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Vasorelaxant activities of the putative endocannabinoid virodhamine in rat isolated small mesenteric artery

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Abstract

Virodhamine is a recently identified novel endocannabinoid. Cannabinoids may evoke vasorelaxation through novel receptors in the vasculature and/or through release of vasodilator peptides from sensory nerve endings. Virodhamine induced endothelium-dependent relaxation in the rat isolated small mesenteric artery mounted in a myograph and precontracted with methoxamine. Desensitization of vanilloid receptors by capsaicin did not affect relaxation responses to virodhamine. The CB_1 receptor antagonist SR 141716A (3 μM), but not the more CB_1-selective blocker AM 251 (1 μM), attenuated the response, while two CB_2 receptor antagonists, SR 144528 (1 μM) and AM 630 (10 μM), had no effect. The novel antagonist for the putative endothelial 'abnormal-cannabidiol receptor', O-1918 (30 μM), inhibited virodhamine relaxations. Hence virodhamine may activate this novel receptor, which might also recognize SR 141716A. Inhibition of nitric oxide synthase (L-NAME 300 μM) did not affect relaxation to virodhamine but the responses were markedly reduced when tone was induced with 60 mM KCl, suggesting a role for the activation of K^+ channels. The Ca^{2+}-activated K^+ channel (K_{Ca}) blockers, apamin (50 nM) and charybdotoxin (50 nM), inhibited virodhamine vasorelaxation. Combination of these blockers with SR 141716A (3 μM) caused no further inhibition. It was concluded that virodhamine relaxes the rat small mesenteric artery by endothelium-dependent activation of K_{Ca}, perhaps via the putative abnormal-cannabidiol receptor.

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Introduction

Since the proposition was made that anandamide, an ethanol amide of arachidonic acid (Devane et al 1992; Figure 1), is an endocannabinoid, an increasing number of compounds have been added to this new class of agents. These include the fatty acid amide docosatetraenoyl ethanolamide, the amine *N*-arachidonyl dopamine and the glycerol ester 2-arachidonyl glycerol (Barg et al 1995; Mechoulam et al 1995; Bisogno et al 2000). Recently, a novel endocannabinoid *O*-arachidonyl ethanolamine (virodhamine; Figure 1), which is arachidonic acid and ethanolamine joined by an ester linkage, has been identified (Porter et al 2002). It has been shown that virodhamine acts as a partial agonist at the CB_1 receptor and as a full agonist at the CB_2 receptor. Like anandamide and some other CB_1 receptor agonists (Ledent et al 1999), virodhamine causes hypothermia (Porter et al 2002). Nevertheless, other possible in-vivo or in-vitro biological effects of virodhamine have yet to be examined.

Besides their psychoactive effects and actions on body temperature, cannabinoids also modulate vascular tone (for reviews see Randall et al 2002; Hiley & Ford 2004). Indeed, the vasorelaxant actions of anandamide have been extensively studied, and the mechanisms by which it causes vasorelaxation appear to display species and regional differences (Randall et al 2002). Furthermore, there is emerging evidence that anandamide might employ multiple relaxant mechanisms in the same tissue. For instance, in the rat mesenteric artery, activation of the vanilloid TRPV1 receptor on perivascular sensory nerves explains much of the relaxation to anandamide (Zygmunt et al 1999; White et al 2001) but the endothelium and cannabinoid receptors might also play a role (White & Hiley 1997; Wagner et al 1999). Of particular interest is the proposal that anandamide acts as an agonist of a yet-to-be-identified receptor, which is distinct from the currently known CB_1 or CB_2 receptors, in the endothelium of the rat mesenteric artery (Wagner et al 1999;

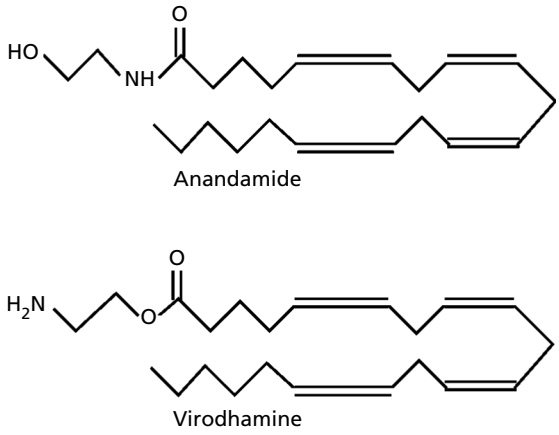

Figure 1 Structures of anandamide and virodhamine.

Járai et al 1999; Begg et al 2003). Since the putative receptor has been partially characterized by the use of abnormal-cannabidiol (abn-cbd), it has been referred to as the 'abn-cbd receptor'. Abn-cbd is an analogue of cannabidiol, a major, non-psychoactive, constituent of marijuana, and it has been found to evoke mesenteric relaxation as well as hypotension in CB_1 and CB_2 receptor-knockout mice (Járai et al 1999). It has been suggested that activation of the abn-cbd receptor, by abn-cbd or anandamide, leads to K^+ channel activation and vasorelaxation in the mesenteric arteries (Wagner et al 1999; Járai et al 1999; Offertáler et al 2003; Begg et al 2003).

In contrast to anandamide, relatively little is known about the effects of other endocannabinoids on vascular tone. Recently, the endocannabinoids 2-arachidonyl glycerol and *N*-arachidonyl dopamine have also been shown to induce vasorelaxation (Kagota et al 2001; O'Sullivan et al 2004). Therefore the potential relaxant effects of virodhamine warrant investigation and, in this study, the vasorelaxant effects of virodhamine have been investigated using rat isolated small mesenteric artery. The involvement of the endothelium and the possible activation of cannabinoid receptors, vanilloid receptors and K^+ channels in the relaxation response to virodhamine have been examined.

Materials and Methods

Myograph studies

Male Wistar rats (300–400 g; Charles River UK Ltd, Kent, UK) were killed with an overdose of sodium pentobarbitone (120 mg kg^{-1}, i.p.; Sagatal, Rhône Mérieux, Harlow, Essex, UK); all animal care and use was in accordance with the UK Animal (Scientific Procedures) Act 1986. The third-order branches of the superior mesenteric artery (internal diameter 364 ± 5 μm; 112 vessels) were removed and cleaned of adherent tissue. Segments (2 mm in length) were mounted in a Mulvany–Halpern type wire myograph (Danish Myo Technology, Aarhus, Denmark) and maintained at 37°C in gassed (95% O_2/5% CO_2) Krebs–Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, D-glucose 5.6; all experiments were carried out in the presence of indometacin (10 μM) as described previously (White & Hiley 1997). In all vessels, the integrity of the endothelium was assessed by precontracting the vessel with 10 μM methoxamine, followed by relaxation with carbachol (10 μM); vessels showing relaxations of greater than 90% were designated as endothelium-intact. When endothelium was not required, it was removed by rubbing the intima with a human hair; carbachol-induced relaxation of less than 10% indicated successful removal.

Experimental protocols

After the test for endothelial integrity, vessels were left for 30 min and then precontracted submaximally with 10 μM methoxamine. This was followed by construction of a cumulative concentration/relaxation curve to virodhamine; a vehicle control for virodhamine was also obtained by adding appropriate volumes of ethanol vehicle to precontracted vessels.

Since preliminary studies indicated considerable variation in the relaxant responses to virodhamine between vessels from different animals, further investigation into the relaxation mechanisms involved was performed using a single, submaximal concentration of virodhamine in a paired experimental design. Cannabinoid receptor antagonists (SR 141716A, AM 251, SR 144528 or AM 630) or other agents (capsaicin, L-NAME, apamin, charybdotoxin or O-1918) were added to the myograph bath 30 min before, and were kept present during, measurement of the responses to 10 μM virodhamine. The effects of virodhamine in the presence of these agents were compared with the control responses obtained in separate vessels from the same rat; consistent repeats of relaxations to the higher, just sub-maximal, concentrations of virodhamine could not be obtained in a given vessel but paired vessels did show consistent responses.

In all experiments where mesenteric arteries were incubated with putative inhibitors, the level of induced tone was normalized to that obtained in the test for endothelial integrity by lowering or increasing the concentration of methoxamine before adding the virodhamine. The mean tension generated by methoxamine in the test for endothelium was 16.6 ± 0.8 mN as compared with 15.9 ± 0.7 mN (49 vessels) when the inhibitors were present. In some cases, the effect of virodhamine was also determined in mesenteric arteries precontracted with high K^+ (60 mM) Krebs–Henseleit solution, which was prepared by equimolar substitution of NaCl for KCl in the Krebs–Henseleit buffer described above. The mean tension generated by 60 mM KCl (11.6 ± 1.3 mN) was similar to the tone induced by 10 μM methoxamine in the same vessel (12.2 ± 1.8 mN; $n = 4$).

Data and statistical analysis

All relaxation responses are expressed as percentage relaxation of the tone induced by 10 μM methoxamine or 60 mM KCl. Values are given as mean ± s.e.m. and

n represents the number of rats. As it was not always possible to fully define concentration/response curves (solubility limitations prevented use of high enough concentrations to determine the maximum responses), potency is expressed as $pEC_{50\%}$ (the negative logarithm of the concentration giving 50% relaxation of the induced tone); these values were determined directly from individual log concentration/response curves. Statistical analysis of concentration/response curves was performed by two-way analysis of variance (StatView 4.5 for Macintosh; Abacus Concepts, Inc., Berkeley, CA). Relaxation to a single concentration of virodhamine was compared by Student's *t*-test or one-way analysis of variance of the whole data set, followed by Bonferroni post-hoc tests. *P* values of less than 0.05 were taken as statistically significant.

Drugs

Methoxamine hydrochloride, carbachol, charybdotoxin, L-NAME (N^G-nitro-L-arginine methyl ester; Sigma Chemical Co., Gillingham, Dorset, UK) and apamin (Calbiochem, Nottingham, UK) were dissolved in deionized water. Indometacin (Sigma) was dissolved in 5% w v^{-1} $NaHCO_3$ solution. Virodhamine (*O*-arachidonyl ethanolamine; Tocris Cookson, Bristol, UK) was supplied in 100% ethanol. Capsaicin (Sigma), SR 141716A (N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide), SR 144528 (N-[(1*S*)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide; generous gifts from Sanofi-Synthélabo, Montpellier, France) and O-1918 ((–)-1,3-dimethoxy-2-(3-3,4-*trans*-*p*-menthadien-(1,8)-yl)-orcinol; a generous gift from Dr George Kunos, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD) were dissolved in 100% ethanol. AM 251 (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) and AM 630 (6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl) methanone; both from Tocris Cookson) were dissolved in 100% dimethyl sulfoxide (Sigma).

Results

Effect of endothelial removal on relaxation to virodhamine

Virodhamine induced concentration-dependent relaxation of endothelium-intact mesenteric arteries precontracted with methoxamine ($pEC_{50\%} = 6.4 \pm 0.3$, n = 4; Figure 2) and removal of the endothelium markedly reduced the response. The ethanol vehicle (up to 0.3% v v^{-1} final concentration in myograph bath) had little relaxant effect (Figure 2).

Effects of capsaicin on relaxation to virodhamine

In endothelium-intact vessels, capsaicin pretreatment (10 μM for 30 min), which causes functional desensitization of the vanilloid receptor system, had no effect on virodhamine-induced relaxation (relaxation to 10 μM virodhamine, control: 56 ± 7%; capsaicin pretreatment 66 ± 5%; n = 4 for both; Figure 3).

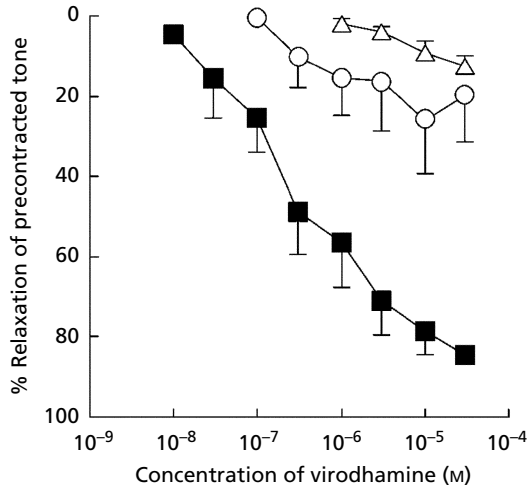

Figure 2 Concentration/response curves for relaxation by virodhamine of methoxamine-induced tone in the isolated small mesenteric artery of the rat. Relaxation to virodhamine was determined in the presence (■) and absence (○) of a functional endothelium; n = 6 for both. Also shown are the effects of the appropriate amounts of vehicle (△), the final concentration of ethanol at the greatest volume used was 0.3% v v^{-1} (n = 7). Values are shown as means and vertical lines represent s.e.m.

Effects of cannabinoid receptor antagonists on relaxation to virodhamine

Figure 4A shows that the cannabinoid CB_1 receptor antagonist SR 141716A (3 μM) significantly inhibited relaxation to 10 μM virodhamine in endothelium-intact vessels. However, another selective CB_1 receptor antagonist, AM 251 (1 μM) had no significant effect (Figure 4A). Neither of the CB_2 receptor antagonists, SR 144528 (1 μM) and AM 630 (10 μM), had any significant effect on virodhamine-induced relaxation (Figure 4B).

Effects of nitric oxide synthase inhibition and K^+ channel blockers on relaxation to virodhamine

Figure 5A shows that the presence of the nitric oxide synthase inhibitor L-NAME (300 μM) alone had no significant effect on relaxation to virodhamine in endothelium-intact vessels. In contrast, the combination of L-NAME (300 μM) and apamin (a blocker of small conductance K_{Ca}; 50 nM) with charybdotoxin (a blocker of intermediate and large conductance K_{Ca}; 50 nM) caused significant inhibition of virodhamine-induced relaxation (Figure 5A). The presence of apamin and charybdotoxin (50 nM for both) also reduced responses to virodhamine (Figure 5B) but addition of SR 141716A (3 μM) to vessels

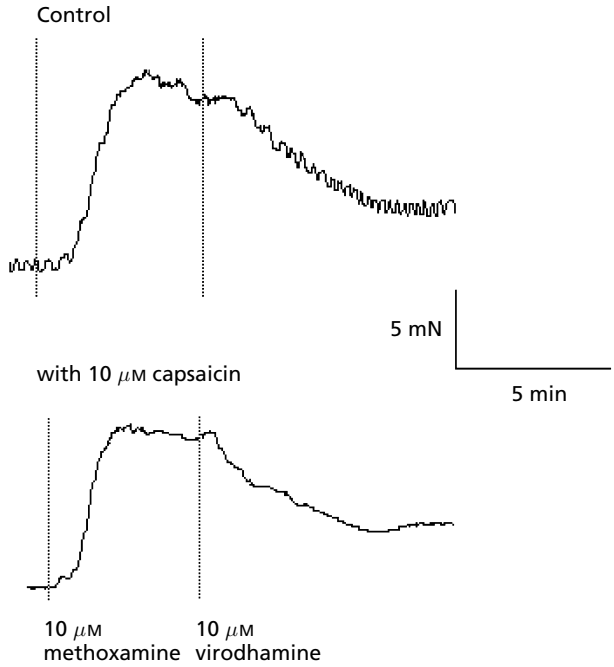

Figure 3 Original recordings showing the relaxation by virodhamine of tone induced by methoxamine in the endothelium-intact, isolated, small mesenteric artery of the rat. Relaxation to virodhamine was elicited in the absence (top panel) and presence (lower panel) of 10 μM capsaicin in separate vessels from the same rat. Vertical lines denote addition of drugs at the concentrations indicated.

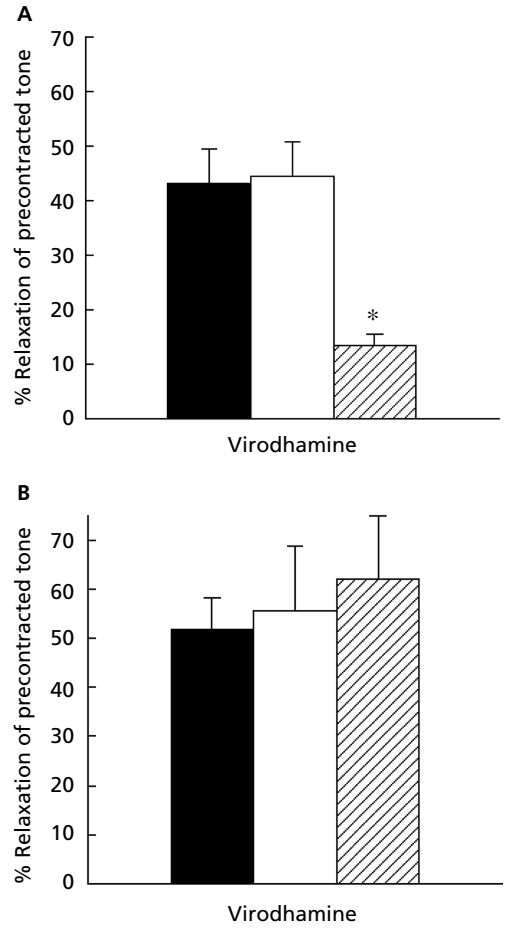

Figure 4 Relaxation by virodhamine of tone induced by methoxamine in the endothelium-intact, isolated, small mesenteric artery of the rat. (A) Relaxation was elicited by 10 μM virodhamine alone (solid column) and in the presence of either 1 μM AM 251 (open column) or 3 μM SR 141716A (hatched column); n = 4 for all. (B) Relaxation was elicited by 10 μM virodhamine alone (n = 10; solid column) and in the presence of either 1 μM SR 144528 (n = 7; open column) or 10 μM AM 630 (n = 4; hatched column). Values are shown as means and vertical lines represent s.e.m. *$P < 0.05$ significant difference from control.

treated with apamin and charybdotoxin caused no further inhibition (Figure 5B).

Relaxation to virodhamine in the presence of the endothelium was also markedly reduced when 60 mM KCl, instead of 10 μM methoxamine, was used to precontract vessels (methoxamine: $62 \pm 15\%$; KCl: $14 \pm 4\%$; n = 4 for both; $P < 0.05$).

Effect of O-1918 on relaxation to virodhamine

In endothelium-intact vessels, the presence of O-1918 (30 μM) significantly reduced the responses to virodhamine (relaxation to 10 μM virodhamine, control: $95 \pm 1\%$; O-1918: $34 \pm 18\%$; n = 4 for both; $P < 0.05$).

Discussion

Recently, Porter et al (2002) proposed that virodhamine is a member of the growing family of putative endocannabinoids in humans and rats. Using cell membranes expressing CB_1 or CB_2 receptors, they showed that virodhamine was a partial agonist at CB_1 receptors and a full agonist at CB_2 receptors. Moreover, it causes hypothermia (Porter et al 2002), which is often associated with CB_1 receptor activation (Ledent et al 1999). The present study shows that virodhamine relaxes the small mesenteric artery of the rat in a manner that is dependent on the presence of a functional endothelium and activation of K_{Ca}, whereas capsaicin-sensitive sensory nerves have no apparent role. Interestingly, virodhamine-induced relaxation appears to involve activation of the recently proposed abn-cbd receptor, which is distinct from the cannabinoid CB_1 or CB_2 receptors, in the endothelium of rat mesenteric artery.

The relaxation to virodhamine is sensitive to SR 141716A, a CB_1 receptor antagonist (Showalter et al 1996), suggesting the potential involvement of CB_1 receptors. However, a more selective CB_1 receptor antagonist, AM 251 (Lan et al 1999), did not affect relaxation to virodhamine. SR 141716A was used at a concentration (3 μM) below those at which it has been shown to cause non-specific effects in the rat mesenteric artery (White & Hiley 1998; Bukoski et al 2002). The observation that micromolar concentrations of SR 141716A were required

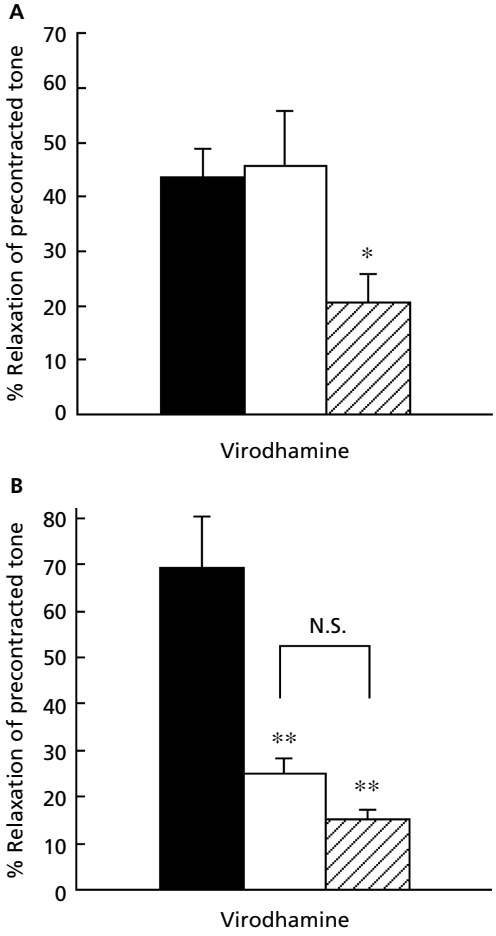

Figure 5 Relaxation by virodhamine of tone induced by methoxamine in the endothelium-intact, isolated, small mesenteric artery of the rat. (A) Relaxation was elicited by 10 μM virodhamine alone (n = 8; solid column) and in the presence of either 300 μM L-NAME (n = 4; open column) or a combination of 300 μM L-NAME, 50 nM apamin and 50 nM charybdotoxin (n = 7; hatched column). (B) Relaxation was elicited by 10 μM virodhamine alone (solid column) and in the presence of either 50 nM apamin plus 50 nM charybdotoxin (open column) or a combination of 3 μM SR 141716A, 50 nM apamin and 50 nM charybdotoxin (hatched column); n = 4 for all. Values are shown as means and vertical lines represent s.e.m. $*P < 0.05$, $**P < 0.01$ significant differences from control. N.S. denotes not significantly different from virodhamine response in the presence of apamin and charybdotoxin alone, as determined by one-way analysis of variance followed by the Bonferroni post-hoc test.

to inhibit responses to virodhamine might indicate the involvement of CB_2 receptors (SR 141716A, CB_1: $K_i = 12.3$ nM, CB_2: $K_i = 702$ nM; Showalter et al 1996). However, the CB_2 receptor antagonists SR 144528 and AM 630, used at concentrations 300-fold greater than their published affinities for CB_2 receptors (Rinaldi-Carmona et al 1998; Ross et al 1999), also had no significant effect on virodhamine relaxations. Consequently, the role of the currently known cannabinoid receptors in virodhamine-induced relaxation remains unclear. In this regard, it is interesting to note that some other cannabinoid receptor agonists, including anandamide and R-(+)-WIN 55,212-2, have recently been shown to cause mesenteric vasorelaxation by mechanisms apparently independent of CB_1 and CB_2 receptors (Ho & Hiley 2003b). Together these results seem to indicate that cannabinoid receptors, if they are present (Darker et al 1998), do not couple to vasorelaxation mechanisms in the rat mesenteric artery.

The presence of the nitric oxide synthase inhibitor L-NAME had no effect on virodhamine-induced relaxation, suggesting that virodhamine does not act by stimulating nitric oxide formation. In contrast, the K_{Ca} inhibitors apamin and charybdotoxin, alone or in combination with L-NAME, significantly attenuated the relaxation to virodhamine; this indicates that activation of K_{Ca} represents an important step in the responses. The observation that virodhamine evoked smaller relaxations when tone was induced by depolarizing K^+ solution (60 mM KCl; which abolishes the electrochemical gradient for K^+ efflux) instead of methoxamine also supports a role for K^+ channels. Nevertheless, it remains to be determined if the K_{Ca} activated are in the endothelium, the smooth muscle or both.

It is noteworthy that the addition of SR 141716A after apamin and charybdotoxin caused no additional effects on the virodhamine-induced relaxation relative to apamin with charybdotoxin alone. It is therefore tempting to propose that the effects of virodhamine to increase the activity of K_{Ca} are mediated through an SR 141716A-sensitive process. One possibility is that virodhamine activates a novel SR 141716A-sensitive receptor, which is positively coupled to K_{Ca}. Indeed, such a receptor, currently referred to as the abn-cbd receptor, has recently been proposed in the endothelium of mesenteric arteries of the rat, as well as mice (Járai et al 1999). Abn-cbd, a synthetic analogue of the plant-derived cannabinoid cannabidiol, has no activity at either CB_1 or CB_2 receptors and yet it causes SR 141716A-sensitive vasorelaxation or systemic depressor responses (Járai et al 1999; Offertáler et al 2003; Ho & Hiley 2003a). Evidence suggests that activation of a non-CB_1, non-CB_2 receptor contributes to the relaxant effect of abn-cbd in mesenteric arteries by increasing the activity of endothelial K_{Ca} (Offertáler et al 2003; Ho & Hiley 2003a; Begg et al 2003). Interestingly, the relaxant effects of virodhamine resemble those of abn-cbd; relaxation induced by both agents is endothelium-dependent, sensitive to SR 141716A and involves activation of K_{Ca} (Offertáler et al 2003; Ho & Hiley 2003a). Virodhamine may therefore act as an agonist of the novel abn-cbd receptor. In support of this, O-1918, another structural analogue of cannabidiol that acts as an antagonist of the abn-cbd site (Offertáler et al 2003; Begg et al 2003), inhibited the relaxation to virodhamine. The concentration of O-1918 used (30 μM) does not bind to cloned CB_1 or CB_2 receptors and has been shown to give an approximate 10-fold rightward shift of the concentration/relaxation curve for abn-cbd in the rat isolated small mesenteric artery (Offertáler et al 2003). Thus, virodhamine could be an endogenous agonist for the putative endothelial abn-cbd receptor.

Further support for this hypothesis might have been obtained by showing that the responses could be

antagonised by cannabidiol, which has been reported to be an antagonist at the putative new receptor in the rat perfused mesenteric bed (Járai et al 1999). However, we have found that cannabidiol is a vasorelaxant of the rat small mesenteric artery in its own right; the presence of 10 μM cannabidiol reduces the tone that can be generated by the addition of methoxamine such that it is insufficient to determine a relaxation/response curve to virodhamine (P. M. Hoi and C. R. Hiley, unpublished observations). This confirms the report of Offertáler et al (2003) that, in the isolated rat mesenteric artery, cannabidiol is a vasorelaxant with a pEC_{50} of 5.66. There is therefore a difference in the behaviour of cannabidiol between the entire mesenteric bed and isolated arteries from that bed which restricts its use as an antagonist at the abn-cbd receptor.

In the presence of inhibitors for nitric oxide synthase and cyclooxygenase (i.e. indometacin, which was present in all experiments in this study), sensitivity of endothelium-dependent relaxation to the K_{Ca} inhibitors apamin and charybdotoxin is often associated with the generation of endothelium-derived hyperpolarizing factors (EDHF; for review, see Busse et al 2002). It follows that virodhamine could act via the release of EDHF. Indeed, based on the same argument, abn-cbd has also been suggested to stimulate the generation of EDHF (Járai et al 1999; Ho & Hiley 2003a). Although the nature of EDHF remains elusive, it is believed that activation of K^+ channels by EDHF causes smooth muscle hyperpolarization, which then reduces Ca^{2+} influx through voltage-gated Ca^{2+} channels and hence vasorelaxation (Busse et al 2002).

Whilst anandamide has been suggested to activate the abn-cbd receptor (Wagner et al 1999; Járai et al 1999; Offertáler et al 2003), mesenteric vasorelaxation to anandamide is predominantly independent of the endothelium (Wagner et al 1999; Harris et al 2002; Ho & Hiley 2003a). In the rat small mesenteric artery, anandamide-induced relaxation is also unaffected by apamin and charybdotoxin (White & Hiley 1997), indicating that the endothelial abn-cbd receptor plays a minor role in the relaxation to anandamide. In contrast, activation of the vanilloid TRPV1 receptor on perivascular sensory nerves is a major mechanism by which anandamide causes mesenteric relaxation (Zygmunt et al 1999; White et al 2001). In this respect, it is important to note that relaxation responses to virodhamine were unaffected by functional desensitization of sensory nerves by the TRPV1 agonist capsaicin, suggesting that activation of these receptors was not involved. Gough et al (2003) have recently shown that virodhamine (up to 100 μM) has only a modest effect on TRPV1 expressed in cultured cells, and so these results strengthen the finding that replacement of the amide head group of anandamide with a carbonyl group, or deletion of the hydroxyl group (cf. Figure 1), decreases its interaction with these receptors (De Petrocellis et al 2000).

At present, no data on the metabolism of virodhamine are available. It is an ester of arachidonic acid and ethanolamine, the opposite of the amide linkage found in anandamide (cf. Figure 1). Consequently, its hydrolysis, perhaps by fatty acid amide hydrolase, which also has esterase activity (Goparaju et al 1998), would result in the same metabolites as anandamide. Given that virodhamine and anandamide produce mesenteric relaxation with different characteristics, it seems unlikely that vasoactive metabolites arising from the hydrolysis of these agents play an important role. Similarly, the possibility that virodhamine is converted to anandamide by spontaneous or enzymatic chemical rearrangement (Markey et al 2000) may also be excluded as a major mechanism for its action. Furthermore, as present experiments were performed in the presence of the cyclooxygenase inhibitor indometacin, prostaglandin synthesis or modification of virodhamine by cyclooxygenase are also unlikely to be involved.

Conclusions

The present study shows that the putative endocannabinoid virodhamine is a vasorelaxant in the rat isolated small mesenteric artery. It causes endothelium-dependent relaxation that involves activation of K_{Ca}, probably by acting on the endothelial abn-cbd receptor. Vanilloid receptor activation has no role in its vasorelaxant effects and it is unlikely that the effects of virodhamine are mediated by production of arachidonic acid derivatives after its metabolic breakdown.

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