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Short communication

Novel antagonist implicates the CB_1 cannabinoid receptor in the hypotensive action of anandamide

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Abstract

In anaesthetised rats, the endogenous cannabinoid anandamide has potent cardiovascular effects that include a brief pressor effect and a more prolonged depressor response. The depressor response is attenuated after transection of the cervical spinal cord or blockade of α-adrenergic receptors by phentolamine, and is dose-dependently inhibited by a selective antagonist of the CB_1 cannabinoid receptor. The pressor component is not affected by any of these interventions. This suggests that the depressor response is due to inhibition of sympathetic tone mediated by CB_1 receptors, whereas the pressor component is due to a peripheral action that does not involve the same receptors or the sympathetic nervous system.

Keywords: Cannabinoid; Blood pressure; Anandamide; CB_1 cannabinoid receptor antagonist

1. Introduction

Cannabis has been widely abused for its potent neurobehavioral effects, but it can also affect other physiological functions, including cardiovascular variables. In man, the most prominent acute effect is tachycardia (Dewey, 1990), while hypotension and bradycardia have been reported after prolonged exposure to marijuana (Benowitz and Jones, 1975). In the rat, a transient pressor response followed by hypotension and bradycardia are the most commonly observed effects of cannabinoids, such as Δ^9-tetrahydrocannabinol (Dewey, 1990). The biological effects of cannabinoids are mediated by specific receptors in the brain and in peripheral tissues (Devane et al., 1988). Two cannabinoid receptors have been cloned: CB_1 that is present predominantly in brain (Matsuda et al., 1990), and CB_2 that is present in peripheral macrophages only (Munro et al., 1993). These receptors bind not only plant-derived cannabinoids, but also the recently identified endogenous ligand, anandamide (Devane et al., 1992), which shares many of the neurobehavioral effects of cannabinoids (Fride and Mechoulam, 1993; Smith et al., 1994). We tested whether anandamide also has cannabinoid-like cardiovascular effects in anaesthetised rats.

2. Materials and methods

Male Sprague-Dawley rats weighing 250–350 g were anaesthetised with urethane, 0.3 g/kg i.p. + 0.8 g/kg i.v., or with pentobarbital sodium, 50 mg/kg i.v. Arterial blood pressure was monitored via an arterial cannula connected to a pressure transducer and physiograph, and heart rate was monitored via a tachograph preamplifier. Drugs were injected via a cannula in the femoral vein. Anandamide (arachydonyl ethanolamide), SR-141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride] and Δ^9-tetrahydrocannabinol were dissolved in the solvent ethanol/emulphor/saline 1:1:18. Intravenous injection of up to 0.5 ml of this solvent caused no change in blood pressure or heart rate. Since the effects of anandamide and SR-141716A were qualitatively similar in animals anaesthetised with either of the two anaesthetics used, results obtained in urethane-anaesthetised animals only are presented.

As there was no tachyphylaxis in the cardiovascular response to repeated (up to four) injections of anandamide at 30-min intervals, a standard dose of anan-

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damide (4 mg/kg) was administered to the same animal before and 10 min after an antagonist, and the effect of the antagonist on the peak effect of anandamide was analyzed by the paired *t*-test. In the experiments with the CB_1 receptor antagonist SR-141716A, the effect of 4 mg/kg anandamide was retested twice, at 10 as well as at 60 min following the administration of the antagonist. The degree of blockade observed was identical at the two time points, and the data presented reflect the measurements made at 60 min.

Anandamide and SR-141716A were synthesized and provided to us by Dr. Raj Razdan. Other drugs and chemicals were from the usual commercial sources.

3. Results

In urethane-anaesthetised rats, bolus i.v. injections of anandamide elicit bradycardia and a triphasic blood pressure response. These effects are highly reproducible on repeated injections at 30-min intervals, indicating the lack of significant tachyphylaxis. The minimal effective dose of anandamide is 0.2 mg/kg, and the effects are dose dependent up to 20 mg/kg (not shown), which is comparable to the dose range at which anandamide elicits neurobehavioral effects (Smith et al., 1994). Immediately upon injection of anandamide, there is a dramatic drop in heart rate which then gradually returns toward control levels over the next 3–5 min. Coincident with the peak of the bradycardic response there is a transient sharp decrease in blood pressure that lasts 5–10 s (phase I in Table 1, see also Fig. 1). This is followed by a short-lasting (30–60 s) pressor response (phase II), before blood pressure decreases again during phase III that lasts for up to 6 min. The bradycardia and the initial brief hypotension are completely blocked by methylatropine (Table 1) or by bilateral cervical vagotomy (not shown). This indicates that both effects are due to vagal activation of the heart, the brief hypotension being secondary to the decreased cardiac output caused by the extreme slowing of the heart. Interestingly, Δ^9-tetrahydrocannabinol (5 mg/kg i.v.) does not elicit the transient sharp drop in heart rate and blood pressure, but evokes a brief pressor response similar to that seen with anandamide, and a subsequent depressor response which is similar to but much longer lasting than the one elicited by anandamide (not shown).

To test whether the brief pressor and subsequent depressor effects of anandamide are mediated via the sympathetic nervous system, animals were treated with the α-receptor blocker phentolamine to remove vasoconstrictor tone, or were subjected to cervical spinal cord transection to eliminate sympathetic outflow to the periphery. Both phentolamine (Table 1) and cord transection (Fig. 1A) caused a pronounced and sustained decrease in basal blood pressure, and phentolamine completely blocked the pressor response to the i.v. injection of 20 μg/kg phenylephrine (not shown). However, neither phentolamine nor cord transection inhibited the pressor component of the response to anandamide, indicating that this effect is not sympathetically mediated. In contrast, the prolonged depressor component was nearly eliminated by phentolamine (Table 1) or was completely blocked by cord transection (Fig. 1A). That this inhibition was not due to maximal vasodilation is indicated by the ability of the peripheral vasodilator, sodium nitroprusside, to cause a further marked reduction in blood pressure (Fig. 1A).

Recently, a novel antagonist has been developed that binds to and blocks the effects mediated by the CB_1 cannabinoid receptor with high potency, but has much lower affinity for the CB_2 receptor (Rinaldi-Carmona et al., 1994). In rats, the antagonist SR-141716A was found to cause half-maximal inhibition of cannabinoid-induced neurobehavioral effects at doses

Table 1
The effect of anandamide on blood pressure (BP) and heart rate (HR) in the absence or presence of various antagonists

Antagonist		Basal		After anandamide			
					Phase I	Phase II	Phase III
None	(n = 9)	BP	107 ± 3	ΔBP	−38 ± 8	+35 ± 3	−40 ± 2
		HR	341 ± 6	ΔHR	−211 ± 23	−129 ± 44	−16 ± 7
Methylatropine	(n = 7)	BP	107 ± 4	ΔBP	0 ± 1 [a]	+37 ± 4	−32 ± 4
1 mg/kg		HR	398 ± 13 [a]	ΔHR	0 ± 3 [a]	−8 ± 4 [a]	+9 ± 6
Phentolamine	(n = 5)	BP	61 ± 4 [a]	ΔBP	−5 ± 2 [a]	+50 ± 10	−10 ± 4 [a]
1 mg/kg		HR	349 ± 26	ΔHR	−87 ± 47 [a]	−24 ± 14	−52 ± 29
SR-141716A	(n = 6)	BP	105 ± 7	ΔBP	−48 ± 10	+48 ± 9	−16 ± 4 [a]
1 mg/kg		HR	362 ± 28	ΔHR	−284 ± 225	−140 ± 36	−5 ± 4
SR-141716A	(n = 5)	BP	93 ± 9	ΔBP	−50 ± 11	+55 ± 9	−2 ± 5 [a]
10 mg/kg		HR	365 ± 21	ΔHR	−275 ± 48	−128 ± 38	−12 ± 12

Anandamide, 4 mg/kg, was injected as an i.v. bolus to urethane-anaesthetised rats. Basal BP (mm Hg) and HR (beats/min) values, as well as peak changes caused by anandamide, are shown. Anandamide was retested 10 min (methylatropine, phentolamine) or 1 h (SR-141716A) following the administration of the antagonist. [a] Significant difference from corresponding value in the absence of antagonist in the same animals.

of 0.1–0.4 mg/kg (Rinaldi-Carmona et al., 1994). We used SR-141716A to test the involvement of CB_1 cannabinoid receptors in the cardiovascular response to anandamide. At doses of 1 and 10 mg/kg i.v., SR-141716A did not influence the bradycardia and the first two phases of the blood pressure response to 4 mg/kg of anandamide (Table 1). However, the prolonged depressor response was dose-dependently inhibited (Table 1), the inhibition being complete with the higher dose of the antagonist (Fig. 1B). At the 1 mg/kg dose the antagonist alone had no effect on blood pressure or heart rate, while at the 10 mg/kg dose it caused a moderate decrease in blood pressure (-8 ± 3 mm Hg) and increase in heart rate ($+18 \pm 8$ beats/min) that lasted about 30 min. These findings suggest that the prolonged depressor response to anandamide, but not the first two components of its effect, is mediated by the CB_1 receptor.

The ability of SR-141716A to inhibit the cardiovascular effects of Δ^9-tetrahydrocannabinol was also tested. Since the hypotensive phase in the effect of Δ^9-tetrahydrocannabinol lasts more than 2 h, control Δ^9-tetrahydrocannabinol responses and responses following treatment with SR-141716A were tested in separate animals. In five control animals, i.v. injection of 5 mg/kg Δ^9-tetrahydrocannabinol caused an initial brief pressor response that peaked at $+24 \pm 5$ mm Hg, and a subsequent prolonged depressor response, with a maximal decrease of -22 ± 5 mm Hg. Coincident with the depressor response, there was also a gradual decrease in heart rate that peaked at -75 ± 11 beats/min, 30 min after the injection of Δ^9-tetrahydrocannabinol. In five other animals, 1 mg/kg of SR-141716A was first injected and caused no change in blood pressure or heart rate. When 5 mg/kg of Δ^9-tetrahydrocannabinol was injected 60 min later, the pres-

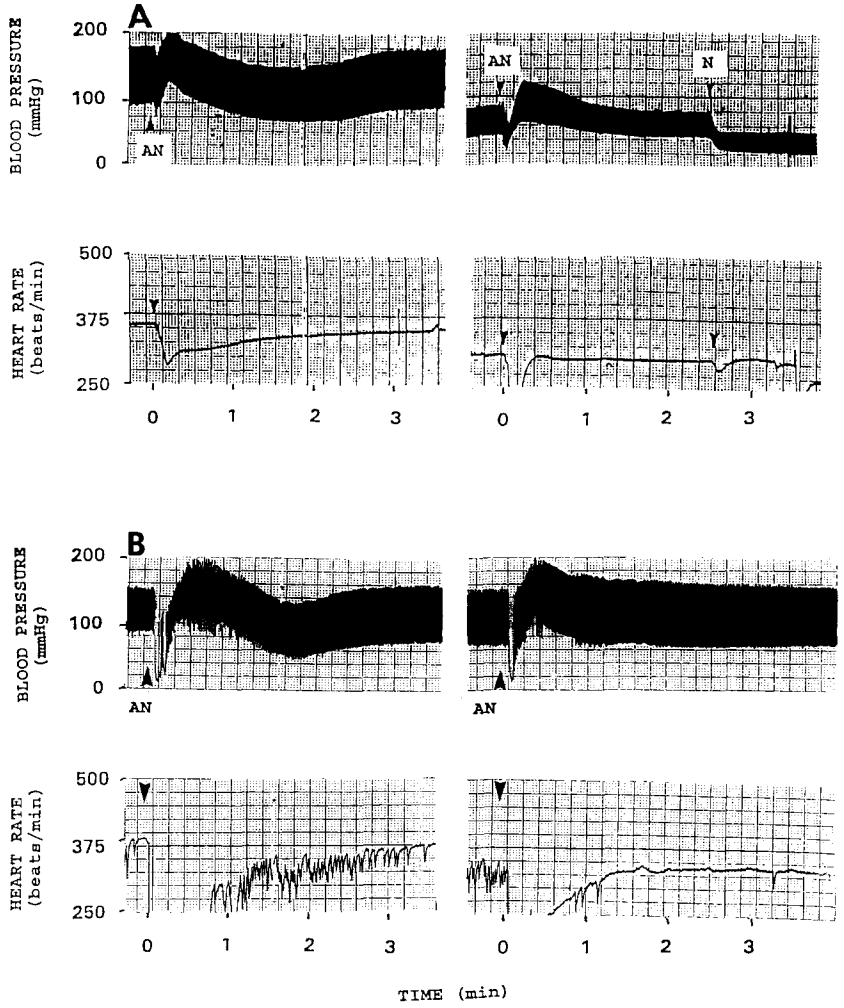

Fig. 1. (A) The effect of anandamide on blood pressure and heart rate in a urethane-anaesthetised rat before (left) and after transection of the cervical spinal cord at the C1/C2 level (right). Anandamide (AN) was injected i.v. in a bolus dose of 4 mg/kg. Note that sodium nitroprusside (N, 10 μg/kg i.v.) causes a marked reduction of blood pressure during the plateau phase of the anandamide response after cord transection. Similar observations were made in three additional animals. (B) SR-141716A blocks the hypotensive response to anandamide. Anandamide, 4 mg/kg i.v., was tested before (left) and 1 h after the i.v. injection of 10 mg/kg SR-141716A in a urethane-anaesthetised rat. The results of similar additional experiments are summarized in Table 1.

sor response was essentially unchanged ($+27 \pm 6$ mm Hg), while the depressor response was significantly reduced, as compared to control (-6 ± 2 mm Hg, $P < 0.005$). The delayed bradycardic response was also significantly attenuated (-24 ± 8 beats/min, $P < 0.005$).

4. Discussion

The results presented indicate that the endogenous cannabimimetic substance anandamide causes pronounced changes in blood pressure and heart rate. Whereas the transient but powerful vagal activation caused by anandamide is not observed after Δ^9-tetrahydrocannabinol, the pressor and subsequent depressor effects of anandamide are similar to but shorter lasting than the effects of Δ^9-tetrahydrocannabinol, and are mediated by similar mechanisms. As for the transient phase I effects, it is not yet clear whether anandamide elicits the underlying vagal activation in the CNS or at peripheral afferent terminals, although the resistance of these transient effects to SR-141716A makes it clear that CB_1 cannabinoid receptors are not involved. The persistence of the pressor response to anandamide after α-receptor blockade or spinal cord transection indicates that this effect is peripherally mediated by a mechanism that does not involve the sympathetic nervous system. Lack of inhibition of the pressor response by the CB_1 receptor antagonist could suggest a non-receptor mechanism such as direct stimulation of vascular smooth muscle. However, Δ^9-tetrahydrocannabinol and the structurally unrelated anandamide both produce a pressor response, which could suggest the involvement of a specific cannabinoid receptor distinct from CB_1. One possible target could be the CB_2 cannabinoid receptor, which is known to interact with both anandamide and Δ^9-tetrahydrocannabinol (Munro et al., 1993). However, testing the involvement of this receptor must await the development of a specific CB_2 receptor antagonist.

On the other hand, the marked reduction of the prolonged depressor response to anandamide after α-receptor blockade or cervical cord transection strongly suggests that the action of anandamide involves a reduction of sympathetic tone to the vasculature, a mechanism similar to that proposed earlier for the hypotensive action of Δ^9-tetrahydrocannabinol (Vollmer et al., 1974). Anandamide could produce such an effect by acting at a site in the central nervous system, at sympathetic ganglia, or at postganglionic sympathetic nerve terminals to reduce neurotransmitter release, and the present findings are compatible with either one of these alternatives. The potency of SR-141716A to inhibit this effect is similar to its potency for inhibition of cannabinoid-induced neurobehavioral effects (Rinaldi-Carmona et al., 1994), which indicates that the delayed hypotension is mediated by the CB_1 cannabinoid receptor. The expression of significant amounts of the mRNA for the CB_1 receptor is limited to the brain (Munro et al., 1993), which would favour a CNS site for the hypotensive action of anandamide. However, mRNA levels not readily detectable by standard Northern blot techniques may be present in sympathetic neurons and may be sufficient to account for functionally relevant levels of receptors in sympathetic ganglia or postganglionic sympathetic terminals.

SR-141716A caused similar inhibition of the prolonged hypotensive and the parallel bradycardic response to Δ^9-tetrahydrocannabinol, which suggests that both effects are probably due to a CB_1 receptor-mediated reduction in sympathetic tone. Although an analogous component in the effect of anandamide on heart rate is not evident, such an effect may have been masked by the much more pronounced and acute vagal bradycardia caused by anandamide, which is not mediated by CB_1 receptors.

If the site of the delayed hypotensive action of anandamide is in the CNS, the localization of such a site within the brain is unclear at present. In the hypothalamus and lower brainstem, which contain many of the structures prominently involved in the central control of sympathetic tone and blood pressure, the distribution of cannabinoid receptors is relatively sparse (Herkenham et al., 1990), and in preliminary experiments we found that intracisternal injection of up to 80 μg/kg anandamide causes no changes in blood pressure or heart rate. However, the cerebellum is rich in cannabinoid receptors (Herkenham et al., 1990), and it has several regions that have been implicated in the modulation of sympathetic tone and blood pressure (Chida et al., 1990; Paton and Gilbey, 1992), where anandamide may be synthesized and released from 'anandaergic' neurons (Devane and Axelrod, 1994). Whether or not such regions are sites of the hypotensive action of cannabinoids and anandamide, remains to be tested.

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References

Benowitz, N.L. and R.T. Jones, 1975, Cardiovascular effects of prolonged delta-9-tetrahydrocannabinol ingestion, Clin. Pharmacol. Ther. 18, 287.

Chida, K., C. Iadecola and D.J. Reis, 1990, Lesions of rostral ventrolataral medulla abolish some cardio- and cerebrovascular components of the cerebellar fastigial pressor and depressor responses, Brain Res. 508, 93.

Devane, W.A., F.A. Dysarz, M.R. Johnson, L.S. Melvin and A.C. Howlett, 1988, Determination and characterization of a cannabinoid receptor in rat brain, Mol. Pharmacol. 34, 605.

Devane, W.A. and J. Axelrod, 1994, Enzymatic synthesis of anandamide, an endogenous ligand for the cannabinoid receptor, by brain membranes, Proc. Natl. Acad. Sci. USA 91, 6698.

Devane, W.A., L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger and R. Mechoulam, 1992, Isolation and structure of a brain constituent that binds to the cannabinoid receptor, Science 258, 1946.

Dewey, W.L., 1990, Cannabinoid pharmacology, Pharmacol. Rev. 38, 151.

Fride, E. and Mechoulam, R., 1993, Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent, Eur. J. Pharmacol. 231, 113.

Herkenham, M., A.B. Lynn, M.D. Little, M.R. Johnson, L.S. Melvin, B.R. De Costa and K.C. Rice, 1990, Cannabinoid receptor localization in brain, Proc. Natl. Acad. Sci. USA 87, 1932.

Matsuda, L.A., S.J. Lolait, M.J. Brownstein, A.C. Young and T.I. Bonner, 1990, Structure of a cannabinoid receptor and functional expression of the cloned cDNA, Nature 346, 561.

Munro, S., K.L. Thomas and M. Abu-Shaar, 1993, Molecular characterization of a peripheral receptor for cannabinoids, Nature 365, 61.

Paton, J.F. and M.P. Gilbey, 1992, Effect of anesthetic on sympathetic responses evoked from cerebellar uvula in decerebrate cats, Am. J. Physiol. 263, H1285.

Rinaldi-Carmona, M., F. Barth, M. Heaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Neliat, D. Caput, P. Ferrara, P. Soubrie, J.C. Breliere and G. Le Fur, 1994, SR141716A, a potent and selective antagonist of the brain cannabinoid receptor, FEBS Lett. 350, 240.

Smith, P.B., D.R. Compton, S.P. Welch, R.K. Razdan, R. Mechoulam and B.R. Martin, 1994, The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice, J. Pharmacol. Exp. Ther. 270, 219.

Vollmer, R.R., I. Cavero, R.J. Ertel, T.A. Solomon and J.P. Buckley, 1974, Role of the central autonomic nervous system in the hypotension and bradycardia induced by (−)-Δ^9-*trans*-tetrahydrocannabinol, J. Pharm. Pharmacol. 26, 186.