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Short communication

Endothelium and cannabinoid receptor involvement in levcromakalim vasorelaxation

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Abstract

Levcromakalim was more potent at relaxing rat small mesenteric arteries with endothelium (EC_{50}, 84 ± 10 nM) than denuded vessels (EC_{50}, 779 ± 101 nM). The cannabinoid receptor antagonist SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride; 1 μM) shifted the levcromakalim concentration/response curve 7.6-fold rightwards in intact vessels but had no effect in de-endothelialised vessels. Similar effects occurred with pinacidil. Combination of the K^+ channel blockers apamin (1 μM) and charybdotoxin (100 nM) shifted the levcromakalim concentration/response curve 3-fold rightwards only in intact vessels. It is concluded that levcromakalim and pinacidil relax mesenteric arteries partly by releasing a relaxing factor from endothelium, possibly an endogenous cannabinoid. © 1997 Elsevier Science B.V.

Keywords: Levcromakalim; Endothelium; Cannabinoid receptor

1. Introduction

Potassium channel activators, such as levcromakalim and pinacidil, relax smooth muscle by opening ATP-sensitive K^+ channels (K_{ATP}) which also occur on endothelial cells (Katnik and Adams, 1997), suggesting that K^+ channel activators could modulate the release of endothelial factors. One factor, endothelium-derived hyperpolarising factor (EDHF), activates K^+ channels other than K_{ATP} to cause vasorelaxation as its effects are not sensitive to glibenclamide (Garland and McPherson, 1992), a K_{ATP} blocker, but can be blocked by a combination of apamin and charybdotoxin (Zygmunt and Högestätt, 1996). Furthermore, Randall et al. (1996) proposed EDHF is an endogenous cannabinoid as its effects are blocked by the cannabinoid receptor antagonist SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride).

The rat small mesenteric artery is extensively used to investigate vasodilators, including EDHF (Garland and McPherson, 1992), and this study was intended to see if there was endothelium-dependence in the vasorelaxation to K^+ channel activators and if there was interaction with cannabinoid-mediated events.

2. Materials and methods

2.1. Rat mesenteric artery preparation

Male Wistar rats (250–350 g; Tucks, Rayleigh, Essex) were killed by a blow to the head and third generation mesenteric arteries (~ 320 μm diameter) were mounted in a myograph (model 500A, JP Trading, Aarhus) in Krebs–Henseleit solution at 37°C (White and Hiley, 1997) containing 10 μM indomethacin to block synthesis of prostanoids. When required, endothelium was removed by rubbing the intima of mounted vessels with a human hair.

Vessels were precontracted with 10 μM methoxamine and presence or absence of endothelium was confirmed by measuring relaxation of precontracted vessels by 10 μM carbachol (endothelium-intact, > 90% relaxation; endothelium-denuded < 5% relaxation). After washing, vessels were incubated, except when stated, for 30 min in Krebs–Henseleit solution containing 100 μM L-nitroarginine methyl ester to abolish nitric oxide production; L-nitroarginine methyl ester was then present for the rest of the experiment. Vessels were then precontracted with 1–5

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μM methoxamine (to produce the same level of tone as in the absence of L-nitroarginine methyl ester) and relaxed by cumulative addition of levcromakalim, pinacidil or *S*-nitroso-*N*-acetylpenicillamine.

When required, apamin (1 μM) and charybdotoxin (100 nM), or SR 141716A (1 μM) were added to the bath 30 min before construction of a concentration/relaxation curve.

Preliminary studies showed that three cumulative concentration/effect curves, separated by 60 min rest periods, could be obtained on a single artery without significant change in the parameters describing the levcromakalim effect (EC_{50}s: 46 ± 16, 47 ± 16, 37 ± 7 nM; maximum responses as reduction of methoxamine-induced tone: 89 $\pm$ 3%, 94 $\pm$ 2% 86 $\pm$ 4%; $n = 4$). Thus all experiments were paired by using each vessel as its own control.

2.2. Data analysis

Values are the mean $\pm$ s.e. of 8 determinations except where another value of n is stated. Concentration/effect curves were fitted to a logistic equation (McCulloch et al., 1997) in order to determine EC_{50} values. Statistical comparisons were by Student's paired *t*-test.

2.3. Drugs

Levcromakalim (SmithKline Beecham, Betchworth, Surrey) and pinacidil (RBI, Natick, MA) were dissolved in 70% (v/v) ethanol to give 10 mM solutions. SR 141716A (RBI, supplied under the NIMH Chemical Synthesis Program, Contract N01MH30003) was dissolved in ethanol to give a 10 mM solution. Carbachol, L-nitroarginine methyl ester, methoxamine, charybdotoxin (all from Sigma, Poole, Dorset), *S*-nitroso-*N*-acetylpenicillamine and apamin (both from Calbiochem/Novabiochem, Nottingham) were dissolved in distilled water. Indomethacin (Sigma) was dissolved in 5% (w/v) $NaHCO_3$ solution. Dilutions were made in distilled water. A maximum volume of 10 μl of a solution was added to the 10 ml myograph bath.

3. Results

Levcromakalim caused concentration-dependent relaxation of intact vessels (Fig. 1) with an EC_{50} of 84 ± 10 nM as compared to 779 ± 101 nM in endothelium-denuded vessels. The cannabinoid receptor antagonist SR 141716A (1 μM) shifted the levcromakalim log concentration/response curve 7.6-fold rightwards in the presence of endothelium (EC_{50} 648 ± 104 nM) but had no significant effect in its absence (EC_{50} 747 ± 34 nM; Fig. 1). SR 141716A (1 μM) did not change basal tension of rat small mesenteric arteries (data not shown) or significantly affect tone in pre-constricted arteries (2.8 ± 1.6% relaxation of methoxamine-induced tone).

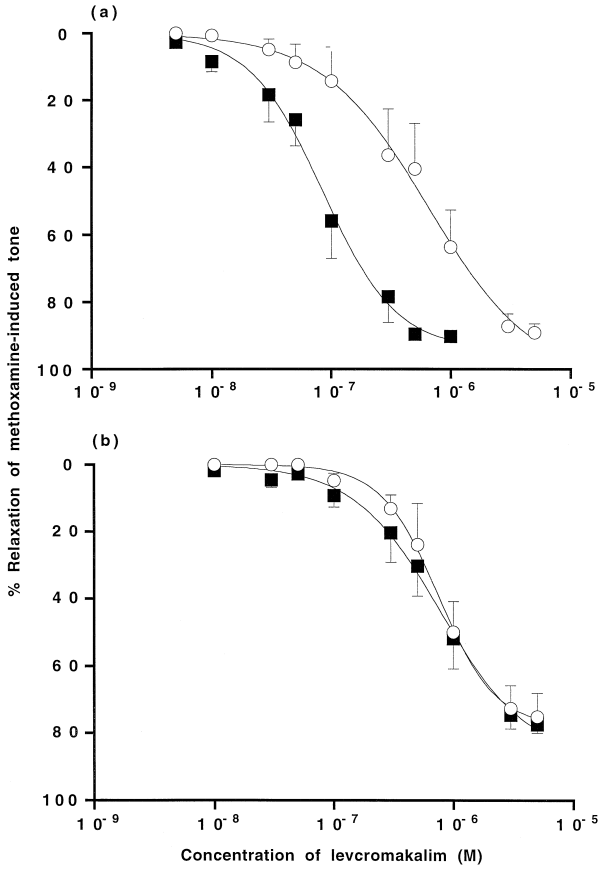

Fig. 1. Vasorelaxation to levcromakalim in endothelium-intact (a) and endothelium-denuded (b) rat small mesenteric artery in the absence (closed square) and presence (open circle) of SR 141716A (1 μM).

Pinacidil also relaxed precontracted vessels (EC_{50}: with endothelium, 0.84 ± 0.09 μM; without endothelium, 7.35 $\pm$ 1.28 μM). SR 141716A (1 μM) caused a 5.3-fold rightward parallel shift of the concentration/response curve in intact vessels (EC_{50}, 4.43 ± 0.05 μM), but again had no effect in denuded preparations (EC_{50}, 7.07 ± 1.01 μM). In the absence of L-nitroarginine methyl ester in vessels with endothelium, levcromakalim (EC_{50}, 248 ± 75 nM; $P < 0.05$) and pinacidil (EC_{50}, 2.41 ± 0.25 μM; $P < 0.001$) were significantly less potent than in its presence.

In the presence of endothelium, a combination of apamin (1 μM) with charybdotoxin (100 nM) significantly reduced the potency of levcromakalim from 0.42 ± 0.04 μM (control) to 2.27 ± 0.23 μM; a 5.3-fold shift in the concentration effect curve (Fig. 2). Fig. 2 shows that the toxin combination did not affect vasorelaxation to levcromakalim in the absence of endothelium (EC_{50}: control, 1.92 ± 0.23 μM; apamin + charybdotoxin, 2.13 ± 0.06 μM).

Removal of endothelium did not affect the maximal response to the nitric oxide donor, *S*-nitroso-*N*-acetylpenicillamine (91 $\pm$ 3% and 89 $\pm$ 2% relaxation of methoxamine-induced tone with and without endothelium, respectively), but slightly increased the potency (EC_{50}: with

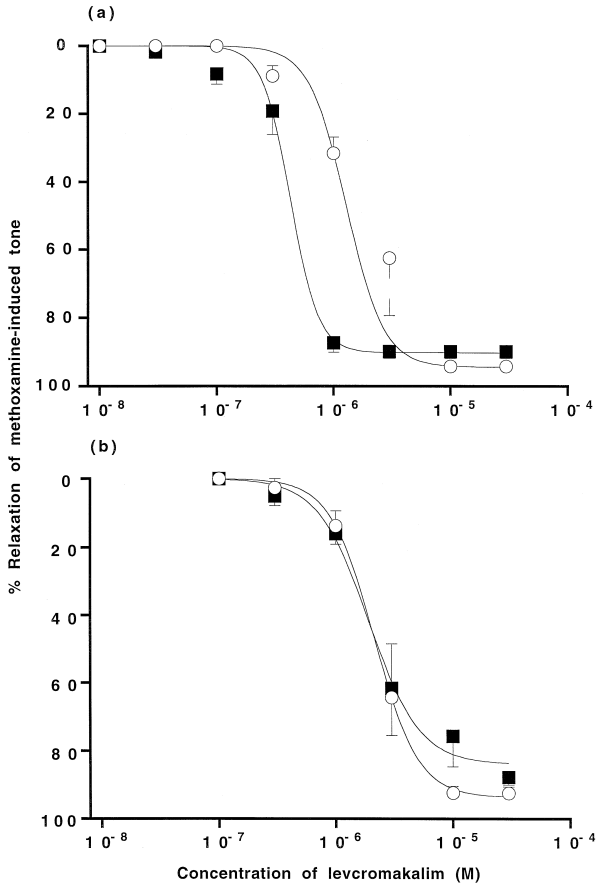

Fig. 2. Vasorelaxation to levcromakalim in endothelium-intact (a) and endothelium-denuded (b) rat small mesenteric artery in the absence (closed square) and presence (open circle) of a combination of apamin (1 μM) and charybdotoxin (100 nM).

endothelium, 160 ± 30 nM; without endothelium, 70 ± 6 nM; $n = 4$; $P < 0.01$). This shows endothelial destruction in itself does not reduce the ability of the vessel to relax.

4. Discussion

It is clear from these results that vasorelaxation to levcromakalim and pinacidil shows endothelium-dependence in the rat small mesenteric artery since both are more potent when the endothelium is intact. This suggests both that the K^+ channel activators act on vascular smooth muscle and that they interact with the endothelium to enhance the vasorelaxant response. One way this could happen is by releasing a relaxing factor which, in the presence of indomethacin and L-nitroarginine methyl ester, is not either a prostanoid or nitric oxide but could be EDHF.

Recently, Randall et al. (1996) showed that EDHF released in the mesenteric bed is sensitive to the cannabinoid receptor antagonist SR 141716A, and suggested that EDHF could be an endocannabinoid. In the present study, SR 141716A only affected relaxations to the K^+ channel activators in intact vessels. Therefore the antagonism was not due to non-receptor-mediated effects on vascular smooth muscle. The similar shifts produced in the concentration/response curves to the two K^+ channel activators suggests that SR 141716A is acting on the same receptor for each and are consistent with an affinity, estimated from the Schild equation, of 150–430 nM. This is similar to the affinity of SR 141716A reported by Showalter et al. (1996) for the cannabinoid CB_2 receptor (702 nM), rather than the cannabinoid CB_1 receptor (12 nM). Furthermore, SR 141716A (1 μM) alone had no effect on either basal or methoxamine-induced tone which suggests that it does not act through inhibition of basal release of a relaxant factor. The effects of SR 141716A support the involvement of a cannabinoid receptor in the endothelium-dependent effects of K^+ channel activators, but the lack of other commercially available cannabinoid receptor antagonists prevented further testing of this hypothesis.

Combination of apamin with charybdotoxin abolishes the vasorelaxant effect of EDHF in the rat small mesenteric artery (Chen and Cheung, 1997; White and Hiley, 1997). Here we found that this combination of potassium channel blockers also inhibited relaxations to levcromakalim in vessels with endothelium, but had no effect in de-endothelialised preparations. Thus the combination has no effect on the direct action of levcromakalim on the K_{ATP} of vascular smooth muscle; this is expected since K_{ATP} are not sensitive to these blockers. It is therefore likely that apamin and charybdotoxin together inhibit the actions of an endothelial factor, probably EDHF, released by levcromakalim. It is notable that, in the presence of apamin and charybdotoxin, levcromakalim has the same potency as in de-endothelialised preparations which would be expected if the endothelium-dependent component had been abolished. Other putative EDHF blockers such as clotrimazole inhibit levcromakalim vasorelaxation in rat hepatic artery with endothelium (Zygmunt et al., 1996), but these agents are less selective and may interact directly with potassium channels on the smooth muscle, whereas, here, SR 141716A selectively inhibited the endothelium-dependent component.

Thus K^+ channel activators, acting on endothelial K_{ATP}, may hyperpolarise the endothelium, and thus increase calcium influx and enhance release of endothelium-derived factors. Indeed, Groschner et al. (1994) have shown that activation of potassium channels contributes to bradykinin-induced increases in intracellular calcium in endothelial cells. In the present study, inhibition of nitric oxide production by L-nitroarginine methyl ester increased the potency of the K^+ channel activators, suggesting that endothelium-derived nitric oxide decreases their effects as has also been shown in the rat perfused mesenteric bed (McCulloch and Randall, 1996). The interaction of nitric oxide to reduce EDHF action (McCulloch et al., 1997) explains why this endothelial effect would only be seen when a single relaxing factor was being studied in isola-

tion, as was done here. It should be noted that McCulloch and Randall (1996) showed that removal of endothelium potentiated the actions of levcromakalim, unlike in the present study, and that there was no significant difference between relaxations to levcromakalim in the absence or presence of endothelium with L-nitroarginine methyl ester. This may reflect greater EDHF release in the isolated mesenteric artery and hence a larger EDHF-mediated component of relaxation to levcromakalim. Indeed, L-nitroarginine methyl ester does not affect the maximal relaxation to carbachol in the isolated artery (Garland and McPherson, 1992) but that in the perfused bed is significantly reduced (Adeagbo and Triggle, 1993).

In conclusion, these results suggest that vasorelaxation to the two K^+ channel activators investigated is partially mediated by the endothelium. Under the conditions used, this cannot be by either nitric oxide or a prostanoid but might involve release of an endogenous compound acting at cannabinoid receptors.

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References

Adeagbo, A.S.O., Triggle, C.R., 1993. Varying extracellular K^+: A functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. J. Cardiovasc. Pharmacol. 21, 423–429.

Chen, G.F., Cheung, D.W., 1997. Effect of K^+-channel blockers on ACh-induced hyperpolarization and relaxation in mesenteric arteries. Am. J. Physiol. 272, H2306–H2312.

Garland, C.J., McPherson, G.A., 1992. Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. Br. J. Pharmacol. 105, 429–435.

Groschner, K., Graier, W.F., Kukovetz, W.R., 1994. Histamine induces K^+, Ca^{2+}, and Cl^- currents in human vascular endothelial cells: Role of ionic currents in stimulation of nitric oxide biosynthesis. Circ. Res. 75, 304–314.

Katnik, C., Adams, D.J., 1997. Characterization of ATP-sensitive potassium channels in freshly dissociated rabbit aortic endothelial cells. Am. J. Physiol. 272, H2507–H2511.

McCulloch, A.I., Randall, M.D., 1996. Modulation of vasorelaxant responses to potassium channel openers by basal nitric oxide in the rat isolated superior mesenteric arterial bed. Br. J. Pharmacol. 117, 859–866.

McCulloch, A.I., Bottrill, F.E., Randall, M.D., Hiley, C.R., 1997. Characterization and modulation of EDHF-mediated relaxations in the rat isolated superior mesenteric arterial bed. Br. J. Pharmacol. 120, 1431–1438.

Randall, M.D., Alexander, S.P.H., Bennett, T., Boyd, E.A., Fry, J.R., Gardiner, S.M., Kemp, P.A., McCulloch, A.I., Kendall, D.A., 1996. An endogenous cannabinoid as an endothelium-derived vasorelaxant. Biochem. Biophys. Res. Commun. 229, 114–120.

Showalter, V.M., Compton, D.R., Martin, B.R., Abood, M.E., 1996. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB_2): Identification of cannabinoid receptor subtype selective ligands. J. Pharm. Exp. Ther. 278, 989–999.

White, R., Hiley, C.R., 1997. Comparison of the vasorelaxation caused by endothelium-derived hyperpolarising factor (EDHF) and anandamide in the rat small mesenteric artery of the rat. Br. J. Pharmacol. 122, 23P.

Zygmunt, P.M., Högestätt, E.D., 1996. Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery. Br. J. Pharmacol. 117, 1600–1606.

Zygmunt, P.M., Edwards, G., Weston, A.H., Davis, S.C., Högestätt, E.D., 1996. Effects of cytochrome P450 inhibitors on EDHF-mediated relaxation in the rat hepatic artery. Br. J. Pharmacol. 118, 1147–1152.