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2-Arachidonoylglycerol, a candidate of endothelium-derived hyperpolarizing factor

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Abstract

We investigated whether 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, is involved in acetylcholine- and calcium ionophore A23187-induced relaxations in the presence of N^G-nitro-L-arginine methyl ester (L-NAME) and indomethacin, which is considered to be mediated by endothelium-derived hyperpolarizing factor (EDHF). In rabbit mesenteric arterial rings pre-constricted with noradrenaline, 2-arachidonoylglycerol caused concentration-dependent relaxation. The 2-arachidonoylglycerol-induced relaxations were not affected by endothelium removal. *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-caroxamide (SR141716A) and 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morholinyl-1*H*-pyrazole-3-carboxamide (AM281), cannabinoid CB_1 receptor antagonists, significantly attenuated 2-arachidonoylglycerol-induced relaxation and the acethylcholine-induced relaxation only slightly, but not the calcium ionophore A23187-induced relaxation. On the other hand, charybdotoxin plus apamin, K^+ channel blockers, significantly attenuated acetylcholine and calcium ionohore A23187-induced relaxations but not 2-arachidonoylglycerol-induced relaxations. These results suggest that 2-arachidonoylglycerol can cause relaxations via cannabinoid CB_1 receptors, but is not involved in EDHF-mediated relaxations.

Keywords: 2-Arachidonoylglycerol; Cannabinoid; EDHF (endothelium-derived hyperpolarizing factor)

1. Introduction

The vascular endothelium regulates the tonus of the underlying smooth muscle cells by releasing vasorelaxing factors, such as nitric oxide (NO), prostacyclin, or an endothelium-derived hyperpolarizing factor (EDHF). EDHF is commonly thought to hyperpolarize vascular smooth muscle cells via activation of the K^+ channel, thereby producing vasorelaxation. Its physiological or pathophysiological significance is not well understood, but EDHF seems to be the major determinant of vascular calibre in small resistance arteries (Garland et al., 1995) and may also act as a back-up relaxing factor for preserving the tones when NO production is suppressed (Kagota et al., 1999). The chemical form of EDHF has not yet been identified, but several substances have been offered as candidates. Of these, anandamide (*N*-arachidonylethanolamide), an endogenous cannabinoid receptor ligand, seems promising (Randall and Kendall, 1998). Anandamide has been suggested to represent an EDHF because anandamide-induced vasorelaxations are sensitive to K^+ channel blockers in the rat mesenteric artery (Randall and Kendall, 1998). However, whether or not it is an EDHF has been a matter of much dispute.

2-Arachidonoylglycerol has been found as a new endogenous cannabinoid receptor ligand in mammalian tissues (Mechoulam et al., 1995; Sugiura et al., 1995). 2-Arachidonoylglycerol has also been reported to possess higher binding affinity toward the cannabinoid receptor, to be more abundant in the brain than anandamide, and to have a more physiological biosynthetic pathway (Sugiura et al., 1995). Furthermore, 2-arachidonoylglycerol can de-

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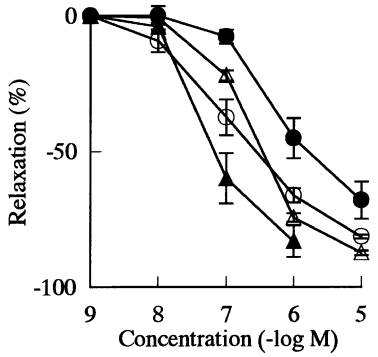

Fig. 1. Concentration–relaxation response curves for 2-arachidonoylglycerol, anandamide, acetylcholine and calcium ionophore A23187 in rabbit mesenteric arterial rings preconstricted with noradrenaline in the presence of N^G-nitro-L-arginine methyl ester (100 μM) and indomethacin (10 μM). (○) 2-Arachidonoylglycerol; (●) anandamide; (△) Acetylcholine; (▲) Calcium ionophore A23187.

crease blood pressure when administered to rats (Mechoulam et al., 1998) and is released from vascular endothelial cells on stimulation with thrombin (Sugiura et al., 1998). Thus, 2-arachidonoylglycerol may play a physiologically important role not only in the central nervous system but also in the vascular system (Di Marzo, 1998).

To elucidate whether 2-arachidonoylglycerol is an EDHF, we compared the properties of 2-arachidonoylglycerol-induced relaxations using K^+ channel blockers and cannabinoid CB_1 receptor antagonists in rabbit mesenteric arteries, with those of acetylcholine-induced nitric oxide- and prostaglandin-independent relaxation, namely EDHF-mediated relaxations.

2. Materials and methods

2.1. Aortic preparations

Male Japanese white rabbits (2–3 kg, Shimizu Laboratory Animals, Kyoto, Japan) were anesthetized with sodium pentobarbitone (50 mg/kg). The second-order branches of the superior mesenteric artery were removed and immediately placed in Krebs–Henseleit solution (118.4 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$, 11.1 mM glucose). The vessels were cleaned of adherent tissues and cut into 3-mm rings, taking care not to damage the endothelium. In some rings, the endothelium was mechanically removed by gentle rubbing with moistened cotton. Each ring was fixed vertically under a resting tension of 0.3 g in a 5-ml organ bath filled with the solution (37°C, pH 7.4) described above, which was continuously aerated with a gas mixture of 95% O_2 and 5% CO_2. The rings were allowed to equilibrate for 60 min before the experiment was started. Isometric tension change was measured with a force-displacement transducer (Model T-7; NEC San-Ei, Tokyo, Japan) coupled to a dual channel chart recorder (Model 8K21; NEC San-Ei).

2.2. Vascular relaxation studies

The mesenteric rings were incubated in the presence or absence of a K^+ channel blocker, charybdotoxin (0.1 μM), apamin (1 μM) or glibenclamide (10 μM), or a cannabinoid CB_1 receptor antagonist, AM281 (1 and 3 μM) or SR141716A (1 and 3 μM). Each ring was preconstricted with noradrenaline (0.1–1.0 μM) to generate approximately 80% maximal contraction. Once a stable contraction was obtained, acetylcholine (1 nM–10 μM), calcium ionophore A23187 (1 nM–0.3 μM), anandamide (0.1–10 μM) or 2-arachidonoylglycerol (0.1–10 μM) was cumulatively added to the bath medium. Endothelium-denuded aortic rings were also used and treated similarly. Denudation of the endothelium was confirmed pharmacologically by the disappearance of an acetylcholine (1 μM)-induced relaxation response. Each relaxation response obtained was expressed as a percentage of the maximal relaxation evoked by papaverine (100 μM). Indomethacin (10 μM), a cyclooxygenase inhibitor, and

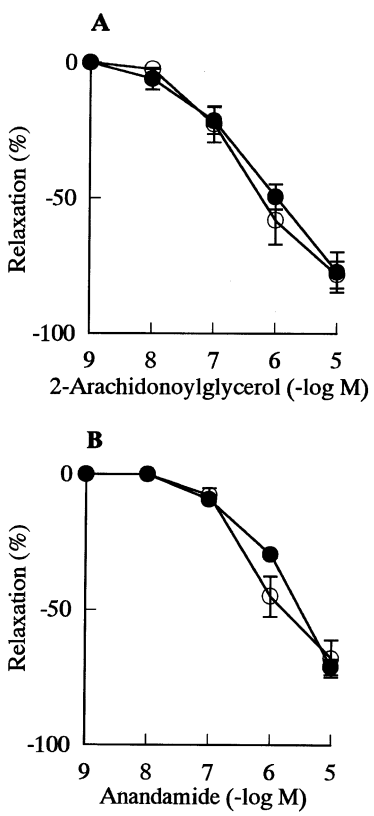

Fig. 2. Effect of endothelium removal on relaxations induced by 2-arachidonoylglycerol (A) and anandamide (B) in rabbit mesenteric arterial rings preconstricted with noradrenaline in the presence of N^G-nitro-L-arginine methyl ester (100 μM) and indomethacin (10 μM). (○) with endothelium; (●) without endothelium.

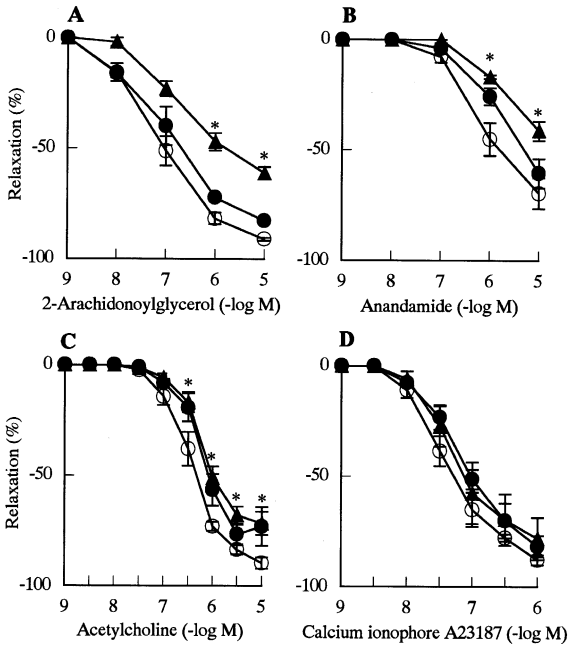

Fig. 3. Effects of SR141716A on relaxations induced by 2-arachidonoylglycerol (A), anandamide (B), acetylcholine (C) and calcium ionophore A23187 (D) in rabbit mesenteric arterial rings preconstricted with noradrenaline in the presence of N^G-nitro-L-arginine methyl ester (100 μM) and indomethacin (10 μM). (○) Control; (●) in the presence of SR141716A (1 μM, 30 min); (△) (3 μM, 30 min). $^*P < 0.05$, SR141716A (3 μM) vs. control.

N^G-nitro-L-arginine methyl ester (L-NAME) (100 μM), a nitric oxide synthase inhibitor, were present in the Krebs–Henseleit solution during all the experiments.

2.3. Drugs

Drugs used in the present experiments were as follows: acetylcholine chloride (Daiichi Pharmaceutical, Tokyo, Japan); anandamide (Tocris Cookson, Ballwin, MO, USA); calcium ionophore A23187, apamin, indomethacin and L-NAME (Sigma, MO, USA); glibenclamide (Hoechst Japan, Tokyo, Japan); charybdotoxin (Peptide Ins., Osaka, Japan); AM281 (Tocris Cookson); noradrenaline (Sankyo, Tokyo, Japan); papaverine hydrochloride (Nacalai Tesque, Kyoto, Japan). SR141716A was kindly provided by Sanofi Recherch (Montpellier, France). 2-Arachidonoylglycerol was synthesized in our laboratory. Other chemicals of analytical reagent grade were purchased from Nacalai Tesque.

2.4. Statistical analysis

All data are expressed as means ± S.E.M. for eight experiments. Statistical analysis was performed using Student's *t*-test. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Relaxations in response to endogenous ligands stimulating cannabinoid

In the presence of indomethacin and L-NAME, 2-arachidonoylglycerol (1 nM–10 μM) and anandamide (1 nM–10 μM) as well as acetylcholine (1 nM–10 μM) and calcium ionophore A23187 (1 nM–1 μM) produced concentration-dependent relaxations in endothelium-intact mesenteric arterial rings pre-constricted with noradrenaline (Fig. 1). The relaxation responses to 2-arachidonoylglycerol and anandamide were comparable to that of acetylcholine (2-arachidonoylglycerol: pEC_{50} [negative logarithm of the EC_{50}] = 6.52 ± 0.08, R [relaxation response at 10 μM] = 87.1 ± 1.6%; anandamide: pEC_{50} = 5.72 ± 0.09, R = 67.1 ± 4.8%; acetylcholine: pEC_{50} = 6.51 ± 0.03, R = 89.7 ± 1.2%). The degree of relaxation of 2-arachidonoylglycerol was significantly greater than that of anandamide ($P < 0.01$).

In endothelium-denuded mesenteric arterial rings, relaxations in response to 2-arachidonoylglycerol and anandamide were almost the same as in endothelium-intact rings (Fig. 2).

3.2. Effects of cannabinoid CB_1 receptor antagonists

We examined the effects of cannabinoid CB_1 receptor antagonists, AM281 and SR141716A, on the relaxation

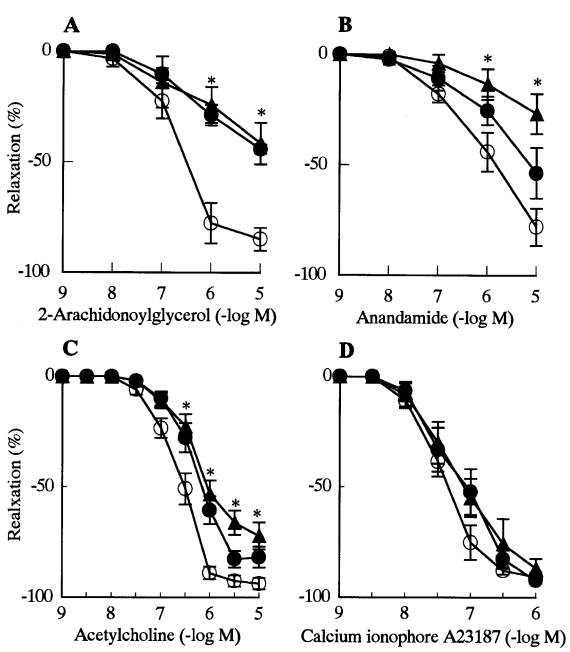

Fig. 4. Effects of AM281 on relaxations induced by 2-arachidonoylglycerol (A), anandamide (B), acetylcholine (C) and calcium ionophore A23187 (D) in rabbit mesenteric arterial rings preconstricted with noradrenaline in the presence of N^G-nitro-L-arginine methyl ester (100 μM) and indomethacin (10 μM). (○) Control; (●) in the presence of AM281 (1 μM, 30 min); (△) (3 μM, 30 min). $^*P < 0.05$, AM281 (3 μM) vs. control.

responses to 2-arachidonoylglycerol, anandamide, acetylcholine and calcium ionophore A23187 in the presence of L-NAME and indomethacin. As shown in Fig. 3A and B, the 2-arachidonoylglycerol- and anandamide-induced relaxations was inhibited by SR141716A slightly at a concentration of 1 μM and significantly at a concentration of 3 μM. The acetylcholine-induced relaxation was also slightly but significantly inhibited by SR141716A (Fig. 3C). However, the calcium ionophore A23187-induced relaxation were not affected by SR141716A at a concentration of 3 μM (Fig. 3D).

The relaxation induced by 2-arachidonoylglycerol and by anandamide was significantly inhibited (Fig. 4A and B) in the experiment using AM281. The acetylcholine-induced relaxation was also slightly but significantly inhibited (Fig. 4C), but the calcium ionophore A23187-induced relaxation was not affected by AM281 (Fig. 4D).

3.3. Effects of K^+ channel blockers

Fig. 5 shows the effects of K^+ channel blockers on the relaxations in response to 2-arachidonoylglycerol, anandamide, acetylcholine or calcium ionophore A23187 in the presence of L-NAME and indomethacin. Ca^{2+}-sensitive K^+ channel blockers, charybdotoxin (0.1 μM) plus apamin (1 μM), affected neither the 2-arachidonoylglycerol- nor anandamide-induced relaxation (Fig. 5A and B), but markedly inhibited the acetylcholine- and calcium ionophore A23187-induced relaxation (Fig. 5C and D). On the other hand, an ATP-sensitive K^+ channel blocker, glibenclamide (10 μM), did not alter the relaxations induced by these cannabinoid receptor antagonists and acetylcholine (data not shown).

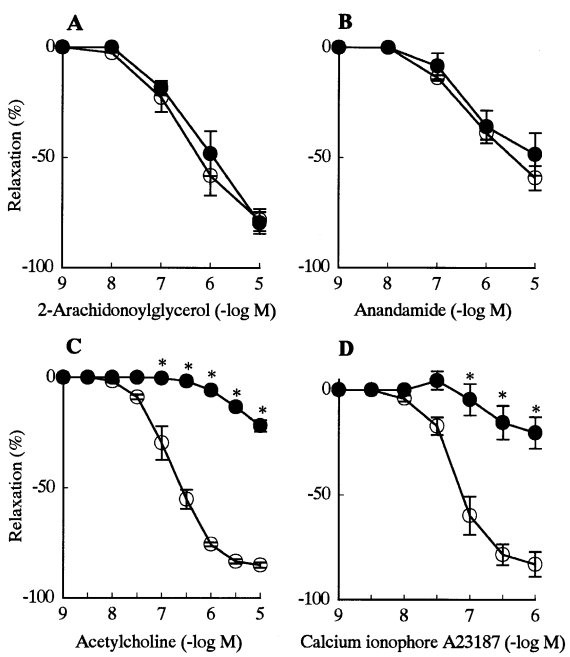

Fig. 5. Effects of charybdotoxin plus apamin, calcium-sensitive K^+ channel blockers, on relaxations induced by 2-arachidonoylglycerol (A), anandamide (B), acetylcholine (C) and calcium ionophore A23187 (D) in rabbit mesenteric arterial rings preconstricted with noradrenaline in the presence of N^G-nitro-L-arginine methyl ester (100 μM) and indomethacin (10 μM). (○) Control; (●) in the presence of charybdotoxin (0.1 μM, 30 min) plus apamin (1 μM, 30 min). $^*P < 0.05$, vs. control.

4. Discussion

Using rabbit mesenteric arteries, we compared the properties of endogenous cannabinoid receptor ligand-induced relaxations and EDHF-mediated relaxations, both induced by acetylcholine and calcium ionophore A23187 in the presence of L-NAME and indomathacin. We demonstrated that the cannabinoid CB_1 receptor antagonists SR141716A and AM281 attenuate the relaxations induced by endogenous cannabinoid receptor ligands, anandamide and 2-arachidonoylglycerol. Also, these cannabinoid CB_1 receptor antagonists could slightly but significantly attenuate acetylcholine-induced relaxations in the presence of L-NAME and indomethacin. Similar inhibitory effects of cannabinoid CB_1 receptor antagonists on both anandamide- and accetylcholine receptor agonist-induced relaxations have been shown to occur in the rat mesenteric artery (Randall and Kendall, 1998; White and Hiley, 1997). We also found that relaxations in response to anandamide and 2-arachidonoylglycerol were endothelium-independent. This finding agrees with evidence that cannabinoid CB_1 receptors have been identified on vascular smooth muscle cells (Sugiura et al., 1998). Therefore, our results raised the possibility that, in rabbit mesenteric arteries, the endogenous cannabinoids behave like EDHF by activating canabinoid CB_1 receptors on the vascular smooth muscle. However, these cannabinoid CB_1 receptor antagonists could not inhibit calcium ionophore A23187-induced relaxations in the presence of L-NAME and indomethacin, i.e. EDHF-mediated relaxations. There is no satisfactory explanation for the difference in the antagonistic effect of anandamide and 2-arachidonoylglycerol against acetylcholine- and calcium ionophore A23187-induced relaxations, although acetylcholine and calcium ionophore A23187 induce endothelium-dependent relaxations via different mechanisms, i.e. receptor-dependent and -independent mechanisms, respectively. As production of 2-arachidonoylglycerol from the endothelium has been shown to occur through stimulation with an acetylcholine receptor agonist, carbachol, in the rat aorta (Mechoulam et al., 1998), only acetylcholine may in part produce 2-arachidonoylglycerol or anandamide from endothelium. Collectively, these findings suggest that cannabinoid CB_1 receptors on smooth muscle cells were not involved in EDHF-mediated relaxations induced by acetylcholine and calcium ionophore A23187 in rabbit mesenteric arteries.

Furthermore, the properties of relaxations induced by endogenous cannabinoids and EDHF were also different. Ca^{2+}-sensitive K^+ channel blockers, charybdotoxin and

apamin, which inhibit EDHF-mediated relaxation, could markedly inhibit acetylcholine- or calcium ionophore A23187-induced relaxation but not 2-arachidonoylglycerol- or anandamide-induced relaxation. Thus, EDHF released from the rabbit mesenteric artery may be a factor other than endogenous cannabinoids. It has been reported that anandamide and EDHF may act via different mechanisms as suggested for the rat mesenteric artery (White and Hiley, 1997; Plane et al., 1997), the rat hepatic artery (Zygmunt et al., 1997) and the rat coronary artery (Fluton and Quilley, 1998).

Some reports have assumed that charybdotoxin and apamin act to inhibit EDHF production/release by blocking the K^+ channel located on the endothelium, i.e. depolarizing the endothelium membrane (Edwards et al., 1998; Doughty et al., 1999). On the other hand, these agents have been reported to have no effect on the acetylcholine-induced increase of endothelial intracellular-free Ca^{2+} concentration but to abolish the EDHF-mediated relaxation in the coronary artery from guinea pigs (Yamanaka et al., 1998) or the skeletal resistance artery from hamsters (Bolz et al., 1999). In the present study, the combination of these K^+ channel blockers could inhibit calcium ionophore A23187-induced EDHF-mediated relaxation in rabbit mesenteric artery. Similar inhibitory effects by these agents on calcium ionophore A23187-induced relaxation have been reported for the rat mesenteric artery (White and Hiley, 1997), the guinea pig basilar artery (Petersson et al., 1998) and the rat hepatic artery (Zygmunt et al., 1998). Calcium ionophore A23187 is well known to cause a direct increase in endothelial Ca^{2+} levels and to stimulate release of EDHF. The increased endothelial Ca^{2+} levels caused by calcium ionophore A23187 occur via an electroneutral exchange of Ca^{2+} for H^+ ions (Reed and Lardy, 1972), which is not sensitive to endothelial membrane potential (White and Hiley, 1997). The conclusion, therefore, is that the part of the acetylcholine- or calcium ionophore A23187-induced relaxations that can be inhibited by charybdotoxin and apamin appears to be due to the action of EDHF, and not the reduced production/release of EDHF from the endothelium.

How the endogenous cannabinoids cause vasorelaxation remains to be demonstrated. Previous work has shown that the vasodilator effect of anandamide is associated with breakdown of the amide to arachidonic acid followed by conversion to vasodilatory eicosanoids (Pratt et al., 1998) or stimulation of NO release from the endothelium (Deutsch et al., 1997). In the present study, however, anandamide as well as 2-arachidonoylglycerol caused vasodilation in the presence of L-NAME and indomethacin. Similar results have been reported for anandamide with the rat mesenteric artery (Ihioka and Bukoski, 1999; White and Hiley, 1997). These findings indicate that anandamide and 2-arachidonoylglycerol do not cause the relaxation via the production of vasodilatory eicosanoids or NO. Further studies are necessary to elucidate the mechanism.

In conclusion, endogenous cannabinoids, 2-arachidonoylglycerol and anandamide, can cause relaxations by stimulating cannabinoid CB_1 receptors in rabbit mesenteric arteries. However, these cannabinoids do not contribute to the acetylcholine-induced L-NAME- and indomethacin-resistant relaxations, i.e. EDHF-mediated relaxations.

References

Bolz, S.-S., de Wit, C., Pohl, U., 1999. Endothelium-derived hyperpolarizing factor but not NO reduces smooth muscle Ca^{2+} during acetylcholine-induced dilation of microvessels. Br. J. Pharmacol. 128, 124–134.

Deutsch, D.G., Goligorsky, M.S., Schmid, P.C., Krebsbach, R.J., Shmid, H.H.O., Das, S.K., Dey, S.K., Arreaza, G., Thorup, C., Stefano, G., Moore, L.C., 1997. Production and physiological actions of anandamide in the vasculature of the rat kidney. J. Clin. Invest. 100, 1538–1546.

Di Marzo, V., 1998. 'Endocannabinoids' and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance. Biochim. Biophys. Acta 1392, 153–175.

Doughty, J.M., Plane, F., Langton, P.D., 1999. Charybdotoxin and apamin block EDHF in rat mesenteric artery if selectively applied to the endothelium. Am. J. Physiol. 276, H1107–H1112.

Edwards, G., Dora, K.A., Gardener, M.J., Garland, C.J., Weston, A.H., 1998. K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 396, 269–272.

Fluton, D., Quilley, J., 1998. Evidence against anandamide as the hyperpolarizing factor mediating the nitric oxide-independent coronary vasodilator effect of bradykinin in the rat. J. Pharmacol. Exp. Ther. 286, 1146–1151.

Garland, C.J., Plane, F., Kemp, B.K., Cocks, T.M., 1995. Endothelium-dependent hyperpolarization: a role in the control of vascular tone. Trends Pharmacol. Sci. 16, 23–30.

Ihioka, N., Bukoski, R.D., 1999. A role for *N*-arachidonylethanolamine (anandamide) as the mediator of sensory nerve-dependent Ca^{2+}-induced relaxation. J. Pharmacol. Exp. Ther. 289, 245–250.

Kagota, S., Tamashiro, A., Yamaguchi, Y., Nakamura, K., Kunitomo, M., 1999. Excessive salt or cholesterol intake alters the balance among endothelium-derived released from renal arteries in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 34, 533–539.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J., Vogel, Z., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. 50, 83–90.

Mechoulam, R., Fride, E., Ben-Shabat, S., Meiri, U., Horowitz, M., 1998. Carbacol, an acetylcholine receptor agonist, enhances production in rat aorta of 2-arachidonoyl glycerol, a hypotensive endocannabinoid. Eur. J. Pharmacol. 362, R1–R3.

Petersson, J., Zygmunt, P.M., Jögestätt, P., Högestätt, E.D., 1998. Characterization of endothelium-dependent relaxation in guinea pig basilar artery-effects of hypoxia and role of cytochrome P_{450} monooxygenase. J. Vasc. Res. 35, 285–294.

Plane, F., Holland, M., Waldron, G.J., Garland, C.J., Boyle, J.P., 1997. Evidence that anandamide and EDHF act via different mechanisms in rat isolated mesenteric arteries. Br. J. Pharmacol. 121, 1509–1511.

Pratt, P.F., Hillard, C.J., Edgemond, W.S., Campbell, W.B., 1998. *N*-Arachidonylethanolamide relaxation of bovine coronary artery is not mediated by CB_1 cannabinoid receptor. Am. J. Physiol. 274, H375–H381.

Randall, M.D., Kendall, D.A., 1998. Endocannabinoids: a new class of vasoactive substances. Trends Pharmacol. Sci. 19, 55–58.

Reed, P.W., Lardy, H.A., 1972. A23187: a divalent cation ionophore. J. Biol. Chem. 247, 6970–6977.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K., 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215, 89–97.

Sugiura, T., Kodaka, T., Nakane, S., Kishimoto, S., Kondo, S., Waku, K., 1998. Detection of an endogenous cannabimimetic molecule, 2-arachidonoylglycerol, and cannabinoid CB_1 receptor mRNA in human vascular cells: is 2-arachidonoylglycerol a possible vasomodulator? Biochem. Biophys. Res. Commun. 243, 838–843.

White, R., Hiley, C.R., 1997. A comparison of EDHF-mediated and anandamide-induced relaxations in the rat isolated mesenteric artery. Br. J. Pharmacol. 122, 1573–1584.

Yamanaka, A., Ishikawa, T., Goto, K., 1998. Characterization of endothelium-dependent relaxation independent of NO and prostaglandins in guinea pig coronary. J. Pharmacol. Exp. Ther. 285, 480–489.

Zygmunt, P.M., Högestätt, E.D., Waldeck, K., Edwards, G., Kirkup, A.J., Weston, A.H., 1997. Studies on the effects of anandamide in rat hepatic artery. Br. J. Pharmacol. 122, 1679–1686.

Zygmunt, P.M., Plane, F., Paulsson, M., Garland, C.J., Högestätt, E.D., 1998. Interactions between endothelium-derived relaxing factors in the rat hepatic artery: focus on regulation of EDHF. Br. J. Pharmacol. 124, 992–1000.