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Vanilloid receptors on capsaicin-sensitive sensory nerves mediate relaxation to methanandamide in the rat isolated mesenteric arterial bed and small mesenteric arteries

*,[1]V. Ralevic, [1]D.A. Kendall, [1]M.D. Randall, *,[2]P.M. Zygmunt, [2]P. Movahed & [2]E.D. Högestätt

[1]School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [2]Department of Clinical Pharmacology, Institute of Laboratory Medicine, Lund University Hospital, SE-221 85 Lund, Sweden

1 In the present study, the vasodilator actions of methanandamide and capsaicin in the rat isolated mesenteric arterial bed and small mesenteric arterial segments were investigated.

2 Methanandamide elicited concentration-dependent relaxations of preconstricted mesenteric arterial beds ($pEC_{50} = 6.0 \pm 0.1$, $E_{max} = 87 \pm 3\%$) and arterial segments ($pEC_{50} = 6.4 \pm 0.1$, $E_{max} = 93 \pm 3\%$).

3 In arterial beds, *in vitro* capsaicin pre-treatment blocked vasorelaxation to 1 and 3 μM methanandamide, and reduced to $12 \pm 7\%$ vasorelaxation to 10 μM methanandamide. Methanandamide failed to relax arterial segments pre-treated *in vitro* with capsaicin.

4 In arterial beds from rats treated as neonates with capsaicin to cause destruction of primary afferent nerves, methanandamide at 1 and 3 μM did not evoke vasorelaxation, and relaxation at 10 μM methanandamide was reduced to $26 \pm 4\%$.

5 Ruthenium red (0.1 μM), an inhibitor of vanilloid responses, attenuated vasorelaxation to methanandamide in arterial beds ($pEC_{50} = 5.6 \pm 0.1$, $E_{max} = 89 \pm 1\%$). Ruthenium red at 1 μM abolished the response to 1 μM methanandamide, and greatly attenuated relaxation at 3 and 10 μM methanandamide in arterial beds. In arterial segments, ruthenium red (0.15 μM) blocked vasorelaxation to methanandamide, but not to CGRP.

6 In arterial segments, the vanilloid receptor antagonist capsazepine (1 μM) inhibited, and the calcitonin gene-related peptide (CGRP) receptor antagonist $CGRP_{8-37}$ (3 μM) abolished, methanandamide-induced relaxations. $CGRP_{8-37}$, but not capsazepine, attenuated significantly relaxation to exogenous CGRP.

7 These data show that capsaicin and ruthenium red attenuate vasorelaxation to methanandamide in the rat isolated mesenteric arterial bed and small mesenteric arterial segments. In addition, $CGRP_{8-37}$ and capsazepine antagonize responses to methanandamide in mesenteric arterial segments. In conclusion, vanilloid receptors on capsaicin-sensitive sensory nerves play an important role in the vasorelaxant action of methanandamide in the rat isolated mesenteric arterial bed and small mesenteric arterial segments.

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Abbreviations: CB, cannabinoid receptor; CGRP, calcitonin gene-related peptide; mAEA, methanandamide; VR, vanilloid receptor

Introduction

There is growing interest in the cardiovascular actions of endogenous cannabinoids (see Randall & Kendall, 1998a). Zygmunt and colleagues recently reported that anandamide and its stable analogue methanandamide at submicromolar concentrations elicit vasodilatation of isolated small arteries by activation of vanilloid receptors on sensory nerves and the release of calcitonin gene-related peptide (CGRP; Zygmunt *et al*., 1999). Furthermore, anandamide and methanandamide were shown to be agonists at the recombinant rat VR1 receptor (Zygmunt *et al*., 1999), findings recently confirmed by Smart *et al.* (2000) using the human VR1 clone. Other cannabinoid receptor ligands, such as HU 210 and WIN 55,212-2, could not mimic the action of anandamide in these bioassay systems (Zygmunt *et al*., 1999; Smart *et al*., 2000), and the CB_1 receptor antagonist SR141716A (0.3 μM) was unable to inhibit anandamide-induced vasorelaxation (Zygmunt *et al*., 1999), indicating no role of CB_1 and CB_2 receptors.

The vascular effects of anandamide in the rat isolated mesenteric arterial bed appear, however, to be more complex. Both CB_1 receptor-dependent and -independent vasodilatation have been proposed (Randall *et al*., 1996; Wagner *et al*., 1999). Clear differences exist between this preparation and isolated small mesenteric arteries. For example, in the perfused mesenteric arterial bed, the vasodilator response to anandamide is partly endothelium-dependent and abolished by the combination of charybdotoxin plus apamin (Randall & Kendall, 1998b; Wagner *et al*., 1999), whilst in isolated mesenteric arterial segments the anandamide-induced vasorelaxation is endothelium-independent and insensitive to this combination of toxins (White & Hiley, 1997).

This study investigated whether vasodilator effects of methanandamide in the isolated arterial bed and arterial segments of the rat mesentery are mediated *via* actions at vanilloid receptors on sensory nerves. This cannabinoid was

*Authors for correspondence;
E-mail: vera.ralevic@nottingham.ac.uk,
Peter.Zygmunt@klinfarm.lu.se

chosen for use as, unlike anandamide, it is relatively resistant to enzymatic breakdown. Whilst we have shown an involvement of VR1 in relaxation to anandamide in isolated small mesenteric arterial segments, Zygmunt *et al.* (1999) reported differences in responsiveness to vasoactive agents between isolated small mesenteric arterial segments and the isolated mesenteric arterial bed indicates that it is not always possible to extrapolate results between these preparations. Mesenteric arterial beds and small mesenteric arterial segments were treated *in vitro* with either capsaicin, in order to cause desensitization of vanilloid receptors and/or depletion of sensory neurotransmitter, or with the channel blocker and inhibitor of vanilloid responses, ruthenium red. In addition, rats were treated as neonates with capsaicin in order to cause destruction of sensory nerves. The selective vanilloid receptor antagonist capsazepine and the CGRP receptor antagonist $CGRP_{8-37}$ were used to investigate further the mechanism of methanandamide vasorelaxation in rat small mesenteric arterial segments.

Methods

Wistar rats of either sex (200–300 g) were used for isolation of the mesenteric arterial beds and small mesenteric arterial segments (200 μm outer diameter). Rats were killed by decapitation after exposure to CO_2. Mesenteric beds were isolated and perfused as described previously (Ralevic *et al.*, 1996). In brief, the abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was cut, blood flushed from the preparation with 0.5 ml of Krebs' solution and the gut dissected carefully away from the mesenteric vasculature. The preparation was mounted on a stainless steel grid (7×5 cm) in a humid chamber and perfused at a constant flow rate of 5 ml min^{-1} using a peristaltic pump (model 7554-30, Cole-Parmer Instrument Co., Chicago, IL, U.S.A.). The perfusate was Krebs' solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.4, $NaHCO_3$ 16, $MgSO_4$ 0.6, $CaCl_2$ 2.5 and glucose 7.8, gassed with 95% O_2, 5% CO_2 and maintained at 37°C. Preparations were allowed to equilibrate for 30 min before experimentation. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (model P23XL, Viggo-Spectramed, Oxnard, CA, U.S.A.) on a side arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass Instrument Co., Quincy, MA, U.S.A.). Small mesenteric arterial segments (1–2 mm long) were isolated, cut into ring segments and mounted in tissue baths under a passive load of 2 mN mm^{-1} (Zygmunt *et al.*, 1997). The tissue bath contained gassed (95% O_2, 5% CO_2) physiological salt solution (37°C; pH = 7.4) of the following composition (mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.5 and glucose 6.0.

Experimental protocol

After equilibration, methoxamine (10–50 μM) was added in order to raise the tone of the perfused mesenteric arterial beds (by 40–90 mmHg) above baseline. The effect of addition of cumulative concentrations of methanandamide (0.3–10 μM) were investigated, as administration of bolus doses of methanandamide evoked very weak vasorelaxations. Capsaicin (0.005–5 nmol) and CGRP (5–500 pmol) were applied as bolus doses. Isolated small mesenteric arterial segments were contracted with phenylephrine (1–3 μM). The concentration of phenylephrine was titrated for each vascular segment to give a contraction equivalent to 70–90% of the maximal response (Zygmunt *et al.*, 1997). When stable contractions were obtained, methanandamide (0.01–10 μM), capsaicin (1–1000 nM) or CGRP (0.1–100 nM) was added cumulatively to determine concentration-response relationships.

One group of mesenteric arterial beds/small mesenteric arterial segments were pretreated with capsaicin (10 μM) for 1 h, followed by 30 min washout, in order to cause vanilloid receptor desensitization and/or sensory neurotransmitter depletion (Zygmunt *et al.*, 1999). Capsaicin was added either to the perfusion solution (arterial beds; luminal exposure) or directly into the tissue bath (arterial segments; luminal and adventitial exposure). As this treatment was found to cause only partial inhibition of responses to methanandamide in the mesenteric arterial beds, another group of mesenteric arterial beds was immersed in gassed Krebs' solution containing 10 μM capsaicin, in addition to being flushed with the same solution, followed by 45 min washout. A group of rats were treated with capsaicin as neonates (50 mg kg^{-1}, s.c.) under ice anaesthesia in order to cause selective destruction of sensory nerves. Preparations were exposed to capsazepine, ruthenium red and $CGRP_{8-37}$ for at least 30 min before challenge with agonist. Since in the presence of capsazepine there was a fall in the tone of the mesenteric arterial bed, the effect of this drug was examined in small mesenteric arterial segments only.

Drugs

Methanandamide was from Tocris Cookson. Capsaicin (8-methyl-N-vanillyl-6-nonenamide), methoxamine hydrochloride, L-phenylephrine hydrochloride, human $CGRP_{8-37}$, human/rat α-CGRP (tested on mesenteric arterial segments/arterial bed) were from Sigma Chemical Co. Methanandamide was dissolved in ethanol. Capsaicin (from capsicum fruit) was dissolved in ethanol (arterial segments) or dimethyl sulphoxide (arterial beds). All other drugs were dissolved in distilled water.

Data analysis

Relaxations of the mesenteric arterial beds and small mesenteric arterial segments are expressed as percentage relaxation of the methoxamine- or phenylephrine-induced increase in tone, respectively. Data were compared using Student's test or analysis of variance, followed by Bonferroni Dunn's *post hoc* test (Statview 4.12). A value of $P < 0.05$ was taken to indicate a statistically significant difference. E_{max} denotes the maximal response achieved and pEC_{50} is the negative logarithm of EC_{50}. For mesenteric arterial beds, the negative logarithm of the concentration of methanandamide required to elicit 50% relaxation ($pEC_{50\%}$) was additionally calculated (as after capsaicin pre-treatment, methanandamide vasorelaxation did not reach a maximum).

Results

Effects of methanandamide and capsaicin on rat mesenteric arterial segments

Methanandamide (0.3–10 μM) elicited concentration-dependent relaxation of pre-constricted mesenteric arterial beds and small mesenteric arterial segments. In arterial beds, the pEC_{50} and E_{max} values were 6.0 ± 0.1 and $87 \pm 3\%$ ($n = 6$), respectively. The vehicle (ethanol) had no effect on tone of the mesenteric arterial beds at a concentration equivalent to that used for 10 μM methanandamide (data not shown). In

mesenteric arterial segments, the corresponding values for methanandamide were 6.4 ± 0.1 and $93 \pm 3\%$ ($n=11$), respectively. Capsaicin elicited graded relaxations of the mesenteric arterial bed ($pD_2 = 10 \pm 0.2$, $E_{max} = 99 \pm 1\%$; $n=8$) and small mesenteric arterial segments ($pEC_{50} = 8.3 \pm 0.1$; $E_{max} = 100 \pm 1\%$; $n=4$). Responses to capsaicin in the mesenteric arterial bed were unaffected by removal of the endothelium ($n=2$; data not shown).

Effect of in vitro *capsaicin pre-treatment on responses to methanandamide and capsaicin*

In mesenteric arterial beds, pre-treatment with capsaicin attenuated relaxations induced by methanandamide; after 1 h luminal perfusion with capsaicin (10 μM), the concentration-response curve was shifted 8 fold to the right. The negative logarithm of the concentration of methanandamide required to elicit 50% relaxation was 5.2 ± 0.2 after capsaicin pre-treatment and 5.9 ± 0.1 in controls ($n=4$; $P<0.01$; Figure 1a). After 2 h of capsaicin (10 μM) immersion and luminal perfusion, relaxations to methanandamide were virtually abolished at 1 and 3 μM, and significantly reduced from $87 \pm 3\%$ to $12 \pm 7\%$ at 10 μM ($n=4-6$; Figure 1a). After this treatment, preparations relaxed maximally to CGRP (50 pmol).

Relaxant responses of the mesenteric arterial bed to capsaicin were virtually abolished after both 1 h luminal perfusion ($n=4$) and 2 h luminal perfusion and superfusion

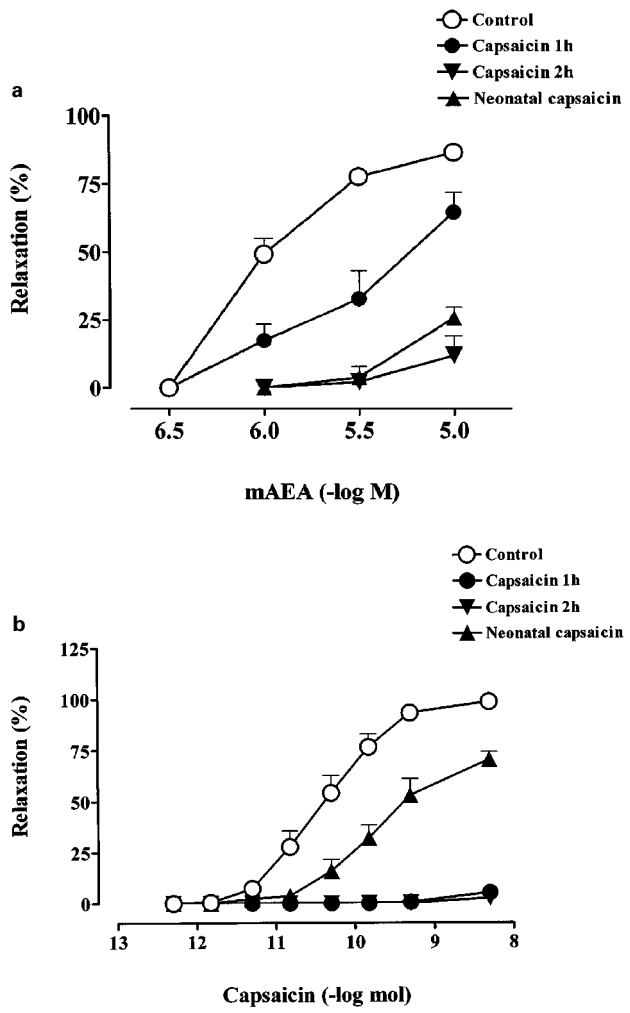

Figure 1 Effects of neonatal and *in vitro* capsaicin treatment on relaxant responses to methanandamide (mAEA) and capsaicin in rat isolated mesenteric arterial bed preconstricted with methoxamine. (a) Concentration-response curves to methanandamide under control conditions ($n=4$), after 10 μM capsaicin perfusion for 1 h ($n=4$), after 10 μM capsaicin perfusion and immersion for 2 h ($n=4$), and with neonatal capsaicin treatment ($n=4$). The concentration of methanandamide required to elicit 50% relaxation was significantly greater after 1 h capsaicin treatment, and responses were virtually abolished by 2 h capsaicin treatment and in mesenteric arterial beds taken from rats treated as neonates with capsaicin. (b) Dose-response curves to capsaicin under control conditions ($n=8$), after 1 h capsaicin pre-treatment ($n=4$), after 2 h capsaicin pre-treatment ($n=3$), and after neonatal capsaicin treatment ($n=4$). Data are shown as means and bars indicate s.e.mean.

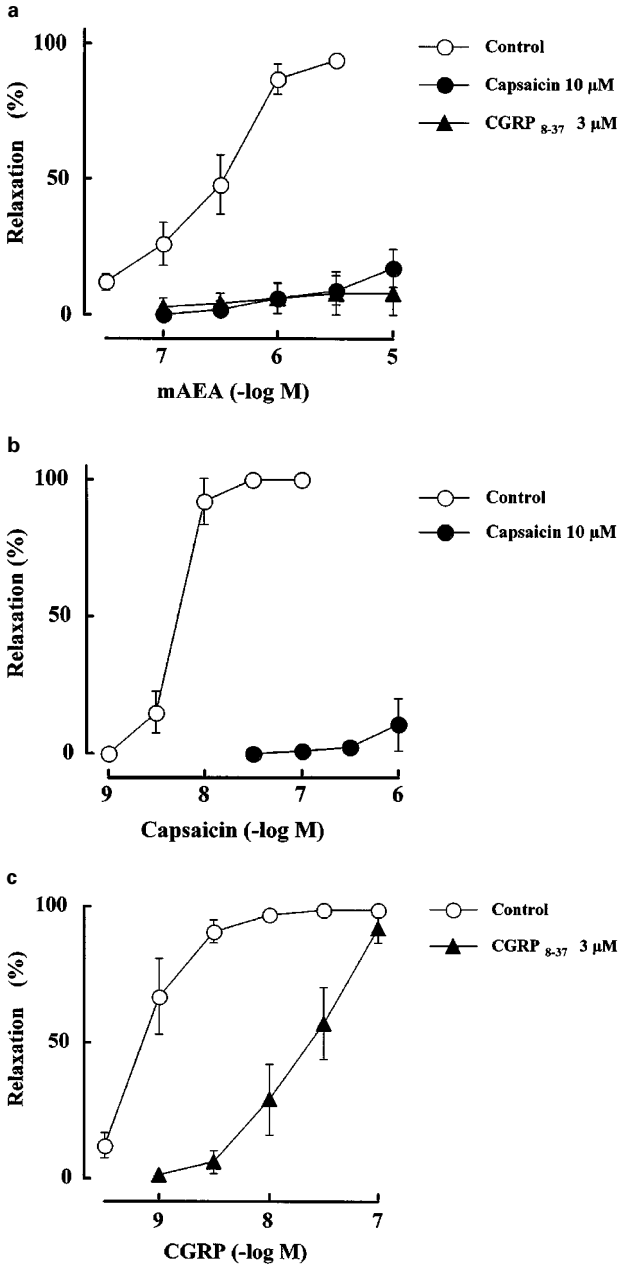

Figure 2 Effects of capsaicin treatment (10 μM, 1 h) or the calcitonin gene-related peptide (CGRP) receptor antagonist $CGRP_{8-37}$ on relaxant responses to methanandamide (mAEA), capsaicin and CGRP in rat isolated small mesenteric arterial segments pre-contracted with phenylephrine. (a) Effect of capsaicin pre-treatment and $CGRP_{8-37}$ on relaxant responses to mAEA. (b) Effect of capsaicin pre-treatment on relaxant responses to capsaicin. (c) Effect of $CGRP_{8-37}$ on relaxant responses to CGRP. Data are shown as means and bars indicate s.e.mean.

with 10 μM capsaicin ($n=3$; Figure 1b). Small relaxations were observed at the highest doses of capsaicin used (12 ± 7, 26 ± 1 and 32 ± 2% at 0.05, 0.5 and 5 μmol, respectively, $n=3$) at 2 h after capsaicin pre-treatment.

In small mesenteric arterial segments, capsaicin pre-treatment (10 μM for 1 h) virtually abolished both methanandamide-induced (Figure 2a) and capsaicin-induced (Figure 2b) relaxations. The selective CGRP receptor antagonist $CGRP_{8-37}$ (3 μM) also abolished methanandamide relaxations (Figure 2a). Capsaicin pre-treatment had no effect on relaxations induced by CGRP (data not shown; $n=6$), whereas $CGRP_{8-37}$ attenuated significantly these responses (Figure 2c).

Effect of neonatal capsaicin treatment on responses to methanandamide and capsaicin

In mesenteric arterial beds from rats treated as neonates with capsaicin (50 mg kg^{-1}), vasorelaxant responses to methanandamide were virtually abolished at 1 and 3 μM, and significantly reduced from 87 ± 3% to 26 ± 4% at 10 μM ($n=4-6$; Figure 1a). Relaxations to CGRP (5–500 pmol) were unchanged; responses at 5, 50 and 500 pmol CGRP were 7 ± 4, 42 ± 7 and 86 ± 5% in untreated mesenteric arterial beds ($n=6$) and 8 ± 4, 58 ± 8 and 90 ± 3% in mesenteric arterial beds from neonatally capsaicin-treated rats ($n=8$), respectively.

Capsaicin elicited small contractions followed by slow relaxation in rats treated with capsaicin as neonates (Figure 1b). A maximal response to capsaicin was not reached so a pD_2 value could not be calculated. The relaxations were not reproducible in the same preparation due to marked desensitization ($n=2$, data not shown).

Effect of ruthenium red on responses to methanandamide and capsaicin

Ruthenium red (0.1 μM), a channel blocker and inhibitor of vanilloid responses, attenuated significantly the relaxant responses to methanandamide in mesenteric arterial beds (Figure 3a); the pEC_{50} and E_{max} values were 5.6 ± 0.1 ($P<0.01$) and 89 ± 1% in the presence of ruthenium red, respectively ($n=6$). Ruthenium red (0.1 μM) also inhibited responses to capsaicin ($pD_2=10\pm0.1$, $P<0.01$; $E_{max}=98\pm1$%; $n=6$; Figure 3b). At 1 μM ruthenium red, responses to both methandamide and capsaicin were virtually abolished (Figure 3a,b). Likewise, ruthenium red (0.15 μM) almost abolished relaxations to methanandamide in small mesenteric arterial segments (Figure 4a). Ruthenium red had no effect on relaxations evoked by exogenous CGRP in mesenteric arterial

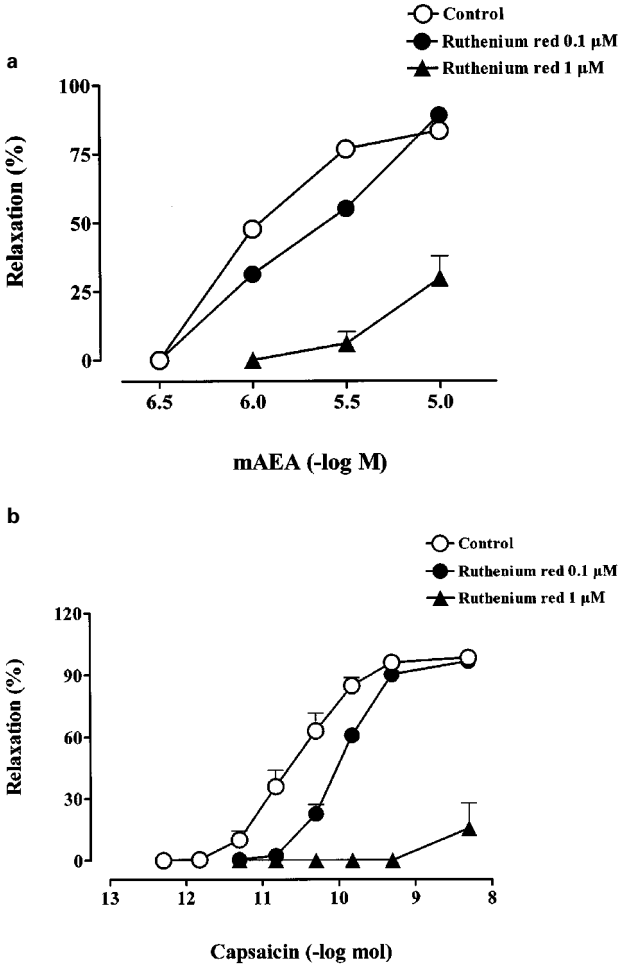

Figure 3 Effect of ruthenium red on relaxant responses to methanandamide (mAEA) and capsaicin in the rat isolated mesenteric arterial bed pre-constricted with methoxamine. (a) Concentration-response curves to mAEA under control conditions ($n=4$), and in the presence of ruthenium red at 0.1 μM ($n=6$) and 1 μM ($n=4$). (b) Dose-response curves to capsaicin under control conditions ($n=6$), and in the presence of ruthenium red at 0.1 μM ($n=6$) and 1 μM (o, $n=4$). Data are shown as means and bars indicate s.e.mean.

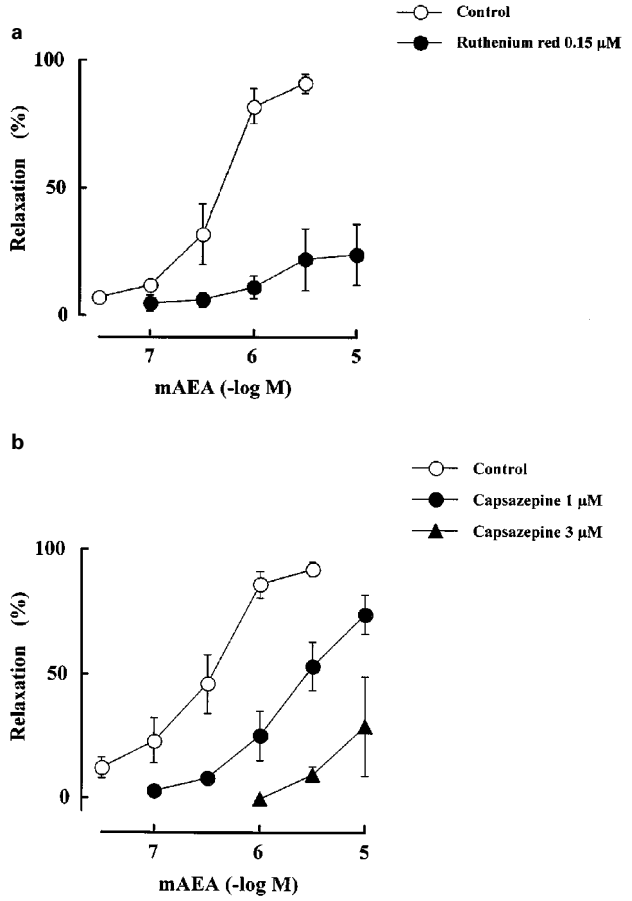

Figure 4 Effects of ruthenium red and the vanilloid receptor antagonist capsazepine on vasorelaxation induced by methanandamide (mAEA) in rat isolated small mesenteric arterial segments contracted with phenylephrine. (a) Responses to mAEA under control conditions and in the presence of 0.15 μM ruthenium red. (b) Responses to mAEA under control conditions and in the presence of 1 μM capsazepine ($n=9$) and 3 μM capsazepine ($n=4$). Data are shown as means and bars indicate s.e.mean.

beds and small mesenteric arterial segments ($n=4-6$; data not shown).

Effect of capsazepine on responses to methanandamide and CGRP

In small mesenteric arterial segments, the selective vanilloid VR1 receptor antagonist capsazepine (1 and 3 μM) significantly attenuated relaxations evoked by methanandamide (Figure 4b), whereas those induced by CGRP were unaffected ($n=6$; data not shown).

Discussion

The results of this study show clearly that in both the rat isolated mesenteric arterial bed and small mesenteric arterial segments the vasorelaxant response to methanandamide is mediated predominantly *via* activation of vanilloid receptors on sensory nerves.

The concentration-response curve to methanandamide was steep in both the mesenteric arterial bed and in small mesenteric arterial segments, but the sensitivity to methanandamide was significantly greater in the arterial segments. This difference in sensitivity may be related to the density of sensory nerves in different parts of the mesenteric vasculature or to differences in the route of administration of methanandamide, i.e. bath application of methanandamide with ready access to the adventitial surface (small mesenteric arterial segments) versus luminal application (mesenteric arterial bed). The fact that vascular responses were recorded under isobaric conditions in the perfused arterial bed, but under isometric conditions in arterial segments, might also contribute to the difference in sensitivity to methanandamide. Such factors are known to influence sensitivity to vasoconstrictor agents (Dunn *et al*., 1994).

Capsaicin pre-treatment for 1 h *in vitro* inhibited methanandamide-induced relaxations in both the mesenteric arterial bed and small mesenteric arterial segments, indicating an important role of sensory nerves in these responses. Quantitative differences in the inhibition between the mesenteric arterial beds and small mesenteric arterial segments were, however, observed. In small mesenteric arterial segments, this protocol of capsaicin pre-treatment virtually abolished methanandamide relaxations. In contrast, there was only a modest, 8 fold shift in the concentration-response curve to methanandamide in the mesenteric arterial beds. The greater effectiveness of capsaicin pre-treatment in blocking methanandamide responses in the mesenteric arterial segments may be related to a more complete neurotransmitter depletion achieved with tissue bath application of capsaicin, whilst intraluminal perfusion with capsaicin in the mesenteric arterial bed may lead to an incomplete vanilloid receptor desensitization and/or neurotransmitter depletion. Indeed, after a more severe pre-treatment of the mesenteric arterial beds (immersion as well as perfusion with 10 μM capsaicin for 2 h), relaxations to methanandamide were virtually abolished, identifying a clear relationship between sensory nerves and vasorelaxant responses to methanandamide.

In mesenteric arterial beds from rats treated as neonates with capsaicin, methanandamide relaxation was abolished at the lowest concentrations and profoundly inhibited at the highest concentration used (10 μM), indicating a major role of sensory nerves. However, methanandamide at 10 μM still caused 25% vasorelaxation in the mesenteric arterial bed. The dose of capsaicin that was administered to the neonates (50 mg kg^{-1}) has been shown to cause 79% reduction in immunoreactive CGRP content of the rat superior mesenteric artery (Wharton *et al*., 1986), raising the possibility that the sensory denervation was incomplete in the present study. We therefore investigated the vasodilator effects of capsaicin and electrical field stimulation in the presence of guanethidine to stimulate selectively sensory nerves in the mesenteric arterial bed (Kawasaki *et al*., 1988; Ralevic *et al*., 1996). Although the vasorelaxant response to electrical field stimulation was abolished in preparations from capsaicin-treated animals (data not shown), capsaicin elicited potent dose-dependent vasorelaxation. Moreover, the relaxant response to capsaicin showed pronounced desensitization upon repeated application, which indicates that this was not due to non-specific actions of capsaicin as these do not desensitize (Maggi & Meli, 1988). Thus, it appears that in this preparation an incomplete sensory denervation contributed to the residual relaxant response to methanandamide.

In the mesenteric arterial bed, neonatal capsaicin treatment was less effective than *in vitro* capsaicin pre-treatment (for 1 h) when capsaicin was used to elicit vasodilatation, whereas the reverse was seen when methanandamide was used as the agonist. The sensory nerves remaining after neonatal capsaicin treatment will probably exhibit normal (high) sensitivity to capsaicin (i.e. no desensitization). It is therefore not surprising that the full agonist capsaicin was able to cause a near maximal vasodilatation in rats treated with capsaicin as neonates, whereas the partial agonist methanandamide (Zygmunt *et al*., 1999; Smart *et al*., 2000) produced only a small response, at least over the concentration interval tested. Another complicating factor, which makes a direct comparison of results with capsaicin and methanandamide difficult, is the fact that capsaicin was administered as a bolus, whereas methanandamide was infused at different concentrations.

Additional possible mechanisms of anandamide/methanandamide-induced vasorelaxation include CB_1 receptor-dependent (SR141716A-sensitive) and -independent actions at the endothelium and vascular smooth muscle (Zygmunt *et al*., 1997; Pratt *et al*., 1998; Chaytor *et al*., 1999; Fimiani *et al*., 1999; Gebremedhin *et al*., 1999; Jarai *et al*., 1999; Mombouli *et al*., 1999; Wagner *et al*., 1999). The role of CB_1 receptors in anandamide-induced vascular responses is, however, somewhat unclear, since SR141716A at micromolar concentrations (3 μM and above) has a number of CB_1 receptor-independent cellular effects (White & Hiley, 1998: Mombouli *et al*., 1999), including inhibition of gap junctions (Chaytor *et al*., 1999) and capsaicin-induced vasorelaxation (Zygmunt *et al*., 1999). The findings of the present study indicate that at least the principal mechanism of methanandamide-induced relaxation in rat mesenteric arteries involves sensory nerves. In this respect, it is interesting that in blood vessels receiving little or no sensory innervation, vascular responses to anandamide are weak or absent (Chataigneau *et al*., 1998).

The channel blocker and inhibitor of vanilloid responses ruthenium red potently inhibited responses to methanandamide and capsaicin in both the mesenteric arterial bed and in the mesenteric arterial segments. Although ruthenium red can have actions other than inhibition of vanilloid responses, its effect in both of these mesenteric arterial preparations was selective for sensory nerves, as ruthenium red did not affect responses to exogenous CGRP. A quantitative difference between the effectiveness of ruthenium red inhibition in the mesenteric arterial bed and small mesenteric arterial segments was observed, with a 10 fold greater concentration required in the mesenteric arterial bed to achieve the same degree of inhibition of responses to methanandamide and capsaicin,

again highlighting differences between these bioassay systems. In small mesenteric arterial segments, inhibition of methanandamide responses by the competitive vanilloid receptor antagonist capsazepine further suggests that methanandamide-induced vasorelaxation is mediated by activation of vanilloid receptors on sensory nerves (Zygmunt *et al*., 1999). In addition, block of responses to methanandamide by the selective CGRP receptor antagonist $CGRP_{8-37}$ indicates an action involving release of CGRP.

In conclusion, this study shows that methanandamide induces vasorelaxation of the rat mesenteric arterial bed and small mesenteric arterial segments by activation of vanilloid receptors on sensory nerves and release of the vasodilator neuropeptide CGRP.

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