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Potentiation of anandamide hypotension by the transport inhibitor, AM404

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Abstract

The putative endogenous cannabinoid, anandamide (0.2–2 mg/kg i.v.), decreased systemic blood pressure dose-dependently in anesthesized guinea pigs. These effects were prevented by the CB1 cannabinoid receptor antagonist SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide · HCl] at the dose of 0.2 mg/kg i.v. The vasodepressor responses to anandamide were significantly potentiated and prolonged by a novel inhibitor of carrier-mediated anandamide transport, *N*-(4-hydroxyphenyl) arachidonylethanolamide (AM404) (10 mg/kg, i.v.). These results suggest that anandamide transport participates in terminating the vascular actions of anandamide. © 1997 Elsevier Science B.V.

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The primary psychoactive ingredient of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC), produces profound vasodilatory and hypotensive effects which are thought to result from the activation of CB1 cannabinoid receptors (Varga et al., 1995). Although it is still unclear whether these effects are initiated in the central nervous system or the periphery, pharmacological evidence indicates that CB1 cannabinoid receptors expressed in vascular smooth muscle may be involved (Randall et al., 1996).

Anandamide (arachidonylethanolamide) is a putative endogenous cannabinoid which displays many pharmacological properties of Δ^9-THC (Devane et al., 1992), including the ability to affect systemic blood pressure. Anandamide produces hypotension in rats and vasorelaxation in isolated vascular preparations (Varga et al., 1995; Randall et al., 1996). The involvement of CB1 cannabinoid receptors in these responses was indicated by their sensitivity to the selective CB1 antagonist, SR141716A (Varga et al., 1995; Randall et al., 1996). Interestingly, SR141716A was also found to prevent endothelium-dependent vasorelaxations induced by carbachol and by calcium ionophore in the isolated mesentery and the vasodilation induced by bradykinin in the hindlimb vasculature, suggesting that endogenous anandamide might act as an endothelium-derived vasorelaxant (Randall et al., 1996).

The pathways of anandamide formation and inactivation in vascular tissue are not yet known. In brain, anandamide may be produced in and released from depolarized neurons (Di Marzo et al., 1994). After release, the biological actions of anandamide may be rapidly terminated by transport into neurons and astrocytes, followed by enzymatic hydrolysis (Di Marzo et al., 1994; Desarnaud et al., 1995). Anandamide transport fulfills several key criteria of a carrier-mediated process, including temperature dependence, high affinity and selectivity (Beltramo et al., 1997). In addition, this transport is inhibited competitively and effectively by a novel anandamide analog, *N*-(4-hydroxyphenyl) arachidonylethanolamide (AM404), which also enhances receptor-mediated anandamide responses in vitro and prolongs anandamide antinociceptive activity in vivo. In contrast and underscoring its selectivity on transport, AM404 does not activate CB1 cannabinoid receptors and does not significantly inhibit anandamide hydrolysis (Beltramo et al., 1997).

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Table 1
Dose-dependent effects of anandamide on systemic blood pressure in anesthetized guinea pigs, and potentiation of these effects by the anandamide transport inhibitor AM404 (AM, 10 mg/kg, i.v.). Anandamide hypotension was in all likelyhood mediated by CB1 cannabinoid receptors, as indicated by its sensitivity to SR141716A (SR, 0.2 mg/kg, i.v.). Results are expressed as mean decrease in systemic pressure ± S.E.M. from 3–7 separate experiments. Asterisks indicate a statistical difference with respect to 5 mg/kg anandamide ($P < 0.05$, Student's t test)

	Dose (mg/kg)						
	0.5	1	2	3	5	5 + AM	5 + SR
mmHg (mean decrease ± SEM)	5.1 ± 1	7.8 ± 0.8	10.4 ± 0.7	18.1 ± 5	22.8 ± 3	36.6 ± 3 *	13.8 ± 1 *

To determine whether carrier-mediated transport participates in terminating the vascular actions of anandamide, we have examined the effects of AM404 on anandamide-induced hypotension in guinea pigs. Male guinea pigs (400–500 g, Charles River, Italy) were anesthetized with pentobarbitone (40 mg/kg, i.p.) and Hypnorm™ (0.5 ml/kg i.m.). To remove autonomic influences, all animals were subjected to bilateral cervical vagotomy and received 2 mg/kg i.v. of the ganglionic blocker, pancuronium. Systemic blood pressure was monitored through a cannula inserted in the carotid artery and connected to a pressure transducer (Ugo Basile, Varese, Italy). Drugs were injected in the jugular vein. Bolus i.v. injections of anandamide elicited a transient and monophasic decrease in systemic arterial pressure. The minimal effective dose of anandamide was 0.5 mg/kg i.v. and the responses were dose-dependent up to 5 mg/kg (Table 1), which is comparable to the dose range at which i.v. anandamide elicits in rodents behavioral effects characteristic of cannabinoid drugs (Fride and Mechoulam, 1993). The responses were highly reproducible when anandamide was administered repeatedly at 30 min intervals, suggesting the lack of significant tachyphylaxis (data not shown). To test whether CB1 cannabinoid receptors participate in the depressor effects of anandamide, we treated five animals with the selective CB1 antagonist, SR141716A (Rinaldi-Carmona et al., 1994). The drug caused half-maximal inhibition of anandamide-induced hypotension at a dose (0.2 mg/kg, i.v.) similar to those reported for half-maximal inhibition of cannabinoid-induced behavioral responses (Rinaldi-Carmona et al., 1994; Table 1). When applied alone, SR141716A exerted only a minor vasopressor effect, which was not statistically significant (data not shown). Palmitylethanolamide, a saturated acylethanolamide that does not bind to CB1 receptors, also had no effect on systemic blood pressure (3 mg/kg, i.v.; mean pressure change: 2.5 mmHg, $n = 2$).

We examined next whether the anandamide responses were affected by the transport inhibitor, AM404. In five animals, we administered AM404 (10 mg/kg, i.v.) 60 min before a maximal dose of anandamide (5 mg/kg, i.v.). AM404 significantly enhanced the vasodepressor response to anandamide (Table 1) and prolonged its duration (from 5–6 to 20–30 min). In comparison, AM404 had only a little vasopressor effect when injected alone (mean pressure change: 12 ± 6 mmHg, $n = 5$).

Our results indicate that anandamide decreases systemic blood pressure in guinea pigs by a mechanim independent of sympathetic or vagal activity, but dependent on CB1 cannabinoid receptor activation. They also suggest that the vascular actions of anandamide may be terminated, at least in part, through a transport mechanism sensitive to inhibition by AM404 (Beltramo et al., 1997). Thus, AM404 may be useful in defining the possible roles of endogenous anandamide in the regulation of vascular tone and might lead to the development of novel agents for the treatment of cardiovascular diseases.

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