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Cannabinoid CB_1 receptor and endothelium-dependent hyperpolarization in guinea-pig carotid, rat mesenteric and porcine coronary arteries

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1 The purpose of these experiments was to determine whether or not the endothelium-dependent hyperpolarizations of the vascular smooth muscle cells (observed in the presence of inhibitors of nitric oxide synthase and cyclo-oxygenase) can be attributed to the production of an endogenous cannabinoid.

2 Membrane potential was recorded in the guinea-pig carotid, rat mesenteric and porcine coronary arteries by intracellular microelectrodes.

3 In the rat mesenteric artery, the cannabinoid receptor antagonist, SR 141716 (1 μM), did not modify either the resting membrane potential of smooth muscle cells or the endothelium-dependent hyperpolarization induced by acetylcholine (1 μM) (17.3 ± 1.8 mV, $n=4$ and 17.8 ± 2.6 mV, $n=4$, in control and presence of SR 141716, respectively). Anandamide (30 μM) induced a hyperpolarization of the smooth muscle cells (12.6 ± 1.4 mV, $n=13$ and 2.0 ± 3.0 mV, $n=6$ in vessels with and without endothelium, respectively) which could not be repeated in the same tissue, whereas acetylcholine was still able to hyperpolarize the preparation. The hyperpolarization induced by anandamide was not significantly influenced by SR 141716 (1 μM). HU-210 (30 μM), a synthetic CB_1 receptor agonist, and palmitoylethanolamide (30 μM), a CB_2 receptor agonist, did not influence the membrane potential of the vascular smooth muscle cells.

4 In the rat mesenteric artery, the endothelium-dependent hyperpolarization induced by acetylcholine (1 μM) (19.0 ± 1.7 mV, $n=6$) was not altered by glibenclamide (1 μM; 17.7 ± 2.3 mV, $n=3$). However, the combination of charybdotoxin (0.1 μM) plus apamin (0.5 μM) abolished the acetylcholine-induced hyperpolarization and under these conditions, acetylcholine evoked a depolarization (7.7 ± 2.7 mV, $n=3$). The hyperpolarization induced by anandamide (30 μM) (12.6 ± 1.4 mV, $n=13$) was significantly inhibited by glibenclamide (4.0 ± 0.4 mV, $n=4$) but not significantly affected by the combination of charybdotoxin plus apamin (17.3 ± 2.3 mV, $n=4$).

5 In the guinea-pig carotid artery, acetylcholine (1 μM) evoked endothelium-dependent hyperpolarization (18.8 ± 0.7 mV, $n=15$). SR 141716 (10 nM to 10 μM), caused a direct, concentration-dependent hyperpolarization (up to 10 mV at 10 μM) and a significant inhibition of the acetylcholine-induced hyperpolarization. Anandamide (0.1 to 3 μM) did not influence the membrane potential. At a concentration of 30 μM, the cannabinoid agonist induced a non-reproducible hyperpolarization (5.6 ± 1.3 mV, $n=10$) with a slow onset. SR 141716 (1 μM) did not affect the hyperpolarization induced by 30 μM anandamide (5.3 ± 1.5 mV, $n=3$).

6 In the porcine coronary artery, anandamide up to 30 μM did not hyperpolarize or relax the smooth muscle cells. The endothelium-dependent hyperpolarization and relaxation induced by bradykinin were not influenced by SR 141716 (1 μM).

7 These results indicate that the endothelium-dependent hyperpolarizations, observed in the guinea-pig carotid, rat mesenteric and porcine coronary arteries, are not related to the activation of cannabinoid CB_1 receptors.

Keywords: Anandamide; cannabinoid; electrophysiology; (EDHF) endothelium-derived hyperpolarizing factor; potassium channels

Introduction

Endothelial cells control the underlying vascular smooth muscle by producing relaxing and contracting factors (Furchgott & Vanhoutte, 1989). Among the relaxing factors released in response to vasodilators, such as acetylcholine, are nitric oxide (NO) (Furchgott & Zawadzki, 1980; Palmer *et al*., 1987), prostacyclin (Moncada & Vane, 1979) and an unidentified endothelium-derived hyperpolarizing factor (EDHF), which induces hyperpolarization of vascular smooth muscle cells by opening potassium channels (Chen *et al*., 1988; 1991; Félétou & Vanhoutte, 1988; Taylor & Weston, 1988; Corriu *et al*., 1996). Randall *et al*. (1996) showed that, in the rat isolated and perfused mesenteric arterial bed, anandamide (an arachidonic acid derivative suspected to be the endogenous ligand for the cannabinoid CB_1 receptor, Devane *et al*., 1992; Di Marzo *et al*., 1994) induces dilatation which mimics the endothelium-dependent responses that are resistant to the combined inhibition of nitric oxide synthase and cyclo-oxygenase. Therefore, these authors postulated that anandamide may represent an EDHF-like substance. The present experiments were designed to determine whether or not the endothelium-dependent hyperpolarizations observed in the

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guinea-pig carotid, rat mesenteric and porcine coronary arteries (in the presence of inhibitors of nitric oxide synthase and cyclo-oxygenase) can be attributed to the production of an endogenous cannabinoid.

Some of these data have been previously presented in Maastricht for the 7th symposium on 'Mechanisms of Vasodilatation' and in Edinburgh at the joint meeting of the British and French Pharmacological Societies (Chataigneau *et al*., 1997).

Methods

Electrophysiological experiments

Male Sprague Dawley rats (200–220 g) and Hartley guinea-pigs (250–350 g), were anaesthetized by intraperitoneal administration of pentobarbitone (200 mg kg^{-1} and 300 mg kg^{-1}, respectively) and exsanguinated. Branches (second to fourth order, 400 to 250 μm) of the mesenteric arteries and the carotid arteries were dissected free of connective tissues. Large-White pigs of either sex (20–25 kg) were anaesthetized with an intramuscular injection of a combination of tilamine plus zolepam (25 mg kg^{-1}) and exsanguinated. The heart was then removed and the coronary arteries were dissected. Carotid and mesenteric arteries were pinned to the bottom of an organ chamber (0.5 ml in volume) with the adventitia upward, the coronary arteries were slit open and pinned with the intimal surface upward. The isolated vascular segments were superfused at a constant flow (2 ml min^{-1}) with a modified Krebs-Ringer bicarbonate solution (37°C, aerated with a 95% O_2/5% CO_2 gas mixture, pH 7.4) of the following composition (in mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, calcium-disodium EDTA 0.026 and glucose 11.1 (control solution). Transmembrane potential were recorded with glass capillary microelectrodes (tip resistance of 30 to 90 MΩ) filled with KCl (3 M) and connected to the headstage of a recording amplifier (intra 767, World Precision Instruments, New Haven, CT) with capacitance neutralization; an Ag/AgCl pellet, in contact with the bathing solution and directly connected to the amplifier, served as the reference electrode. The signal was continuously monitored on an oscilloscope (3091 Nicolet, Madison, WI) and simultaneously recorded on paper (Gould, Valley View, OH) and on a video recorder (TEAC XR310, Tokyo, Japan). Successful impalements were signalled by a sudden negative drop in potential from the baseline (zero potential reference) followed by a stable negative potential for at least 3 min. The incubation times were at least 30 min with the various potassium channel inhibitors studied and 60 min with the cannabinoid receptor antagonist, SR 141716. Acetylcholine was infused for no longer than 5 min to avoid desensitization of the preparation. With the exception of the experiments designed to study the reproducibility of the effects of anandamide, each preparation was exposed only once to one concentration of a single cannabinoid agonist. All experiments were performed in the presence of N^{ω}-L-nitro-arginine (L-NOARG) and indomethacin to inhibit nitric oxide synthase and cyclo-oxygenase, respectively. In most experiments, care was taken to maintain the endothelium as intact as possible; in some experiments performed in the rat mesenteric artery, the endothelium was destroyed by a rapid infusion of saponin (1 mg ml^{-1}) through the lumen of the blood vessel (Corriu *et al*., 1996). Endothelial cell removal was considered to be successful when the hyperpolarization induced by acetylcholine (μM) did not exceed 5 mV (average: 0.8 ± 0.7 mV, $n = 6$).

Relaxation experiments

Rings of porcine coronary arteries were suspended in organ chambers filled with control solution for isometric tension recording. The tissues were connected to a force transducer (Gould, France) and changes in tension were recorded on a polygraph (Gould). Rings were stretched step by step until optimal and reproducible contraction to KCl (40 mM added to the bath) was achieved (basal tension 6 to 8 g). A maximal contraction was then obtained with 60 mM KCl. After repeated rinsing, rings were subjected to a 45 min period of equilibration. In some experiments the endothelium was mechanically removed by gentle rubbing.

Drugs

The drugs used were: acetylcholine chloride, indomethacin, N^{ω}-L-nitro-arginine (L-NOARG) (Sigma, La Verpillère, France); charybdotoxin, apamin (Latoxan, Rosans, France); cromakalim, glibenclamide and SIN-1 (morpholino-3 sydnonimine, HCl) were synthesized at the Institut de Recherches Servier (Suresnes, France); SR 141716 (N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; gift from Sanofi, Montpellier, France); HU-210 ((6a**R**)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol), palmitoylethanolamide (Tocris Cookson inc., St Louis, MO, U.S.A.); U-46619 (9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$, Cayman Chemical, Ann Arbor, MI, U.S.A.).

SR 141716, HU-210 and palmitoylethanolamide were dissolved in ethanol and stock solutions were prepared in such a way that the final concentration of ethanol in the bath did not exceed 0.1% v/v. Indomethacin was dissolved in deionized water with an equimolar concentration of Na_2CO_3. SIN-1 was prepared in methanol. Glibenclamide was prepared in dimethylsulphoxide. Cromakalim was dissolved in equivalent volumes of deionized water and propylene glycol. The other drugs were dissolved in deionized water. In these studies, anandamide came from three different sources. The anandamide originating from Sigma or synthesized in the Institute by Dr Iliou was dissolved in ethanol. The third source of anandamide, a kind gift from Dr Bennett (Nottingham, U.K.), was solubilized in inert oil and water emulsion prepared by Dr Boyd (Nottingham, U.K.). As the effects of anandamide from these three different sources were qualitatively and quantitatively similar and as the various vehicles did not affect the response, the results have been pooled for the sake of clarity.

Statistics

Data are shown as mean ± s.e.mean; n indicates the number of cells in which membrane potential was recorded. Statistical analysis was performed by Student's t test for paired or unpaired observations. Significant differences for P less than 0.05, 0.01 and 0.001 are indicated with asterisks (*, **, ***, respectively).

Results

Rat mesenteric artery

In the presence of L-NOARG (100 μM) and indomethacin (5 μM), the resting membrane potential of the smooth muscle cells of the rat mesenteric arteries with endothelium averaged

-55.5 ± 1.5 mV ($n=14$). Treatment of the preparations with SR 141716 (1 μM) did not modify the resting membrane potential (-53.8 ± 1.7 mV, $n=8$). Acetylcholine (1 μM) induced an endothelium-dependent hyperpolarization (17.3 ± 1.8 mV, $n=4$) which was not altered by SR 141716 (1 μM; 17.8 ± 2.6 mV, $n=4$).

In the rat mesenteric artery, anandamide (0.1, 5 and 30 μM) induced a hyperpolarization (4.0 ± 1.2, $n=3$, 2.8 ± 1.7, $n=4$ and 12.6 ± 1.4 mV, $n=13$, respectively). Anandamide (30 μM) did not significantly hyperpolarize the rat isolated mesenteric artery when the endothelial cells were removed (2.0 ± 3.0 mV, $n=6$). HU-210 and palmitoylethanolamide (30 μM) did not significantly influence the membrane potential of the rat mesenteric artery (Figure 1). The hyperpolarization to anandamide (30 μM) could not be reproduced in the same preparation even after 30 min of washout, although acetylcholine in the same preparation still evoked an endothelium-dependent hyperpolarization (Figure 2). The hyperpolarizations induced by anandamide (5 μM and 30 μM) were not significantly affected by the presence of 1 μM SR 141716 (5.0 ± 1.5, $n=4$ and 17.3 ± 2.3 mV, $n=4$ respectively; Figure 3).

The endothelium-dependent hyperpolarization to acetylcholine (1 μM) was not altered by glibenclamide (1 μM) but was reversed to a depolarizing response by the combination of charybdotoxin (0.1 μM) plus apamin (0.5 μM) (Figure 4a). The hyperpolarization induced by anandamide (30 μM) was significantly inhibited by glibenclamide (1 μM), whereas it was not affected by the combination of 0.1 μM charybdotoxin plus 0.5 μM apamin (Figure 4b).

Guinea-pig carotid artery

In the presence of L-NOARG (100 μM) and indomethacin (5 μM), the resting membrane potential of the smooth muscle cells of the guinea-pig carotid artery averaged -57.5 ± 0.9 mV ($n=40$). SR 141716 (10 nM to 10 μM) produced a significant

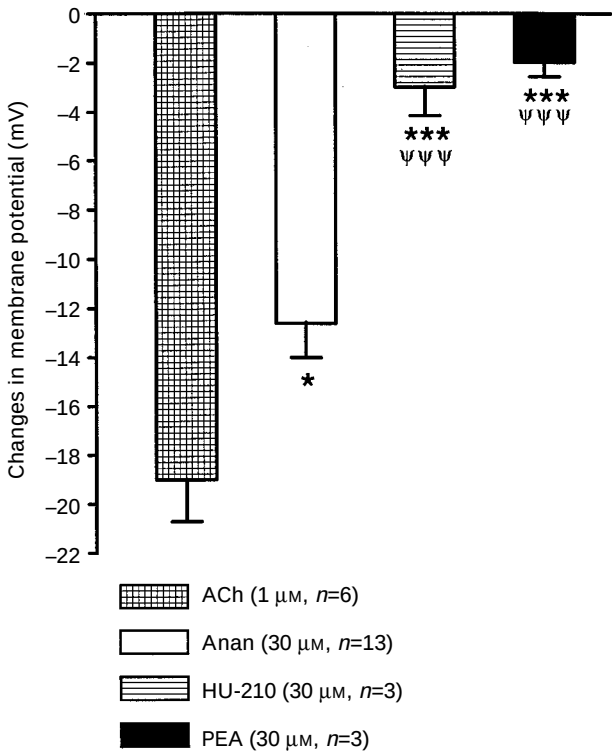

Figure 1 Comparison of the effects of acetylcholine (ACh; 1 μM), anandamide (Anan; 30 μM), HU-210 (30 μM) and palmitoylethanolamide (PEA; 30 μM) in rat isolated mesenteric arteries with endothelium in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Anandamide induced a significant hyperpolarization of the smooth muscle cells whereas HU-210 and palmitoylethanolamide were ineffective. Data are shown as means$\pm$s.e.mean. Asterisks indicate a statistically significant difference from acetylcholine (*$P<0.05$ and ***$P<0.001$). ΨIndicates a statistically significant difference between the cannabinoid agonists and anandamide (ΨΨΨ$P<0.001$).

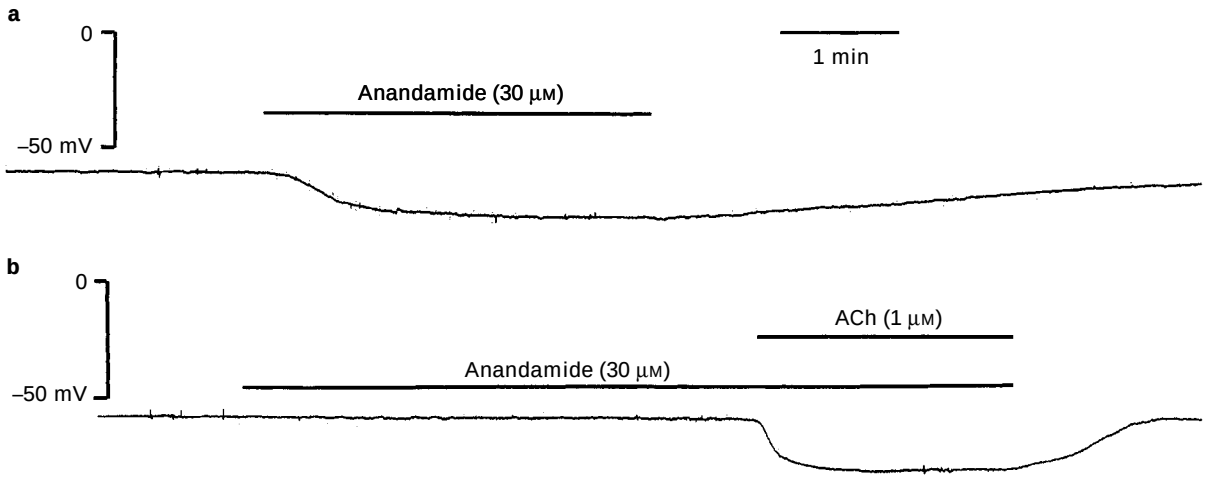

Figure 2 Original recording of the smooth muscle membrane potential, in the rat isolated mesenteric artery with endothelium in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Continuous recording from the same smooth muscle cell. (a) The first administration of anandamide elicited hyperpolarization. (b) The subsequent application of anandamide, after a washout period (30 min), did not induce changes in membrane potential whereas acetylcholine (ACh, 1 μM) was still able to evoke endothelium-dependent hyperpolarization.

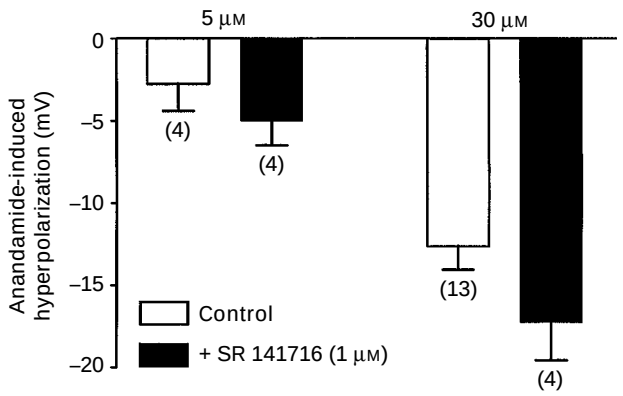

Figure 3 Effects of SR 141617 (1 μM) on the hyperpolarizations elicited by anandamide (5 and 30 μM) in rat isolated mesenteric arteries with endothelium in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Data are shown as mean $\pm$ s.e.mean.

concentration-dependent hyperpolarization (Figure 5a). Acetylcholine (1 μM) induced an endothelium-dependent hyperpolarization (18.8 ± 0.7 mV, $n=15$) which was inhibited significantly, in a concentration-dependent manner, by SR 141716 (Figure 5b). The hyperpolarization induced by cromakalim and SIN-1 (10 μM) was inhibited at the highest concentration of SR 141716 tested (10 μM, Table 1).

Anandamide at 0.1, 0.3, 1 and 3 μM did not affect the membrane potential of the smooth muscle cells of the guinea-pig carotid artery ($n=2-4$; data not shown). However, at 30 μM the cannabinoid agonist induced a hyperpolarization which was significantly smaller (5.6 ± 1.3 mV, $n=10$) and had a slower time course than the response to acetylcholine (time to reach the nadir of the hyperpolarization in seconds: 228 ± 40, $n=8$ and 76 ± 8, $n=15$, for anandamide and acetylcholine, respectively). The anandamide (30 μM)-induced hyperpolarization was not affected by SR 141716 (1 μM; 5.3 ± 1.5 mV, $n=3$) and was not reproducible on the same preparation even after a 30 min washout period (data not shown).

Porcine coronary artery

In the presence of L-NOARG (30 μM) and indomethacin (10 μM), the resting membrane potential of the smooth muscle cells of the porcine coronary artery was not affected significantly by SR 141716 (3 μM) (-49.3 ± 0.6 and -48.6 ± 0.9 mV in control conditions and in presence of the cannabinoid antagonist, respectively; $n=6$). Bradykinin (10 nM) induced an endothelium-dependent hyperpolarization which was not significantly affected by SR 141716 (12.4 ± 2.1 and 12.2 ± 1.9 mV in control conditions and in presence of the cannabinoid antagonist respectively; $n=6$). Anandamide (30 μM) did not significantly affect the membrane potential of the smooth muscle cells of the porcine coronary artery (data not shown).

In isolated rings of porcine coronary arteries, contracted with U-46619 (30 – 100 nM), bradykinin (0.1 nM – 1 μM) induced relaxation. The concentration-response curve was shifted to the right by the presence of L-NOARG (100 μM) and indomethacin (5 μM; pD_2: $8.84 + 0.13$ and $8.09 + 0.46$ in control and presence of L-NOARG and indomethacin, respectively; $P<0.05$, $n=5$). SR 141716 (1 μM) did not affect significantly the endothelium-dependent relaxation to bradykinin either under control conditions or in the presence of the inhibitors of nitric oxide synthases and cyclo-oxygenases

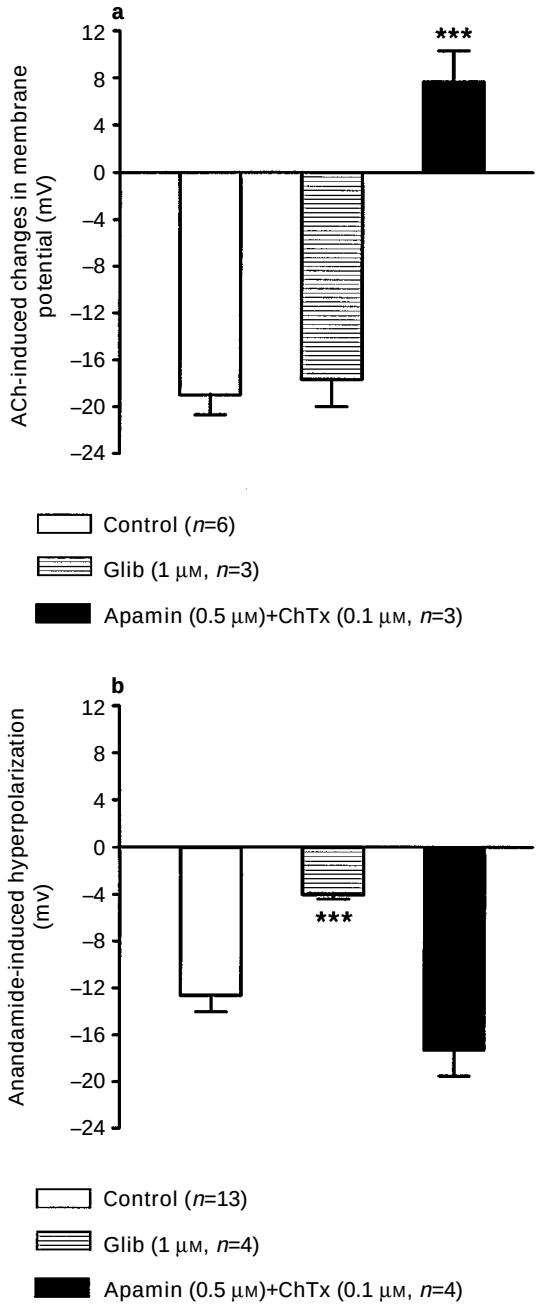

Figure 4 Effects of potassium channel blockers, glibenclamide (Glib), apamin and charybdotoxin (ChTx), on the hyperpolarizations elicited by (a) acetylcholine (ACh, 1 μM) and (b) anandamide (30 μM) in rat isolated mesenteric arteries with endothelium in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Data are shown as mean $\pm$ s.e.mean. For the sake of clarity, the control values have been pooled. Asterisks indicate a statistically significant difference from control values (***$P<0.001$).

(Figure 6). Anandamide (100 nM – 30 μM) did not modify tension in rings with or without endothelium (data not shown).

Discussion

This study demonstrated that endothelium-dependent hyperpolarizations in the guinea-pig carotid, rat mesenteric and porcine coronary arteries are not mediated by cannabinoid-like substance(s) confirming recent findings in the rat mesenteric artery (Plane *et al.*, 1997).

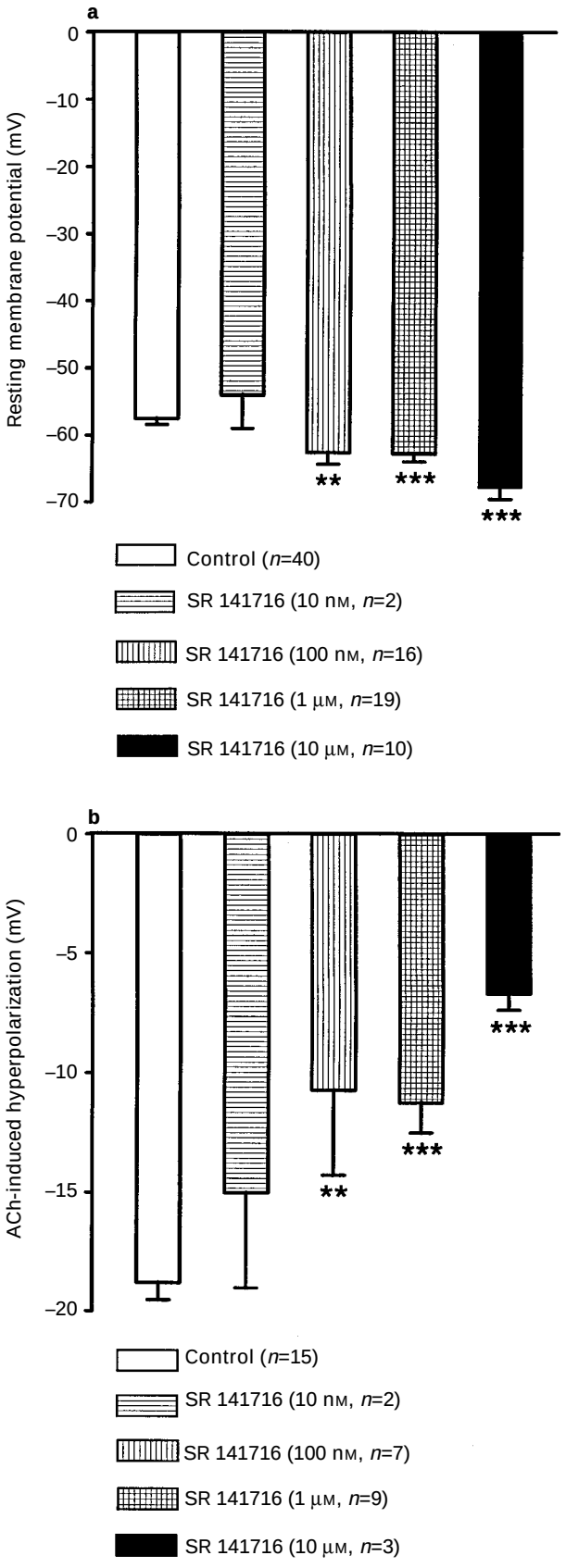

Figure 5 Effects of SR 141716 on the membrane potential (a) and the endothelium-dependent hyperpolarization elicited by acetylcholine (ACh, 1 μM, b) in guinea-pig isolated carotid artery in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Data are shown as mean$\pm$s.e.mean. For the sake of clarity, the control values have been pooled. Asterisks indicate a statistically significant difference from control values (**$P<0.01$ and ***$P<0.001$).

Table 1 Effect of SR 141716 on hyperpolarization to SIN-1 (10 μM) and cromakalim (10 μM) of the smooth muscle cells of the guinea-pig isolated carotid artery in the presence of L-NOARG (100 μM) and indomethacin (5 μM)

	Hyperpolarization (mV)	
	SIN-1	*Cromakalim*
Control	-11.0 ± 1.1 ($n=9$)	-22.5 ± 2.2 ($n=6$)
SR 141716 (0.1 μM)	-9.3 ± 2.2 ($n=3$)	-17.3 ± 3.6 ($n=6$)
SR 141716 (1 μM)	-12.3 ± 3.3 ($n=3$)	-18.5 ± 3.5 ($n=4$)
SR 141716 (10 μM)	-3.7 ± 1.9 ($n=3$)**	-13.5 ± 3.4 ($n=4$)*

Data are shown as means$\pm$s.e.mean. Asterisks indicate a statistically significant difference from control (*$P<0.05$ and **$P<0.01$).

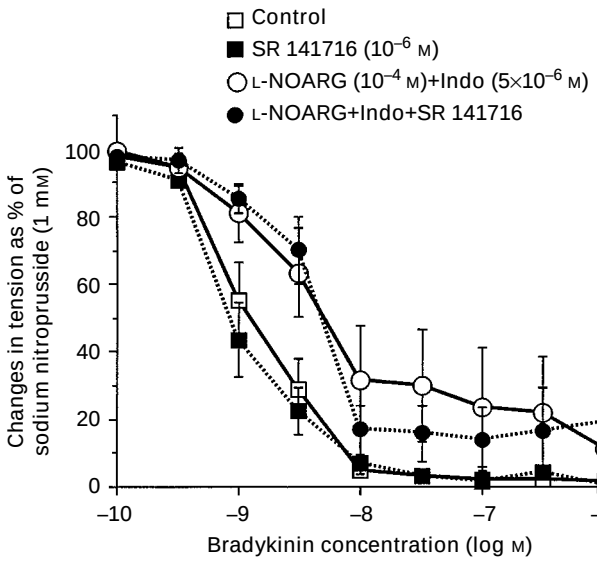

Figure 6 Effect of SR 141716 (1 μM) on the endothelium-dependent relaxations obtained in porcine isolated coronary arteries with endothelium untreated or treated with combination of L-nitro-arginine (L-NOARG, 100 μM) plus indomethacin (Indo, 5 μM). Data are shown as mean ($n=5$) and vertical lines indicate s.e.mean.

In the guinea-pig carotid artery, the endothelium-dependent hyperpolarization induced by acetylcholine was inhibited by SR 141716, in a concentration-dependent manner. This observation could suggest a specific inhibitory effect of SR 141716 against the response to acetylcholine, as the endothelium-independent hyperpolarizations produced by the activation of ATP-sensitive potassium channels in response to either SIN-1 (NO donor) or cromakalim (Corriu *et al*., 1996) were only inhibited at the highest concentration of the CB_1 cannabinoid antagonist tested. However, SR 141716 produced a direct concentration-dependent hyperpolarization. In the guinea-pig carotid artery, as in other tissues such as the rabbit mesenteric artery, the amplitude of the endothelium-dependent hyperpolarization is inversely related to the absolute value of the resting membrane potential of the vascular smooth muscle cells (Murphy & Brayden 1995; Chataigneau *et al*., 1998). The different sensitivity to SR 141716, of the hyperpolarizations produced either by the opening of ATP-dependent potassium channels or by the potassium channels involved in the EDHF-response, could be explained by the intrinsic properties of these channels, as the former are voltage-independent

(Edwards & Weston, 1993) and the latter supposedly are voltage-dependent (Petersson *et al*., 1997). In the rat mesenteric and the porcine coronary artery, SR 141716 up to 1 μM (an effective and specific concentration, Rinaldi-Carmona *et al*., 1994) did not affect either the resting membrane potential of the vascular smooth muscle cells or the endothelium-dependent hyperpolarizations. In the porcine coronary artery, the endothelium-dependent relaxation to bradykinin, resistant to inhibitors of nitric oxide synthase plus cyclo-oxygenase, is also not affected by the CB_1 receptor blocker. This then indicates that these relaxations (generally attributed to the release of EDHF, Vanhoutte & Félétou, 1996) and the endothelium-dependent hyperpolarizations do not involve the activation of CB_1 receptors.

Even at a high concentration, anandamide did not alter the membrane potential of the porcine coronary artery smooth muscle cells. However, anandamide elicited hyperpolarizations in the smooth muscle cells of the guinea-pig carotid and rat mesenteric arteries (modest amplitude in the former blood vessel). These hyperpolarizations were not inhibited by SR 141716 in either vessel. Furthermore, in the rat mesenteric artery, HU-210, a synthetic CB_1 receptor agonist, and palmitoylethanolamide, a preferential CB_2 receptor agonist, did not induce hyperpolarization. These results indicate that in our experimental conditions, the responses produced by anandamide are probably not dependent on CB_1 receptor activation. Interestingly, in the rat mesenteric artery, the hyperpolarization induced by anandamide was endothelium-dependent confirming data obtained in the rat hepatic artery (Zygmunt *et al*., 1997). This observation indicates that the cannabinoid agonist cannot be EDHF. As the experiments were performed in the presence of inhibitors of nitric oxide synthase and cyclo-oxygenase, the endothelium-dependent hyperpolarization produced by anandamide cannot be attributed to the release of nitric oxide or prostacyclin. The release of EDHF by anandamide could have reconciled the data obtained in the present study with those of Randall *et al*. (1996). However, the hyperpolarization produced by anandamide in the rat mesenteric artery cannot even be attributed to the release of EDHF. Indeed, the hyperpolarization produced by anandamide did not mimic the one produced by acetylcholine in terms of amplitude or time-course. Furthermore, the endothelium-dependent hyperpolarization obtained in response to acetylcholine was not altered by glibenclamide but was abolished by the combination of charybdotoxin plus apamin. These results are consistent with those from previous studies indicating that ATP-sensitive potassium channels are not involved in EDHF-mediated response in blood vessels such as the guinea-pig carotid (Corriu *et al*., 1996), rat mesenteric (Fukao *et al*., 1997; McCulloch *et al*., 1997) and guinea-pig basilar (Petersson *et al*., 1997) arteries. Instead, in these blood vessels, EDHF activates a class of potassium channel(s) sensitive to the combination of the two toxins charybdotoxin and apamin (Garland & Plane, 1996; Corriu *et al*., 1996; Zygmunt & Högestätt, 1996; Petersson *et al*., 1997). Conversely, the hyperpolarization induced by anandamide was inhibited by glibenclamide but was not significantly affected by the combination of charybdotoxin plus apamin. This rules out the hypothesis that anandamide and EDHF share a common mechanism of action. In the rat mesenteric and guinea-pig carotid arteries, the hyperpolarization to anandamide was not repeatable in the same preparation whereas in these tissues the hyperpolarization to acetylcholine could be repeated at least 5 times. Furthermore, in tissues which were unable to respond to anandamide, acetylcholine still evoked hyperpolarization, confirming that the endothelium-dependent hyperpolarization is not mediated by anandamide and that anandamide does not release EDHF.

Anandamide is principally metabolized by amidohydrolase (into arachidonic acid and ethanolamine, Desarnaud *et al*., 1995) and into potentially bioactive metabolites, either directly or after degradation into arachidonic acid by various enzymes including cyclo-oxygenase, lipoxygenase and cytochrome P_{450} mono-oxygenase (Bornheim *et al*., 1993; 1995; Hampson *et al*., 1995). In bovine coronary arteries, anandamide induces endothelium-dependent relaxation as a result of its conversion to vasodilator eicosanoids such as PGI_2 or the epoxyeicosatrienoic acids (Campbell *et al*., 1997). Arachidonic acid by itself, at concentrations up to 100 μM does not cause any significant changes in the membrane potential of vascular smooth muscle cells of the rat mesenteric artery (Fukao *et al*., 1997). In the present study, experiments were performed in the presence of indomethacin to prevent the production of prostacyclin from arachidonic acid produced by anandamide degradation. However, in rat mesenteric artery 11,12 epoxyeicosatrienoic acid, a metabolite of arachidonic acid through the cytochrome P_{450} mono-oxygenase pathway, induces endothelium-independent hyperpolarization of the smooth muscle cells by activation of a glibenclamide-sensitive potassium conductance (Fukao *et al*., 1997). Therefore, the results obtained in the present study in the rat mesenteric artery are consistent with the following hypothesis: anandamide is degraded, in an endothelium-dependent manner, into a bioactive metabolite, possibly an epoxyeicosatrienoic acid, which produces hyperpolarization of the vascular smooth muscle cells by activating ATP-sensitive potassium channels.

However, there are some apparent discrepancies between the results of the present study and the work published by Plane *et al*. (1997). These authors found that in the rat mesenteric artery, anandamide induced relaxation and repolarization (pD_2 values: 8.0) which were not affected by apamin but fully inhibited by charybotoxin, while in our study anandamide was much less potent and its effects were blocked by glibenclamide and unaffected by the combination of apamin plus charybdotoxin. The major difference between the two studies is that Plane *et al*. (1997) studied depolarization and relaxation in tissues under tension and subsequently depolarized and contracted by phenylephrine, whereas we studied the effect of anandamide in resting tissues. Hyperpolarizations in response to vasodilators such as exogenous nitric oxide and prostacyclin are highly dependent on the contractile status of the tissue and the amount of stretch imposed to the isolated artery (Garland & McPherson, 1992; Parkington *et al*., 1993). The same appears to be true for anandamide in the isolated mesenteric artery of the rat. Nevertheless, the present study and the work by Plane *et al*. (1997) showed that, in different experimental conditions, in the rat isolated mesenteric artery anandamide does not mimic the endothelium-dependent hyperpolarization to acetylcholine.

In conclusion, the present results make it highly unlikely that the endothelium-dependent hyperpolarizations observed in the different tissues studied are related to the activation of CB_1 cannabinoid receptors by anandamide.

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