.90$; $PaCO_2$ values $P=0.85$; PaO_2 values $P=0.99$.

3.2. Cannabidiol dissolved in Tween 80/saline mixture

In order to determine whether higher doses of cannabidiol might have cardiovascular and ventilatory actions, independent of those caused by the methanol solvent, we undertook additional experiments in which commercial cannabidiol was evaporated and re-dissolved in a Tween 80/saline mixture. By this means we were able to study the effect of bolus injections of (±) cannabidiol in doses up to 6360 nmol (2000 μg i.a.), the maximum dose that it was possible to test during this investigation, without the complication of methanol-induced hypotension and asso-

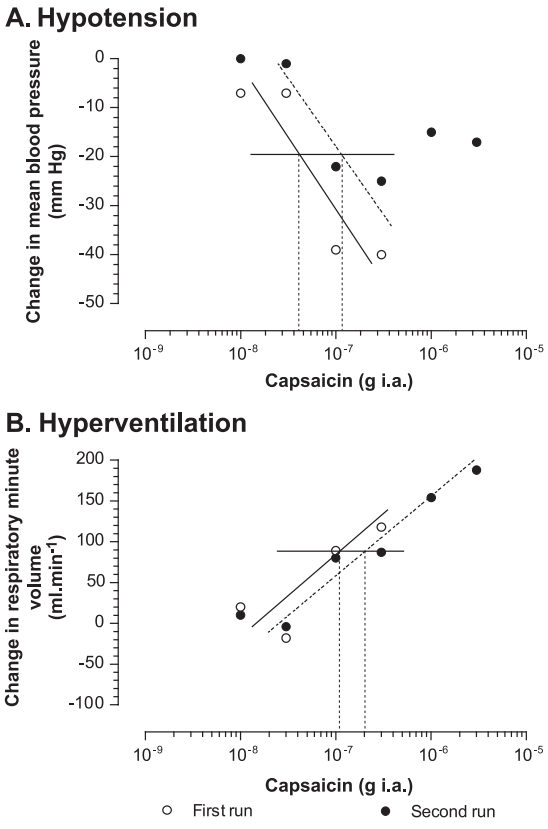

Fig. 3. Log dose–response plots for capsaicin from an individual experiment (455g rat) demonstrating the trend to a rightward shift in the response curves when the sequence of increasing doses of capsaicin was repeated after an interval of 60 min. An injection of 0.1 ml 10% methanol in saline was made 1 min before each dose of capsaicin on the second run, as a control for 10 μg cannabidiol, which was used in other experiments. The desensitization (rightward shift in the curves) was slightly greater for the hypotension, and very marked desensitization occurred in the depressor, but not the hyperventilatory, response to higher doses of capsaicin. Lines were fitted to the data by eye, omitting the highest doses which markedly reduced the mean blood pressure response. Dotted lines indicate the apparent ED_{50} values, the dose causing half the estimated maximum change in mean blood pressure (upper) and respiratory minute volume (lower panel).

ciated hyperventilation; Tween 80/saline in the amounts used to dissolve cannabidiol had no effect on mean blood pressure or respiratory minute volume. As illustrated in Fig. 1, and summarised in Fig. 2 (right-hand panels), the higher doses of cannabidiol had no effect on either mean blood pressure (upper panels) or respiratory minute volume (lower panels). Statistical comparison of blood pressure changes in response to injecting cannabidiol 1–1000 μg (3–3180 nmol), saline or the corresponding Tween 80/saline solvent mixtures showed there was no significant difference between individual groups (Kruskal–Wallis test on 13 groups where individual group $n=2–6$; $P=0.07$). Corresponding values for respiratory minute volume from the groups also showed a similar lack of responsiveness to cannabidiol (Kruskal–Wallis test on 12 groups, groups $n=2–6$; $P=0.53$).

3.3. Anandamide and capsaicin

We investigated whether cannabidiol modulates vasorespiratory responses to anandamide and capsaicin. These vanilloid VR1 receptor agonists induced dose-dependent hypotensive hyperventilatory responses, as had been previously reported, (Gauldie et al., 2001; Smith and McQueen, 2001) and is shown for intra-arterial and intra-venous capsaicin in Fig. 1. Preliminary experiments confirmed what is already known, namely that responses to capsaicin are liable to desensitization, which meant that repeating a dose–response plot (0.01, 0.03, 0.1, 0.3 and 1 μg i.a., with 5–10 min between successive doses) after an interval of 20 min gave vasorespiratory responses that were reduced (see Fig. 3).

To avoid the possibility of confusing agonist-induced desensitization with inhibitory effects of (±) cannabidiol, and to ensure that a reasonable amount of cannabidiol was present when the agonist was injected, a protocol was devised for investigating the effects of cannabidiol on

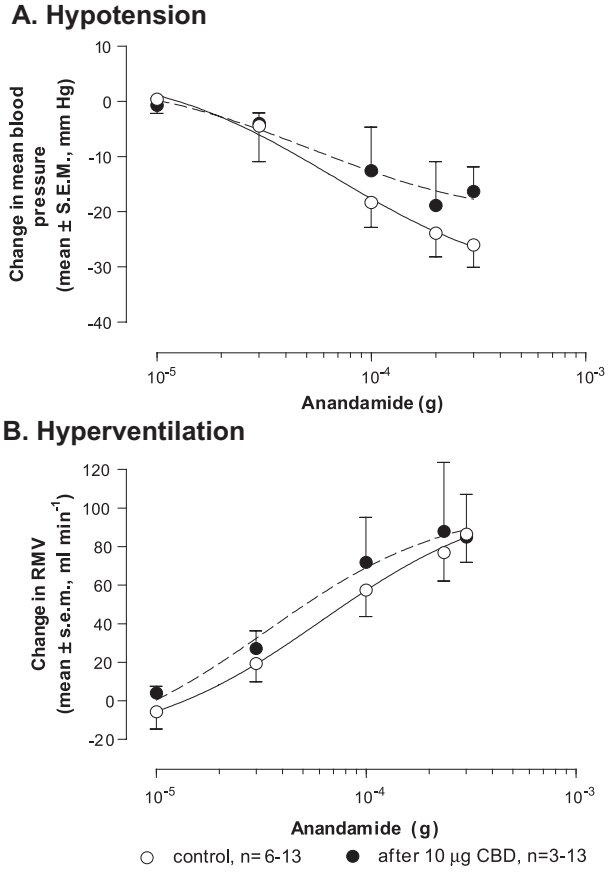

Fig. 4. Log dose–response curves generated from pooled data for anandamide before and 1–3 min after administration of cannabidiol 10 μg i.a. There was no statistically significant difference between responses to anandamide obtained before or after cannabidiol, either for hypotension or hyperventilation ($P>0.05$, two-way ANOVA), indicating that cannabidiol doesn't affect the vasorespiratory responses evoked by anandamide.

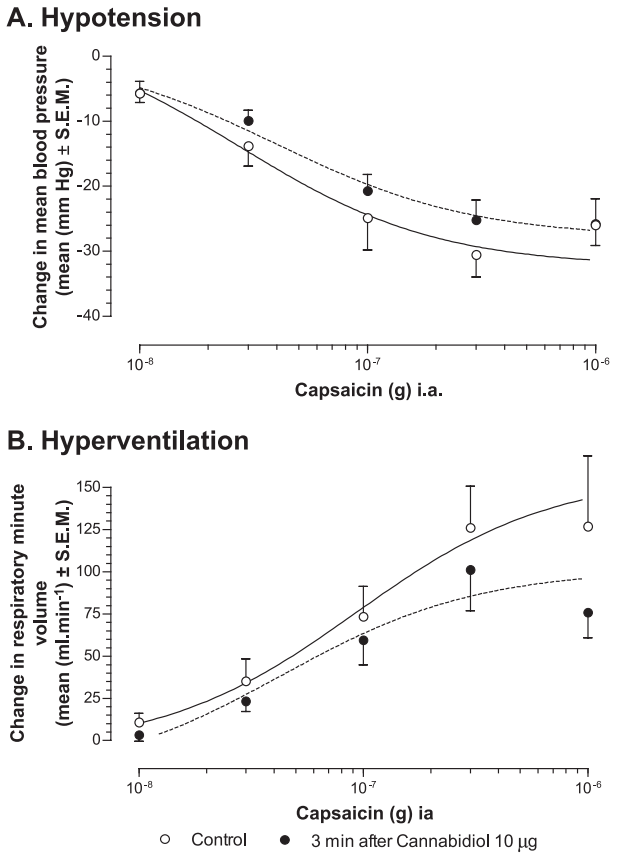

Fig. 5. Log dose–response curves generated from pooled data for capsaicin before and 1–3 min after cannabidiol 10 μg i.a. There was no statistically significant difference between responses to capsaicin obtained before or after cannabidiol, either for hypotension or hyperventilation ($P>0.05$, two-way ANOVA), indicating that cannabidiol doesn't affect the vasorespiratory responses evoked by capsaicin.

responses to capsaicin or anandamide. This involved obtaining a response to the lowest dose of agonist, then after an interval of 5–10 min cannabidiol was injected (32 nmol; 10 μg i.a.) and the dose of agonist repeated 1–3 min later. In most experiments, a 2 min interval was used, but in some, the period was shortened to 60 s because of uncertainty about the speed at which the drug would be metabolised. Evidence suggests that 80% of a dose of cannabidiol will be present in rat blood after 30 s, but only 50% at 60 s, and approximately 20% 5 min post-injection, but this varies between individual animals (Alozie et al., 1980). This procedure was continued for increasing doses of agonist, avoiding the higher doses of capsaicin (>3–10 μg), which caused pronounced loss of vasodepressor responses (see Fig. 3).

Pooled log dose–response data for the agonists before and 1–3 min after an injection of cannabidiol (32 nmol, 10 μg i.a.) are summarised in Fig. 4 (anandamide) and Fig. 5 (capsaicin). We estimated that this dose of cannabidiol would give a maximum transient peak concentration of approximately 30 μM in the local vasculature, based on 0.1 ml drug solution being diluted to approximately 1 ml in the first few seconds following injection. The bolus reached the leg vasculature 2–3 s following an i.a. injection, and cannabidiol would be rapidly metabolised over the following 5 min (Alozie et al., 1980; Siemens et al., 1980). Responses to either anandamide or capsaicin were not significantly affected by cannabidiol, although there was a slight rightward shift of the mean blood pressure curves following cannabidiol, probably reflecting a degree of agonist-induced desensitization (see above). Statistical analysis by two-way ANOVA indicated that vasodepressor responses to anandamide (10–300 μg; 29–863 nmol) or capsaicin (0.01–1 μg; 0.03–3.2 nmol) were unaffected by cannabidiol 10 μg i.a. ($P=0.31$ and 0.28, respectively, for individual dose comparisons before and after cannabidiol).

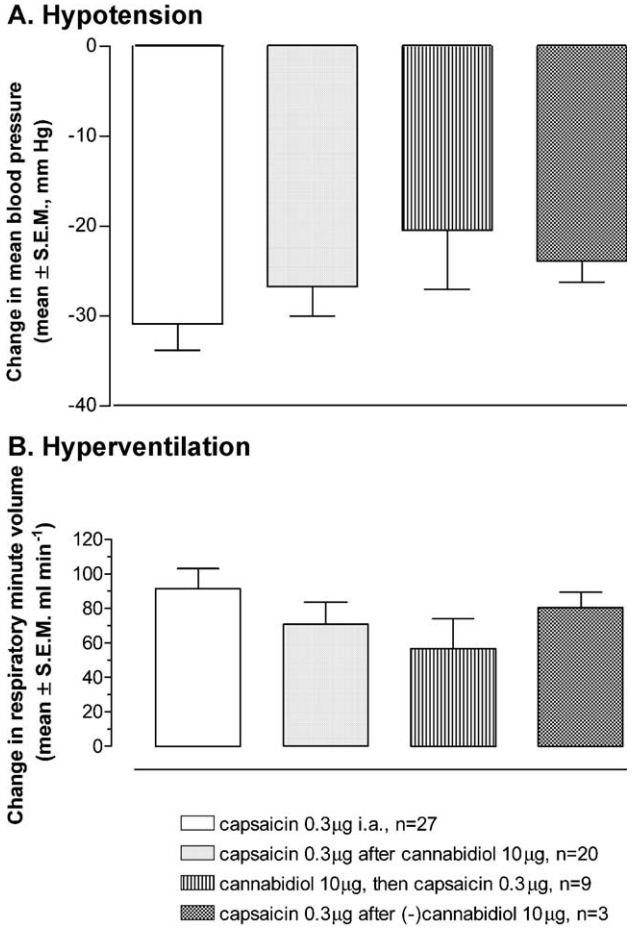

Fig. 6. Hypotension (A) and hyperventilation (B) caused by a single repeatable mid-range dose of capsaicin (0.3 μg i.a., 1 nmol) in separate groups of animals. Responses to capsaicin were studied: before and 1–3 min following (±) cannabidiol (10 μg); after pre-treatment with (±) cannabidiol (10 μg); and before and after (−) cannabidiol (10 μg). Administration of cannabidiol before capsaicin (data in third column) eliminated the possibility of capsaicin-induced desensitization, which would complicate interpretation of the results. There was no significant difference between group values showing that cannabidiol did not influence either the blood pressure or respiratory responses evoked by capsaicin (Kruskal–Wallis test; $P=0.43$ for blood pressure and $P=0.28$ for respiratory minute volume).

Similarly, two-way ANOVA showed that cannabidiol (10 μg) had no statistically significant effect on the hyperventilation evoked by either capsaicin ($P=0.11$) or anandamide ($P=0.57$). A higher dose of capsaicin (3 μg i.a. or i.v.) was also unaffected by a very high dose of cannabidiol (3180 nmol; 1000 μg i.a. or i.v.) injected 2 min earlier, as shown in Fig. 1.

3.4. Cannabidiol injected before anandamide or capsaicin

Additional experiments were undertaken in which (±) cannabidiol 10 μg was injected i.a. 1–3 min before a single mid-range dose of capsaicin (10 nmol; 0.3 μg i.a., see Fig. 6); anandamide was not studied. The blood pressure and respiratory minute volume responses to capsaicin after pre-treatment with cannabidiol were compared with those from other experiments in which the same dose of capsaicin was given before and after (±) cannabidiol or (−) cannabidiol. There was no significant difference between group means for the hypotension or hyperventilation evoked by this dose of capsaicin ($P=0.43$ and 0.28, respectively, Kruskal–Wallis test).

3.5. Apparent ED_{50} values

Apparent ED_{50} values were estimated for changes in mean blood pressure and respiratory minute volume before and after cannabidiol (32 nmol, 10 μg i.a.) from log dose–response plots obtained from individual experiments and the results are summarised in Table 2. The increase in ED_{50} values obtained reflect the slight desensitization that occurs with repeated dosing with capsaicin (see Fig. 3), and there was no significant change in response to capsaicin in a separate series of experiments in which the dose of cannabidiol was administered 1–3 min before each dose of capsaicin.

4. Discussion

The main finding from this study is that cannabidiol in doses up to 2 mg (5 mg kg^{-1}; 6360 nmol) i.a. or i.v. had no appreciable effect on blood pressure or ventilation in rats anaesthetised with pentobarbital, nor did the cannabinoid affect vanilloid VR1-mediated hypotension and hyperventilation evoked by capsaicin or anandamide. This result was unexpected because it has been reported that cannabidiol is a full agonist on vanilloid human VR1 receptors overexpressed in human embryonic kidney (HEK) cells (Bisogno et al., 2001), the cannabinoid being about 100-fold less potent than capsaicin. These authors also found that cannabidiol desensitized vanilloid human VR1 receptors to the action of capsaicin. Clearly there are major differences between their in vitro studies on cells and our in vivo vasorespiratory experiments in rats, including the presence of an anaesthetic agent, drug uptake and metabolism, and the number of vanilloid VR1 receptors exposed to cannabidiol. There may also be fundamental differences between the functional properties of vanilloid VR1 receptors in rats and humans, even though vanilloid human VR1 and vanilloid rat VR1 are 92% homologous (Hayes et al., 2000). Indeed, clear differences in both agonist potencies and antagonist sensitivity between vanilloid human VR1 and vanilloid rat VR1 have been reported (Smart et al., 2001), and preliminary data indicates that cannabidiol is without effect on recombinant vanilloid rat VR1 activity (D. Smart, unpublished observations).

Fairly high doses of cannabidiol (100–300 μg i.a.) caused hypotension and hyperventilation in our initial experiments, but this was clearly an artefact associated with the drug vehicle, 100% methanol, as can be seen from the summary in Fig. 2. In later experiments we were able to study bolus doses of up to 2000 μg cannabidiol (5 mg kg^{-1}), without the vascular and respiratory effects of methanol, by using a

Table 2
Mean "apparent" mean ED_{50} values ± 95% CI determined from individual experiments in which log dose–response plots for capsaicin and anandamide were obtained before and again after cannabidiol (10 μg injected 1–3 min before each dose of agonist)

	Capsaicin alone (nmol)	Capsaicin after cannabidiol nmol)	Cannabidiol first, then capsaicin (nmol)	Anandamide alone (nmol)	Anandamide after cannabidiol (nmol)
$n=$	17	17	5	8	8
Mean blood pressure					
Mean ED_{50}	0.38	1.62*	0.26	335	1140*
95% CI	0.06–0.71	0.41–2.82	0.04–0.48	245–426	709–1572
Respiratory minute volume					
Mean ED_{50}	0.60	1.01	0.28	348	543*
95% CI	0.18–1.02	0.32–1.7	0.2–0.37	271–424	431–654

The mean changes in mean blood pressure at the ED_{50} for capsaicin were −21 ± 1.8 mm Hg and 92 ± 17 ml min^{-1} for respiratory minute volume; corresponding values for anandamide were −18 ± 4.2 and 76.9 ± 14.8. The dose ratios for both agonists showed that there was a slight statistically significant decrease in the depressor responses following cannabidiol, and in the respiratory minute volume response for anandamide. In a separate series of experiments, cannabidiol was injected before capsaicin: i.e. there was no preliminary or "control" curve for the agonist. The apparent ED_{50} values for mean blood pressure and respiratory minute volume from these experiments were not significantly different from the pre-cannabidiol capsaicin "controls" ($P>0.05$). n = number of individual experiments.

*$P<0.05$ versus pre-cannabidiol: Mann–Whitney test for anandamide; Kruskal–Wallis test for the capsaicin group data.

Tween 80/saline mixture to dissolve the cannabinoid. Even these very high doses of cannabidiol, injected i.a. or i.v., lacked any overt vanilloid VR1-like activity, and also had no discernable effect on the vanilloid VR1 receptor agonist-mediated actions of capsaicin or anandamide. Overall, the results obtained do not support the hypothesis that cannabidiol is an agonist at vanilloid VR1 receptors in anaesthetised rats. Further studies could be performed in conscious animals to eliminate the possibility that the anaesthetic, pentobarbital, inhibits the actions of cannabidiol on vanilloid rat VR1 receptors in vivo. However, we consider this unlikely because deepening the level of anaesthesia substantially with additional pentobarbital does not change the responsiveness to the vanilloid VR1 receptor agonist capsaicin (Smith and McQueen, 2001).

One problem associated with studying vanilloid VR1 receptors in vivo is that the agonists, capsaicin and anandamide, both desensitize the preparation (Cortright et al., 2001). This makes interpretation of log dose–response plots complicated, because rightward shifts can result from agonist-induced desensitization, rather than from the effects of a putative modulator such as cannabidiol: i.e. repeating the doses of agonist in the absence of any other procedure gives a reduced response. In addition, it is not feasible to generate full log dose–response curves from which to calculate absolute ED_{50} values, even with a 15–30 min interval between successive doses of capsaicin, because near-maximal doses cause prolonged desensitization. This meant using responses to lower doses to derive an "apparent" maximum, and from these dose–response plots an "apparent ED_{50}" (not achieving steady state conditions) was determined, as shown in Fig. 3. Despite these precautions, there was still some desensitization (see Fig. 3), so we used single mid-range doses of agonist before and after cannabidiol. We also investigated whether administering cannabidiol before a single dose of capsaicin or anandamide influenced the response to the vanilloid VR1 receptor agonists. The results overall showed that cannabidiol did not significantly affect either the vasodepression or the hyperventilation induced by either of the vanilloid VR1 receptor agonists.

The commercial cannabidiol we used in this study was a racemic mixture, whereas Bisogno et al. (2001) used (−) cannabidiol and (+) cannabidiol in their experiments. We speculated whether the lack of vasorespiratory responses in our experiments might be attributable to use of the racemic mixture. For example, if the (+) and (−) isomers of cannabidiol had exactly opposite effects, then the racemic mixture would show no overall effect on mean blood pressure and respiratory minute volume. This possibility seemed highly improbable, particularly since both enantiomers were equieffective as vanilloid VR1 receptor agonists on vanilloid human VR1 receptors expressed in HEK cells in vitro (Bisogno et al., 2001). Nevertheless, we investigated the actions of a sample of (−) cannabidiol kindly provided by Prof. Mechoulam. The results obtained with (−) cannabidiol were no different from those with the racemate: the cannabinoid had no effect on mean blood pressure or respiratory minute volume, nor did it influence responses to capsaicin.

The EC_{50} for cannabidiol stimulation of vanilloid human VR1 receptors expressed in HEK cells in vitro was 3 μM, and the maximal effect of cannabidiol was similar to that of the vanilloid VR1 receptor agonist capsaicin (Bisogno et al., 2001). We estimate that a bolus injection of 10 μg cannabidiol in our experiments would give a peak concentration in the first 5 s post-injection of approximately 30 μM, with 2000 μg giving approximately 6 mM cannabidiol. This may be an underestimate, but we have no way of knowing what the concentration of cannabidiol actually is in vivo at the vanilloid VR1 receptors in the hind limb during the first 5 s following a bolus injection, which is when vanilloid VR1 receptor agonists such as capsaicin activate the receptors and initiates reflex changes in blood pressure and ventilation. Measurement of cannabidiol levels in blood or plasma samples would not provide a definitive answer, even had we been able to undertake such measurements. It is improbable that more than 10% of the cannabidiol was metabolised in the first 10 s following injection, because experiments with radiolabelled cannabidiol showed that about 80% of the dose remains in rat plasma 30 s after a bolus i.v. injection (Alozie et al., 1980). We consider it unlikely that our estimate of cannabidiol concentration near the site of injection will be out by much more than a factor of 10, but the important point is that there was no evidence from our experiments that cannabidiol had any rapid onset short-acting capsaicin-like actions whatsoever, even following the highest doses studied. In contrast, capsaicin was effective in all our experiments, with a mean apparent ED_{50} for vasodepression of 0.4 nmol (Table 2); applying the same estimate for local concentration as used for cannabidiol gives a value of 0.4 μM. cannabidiol was about 100-fold less potent than capsaicin as a vanilloid VR1 receptor agonist on HEK cells in vitro (Bisogno et al., 2001), so if the potency ratio applied in vivo we would expect to see evidence of vanilloid VR1-like reflex cardiovascular and respiratory effects with cannabidiol at local concentrations of 40 μM, whereas we did not detect any such effects, even following doses as high as 2000 μg (5 mg kg^{-1})—estimated peak tissue concentration of approximately 6 mM cannabidiol.

Consistent with our negative findings on rat mean blood pressure, others have reported that cannabidiol does not cause hypotension in anaesthetised dogs (Adams et al., 1977) or mice (Jarai et al., 1999), nor does it affect anandamide-induced hypotension in anaesthetised mice (Jarai et al., 1999). Abnormal-cannabidiol, a structural analogue of cannabidiol in which the free phenolic group is transposed with the pentyl side chain, was recently shown to elicit endothelium-dependent mesenteric vasodilatation that could be inhibited by cannabidiol (Jarai et al., 1999). This effect was

obtained in mice lacking both CB_1 and CB_2 receptors, and suggests the existence of a novel endothelial ''anandamide receptor'' for which abnormal-cannabidiol is a selective agonist and at which cannabidiol is an antagonist (Jarai et al., 1999; Wagner et al., 1999). We have previously demonstrated that vanilloid VR1 receptors mediate the cardiorespiratory responses to intra-arterially administered anandamide and capsaicin in the anaesthetised rat (Gauldie et al., 2001; Smith and McQueen, 2001). Offertaler et al. (2003) report that actions at vanilloid VR1 receptors do not account for abnormal-cannabidiol-induced hypotension because the selective vanilloid VR1 receptor antagonist, capsazepine, had no effect on the vasodepressor action of abnormal-cannabidiol in anaesthetised mice.

Cannabidiol has been reported to inhibit the hydrolysis of anandamide in vitro (Watanabe et al., 1998) and we anticipated responses to anandamide being enhanced following administration of cannabidiol. The finding that responses to anandamide were not potentiated suggests that any effect cannabidiol might have on the hydrolysis of anandamide in vivo is too small to detect under the conditions of our experiments. Cannabidiol has also been shown to be a potent antioxidant in vitro (Hampson et al., 1998) and in vivo (Hampson et al., 2000). This action, together with inhibition of anandamide uptake and hydrolysis, is likely to stimulate further research on cannabidiol, particularly in relation to potential antipsychotic, anxiolytic, neuroprotective and anti-arthritic effects of this compound (Mechoulam and Hanus, 2002; Straus, 2000).

In conclusion, cannabidiol lacks agonist actions at vanilloid VR1 receptors in anaesthetised rats, and this cannabinoid does not modulate the agonist activity of capsaicin or anandamide at vanilloid VR1 receptors. Cannabidiol may have important actions at other sites, such as putative non-CB_1/CB_2 anandamide sensitive receptors.

Acknowledgements

We are very grateful to Professor Mechoulam of the Hebrew University, Jerusalem, Israel for supplying (−) cannabidiol.

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