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Endothelial-dependent sexual dimorphism in vascular smooth muscle: role of Mg^{2+} and Na^+

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1 In isolated aortae of the male rat $[Mg^{2+}]_o$ withdrawal and concomitant reduction in $[Na^+]_o$ (to 84 mM) induced significant increases of basal tone, but, surprisingly, this did not occur in intact aortae removed from female rats. Such tension development, however, was observed in endothelium-denuded aortic preparations from both sexes. These observed gender-related differences were not dependent on animal strain or types of tissue preparations.

2 No tension development was observed in aortae obtained from castrated males treated with oestradiol. Aortic tissues of sexually-immature male and female rats exhibited marked tension development when exposed to 0 mM $[Mg^{2+}]_o$ and low $[Na^+]_o$.

3 Tension development in Mg^{2+}-free, low-Na^+ media was not tachyphylactic and completely dependent on extracellular Ca^{2+}; addition of 1.2 mM Mg^{2+} to the Mg^{2+} and Na^+-deficient incubation media relaxed the increase in tension to a normal basal level.

4 Two known endothelial-derived relaxant factor (EDRF) inhibitors, methylene blue and haemoglobin, induced tension development in female aortae with intact endothelium exposed to Mg^{2+}-Na^+ deficient media, while use of a specific inhibitor of EDRF-derived nitric oxide, *viz.*, N^G-monomethyl-L-arginine (L-NMMA), resulted in potentiation of tension development in male, but not in female, aortae. This effect of L-NMMA was antagonized by L-arginine.

5 The Ca ionophore, A23187, partially relaxed contractile responses in male aortae (with intact endothelium) which were followed by potentiated contractions. Endothelium-dependent vasodilator responses to A23187 (10^{-10}–10^{-6} M) of aortic rings from male or female rats in normal Krebs-Ringer bicarbonate solution were not different.

6 These results suggest that: (a) as in vascular smooth muscle cells, Mg^{2+} plays an important role in Ca^{2+} homeostasis in endothelial cells, probably via Na^+-Ca^{2+} exchange; and (b) sex steroid hormones, probably the female sex hormone, 17-β-oestradiol, may regulate contractile responses of intact vascular smooth muscle by modifying endothelium functions through such Mg^{2+}-regulated internal Na^+-dependent Ca^{2+} entry. These data may help to explain why female subjects, despite Mg deficiency, unlike male subjects, are protected against ischaemic heart disease and cerebrovascular disease until menopause.

Keywords: Gender differences; magnesium regulated Na^+-Ca^{2+} exchange; endothelial-derived factors; vascular smooth muscle

Introduction

Gender-related differences in haemodynamic characteristics have received considerable scientific attention because women, prior to menopause, are known to be less susceptible to numerous cardiovascular disorders when compared to men (for reviews, see Altura & Altura, 1977; Caplan *et al.*, 1986; Lerner & Kannel, 1986). The precise mechanism(s) of sex steroids in the modulation of cardiovascular function, however, remains to be elucidated.

Recently, Maddox *et al.* (1987) reported that there is an endothelium-dependent gender difference in responses of rat aortae to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). *In vivo* and *in vitro* studies have demonstrated that female hormones potentiate endothelium-dependent relaxations to acetylcholine (Williams *et al.*, 1990; Gisclard *et al.*, 1988). Sex steroid hormones may influence vascular reactivity (Altura & Altura, 1977) via this previously unrecognized property of endothelial cells which generates and releases endothelium-derived relaxant factor(s) (EDRF) (Furchgott & Vanhoutte, 1989).

Intracellular, free calcium ions ($[Ca^{2+}]_i$) are typically thought of as playing a critical role in synthesis and/or release of EDRF from endothelial cells (Long & Stone, 1985). It has been suggested that, in addition to Ca channels, Na^+-Ca^{2+} exchange may participate in the ion transport mechanisms involved in Ca^{2+} homeostasis in both endothelial and vascular smooth muscle cells (Adams *et al.*, 1989).

It has been demonstrated that magnesium (Mg^{2+}) can exert antagonistic effects on Na^+-Ca^{2+} exchange in cardiac and vascular smooth muscle cells (Wakabayashi & Goshima, 1981; Smith *et al.*, 1987), but such data have not been reported for endothelial cells. However, removal of extracellular Mg^{2+} ($[Mg^{2+}]_o$) has been shown to induce $[Ca^{2+}]_o$-dependent vasodilatation by releasing EDRF from endothelial cells (Ku & Ann, 1987; Gold *et al.*, 1990). Such relaxation of vascular smooth muscle is inhibited by dichlorobenzamil (DCB), an amiloride analogue and inhibitor of Na^+-Ca^{2+} exchange (Siegel *et al.*, 1984). This led us to the suggestion that Mg^{2+} may also modulate activity and function of Na^+-Ca^{2+} exchange in endothelial cells. Mg^{2+}-regulated Na^+-Ca^{2+} exchange may have opposite effects on vascular tone: (1) a release of EDRF from endothelium, which leads to relaxation, and (2) activation of contraction in vascular smooth muscle (either of which may be modulated by the presence of sex steroids).

With this in mind, we conducted experiments to examine possible gender differences in contractile responses of vascular tissues to alteration of $[Mg^{2+}]_o$ and $[Na^+]_o$ ions. It was anticipated that such experiments would provide new information on how hormones act on Ca^{2+} translocation pathways involved in the expression of endothelium-dependent relaxation. The data presented here, suggest that the female sex hormone, 17-β-oestradiol, regulates contraction and tone of intact blood vessels by modulating endothelial cell-derived factors through Mg^{2+}-regulated Na^+-dependent Ca^{2+} entry.

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Methods

Animals, preparations and general procedure

The experiments were performed on isolated aortae taken from: (1) adult Wistar and Sprague-Dawley male and female rats (16–24 weeks old and weighing 275–400 g); (2) young (sexually-immature) Wistar male and female rats (2–4 weeks old and weighing 80–100 g); (3) adult Wistar male and female rats treated by castration and replacement of sex steroid hormones. In these experiments, 8-week-old male and female rats were castrated or ovariectomized bilaterally under pentobarbitone sodium anaesthesia (Anthony Products Co, Arcadia, California; 30 mg kg^{-1}, i.p.). After 3 days of recovery, these male and female animals were treated with 17-β-oestradiol benzoate (Squibb and Sons, Inc., Princeton, New Jersey, U.S.A.; 1.5 mg kg^{-1}, i.m.) or testosterone (Schein Pharmaceutical, Inc., Phoenix, Arizona, U.S.A.; 2.5 mg kg^{-1}, i.m.), respectively, every three days for 4 weeks.

All animals were killed by decapitation and exsanguinated. Thoracic aortae were excised and immediately placed in normal Krebs-Ringer bicarbonate (NKRB) solution at room temperature and cleaned of blood, loose connective tissue and fat. Aortic strips were cut helically (2 mm in width by 25 mm in length) for adult animals (Altura & Altura, 1974), and 1 mm in width by 20 mm in length for the sexually-immature animals. Some aortae isolated from adult Wistar rats were cut into rings about 2 mm long. For intact tissue preparations, extreme care was taken to avoid damage of endothelial cells. In every other ring, the endothelium was removed gently with small forceps according to the method of De Mey & Vanhoutte (1982).

The composition of the NKRB was (in mM): NaCl 118, KCl 4.7, $KHPO_4$ 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 10 and $NaHCO_3$ 25. When Mg^{2+}-free, low-Na^+ solutions (Mg-free low-Na KRB) were used, 59 mM NaCl was replaced by an isosmolar amount of sucrose. The residual Na ion concentration in the substituted solutions was 84 mM.

Measurement of vascular reactivities of aortic rings and strips

Thoracic aortic rings, with and without endothelium, and strips isolated from adult rats were arranged isometrically, under resting tensions of 4.0 or 1.5 g, respectively, while 0.5 g of loading tension was used in aortic strip tissues from younger (sexually-immature) Wistar rats. All tissues were initially equilibrated for 2 h in chambers containing 20 ml of NKRB at 37°C and gassed continuously with a 95%O_2:5%CO_2 mixture. The loading tensions were adjusted periodically and maintained throughout the equilibration period. The incubation media were routinely changed every 10–15 min as a precaution against interfering metabolites (Altura & Altura, 1970). The tissues were attached to force-displacement transducers (Grass Model FT 03) connected to Grass Model 7 polygraphs, and isometric tensions of the vascular smooth muscle preparations were recorded. The stable level of tension developed in response to the addition of 80 mM KCl was always measured prior to the collection of data. To examine the functional viability of an intact endothelium, aortic rings were precontracted by ED_{50} doses of $PGF_{2\alpha}$ as described below, and the presence and absence of endothelium was confirmed by testing for relaxation to acetylcholine (5×10^{-7} M) (Furchgott & Vanhoutte, 1989), which generally resulted in 90% relaxation in rat aortae with intact endothelium.

After equilibration in NKRB, the tissues were exposed to Mg^{2+}-free low-Na^+ KRB for variable periods, of from 30 to 120 min, and then observed for tension development (Altura & Altura, 1974). In order to determine if Mg^{2+} and Ca^{2+} were important in low-Na^+-induced alterations of tension development, $MgSO_4$ (1.2 mM) was reintroduced to the Mg^{2+}-free low-Na^+ KRB (to restore normal $[Mg^{2+}]_o$) or $CaCl_2$ was withdrawn from Mg^{2+}-free low-Na^+ KRB, respectively. In these experiments, all observations were repeated at least twice in the same tissue, and each tissue was returned to NKRB after the incubation in modified KRB solutions for at least 30 min to re-establish normal vascular reactivity and tone.

Role of endogenous vasoactive substances

To determine whether the gender-related differences in responses in Mg^{2+}-free, low-Na^+ KRB could be attributed to differences in the endogenous release of specific types of vasoactive amines from the blood vessels (e.g., noradrenaline, acetylcholine, histamine and 5-hydroxytryptamine (5-HT)), adenine nucleotide (ATP), peptides (substance P) or prostaglandins) these vasoactive substances (10^{-6} M) were examined in the modified KRB solutions. Other experiments were conducted by treating tissues with a variety of specific pharmacological antagonists as well as a cyclo-oxygenase inhibitor (i.e., indomethacin) before and during incubation in modified KRB solutions. These antagonists were used in concentrations which produced specific antagonism to their respective agonists and cyclo-oxygenase in rat aortic tissue (i.e., 10^{-7} to 10^{-5} M) (Altura *et al.*, 1976). The drugs used for these studies included noradrenaline bitartrate (Sigma Chem. Co., St. Louis, Missouri, U.S.A.), acetylcholine chloride (Nutritional Biochemicals Co., Cleveland, Ohio, U.S.A.), 5-HT creatinine sulphate (Nutritional Biochemicals Co., Cleveland, Ohio, U.S.A.), histamine hydrochloride and substance P (Sigma Chem. Co., St. Louis, Missouri, U.S.A.), atropine sulphate (Mann Res. Labs, New York, U.S.A.), phentolamine methanesulphonate (Regitine, Ciba-Geigy, Summit, New Jersey, U.S.A.), diphenhydramine HCl (Benadryl, Parke Davis Co; Ann Arbor, Michigan, U.S.A.), propranolol HCl and ATP (Sigma Chem. Co., St. Louis, Missouri, U.S.A.), methysergide maleate (Sandoz Ltd; Basel, Switzerland), and indomethacin (Merck, Rahway, New Jersey, U.S.A.).

Role of endothelium-derived relaxing factor

To examine whether activation or release of EDRF from endothelial cells was involved in the vascular responses to Mg^{2+}-free, low-Na^+ media, three known EDRF inhibitors, i.e., methylene blue (10^{-5} M, Sigma Chem. Co., St. Louis, Missouri, U.S.A.), oxyhaemoglobulin (10^{-5} M, kindly provided by Dr R.F. Furchgott) and N^G-monomethyl-L-arginine (L-NMMA, 3×10^{-4} M, Calbiochem Co., La Jolla, California, U.S.A.), as well as the Ca ionophore A23187 (5×10^{-7} M, Calbiochem Co., La Jolla, California, U.S.A.) (Furchgott & Vanhoutte, 1989), were tested in aortic ring preparations incubated in Mg^{2+}-free, low-Na^+ KRB solutions. In the experiments dealing with L-NMMA, L-arginine (3×10^{-4} M, Calbiochem Co., La Jolla, California, U.S.A.) was used to test whether the effects of L-NMMA could be reversed.

In order to study endothelium-dependent responses in NKRB solutions, the intact and denuded ring preparations of male or female rat aortae were initially precontracted with an ED_{50} dose of $PGF_{2\alpha}$, i.e., $2–4 \times 10^{-7}$ M or $1–3 \times 10^{-6}$ M, respectively, followed by challenge with the Ca ionophore A23187 (10^{-10}–10^{-6} M); concentration-dependent relaxation curves were obtained. The observed differences in ED_{50}s for $PGF_{2\alpha}$ used here resulted from sexual differences in sensitivity to the agent (Maddox *et al.*, 1987). However, concentrations chosen for $PGF_{2\alpha}$ were intended to produce identical levels of contractile force, which is important for comparing vasodilator effectiveness (Winquist *et al.*, 1984), in both male and female animals. Care was taken to contract each ring tested, herein, to similar levels of force (i.e., 800–900 mg) before challenging with A23187.

Statistical analyses

Where appropriate, means ± s.e.mean were calculated and compared for statistical significance by means of Student's *t*

tests, paired t tests or ANOVA using Scheffe's contrast test. P values less than 0.05 were considered significant.

Results

Tension development in response to lowering of $[Mg^{2+}]_o$ and $[Na^+]_o$ with and without castration and/or replacement of sex hormones

Figure 1 shows recordings of typical changes in resting tension of isolated aortic rings from male and female rats when placed in Mg^{2+}-free low-Na^+ KRB solution. Simultaneous $[Mg^{2+}]_o$ withdrawal and lowering the $[Na^+]_o$ concentration from 143 to 84 mM induced significant increases of basal tone in aortic rings isolated from male Wistar rats, but not in tissues from female Wistar rats. Surprisingly, such tension development induced by lowering $[Mg^{2+}]_o$ and $[Na^+]_o$ was observed in endothelial-denuded aortic rings isolated from either sex, in which male tissues clearly exerted greater responses than female tissues (Figure 1). Similarly, sex differences in responsiveness to Mg^{2+}-free low-Na^+ KRB solution were also observed in aortic strip preparations from both adult Wistar and Sprague-Dawley rats as shown in Figure 2. It should be noted that about 14% of intact male aortae (7 out of 51 preparations) did not show significant contractile responses, i.e., the tone induced by $[Mg^{2+}]_o$- and $[Na^+]_o$-deficient media was below 30% maximal K^+-induced contraction in these tissues, and therefore these results were not included in Figure 2. However, little in the way of tension development on lowering $[Mg^{2+}]_o$ and $[Na^+]_o$ ions was observed in aortae from male Wistar rats castrated and treated with 17-β-oestradiol, while castration and treatment with testosterone failed to induce contraction in female Wistar rats exposed to Mg^{2+}-free low-Na^+ KRB solution (Figure 2). Castration without replacement of sex steroids failed to exert any effect on basal tone (data not shown). In contrast to adults, no gender-related differences were observed in the young sexually-immature Wistar rats; relatively greater contractile responses in terms of %K^+-induced maximal contractions (to lowering of $[Mg^{2+}]_o$ and $[Na^+]_o$ ions) were observed compared to the adult rats (Figure 2). Such mechanical changes in base-line tension were not tachyphylactic and

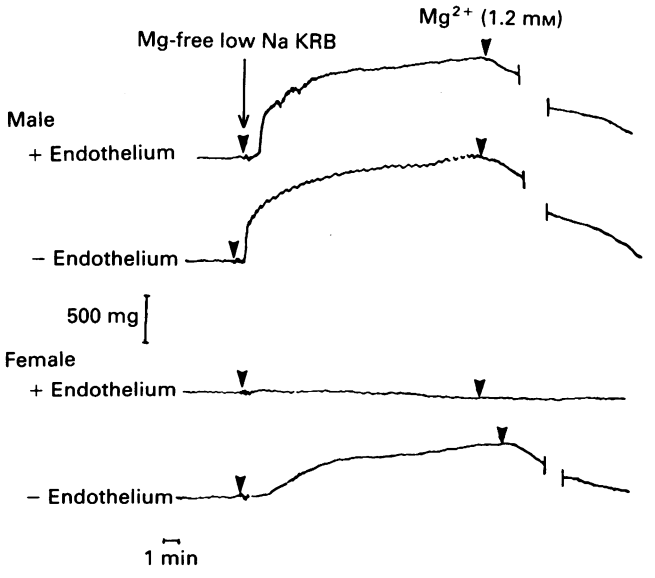

Figure 1 Effects of extracellular magnesium removal and readdition on basal tone of aortae isolated from adult Wistar male and female rats in low-Na containing medium (84 mM NaCl). Intact (+ Endothelium) and endothelium-denuded (− Endothelium) aortic rings were mounted and equilibrated as described in the text. Subsequent replacements of NKRB medium with Mg-free, low-Na KRB medium are indicated by the arrows. Mg^{2+} (1.2 mM) denotes readdition of 1.2 mM $MgSO_4$ to the bathing medium.

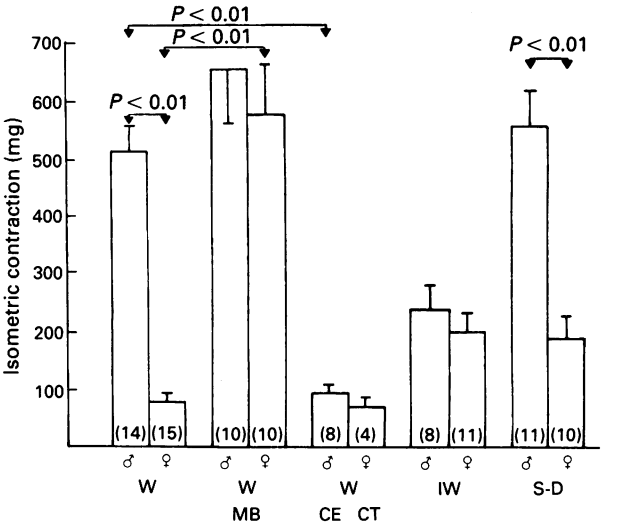

Figure 2 Effects of $[Mg^{2+}]_o$ removal from low-Na KRB medium on basal tone of rat aortic strips. Strip tissues were mounted and equilibrated as described in the text. Columns represent the means with s.e.mean shown by vertical bars; numbers of animals in parentheses; W = adult Wistar rats; MB = methylene blue-treated; CE = castrated and oestradiol-treated; CT = castrated and testosterone-treated; IW = sexually-immature Wistar rats; S-D = adult Sprague-Dawley rats.

could be maintained for at least 2 h. Addition of 1.2 mM $MgSO_4$ to the incubation media relaxed the increase in tension observed to normal resting levels in all of these intact or denuded tissues (Figure 1). Removal of $[Ca^{2+}]_o$ from Mg^{2+}-free low-Na^+ KRB completely abolished tension development in all tissues tested (data not shown, $n = 12$). Lowering of only $[Na^+]_o$ to 84 mM (in the presence of 1.2 mM $[Mg^{2+}]_o$) failed to produce any changes in basal tone in either male or female tissues ($n = 16$).

Failure of specific neurotransmitters and hormones to induce relaxation, and failure of specific pharmacological antagonists as well as cyclo-oxygenase inhibitor to interfere with tonic responses of rat aortae

None of the relaxants when tested over wide concentration ranges (i.e., acetylcholine, substance P, 5-HT, histamine or ATP) (10^{-9}–10^{-6} M), induced relaxation of male aortic tissues incubated in Mg^{2+}-free low-Na^+ KRB solution. Experiments with antiadrenoceptor, anticholinoceptor and antihistamine agents, as well as a cyclo-oxygenase inhibitor (phentolamine, propranolol, atropine, diphenhydramine, and indomethacin, respectively) revealed an inability of these specific pharmacological antagonists and cyclo-oxygenase inhibitor to interfere with the contractile responses observed on lowering of $[Mg^{2+}]_o$ and $[Na^+]_o$ ions (six to eight experiments were performed with each agent). Methysergide, an antagonist of 5-HT, in a concentration of 10^{-6} M, induced slight contractions (8% K^+-induced maximal contractions) followed by relaxation in aortic tissues incubated in Mg^{2+}-free low-Na^+ KRB solution.

Effects of methylene blue, oxyhaemoglobulin and N^G-monomethyl-L-arginine, as well as A23187, on tonic responses of rat aortae

Figures 3 and 4 illustrate recordings of typical changes of resting tension in aortic rings obtained with Mg^{2+}-free, low-Na^+ KRB before and after the addition of methylene blue and A23187. Methylene blue (MB, 10^{-5} M) enhanced tension development in both intact and denuded aortic rings from both male and female rats in Mg^{2+}-free, low-Na^+ KRB solution (Figure 3). In aortic strips, MB also significantly potentiated contractile responses as seen in the ring preparations

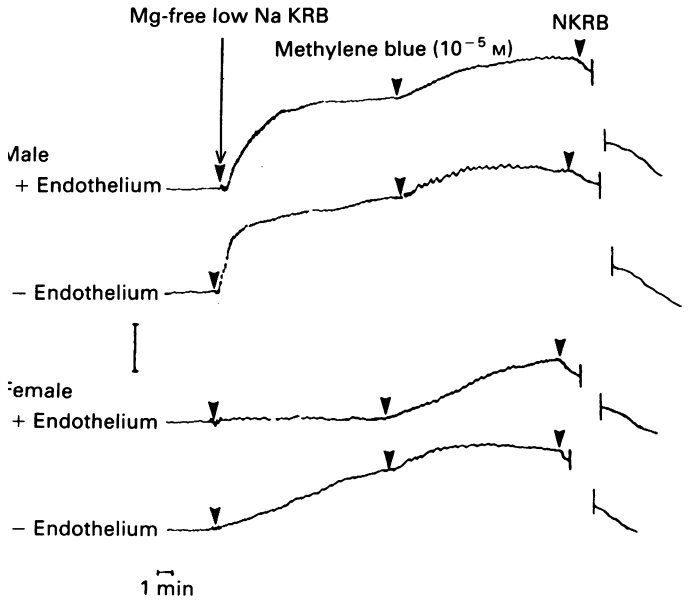

Figure 3 Influence of methylene blue on alterations in tone elicited by reduction $[Mg^{2+}]_o$ and $[Na^+]_o$ in rat aortae. Intact and denuded aortic rings were mounted and equilibrated as described in the text. Conventions similar to these used in Figure 1. At peak tension in Mg-free low-Na KRB, methylene blue (10^{-5} M) was added as indicated by the second set of arrows. This tracing is representative of six different tissues.

(Figure 2). In contrast to MB, L-NMMA treatment (3×10^{-4} M) exerted only potentiating effects on contractile responses of male aortae, and failed to interfere with responses of female aortae (data not shown, $n = 6$). The effects of L-NMMA were completely antagonized by the same concentration of L-arginine (3×10^{-4} M). A23187 (10^{-6} M) produced initial relaxation (about 50% of tonic amplitude) followed by enhanced contraction in intact male tissues; however, no effects of this agent were observed in aortic rings from female rats, or in denuded tissues (Figure 4). Neither methylene blue nor A23187 was found to exert such effects in NKRB solution ($n = 6$). As with methylene blue, addition of oxyhaemoglobulin (10^{-5} M) resulted in a furtherance of contractile amplitude

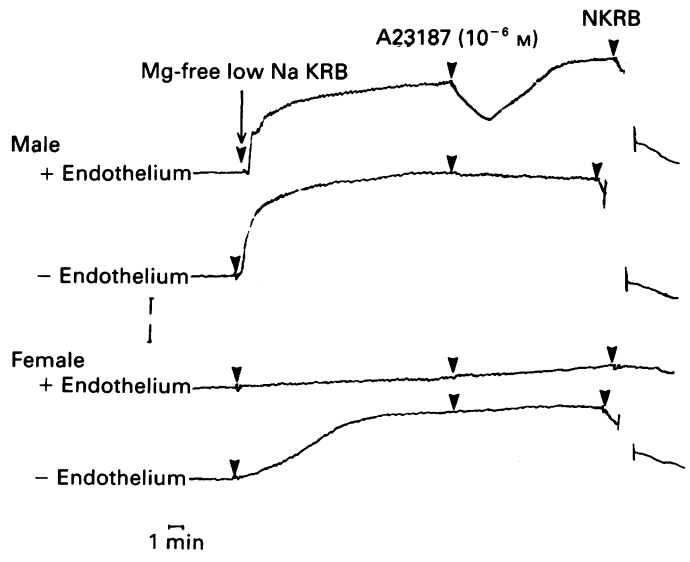

Figure 4 Influence of the Ca ionophore, A23187, on alterations in basal tone elicited by reduction $[Mg^{2+}]_o$ and $[Na^+]_o$ in rat aortae. Intact and denuded aortic rings were mounted and equilibrated as described in the text. Conventions similar to those in Figure 1. At peak tension in Mg-free, low-Na KRB, A23187 (10^{-6} M) was added as indicated by the second set of arrows. This tracing is representative of eight different tissues.

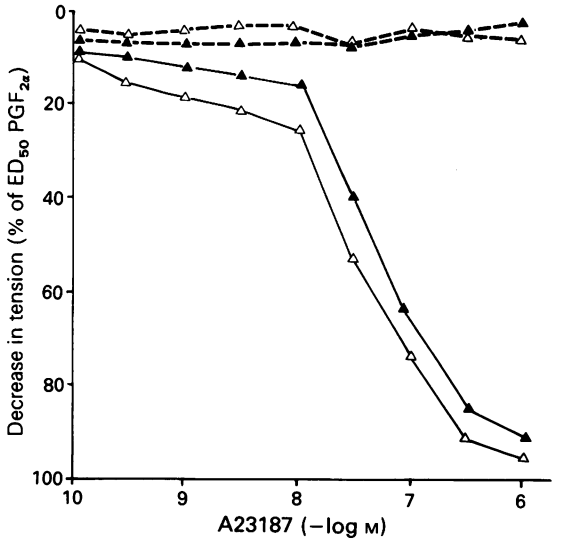

Figure 5 Lack of a gender difference observed on cumulative concentration-response curves to A23187-induced relaxation in aortic rings isolated from adult male and female rats, which were precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (ED_{50}). Data are presented as a percentage of the contractile response to $PGF_{2\alpha}$ and expressed as means with s.e.means shown by vertical bars. $n = 6$ in each gender group. Denuded tissues from both gender groups exerted little or no relaxation response to acetylcholine. Female: ▲——▲ intact rings; ▲---▲ denuded rings; Male: △——△ intact rings; △---△ denuded rings.

(160–190% of K^+-induced maximal contraction) in aortae from both male and female rats in $[Mg^{2+}]_o$- and $[Na^+]_o$-deficient medium (data not shown, $n = 4$).

A23187-induced relaxation in normal Krebs Ringer solution

In both male and female rats, A23187 (10^{-10} to 10^{-6} M) produced cumulative concentration-dependent relaxations in the $PGF_{2\alpha}$-precontracted aortic rings with endothelium; no relaxations were observed in rings without endothelium (Figure 5). The observed differences in magnitudes of relaxation to A23187 between male and female rats were not found to be significant in the present studies ($n = 6$).

Discussion

The results described here with acute removal of Mg^{2+} ions in low-Na^+ media demonstrate two contrasting aortic vascular effects of Mg^{2+} in male and female animals. A marked increase in resting tension occurs in isolated intact aortae of males but not in intact aortae from females when Mg^{2+} and Na^+ concentrations are reduced in the incubation medium. Such contractions are observed in tissues from both male and female rats, when denuded. This gender-related difference is evident in both Wistar and Sprague-Dawley rats in both helical-cut strips and rings of aorta. Therefore, the data suggest a strain- and tissue-type-independence. Endothelial cells in female animals seem to exert predominant effects on the modulation of vascular responses to a lowering of $[Mg^{2+}]_o$ and $[Na^+]_o$, probably by a release of EDRF, because contractile responses were observed in tissues without endothelium in either sex. Indomethacin failed to interfere with such events, suggesting that prostacyclin and other dilator prostanoids are not involved. Two different inhibitors of EDRF, viz., methylene blue and oxyhaemoglobulin, however, potentiated tension in male and female aortae supporting the hypothesis that EDRF released from endothelial cells mediates the gender-related differences observed in intact rat aortae.

The mechanism whereby endothelial function in females is dominant, as observed in the present studies, is not known.

We cannot find any difference between young, sexually-immature male and female rats, suggesting that the unusual change of vascular reactivity and basal tone noted herein is associated with sexual maturity. Since castration and treatment with oestradiol inhibited the cation-related increments in basal tension of male rat aortae, while aortae from castrated-testosterone-treated female rats did not produce contraction, it seems that female sex hormones (probably 17-β-oestradiol) exert predominant effects on the modification of vascular responses to reduction of $[Mg^{2+}]_o$ and $[Na^+]_o$ ions. Several explanations and sites of action of sex steroid hormones seem plausible.

The lack of effect of amine antagonists in male or female tissues in our studies, suggest that sex-related differences of vascular responsiveness in Mg^{2+}-free, low-Na^+ media cannot be attributed to the release of neurotransmitters, from autonomic nerve terminals or endothelial cells (Altura & Altura, 1977; Burnstock, 1987).

Since no difference of vasorelaxation to the Ca ionophore A23187 in NKRB solution was found between male and female animals, the passive mechanical properties of vascular tissues (Fischer & Swain, 1977; Cox & Fischer, 1978) also cannot explain our results. The Ca ionophore A23187 is believed to increase $[Ca^{2+}]_i$ by enhancement of Ca^{2+} influx (Reed & Lardy, 1972), independent of membrane Ca channels and antiporter systems. Thus, our results of endothelium-dependent relaxation to A23187 in NKRB solution suggest further that, after the initial triggering step of Ca^{2+} influx into the vascular endothelial cells, there are no differences in the expression of endothelium-dependent relaxation, including synthesis and/or release of EDRF as well as sensitivity of the vascular smooth muscle to EDRF, between male and female vascular tissues. Since oestrogen has been demonstrated to have no effect on the level of guanosine 3':5'-cyclic monophosphate (cyclic GMP) in vascular smooth muscle (Kishi & Numano, 1982) such a direct mechanism of action is unlikely.

We reported recently, that reductions in $[Na^+]_o$, which cause no contractile effects in normal $[Mg^{2+}]_o$ media, caused a Ca^{2+}-dependent tension development when $[Mg^{2+}]_o$ was simultaneously withdrawn from isolated aortic smooth muscle of male rats (Altura *et al.*, 1990). These studies, concomitant with observations in cultured vascular smooth muscle cells (Smith *et al.*, 1987; 1989), support the idea that there are Mg^{2+}-regulated Na^+-Ca^{2+} exchanges in the plasma membrane in vascular smooth muscle (Altura & Altura, 1982; Smith *et al.*, 1987). Thus, it is not unreasonable to postulate that there is facilitation of Na^+-Ca^{2+} exchange when endothelial cells are exposed to Mg^{2+}-free, low-Na^+ media.

There is substantial evidence that both endothelial cells and vascular smooth muscle cells possess specific oestrogen receptors (Colburn & Buonassisi, 1978; Harder & Coulson, 1979). Female steroid hormones may, in some unknown way, exert direct influences on Mg^{2+}-regulated Na^+-Ca^{2+} exchange to control the intracellular Ca^{2+} concentration of endothelial cells. Since potentiation of tension development of male rat aortae always occurred with removal of endothelial cells, a relatively lower but definite activity of endothelium seems to operate normally also in these tissues. Support for this hypothesis can be derived from the present studies performed with the Ca ionophore, A23187, which results in endothelium-dependent vasodilatation followed by contraction.

Nitric oxide (NO) is an important EDRF, which is formed from L-arginine in endothelial cells (Palmer *et al.*, 1988; Furchgott & Vanhoutte, 1989). L-Arginine metabolism in endothelium can be reversibly inhibited by competition of the NO-synthesizing enzyme using an analogue of L-arginine, L-NMMA (Furchgott & Vanhoutte, 1989; Rees *et al.*, 1989). It is noteworthy, in our present studies, that there was gender dimorphism of vascular responses to L-NMMA when aortic tissues were incubated in Mg^{2+}- and Na^+-deficient media, i.e., L-NMMA potentiated tension development in male, but not in female, aortic tissue. Since such potentiating effects of L-NMMA were completely reversed by L-arginine, the data are compatible with the notion that a certain amount of NO was indeed produced and released from endothelial cells in male aortae by lowering $[Mg^{2+}]_o$ and $[Na^+]_o$, and may be a reason why a small number of the male aortic tissues failed to undergo tension development. It is puzzling as to why addition of L-NMMA to the female aortic smooth muscle, exposed to low $[Mg^{2+}]_o$ and $[Na^+]_o$, does not result in tension development.

Although considerable evidence now exists that low dietary intake of Mg is associated with increased incidence of sudden death ischaemic heart disease (SDIHD) in men below the age of 50 (Seelig, 1980; Turlapaty & Altura, 1980; Altura & Altura, 1985), which is thought to be attributed to coronary vasospasm (for reviews, see Altura & Altura, 1985; 1990), it has not been possible to explain why women ingesting similar, low levels of Mg do not exhibit such an incidence until after the age of 50 (Seelig, 1980). If our results pertain to human coronary vessels, then the loss of oestrogenic hormones in postmenopausal women together with deficits in Mg dietary intake would result in a similar incidence of SDIHD, exactly as is noted clinically.

In conclusion, the results presented here represent the first demonstration of an endothelium-dependent gender-related difference of vascular responsiveness to activation of Na^+-Ca^{2+} exchange, which is regulated by Mg^{2+}, in isolated arteries. Irrespective of the exact mechanism(s) whereby female steroid hormones modulate Mg^{2+}-regulated Na^+-Ca^{2+} exchange, our data could prove valuable in elucidating the precise control mechanisms for sexual dimorphism of vascular responsiveness. These observations may be of importance in explaining why premenopausal females are much less susceptible to cardiovascular disease processes than are males.

References

ADAMS, D.J., BARAKEH, J., LASKEY, R. & VAN BREEMEN, C. (1989). Ion channels and regulation of intracellular calcium in vascular endothelial cells. *FASEB J.*, **3**, 2389–2400.

ALTURA, B.M. & ALTURA, B.T. (1970). Differential effects of substrate depletion on drug-induced contractions of rabbit aorta. *Am. J. Physiol.*, **219**, 1698–1705.

ALTURA, B.M. & ALTURA, B.T. (1974). Magnesium and contraction of arterial smooth muscle. *Microvasc. Res.*, **7**, 145–155.

ALTURA, B.M. & ALTURA, B.T. (1977). Influence of sex hormones, oral contraceptives and pregnancy on vascular muscle and its reactivity. In *Factors Influencing Vascular Reactivity*, ed. Carrier, O. & Shibata, S. pp. 221–254. New York: Igaku-Shoin Press.

ALTURA, B.M. & ALTURA, B.T. (1982). Mg, Na, K interactions and coronary heart disease. *Magnesium*, **1**, 241–265.

ALTURA, B.M. & ALTURA, B.T. (1985). New perspectives on the role of magnesium in the pathophysiology of the cardiovascular system. I. Clinical aspects. *Magnesium*, **4**, 226–244.

ALTURA, B.M. & ALTURA, B.T. (1990). Magnesium and the cardiovascular system: Experimental and clinical aspects updated. In *Metal Ions in Biological Systems*, Vol. 26: *Compendium on Magnesium and Its Role in Biology, Nutrition and Physiology*. ed. Sigel, H. pp. 359–416. New York: Marcel Dekker Inc.

ALTURA, B.M., EDGARIAN, H. & ALTURA, B.T. (1976). Differential effects of ethanol and mannitol on contraction of arterial smooth muscle. *J. Pharmacol. Exp. Ther.*, **197**, 352–361.

ALTURA, B.T., ZHANG, A. & ALTURA, B.M. (1990). Sodium-calcium exchange mechanism in vascular smooth muscle tissue as revealed by manipulating external magnesium. *Magnesium Trace Elements*, **9**, 163–175.

BURNSTOCK, G. (1987). Nervous control of smooth muscle by transmitters, cotransmitters and modulators. *J. Cardiovasc. Pharmacol.*, **10** (Suppl. 12), S74–S81.

CAPLAN, L.R., GORELICK, P.B. & HIER, D.B. (1986). Race, sex and occlusive cerebrovascular disease: a review. *Stroke*, **17**, 648–655.

COLBURN, P. & BUONASSISI, V. (1978). Estrogen-binding sites in endothelial cell cultures. *Science*, **201**, 817–819.

COX, R.H. & FISCHER, G.M. (1978). Effects of sex hormones on the passive mechanical properties of rat carotid artery. *Blood Vessels*, **15**, 266–277.

DE MEY, J.G. & VANHOUTTE, P. (1982). Heterogeneous behavior of the canine arterial and venous wall. Importance of the endothelium. *Circ. Res.*, **51**, 439–447.

FISCHER, G.M. & SWAIN, M.L. (1977). Effect of sex hormones on blood pressure and vascular connective tissue in castrated and non-castrated male rats. *Am. J. Physiol.*, **232**, H617–H621.

FURCHGOTT, R.F. & VANHOUTTE, P.M. (1989). Endothelium-derived relaxing and contracting factors. *FASEB J.*, **3**, 2007–2018.

GISCLARD, V., MILLER, V.M. & VANHOUTTE, P.M. (1988). Effect of 17-β-estradiol on endothelium-dependent responses in the rabbit. *J. Pharmacol. Exp. Ther.*, **244**, 19–22.

GOLD, M.E., BUGA, G.M., WOOD, K.S., BYRNS, R.E., CHAUDHURI, G. & IGNARRO, L.J. (1990). Antagonistic modulatory roles of magnesium and calcium on release of endothelium-derived relaxing factor and smooth muscle tone. *Circ. Res.*, **66**, 355–366.

HARDER, D.R. & COULSON, P.B. (1979). Estrogen receptor and effects of estrogen on membrane electrical properties of coronary vascular smooth muscle. *J. Cell Physiol.*, **100**, 375–382.

KISHI, Y. & NUMANO, F. (1982). A study of the mechanism as an antiarterosclerotic: the inhibitory effect of estrogen on A23187-induced contractions of the aortic wall. *Mech. Ageing Dev.*, **18**, 115–123.

KU, D.D. & ANN, H.S. (1987). Magnesium deficiency produces endothelium-dependent vasorelaxation in canine coronary arteries. *J. Pharmacol. Exp. Ther.*, **241**, 961–966.

LERNER, D.J. & KANNEL, W.B. (1986). Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. *Am. Heart J.*, **111**, 383–390.

LONG, C.J. & STONE, T.W. (1985). The release of endothelium-derived relaxant factor is calcium dependent. *Blood Vessels*, **22**, 205–208.

MADDOX, Y.T., FALCON, J.G., RIDINGER, M., CUNARD, C.M. & RAMWELL, P. (1987). Endothelium-dependent gender differences in the responses of rat aorta. *J. Pharmacol. Exp. Ther.*, **240**, 392–395.

PALMER, R.M.J., ASHTON, D.S. & MONCADA, A. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **333**, 664–666.

REED, P.W. & LARDY, H.A. (1972). A divalent cation ionophore. *J. Biol. Chem.*, **247**, 6970–6972.

REES, D.D., PALMER, R.M.J., HODSON, H.F. & MONCADA, S. (1989). A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.*, **96**, 418–424.

SEELIG, M.S. (1980). *Magnesium Deficiency in the Pathogenesis of Disease*. New York: Plenum.

SIEGEL, P.K.S., CRAGOE, E.J. Jr., TRUMBLE, M.J., KACZOROWSKI, G.J. (1984). Inhibition of Na^+/Ca^{2+} exchange in membrane vesicle and papillary muscle preparations from guinea pig heart by analogs of amiloride. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 3238–3242.

SMITH, J.B., CRAGOE, E.J. & SMITH, L. (1987). Na^+/Ca^{2+} antiport in cultured arterial smooth muscle cells. *J. Biol. Chem.*, **262**, 11988–11994.

SMITH, J.B., ZHENG, T. & SMITH, L. (1989). Relationship between cytosolic free Ca^{2+} and Na^+-Ca^{2+} exchange in aortic muscle cells. *Am. J. Physiol.*, **256**, C147–C154.

TURLAPATY, P.D.M.V. & ALTURA, B.M. (1980). Magnesium deficiency produces spasms of coronary arteries: relationship to etiology of sudden death ischemic heart disease. *Science*, **208**, 198–200.

WAKABAYASHI, S. & GOSHIMA, K. (1981). Kinetic studies on sodium-dependent calcium uptake by myocardial cells and neuroblastoma cells in culture. *Biochim. Biophys. Acta*, **642**, 158–172.

WILLIAMS, J.K., ADAMS, M.R. & KLOPFENSTEIN, H.S. (1990). Estrogen modulates responses of atherosclerotic coronary arteries. *Circulation*, **81**, 1680–1687.

WINQUIST, R.J., BUNTING, P.B., BASKING, E.P. & WALLACE, A.A. (1984). Decreased endothelium-dependent relaxation in New Zealand genetic hypertensive rats. *J. Hypertens.*, **2**, 541–545.

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