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Sex-linked differences in the vasorelaxant effects of anandamide in vascular mesenteric beds: role of oestrogens

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Abstract

Anandamide (0.01 to 10 μM) caused greater concentration-dependent reductions of the contractile-induced responses to noradrenaline in female than in male mesenteric vascular beds isolated from adult Sprague–Dawley rats. Greater relaxant responses in females were also induced by the vanilloid TRPV1 receptor agonist capsaicin (0.01 to 10 μM), whereas no sex differences were observed for the relaxations caused by either acetylcholine or sodium nitroprusside. The effect of anandamide in either sex was reduced by the vanilloid TRPV1 receptor antagonist capsazepine but not by the cannabinoid CB_1 receptor antagonist *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A). In males, the anandamide-induced relaxations were potentiated by in vitro exposure during 5 min to 0.5 μM 17β-oestradiol and unmodified by the protein synthesis inhibitor cycloheximide. The vasorelaxant effects of anandamide in female rats were decreased by ovariectomy. This decrease was prevented by in vivo treatment with 17β-oestradiol-3-benzoate (450 μg/kg i.m., once a week during 3 weeks) and counteracted by in vitro exposure to oestrogen. In vivo treatment with 17β-oestradiol also potentiated anandamide-induced responses in males. In conclusion, this study shows an oestrogen-dependent sensitivity to the vanilloid TRPV1 receptor-mediated vasorelaxant effects of anandamide in the mesenteric vasculature of Sprague–Dawley rats, that could be mediated by both genomic and non-genomic mechanisms.

Keywords: Anandamide; 17β-Oestradiol; Vasorelaxation; Sex difference; (Sprague–Dawley rat)

1. Introduction

Anandamide, arachidonoylethanolamide (Devane et al., 1992), is a member of a group of endogenous lipids with long-chain polyunsaturated fatty acids termed endocannabinoids. The endocannabinoid anandamide activates cannabinoid CB_1 and CB_2 receptors and mimics many of the central and peripheral actions of plant-derived and synthetic cannabinoids (Howlett, 2002). It has been described that anandamide also activates vanilloid TRPV1 receptors which causes relaxation of the vascular smooth muscle through the release of the vasodilator calcitonin gene-related peptide (CGRP) from perivascular sensory nerves (Zygmunt et al., 1999). Moreover, the activation of this receptor by anandamide reduces the transient contractions caused by noradrenaline in the isolated vascular mesenteric beds (Mendizábal et al., 2001; Orliac et al., 2003).

On the other hand, 17β-oestradiol activates the anandamide membrane transporter and inhibits the degradation of the endocannabinoid in human endothelial cells (Maccarrone et al., 2002). In addition, 17β-oestradiol increases the vascular sensitivity to CGRP (Gangula et al., 1999). These observations suggest that oestrogens could play a modulatory role in the vascular effects of anandamide. To support this hypothesis, it has been reported that 17β-oestradiol modulates the responsiveness to vasoactive substances such

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as nitric oxide (Caulin-Glaser et al., 1997; Tolbert and Oparil, 2001), endothelin (Miller et al., 1996; Tan et al., 2003), angiotensin II (Nickenig et al., 2000; Takeda-Matsubara et al., 2002), arachidonic acid metabolites (Fulton and Stallone, 2002) and noradrenaline (Bowyer et al., 2001). In addition, 17β-oestradiol can directly activate potassium channels and inhibit calcium influx in blood vessels (Tepareenan et al., 2003).

Cardiovascular modulatory effects of oestradiol are a matter of continuous interest due to its protective role in premenopausal women (Stampfer et al., 1991), e.g. the incidence of coronary artery disease is higher in men than in women, at least until menopause and this gender difference could be explained by the action of sex steroids (Pugeat et al., 1995). Therefore, the aim of the present study was to investigate whether sex-linked differences as well as oestrogen modulation could eventually exist for the role of anandamide in the regulation of vascular reactivity to a contractile agent such as noradrenaline in the rat vascular mesenteric bed.

2. Materials and methods

2.1. Animals

Male and female Sprague–Dawley rats were housed under a 12:12-h light–dark cycle, at controlled room temperature with food and water ad libitum. Experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of the National Research Council (USA, 1996).

Female rats were randomly chosen and vaginal smears were taken on the day of the experiment and examined microscopically to assess the stage of the oestrous cycle and to confirm that the females used were evenly distributed throughout the cycle according to McCulloch and Randall (1998).

2.2. Treatments

Adult female rats (8–10 weeks, 165–200 g) were either bilaterally ovariectomized or sham-operated through dorsal incision under anaesthesia with a mixture of 40 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride. Some ovariectomized rats received an i.m. injection of either 17β-oestradiol 3-benzoate (450 μg/kg) or vehicle (soja oil) once a week beginning on the day of surgery. In all the cases, experiments were performed 21 days after surgery. When stated, the same oestradiol treatment was performed in intact male rats.

2.3. Mesenteric vascular bed preparation

Adult male (250–350 g) and female (230–300 g) Sprague–Dawley rats were anaesthetized with urethane (1.2 g/kg body weight), the abdomen was opened and the mesenteric vascular bed was cannulated and removed according to McGregor (1965). The isolated mesenteric bed was transferred to a perpex chamber and perfused with Krebs solution at 37 °C bubbled with 95% O_2 plus 5% CO_2 (in mM: NaCl 118; KCl 4.7; $MgCl_2$ 1.2; NaH_2PO_4 1.0; $CaCl_2$ 2.6; $NaHCO_3$ 25.0; glucose 11.1; sodium ethylenediamine tetraacetic acid (Na_2 EDTA) 0.004; ascorbic acid 0.11; final pH 7.4), at the constant flow rate of 2 ml/min maintained by a peristaltic pump. Changes in vascular resistance were measured as changes in perfusion pressure and recorded through a Statham pressure transducer connected to a Grass polygraph.

After an equilibration period of 60 min, perfused mesenteric vascular beds were challenged with bolus injections of noradrenaline. According to previous evidence (Mendizábal et al., 1999) this form of administration of the agonist causes short-lasting, highly reproducible increases in perfusion pressure. Up to nine consecutive bolus administrations of noradrenaline, 15 to 20 min apart, were performed in each preparation. The dose of noradrenaline usually employed, 10 nmol, produced a sub-maximal pressor effect, i.e., 40 to 60 mmHg in either male or female rats.

Concentration–response curves to either anandamide (0.01–10 μM), capsaicin (0.001–10 μM), acetylcholine (0.1–1000 nM) or sodium nitroprusside (0.01–100 nM) were performed by evaluation of the reduction of noradrenaline-induced contractions. Drugs were present in the perfusion fluid at a given concentration, which was then rampered up to generate the cumulative concentration–response effects. The corresponding concentrations of drugs

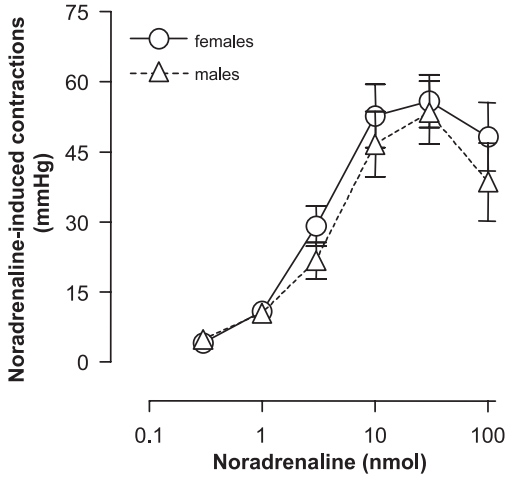

Fig. 1. Dose–response curves to noradrenaline were carried out in mesenteric vascular beds isolated from male rats (open triangles) and age-matched female rats (open circles). Data are presented as the mean ± S.E.M. (n = 8 to 9) of the changes in the perfusion pressure induced by increasing doses of noradrenaline.

were maintained from 15 min before bolus administration of noradrenaline and up to the end of the contractile response, usually attained 3 to 5 min after the addition of noradrenaline. Basal perfusion pressure was not changed by the concentrations of drugs assayed.

In some experiments, concentration–response curves to anandamide were performed in the presence of either the vanilloid TRPV1 receptor antagonist capsazepine (1 μM), the cannabinoid CB_1 receptor antagonist *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A; 1 μM), or the fatty acid amide hydrolase inhibitor phenylmethyl sulfonyl fluoride (PMSF; 200 μM). These drugs were added 20 to 45 min before anandamide and remained up to the end of the experiment. The basal perfusion pressure as well as the noradrenaline-induced pressure responses was not modified by either capsazepine, SR141617A or PMSF.

To determine the effects of oestrogens on the vaso-relaxation induced by anandamide and capsaicin, 0.5 μM 17β-oestradiol, which was the maximal concentration that did not cause per se any effect on the pressor responses to noradrenaline, was added to the perfusion medium 60 min before and up to the end of the concentration–response curves to either anandamide or capsaicin. In some experiments a unique concentration of anandamide (0.1 μM) was assayed; the endocannabinoid was added 15 min before a bolus injection of 10 nmol noradrenaline while 0.5 μM 17β-oestradiol was added either 5 or 60 min prior to the contractile response to noradrenaline. When indicated, the protein synthesis inhibitor cycloheximide (10 μM) was added to the perfusion medium 60 min before 17β-oestradiol. Neither the basal perfusion pressure nor the pressor responses to NA were modified by 10 μM cycloheximide.

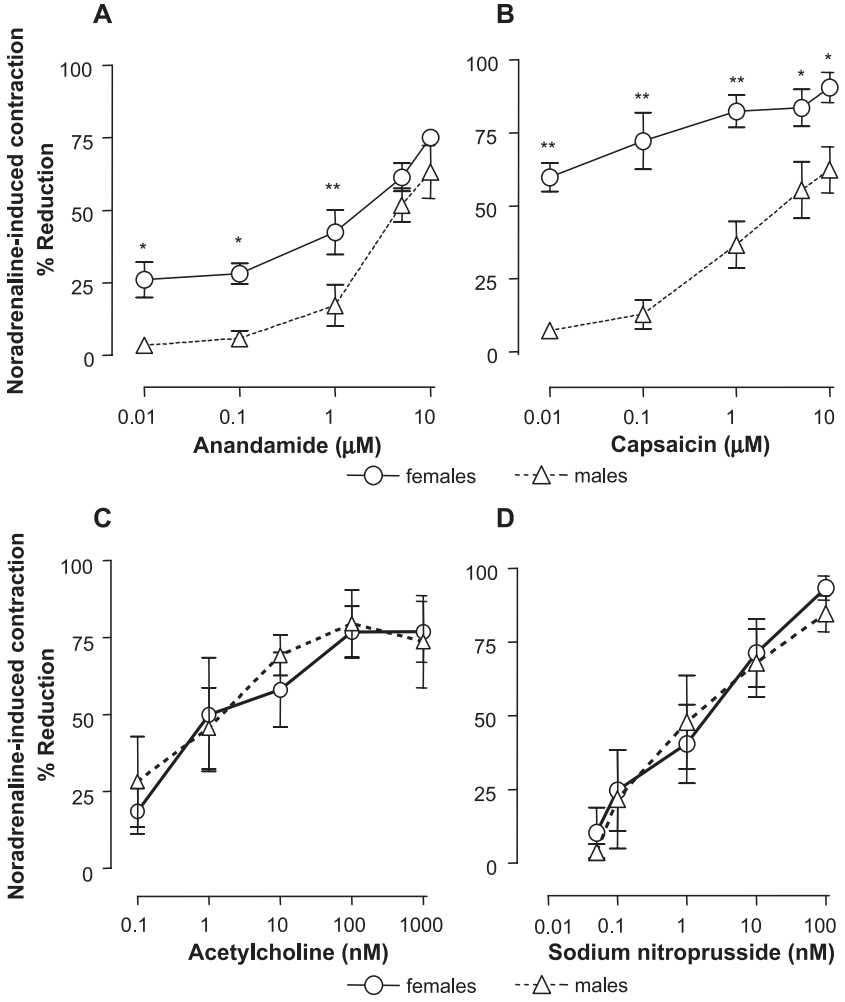

Fig. 2. Effects of anandamide (A), capsaicin (B), acetylcholine (C) and sodium nitroprusside (D) on consecutive noradrenaline-induced contractions in mesenteric vascular beds isolated from either male (open triangles) or female (open circles) rats. Each concentration of the drugs was perfused since 15 min before a bolus administration of 10 nmol noradrenaline up to the end of the contractile response. Data are presented as the mean ± S.E.M. ($n = 4$ to 6) of the percent reductions of the initial contraction to noradrenaline. *$P < 0.05$, **$P < 0.01$ when female were compared to male rats.

Concentration–response curves to anandamide (0.01–10 μM) were also performed in mesenteric vascular beds isolated from the following groups of animals (see Section 2.2): (a) ovariectomized; (b) sham-operated; (c) ovariectomized treated with 17β-oestradiol; (d) ovariectomized treated with 17β-oestradiol vehicle; (e) males treated with 17β-oestradiol; (f) males treated with 17β-oestradiol vehicle.

2.4. 17β-Oestradiol radioimmunoassay

Serum 17β-oestradiol was measured by radioimmunoassay as described by Koreman et al. (1974), after ether extraction and resuspension in buffer, using a specific antiserum kindly provided by Dr. G.D. Niswender (NIDDK). [2, 4, 6, 7-3H(*N*)]-Oestradiol (SA: 74 Ci/mmol) was purchased from NEN. Assay sensitivity was 2.2 pg/ml. Intra- and interassay coefficients of variation were 9.3% and 11.4%, respectively.

2.5. Drugs

(−)-Noradrenaline bitartrate, 17β-oestradiol 3-benzoate, 17β-oestradiol-water soluble, acetylcholine hydrochloride, cycloheximide, phenylmethylsulphonyl fluoride (PMSF), and sodium nitroprusside were obtained from Sigma-Aldrich (St. Louis, MO). Anandamide, capsazepine and capsaicin were purchased from Tocris Cookson, Ellisville, MO. *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141617A)

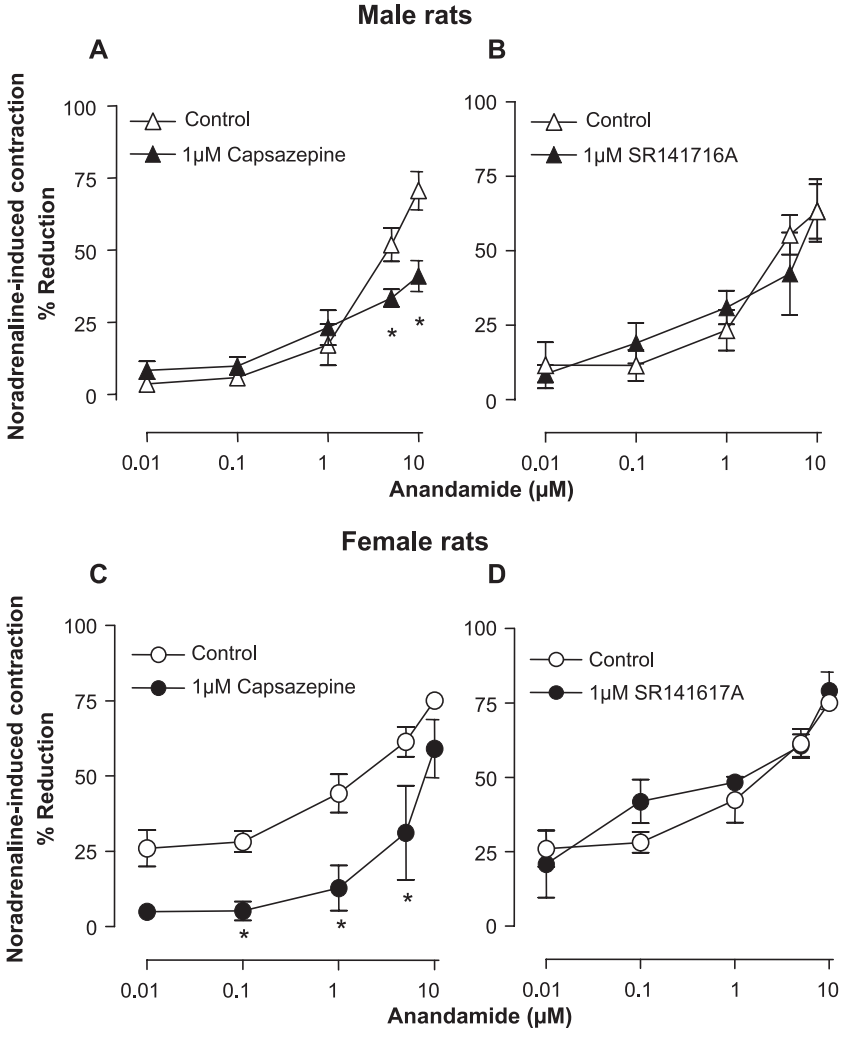

Fig. 3. Effects of the vanilloid TRPV1 receptor antagonist 1 μM capsazepine (filled symbols; A, C) and the cannabinoid CB_1 receptor antagonist 1 μM SR141716A (filled symbols; B, D) on the anandamide-induced reduction of the consecutive contractile responses to noradrenaline in mesenteric vascular beds isolated from either male (upper panels) or female rats (lower panels). Each concentration of anandamide was perfused since 15 min before a bolus administration of 10 nmol noradrenaline up to the end of the contractile response. Controls are depicted in either open triangles (males) or open circles (females). Data are presented as the mean ± S.E.M. ($n = 5$ to 6) of the percent reductions of the initial contraction to noradrenaline. *$P < 0.05$ when compared to the controls.

was a gift from Sanofi Recherche (Montpellier, France). Anandamide, capsaicin and PMSF were dissolved in ethanol. Capsazepine and SR141716A were dissolved in dimethyl sulphoxide. The remaining drugs were dissolved in distilled water. Further dilutions were made in Krebs solution. The maximal concentrations of ethanol (0.1%) and dimethyl sulphoxide (0.1%) employed were devoid of effects on either the basal perfusion pressure or the noradrenaline-induced contractions.

2.6. Statistical analysis

Data were presented as the mean ± S.E.M. Statistical comparisons were made by either two-way analysis of variance followed by Bonferroni's post hoc *t*-test or one-way analysis of variance followed by Newman–Keuls multiple comparison test or Student's *t*-test for paired data. A *P*-value smaller than 0.05 was considered as significant.

3. Results

As shown in Fig. 1, bolus administration of noradrenaline induced dose-dependent increases in the perfusion pressure of mesenteric vascular beds isolated from adult rats. No differences were observed in the responsiveness to noradrenaline between male and age-matched female rats. The vascular contractile responses elicited by 10 nmol noradrenaline were reduced in a concentration-dependent manner by the endocannabinoid anandamide (Fig. 2A) as well as by the vanilloid TRPV1 receptor agonist capsaicin (Fig. 2B). The relaxations induced by anandamide and capsaicin were significantly greater in female than in male rats. On the contrary, no sex differences were observed for the reductions of the noradrenaline-induced contractile responses caused by other vasoactive substances such as the acetylcholine receptor agonist (Fig. 2C) and the nitric oxide donor sodium nitroprusside (Fig. 2D).

In order to study whether the reductions caused by anandamide on the contractile responses to noradrenaline in male and female rats were related to the activation of specific receptors, the effects of a cannabinoid CB_1—as well as of a vanilloid TRPV1—receptor antagonist were tested. As shown in Fig. 3A–D, the vanilloid TRPV1 receptor antagonist 1 μM capsazepine reduced the effects of anandamide on noradrenaline-induced contractions in both male and female rats, whereas the cannabinoid CB_1 receptor antagonist 1 μM SR141716A did not modify the effects of anandamide in either sex.

To study whether the greater effect of anandamide in female mesenteric beds was related to eventual differences in the anandamide metabolism, concentration–response curves to anandamide were performed in the presence of the fatty acid amide hydrolase inhibitor PMSF. As shown in Fig. 4A and B, the concentration of PMSF employed, 200 μM, did not modify the effects of anandamide in vascular mesenteric beds isolated from either male or female rats.

To determine whether oestrogenic hormones were involved in the greater vasorelaxant effect caused by anandamide in female rats, vascular mesenteric beds isolated from both males and females were exposed to a concentration of 17β-oestradiol (0.5 μM) that did not cause per se any effect on the contractile responses to noradrenaline. A 60-min

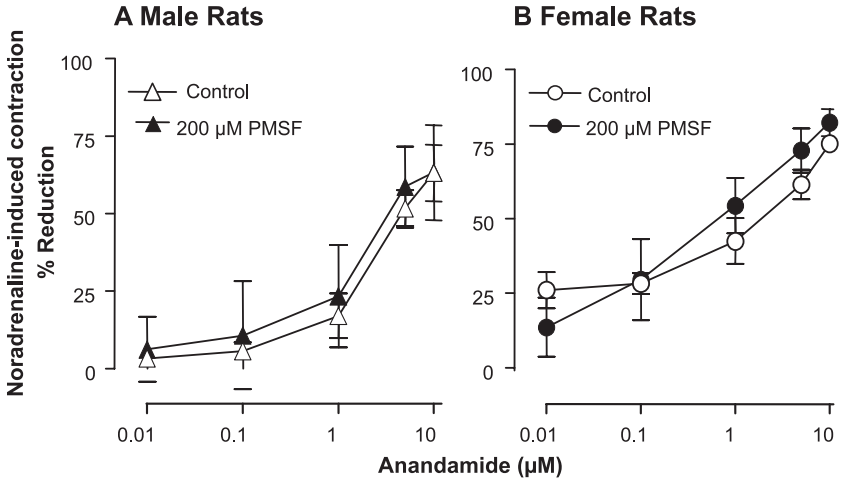

Fig. 4. Effect of the fatty acid amide hydrolase inhibitor PMSF on the anandamide-induced reduction of the consecutive contractile responses to noradrenaline in mesenteric vascular beds isolated from male (triangles, A) and female rats (circles, B). Each concentration of anandamide was perfused since 15 min before a bolus administration of 10 nmol noradrenaline up to the end of the contractile response, either under control conditions (open symbols) or in the presence of 200 μM PMSF (filled symbols). Data are presented as the mean ± S.E.M. ($n = 4$ to 6) of the percent reductions of the initial contraction to noradrenaline.

exposure to 0.5 μM 17β-oestradiol significantly potentiated the reduction caused by either anandamide (Fig. 5A) or capsaicin (Fig. 5B) on the contractile responses elicited by noradrenaline in mesenteric vascular beds from male rats, whereas it did not modify the effect of anandamide in vascular tissues isolated from female rats (Fig. 5C). As shown in Fig 5A and B, the extent of the response to either anandamide or capsaicin in male mesenteric vascular beds exposed to 17β-oestradiol was similar to that observed in preparations from intact females (Fig. 2A,B). The effect of 17β-oestradiol in mesenteric vascular beds from male rats was not modified by the protein synthesis inhibitor 10 μM cycloheximide (Fig. 5A). Moreover, the potentiation caused by 60-min incubation with 0.5 μM 17β-oestradiol on anandamide responses in male rats was also observed when the time of exposure to the oestrogen was reduced to 5 min (Fig. 6).

To further analyze the role of oestrogens in the vasorelaxant effects of anandamide, experiments were performed in vascular tissues isolated from either ovariectomized or sham-operated female rats. As shown in Fig. 7A, the decrease caused by anandamide in the noradrenaline-induced contractions was significantly lower in vascular mesenteric beds isolated from ovariectomized than from sham-operated rats. The effects of anandamide in vascular mesenteric beds isolated from ovariectomized rats were potentiated by either in vitro exposure to 0.5 μM 17β-oestradiol during 60 min (Fig. 7B) or after in vivo chronic

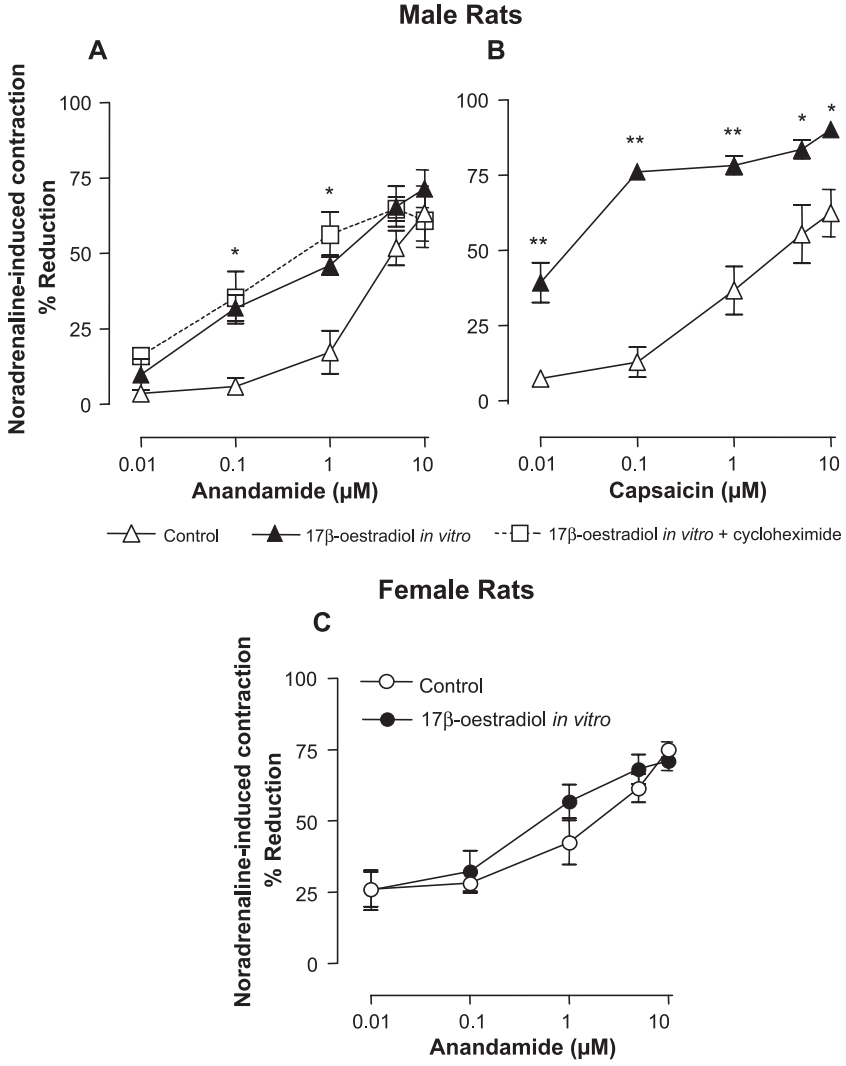

Fig. 5. Effects of 17β-oestradiol on either anandamide-(A, C) or capsaicin-(B) induced reductions of consecutive contractile responses to noradrenaline in mesenteric vascular beds isolated from male (triangles) and female rats (circles). Each concentration of either anandamide or capsaicin was perfused since 15 min before a bolus administration of 10 nmol noradrenaline up to the end of the contractile response. 17β-Oestradiol (0.5 μM, filled symbols) was added to the perfusion medium 60 min before the beginning of the concentration–response curves and remained up to the end of the experiment. In some preparations, the protein synthesis inhibitor 10 μM cycloheximide was added 60 min before 17β-oestradiol (open squares). Controls are depicted in open triangles (males) and open circles (females). Data are presented as the mean ± S.E.M. ($n = 5$ to 6) of the percent reductions of the initial contraction to noradrenaline. *$P < 0.05$, **$P < 0.001$ when compared to control rats.

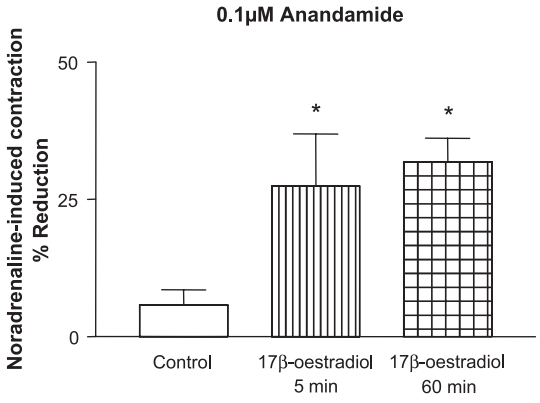

Fig. 6. Effect of in vitro exposure to 17β-oestradiol on the anandamide-induced reductions of consecutive contractile responses to noradrenaline in mesenteric vascular beds isolated from male rats. Anandamide (0.1 μM) was perfused since 15 min before administration of a bolus of 10 nmol noradrenaline. 0.5 μM 17β-Oestradiol was added to the perfusion medium 5 min (stripped bar) or 60 min (grid bar) prior to the contractile response to noradrenaline. Data are presented as the mean ± S.E.M. ($n = 4$ to 6) of the percent reduction of the contraction to noradrenaline obtained prior to anandamide *$P < 0.01$ when compared to control rats.

treatment with 17β-oestradiol (Fig. 7C). In the latter group the vasorelaxant effect of anandamide was similar to that observed in preparations from intact females (Figs. 2A and 7C). Moreover, 17β-oestradiol treatment restored serum oestrogen levels (25.5 ± 2.6 pg/ml in sham-operated proestrous rats; 4.2 ± 1.5 pg/ml in sham-operated dioestrous rats; 1.0 ± 1.0 pg/ml in ovariectomized rats; 30.2 ± 9.0 pg/ml in ovariectomized plus 17β-oestradiol rats; $n = 4–8$ per group; $p < 0.05$ when either sham-operated proestrus or ovariectomized plus 17β-oestradiol rats were compared to ovariectomized rats). In vivo chronic treatment with 17β-oestradiol also potentiated the response to anandamide in intact males (Fig. 8).

4. Discussion

The present study shows that anandamide-induced reductions of transient contractile responses to noradrenaline are higher in isolated vascular mesenteric beds from adult female than from age-matched male Sprague–Dawley rats. These results do not seem to arise from unspecific differences in the contractile machinery between male and female rats as far as sex differences were not observed either for the contractile responses to bolus injections of noradrenaline or for the decrease of noradrenaline-induced contractions caused by the acetylcholine receptor agonist and by the nitric oxide donor sodium nitroprusside.

The fact that the vasorelaxant responses to anandamide in vascular mesenteric beds from rats of either sex were unmodified by exposure to the cannabinoid CB_1 receptor antagonist SR141716A, but reduced by the vanilloid TRPV1 receptor antagonist capsazepine, suggests that there are no differences between sexes in the type of receptor involved in the vasorelaxant effects of anandamide. This is in agreement with previous studies showing that anandamide-induced vasorelaxation in both male (Mendizábal et al., 2001; Orliac et al., 2003) and female (Zygmunt et al., 1999) rat mesenteric arteries is related to vanilloid TRPV1 receptor activation. These observations, as well as the finding that sex-linked differences occurred also for the vasorelaxant responses to the archetypal vanilloid TRPV1 agonist capsaicin, suggest that female hormone environment can increase vanilloid TRPV1 receptor-mediated vasodilation in rat vascular mesenteric bed. The modest antagonistic effect of capsazepine in males could be linked to the concentration employed (1 μM) that was lower than that used by other authors (Vanheel and Van de Voorde, 2001; Andersson et al., 2002).

Vasodilator effects of 17β-oestradiol were reported in animals (Magness and Rosenfeld, 1989) and in postmenopausal women (Gilligan et al., 1995). The vasorelaxant actions of oestrogen involve genomic effects that depend on gene expression followed by protein synthesis as well as non-genomic effects such as alteration of membrane ionic permeability and activation of membrane enzymes (Tostes et al., 2003). In our study, the effect of 17β-oestradiol in vitro could be linked to a non-genomic mechanism because it was rapid in onset and was not modified by the protein synthesis inhibitor cycloheximide. On the other hand, the observations that ovariectomy decreased the vasorelaxant effects of anandamide, which are counteracted by both in vivo and in vitro exposure to 17β-oestradiol, suggest that oestradiol probably interacts with the endocannabinoid through genomic as well as non-genomic mechanisms. Non-genomic effects of oestradiol could be mediated by either α- or β-oestradiol receptors localized at the plasma membrane (Razandi et al., 1999; Norfleet et al., 2000). However, we were unable to confirm the participation of specific receptors in the in vitro effects of 17β-oestradiol by the use of oestrogen receptor antagonists such as tamoxifen and ICI 172,780 because both drugs produced per se inhibitory effects on the contractile responses to noradrenaline (Peroni et al., unpublished observations). Vasorelaxant effects of tamoxifen in the rat isolated mesenteric bed were also reported by Tep-areenan et al. (2003).

The effects of oestradiol in the present study do not appear to be restricted to females as far as exposure to the hormone modulated the effects of anandamide also in male mesenteric vascular beds. In this regard, it has been proposed that oestrogens have significant effects on endocrine and paracrine factors synthetized at the vascular wall in the male cardiovascular system (Gooren and Toorians, 2003).

At the present, it is not clear which step of the cascade of events involved in the vasorelaxant effects of anandamide is the target for oestrogenic modulation. It has been reported that the activity of anandamide at

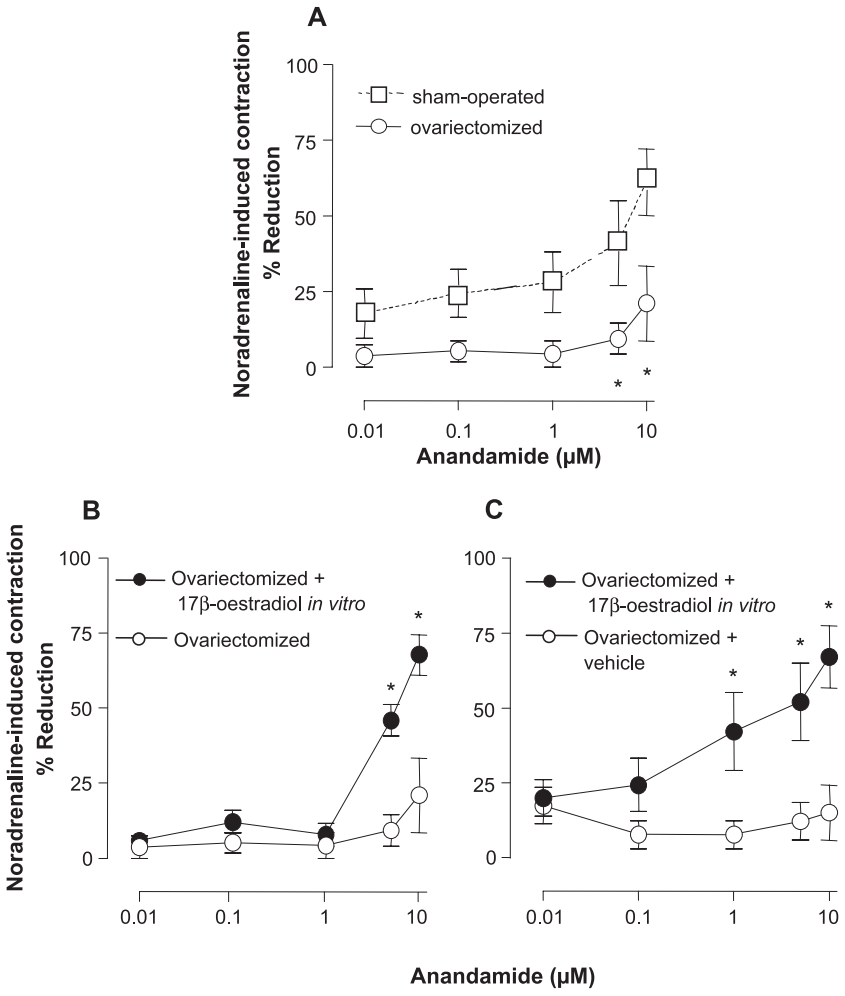

Fig. 7. Effects of anandamide on the consecutive noradrenaline-induced contractions in mesenteric vascular beds isolated from ovariectomized rats. Changes in anandamide effects caused by either in vivo or in vitro exposure to 17β-oestradiol. Each concentration of anandamide was perfused since 15 min before administration of a bolus of 10 nmol noradrenaline up to the end of the contractile response. (A) Responses to anandamide in preparations from ovariectomized (full line) and sham-operated rats (dot line). (B) 0.5 μM 17β-Oestradiol (filled circles) was added to the perfusion medium 60 min before the beginning of the concentration–response curve to anandamide and remained up to the end of the experiment. (C) Ovariectomized rats were treated with an i.m. injection of either 450 μg/kg 17β-oestradiol 3-benzoate (filled circles) or its vehicle (soja oil, open circles) once a week during 3 weeks. Data are presented as the mean ± S.E.M. ($n = 4$ to 6) of the percent reductions of the initial contractile response to noradrenaline. *$P < 0.01$ when compared to either sham-operated (A) or ovariectomized (B) or vehicle-treated ovariectomized (open circles).

vanilloid TRPV1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism (De Petrocellis et al., 2001a). In agreement with this, Andersson et al. (2002) have been shown that anandamide transporter inhibition decreases the vasodilator effect of anandamide in rat mesenteric arteries. 17β-Oestradiol activates the anandamide membrane transporter and inhibits the degradation of anandamide in human endothelial cells (Maccarrone et al., 2002). Moreover, the oestrogen increases the synthesis of nitric oxide (Miller et al., 1996; Caulin-Glaser et al., 1997) which in turn can stimulate the activity of the anandamide transporter (De Petrocellis et al., 2001a). These observations could suggest that the greater vasorelaxant effects of anandamide in the female mesenteric vasculature in this study could be related to an increased availability of the endocannabinoid. Sex-linked differences in the metabolic degradation of anandamide are precluded in our work on the basis that the fatty acid amide hydrolase inhibitor PMSF was devoid of effect on the responses to anandamide in mesenteric vascular beds isolated from both sexes. On the other hand, the possible role of anandamide membrane transporter in the greater relaxant effect of anandamide in females could not be assessed in this study since anandamide transporter inhibitors such as AM 404 and VDM 11 reduced per se the noradrenaline-induced contractions. Hence, the possibility that the greater relaxant effect of anandamide in female rats is due to differential regulation of the anandamide transporter cannot be disregarded at the present.

A possibility that will be explored in future studies is that oestrogenic modulation of the vascular relaxant

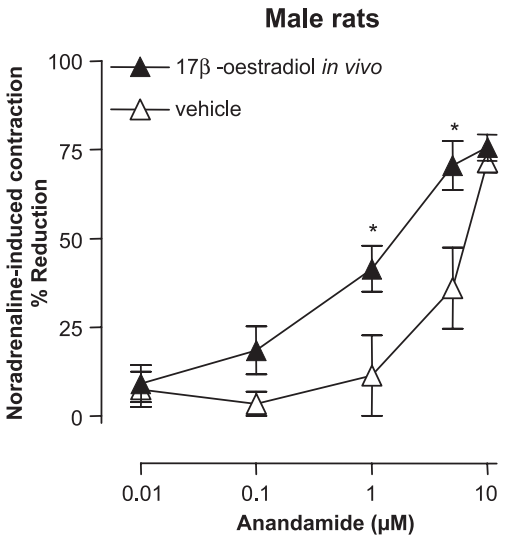

Fig. 8. Effect of in vivo treatment with 17β-oestradiol on anandamide-induced reductions of consecutive contractile responses to noradrenaline in rat mesenteric vascular beds isolated from male rats. Each concentration of anandamide was perfused since 15 min before administration of a bolus of 10 nmol noradrenaline up to the end of the contractile response. Male rats were treated with an i.m. injection of either 450 μg/kg 17β-oestradiol 3-benzoate (filled triangles) or its vehicle (soja oil; open triangles) once a week during 3 weeks. Data are presented as the mean ± S.E.M. ($n = 5$ to 6) of the percent reductions of the initial contraction to noradrenaline. *$P < 0.05$ when compared to vehicle-treated rats.

effects of anandamide in the rat mesenteric vascular bed is related to the vascular sensitivity to the CGRP which is released by vanilloid TRPV1 receptor activation (Holzer, 1991; Caterina et al., 1997; Zygmunt et al., 1999). In this sense, it has been reported that 17β-oestradiol potentiates the vasodilator (Gangula et al., 1999) and hypotensive (Grewal et al., 1999) effects caused by CGRP. Moreover, a variety of signal transduction events, such as the activation of the inositol triphosphate/protein kinase C pathway (Marino et al., 2001) and the stimulation of cAMP-dependent protein kinase (Doolan et al., 2000), were proposed for the non-genomic effects of 17β-oestradiol. Since activation of either protein kinase C (Premkumar and Ahern, 2000) or protein kinase A (De Petrocellis et al., 2001b) facilitates the effects of anandamide at vanilloid TPVR1 receptors, it will be of interest to investigate whether these signal transduction pathways are involved in the effects of oestradiol observed in our study.

Opposite to our present observation in vascular mesenteric beds from Sprague–Dawley rats, no sex-linked differences in the vasorelaxant effects of anandamide were found for mesenteric arteries of Wistar rats (McCulloch and Randall, 1998). This discrepancy could be related to animal strain differences and it is likely to indicate that the choice of the animal strain is critical for studies of the vascular effects of endocannabinoids.

In conclusion, this study is a first physiological evidence of an oestrogen-dependent sensitivity for the vanilloid TRPV1 receptor-mediated vasorelaxant effects of anandamide in the mesenteric vasculature, mediated by both genomic and non-genomic mechanisms. Biochemical as well as molecular studies are necessary to elucidate the signal transduction events involved in the oestrogen modulation of anandamide vasorelaxation.

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