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Short communication

Anandamide and endothelium-derived hyperpolarizing factor act via a common vasorelaxant mechanism in rat mesentery

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Abstract

We have recently proposed that an endocannabinoid, of which anandamide is prototypic, may be an endothelium-derived hyperpolarizing factor (EDHF). In the present study, both anandamide-induced and EDHF-mediated relaxations were insensitive to either charybdotoxin (100 nM) or apamin (500 nM) alone, but were inhibited by these agents in combination. These results point to EDHF and anandamide acting at a common site to cause vasorelaxation via K^+ channel activation, and support our proposal that an endocannabinoid is an EDHF. © 1998 Elsevier Science B.V.

Keywords: Endothelium; EDHF (endothelium-derived hyperpolarizing factor); Anandamide; Charybdotoxin; Apamin; K^+ channel; Cannabinoid

1. Introduction

We have recently proposed that an endocannabinoid may be an endothelium-derived hyperpolarizing factor (EDHF) (Randall et al., 1996). More recently the prototype endocannabinoid, anandamide (Di Marzo et al., 1994), has been shown to cause hyperpolarization in small mesenteric arteries (Plane et al., 1997), although the identity of anandamide as an EDHF was questioned. Anandamide has now been shown to be synthesised by endothelial cells (Deutsch et al., 1997), although in the renal vasculature it appears to cause vasorelaxation via nitric oxide. In our previous studies we have shown that both EDHF-mediated and anandamide-induced vasorelaxations are sensitive to a range of non-selective K^+ channel blockers (Randall et al., 1997; Randall and Kendall, 1997). We have now examined the effects of the more selective potassium channel inhibitors, charybdotoxin (which blocks large conductance Ca^{2+}-activated K^+ channels, BK_{Ca} and also voltage-sensitive K^+ channels), and apamin (which blocks small conductance Ca^{2+}-activated K^+ channels, SK_{Ca}) and their use in combination (Zygmunt et al., 1997) in order to define and compare the actions of anandamide and EDHF.

2. Methods

2.1. Isolated perfused mesentery

Male Wistar rats (250–350 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and, following a mid-line incision, the superior mesenteric artery was cannulated. The arterial vasculature was dissected away from the guts and placed in a jacketed organ bath as previously described (Randall et al., 1997) and perfused at 2 ml min^{-1} with oxygenated Krebs–Henseleit solution containing indomethacin (10 μM) and N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) to inhibit prostanoid and nitric oxide (NO) synthesis respectively. These inhibitors were included in order to define EDHF as the mediator of NO- and prostanoid-independent relaxations to the endothelium-dependent vasorelaxant, carbachol. All experiments were carried out in endothelium intact preparations.

2.2. Experimental protocol

Following a 30 min equilibration period, methoxamine (1–2 μM) was added to the buffer to increase perfusion

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pressure by 80–100 mmHg. Once stable tone had been established, the vasorelaxant effects of carbachol (acting via EDHF) and anandamide were assessed by bolus injection in volumes of 100 μl or less. In preparations receiving the toxins the agents were added to the buffer 15 min before the construction of the dose–response curves. When charybdotoxin and apamin were used in combination there was a reduction in established tone which was restored by addition of methoxamine (10 μM).

2.3. Data and statistical analysis

The dose–response curves were fitted to a logistic equation (Randall et al., 1997) and the ED_{50} and maximal relaxation (R_{max}) values obtained were compared by analysis of variance with Bonferroni's post hoc test.

2.4. Drugs

All drugs were supplied by Sigma (Poole, UK) and dissolved in saline, except anandamide which was synthesised from arachidonoyl chloride and ethanolamine and dissolved in an inert oil/water emulsion by Dr. E.A. Boyd, Department of Pharmaceutical Sciences, University of Nottingham; charybdotoxin and apamin (both from Latoxan, Rosans, France) were a generous gift from Servier, Paris.

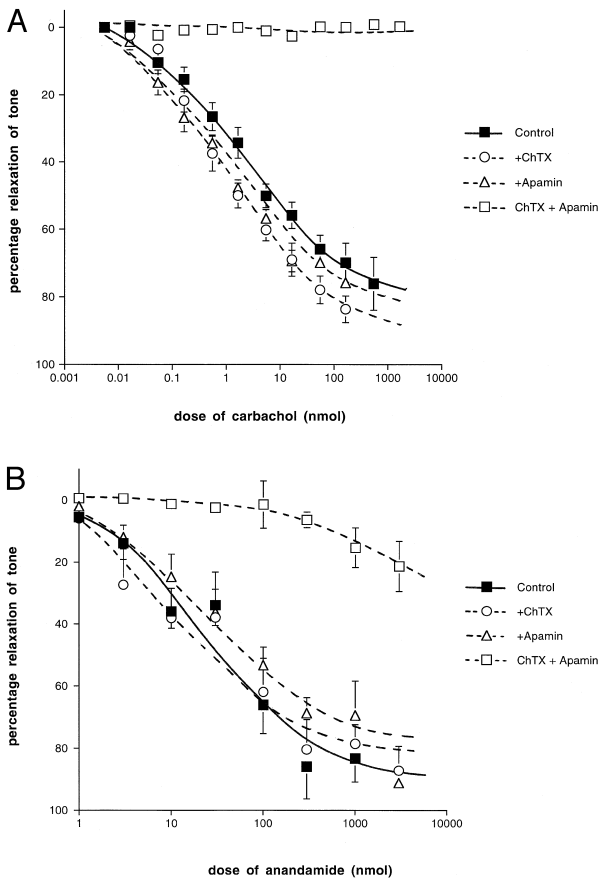

Fig. 1. The vasorelaxant effects of (a) carbachol and (b) anandamide against methoxamine-induced tone in the rat isolated mesentery in the presence of 100 nM charybdotoxin (ChTX) or 500 nM apamin alone and in the presence of the combination of charybdotoxin (100 nM) and apamin (500 nM). In each case, data are given as mean percentage relaxation of established tone with the bars indicating S.E.M. The lines were obtained from the curve fitting procedure.

3. Results

Carbachol (5.5 pmol–546 nmol) caused dose-related relaxations of induced tone, ($ED_{50} = 1.81 \pm 0.71$ nmol and $R_{max} = 74.3 \pm 5.6\%$, $n = 7$). In the presence of either 100 nM charybdotoxin ($n = 6$) or 500 nM apamin ($n = 5$) these responses were unaffected, with respective ED_{50} values of 999 ± 187 pmol and 792 ± 113 pmol and the respective R_{max} values were $81.9 \pm 2.6\%$ and $77.5 \pm 1.3\%$ (Fig. 1a). By contrast, in the presence of the combination of charybdotoxin (100 nM) and apamin (500 nM), the vasorelaxant responses to carbachol ($n = 5$) were abolished (Fig. 1a). In the presence of the toxin combination vasorelaxation to 100 μM papaverine was unaffected ($82.0 \pm 8.3\%$, $n = 4$).

Anandamide (1 nmol–3 μmol) caused dose-related relaxations of tone, with an $ED_{50} = 28.6 \pm 11.5$ nmol and $R_{max} = 88.6 \pm 7.2\%$ ($n = 9$). Neither of the toxins alone affected anandamide-induced vasorelaxation (charybdotoxin, $ED_{50} = 21.1 \pm 12.2$ nmol and $R_{max} = 86.1 \pm 7.4\%$, $n = 5$; apamin, $ED_{50} = 27.7 \pm 12.6$ nmol and $R_{max} 73.3 \pm 8.0\%$, $n = 5$) (Fig. 1b). However, when charybdotoxin and apamin were used in combination, vasorelaxation to anandamide was substantially inhibited (Fig. 1b), with the only significant relaxation occurring at 3 μmol ($21.3 \pm 8.1\%$ relaxation of tone) which was significantly ($P < 0.001$) less than the maximum control response.

4. Discussion

In the present investigation we have shown that EDHF and anandamide share a common site of action at a K^+ channel, adding further weight to our proposal that EDHF is an endogenous cannabinoid.

The major finding was that vasorelaxations to both EDHF and anandamide were abolished by the combination of charybdotoxin and apamin. The finding that this toxin combination abolished EDHF responses is consistent with the findings of Zygmunt et al. (1997) and White and Hiley (1997). Furthermore, neither agent alone opposed EDHF-mediated responses which is also consistent with the studies of Zygmunt et al. (1997) who proposed that only the combination of toxins is effective, due to either apamin causing an allosteric increase in charybdotoxin sensitivity or that the combination blocks the action of EDHF at a novel K^+ channel.

The finding that responses to anandamide were insensitive to charybdotoxin alone contrasts with the study of Plane et al. (1997), who reported that, in third–order small mesenteric arterial segments, charybdotoxin opposed responses to anandamide but not EDHF. Plane et al. also

found that EDHF-mediated, but not anandamide-induced, responses were apamin-sensitive. By contrast in the same preparation, White and Hiley (1997) found that charybdotoxin alone had no effect against responses to anandamide. The explanation for these conflicts within the literature is not clear. In the context of the present study, it should be noted that the mesentery is an intact vascular bed, where induced tone largely resides in the more proximal vessels (Griffith et al., 1988). Accordingly, depressor responses to endothelium-dependent agents largely reflect changes in these proximal vessels. Therefore, it is difficult to make simple comparisons between responses of intact vascular beds and isolated segments obtained from distal sites.

An alternative explanation to the present observations is that anandamide itself releases EDHF, which would account for their sensitivity to the toxins. However, this is unlikely to be the case in the mesentery as anandamide acts independently of the endothelium (Randall et al., 1996; White and Hiley, 1997).

In conclusion, we have shown that EDHF and anandamide have a common site of action at a K^+ channel target sensitive to the combination of charybdotoxin and apamin. Further, the profile of sensitivity to the different toxins points to important parallels in the pharmacology of EDHF and anandamide, which adds support to the proposal that an endocannabinoid may be an EDHF.

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