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Endothelin-1-induced contraction in isolated aortae from normotensive and DOCA-salt hypertensive rats: effect of magnesium

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1 The contractile responses to endothelin-1 and the effect on these of various magnesium concentrations, were studied in isolated aortic rings from normotensive Sprague-Dawley rats and deoxycorticosterone acetate-salt (DOCA-salt) hypertensive rats.

2 Contractions induced by endothelin-1 were smaller in endothelium-denuded aortae from DOCA-salt hypertensive rats than in those from normotensive rats. The absence of calcium in the medium attenuated endothelin-1-induced contractions of aortae from both normotensive and DOCA-salt rats, but the contraction was greater in aortae from DOCA-salt hypertensive rats. Ryanodine (which inhibits the release of intracellular calcium) inhibited endothelin-1-induced contractions in aortae from DOCA-salt hypertensive rats to a greater extent than in aortae from normotensive rats.

3 A high extracellular magnesium concentration (4.8 mM) attenuated endothelin-1-induced contractions in tissues from DOCA-salt hypertensive rats but not in tissues from normotensive rats. In the absence of calcium, a high concentration of magnesium attenuated endothelin-1-induced contraction in aortae from both normotensive and hypertensive rats. In the presence of ryanodine, a high concentration of magnesium did not modify the contraction in preparations from either strain.

4 Absence of magnesium attenuated endothelin-1-induced contractions in aortae from both normotensive and DOCA-salt hypertensive rats. In the absence of calcium, removal of magnesium totally inhibited endothelin-1-induced contraction in tissues from normotensive rats but had no effect in those from hypertensive rats. In the presence of ryanodine, the lack of magnesium inhibited endothelin-1-induced contractions in aortae from DOCA-salt hypertensive rats but increased the sensitivity to endothelin-1 of aortae from normotensive rats.

5 The presence of endothelium did not modify the effect of high magnesium on endothelin-1-induced contractions in aortae from normotensive and DOCA-salt hypertensive rats. Conversely, the attenuating effect of magnesium removal on endothelin-1-induced contractions did not occur when endothelium was present.

6 In conclusion, endothelin-1-induced contraction was blunted in aortae from DOCA-salt hypertensive rats. The blunted response was related to altered calcium utilization during contraction. Changes in extracellular magnesium concentration differentially alter endothelin-1-induced contraction in aortae from normotensive and hypertensive rats, possibly by interfering with calcium utilization during contraction. Magnesium may be required for the contractile response to endothelin-1 and increasing magnesium may limit the vascular effects of endothelin-1 in blood vessels from DOCA-salt hypertensive rats.

Keywords: Mineralocorticoid-salt hypertension; calcium; contractility; vascular smooth muscle; endothelium

Introduction

Endothelin-1 is a potent vasoconstrictor peptide synthesized and released by the vascular endothelium (Yanagisawa *et al.*, 1988). It acts on underlying smooth muscle cells where it can bind to specific endothelin type A (ET_A) and endothelin type B (ET_B) receptors (Lüscher *et al.*, 1993). Both receptors subtypes on muscle cells mediate vasoconstriction through intracellular calcium signalling pathways (Marsden *et al.*, 1989; Touyz *et al.*, 1995). Endothelin-1 also interacts with endothelial ET_B receptors, which induces the synthesis and release of nitric oxide and prostacyclin and mediates vasodilatation (De Nucci *et al.*, 1988). In addition, endothelin-1 exerts mitogenic and hypertrophic effects on vascular smooth muscle cells (Hirata *et al.*, 1989; Bobik *et al.*, 1990).

The possible role of endothelin-1 in the pathogenesis of hypertension has received increasing attention (for review see Schiffrin, 1995). In human essential hypertension, studies show that circulating levels of endothelin-1 are normal or very slightly increased (Davenport *et al.*, 1990; Kohno *et al.*, 1990; Shichiri *et al.*, 1990; Schiffrin & Thibault, 1991). In spontaneously hypertensive rats, endothelin immunoreactivity and gene expression in blood vessels are not modified (Lariviere *et al.*, 1993b; 1995). Mineralocorticoid-salt hypertension in rats is characterized by increased plasma endothelin-1 concentration and, increased immunoreactive endothelin-1 content and gene expression in vascular endothelial cells (Lariviere *et al.*, 1993a,b). Chronic administration of the combined ET_A/ET_B receptor antagonist, bosentan, attenuates the development of mineralocorticoid-salt hypertension (Li *et al.*, 1994). All these findings suggest that endothelin-1 may be involved in the development and maintenance of high blood pressure in deoxycorticosterone acetate-salt (DOCA-salt) hypertensive rats.

Numerous experimental studies have shown that changes in extracellular magnesium concentration affect blood flow, vascular reactivity and blood pressure. Lowering the extracellular magnesium concentration enhances *in vitro* vascular tone and the contractile response to various agonists, whereas increasing the extracellular magnesium concentration exerts opposite effects (Altura & Altura, 1990). The mechanisms by which

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magnesium exerts these vascular effects are not fully understood. Calcium ions play an essential role in the contraction of vascular smooth muscle cells and consequently in the modulation of vascular contractility (Rembold, 1992). It seems now established that the effects of magnesium are related, at least in part, to the natural calcium-antagonistic properties of magnesium, since magnesium ions regulate calcium flux across vascular smooth muscle cell membranes and calcium release from intracellular storage sites (Turlapaty & Altura, 1978; Altura *et al.*, 1987; Zhang *et al.*, 1992). The possible role of magnesium in the pathogenesis and treatment of hypertension has received increasing attention. Intracellular free magnesium concentration is low in clinical and experimental hypertension (Resnick *et al.*, 1984; Ng *et al.*, 1992). Magnesium deficiency in rats induces hypertension, reduces blood vessel lumen (Altura *et al.*, 1984) and increases plasma endothelin concentration (Weglicki *et al*, 1992). Magnesium infusion induces vasodilatation (Ji *et al.*, 1983), and recent studies report that it strongly inhibits endothelin-1-induced vasoconstriction (Kemp *et al.*, 1993). Oral magnesium supplementation attenuates the development of hypertension, particularly in DOCA-salt hypertensive rats (Laurant *et al.*, 1995). Although the mechanisms whereby magnesium exerts its beneficial action in DOCA-salt hypertensive rats are not well known, magnesium effects may be related to physiopathological changes in vascular function (Laurant *et al.*, 1995). Since endothelin-1 may be involved in mineralocorticoid-salt hypertension (Schiffin, 1995), the following study was conducted to examine endothelin-1-induced contraction and the effects of various concentrations of magnesium in isolated aortae from normotensive and DOCA-salt hypertensive rats.

Methods

Animals and experimental setup

Eighteen Sprague Dawley male rats (5 weeks old) (Iffa Credo, L'Arbresles, France) were randomized into two groups. All animals received care that was in compliance with guidelines from the French Ministry of Agriculture. One group was made hypertensive under ether anaesthesia by thoracic subcutaneous pellets implantation of 100 mg DOCA and by administration of 0.9% NaCl in distilled water as drinking water. The normotensive control group was sham-operated. Rats were housed in stainless steel cages at a constant temperature of 23°C, constant humidity (50–60%) and a daily 12 h light-dark cycle. Systolic blood pressure was measured by the tail-cuff method utilizing a sphygmomanometer (PE-300 Narco Biosystem, Houston, TX, U.S.A.) in unanaesthetized prewarmed rats once a week and 24 h before the animals were killed.

Eight weeks after surgery, the rats were killed by decapitation and exsanguinated, and the thoracic aortae were excised, cleaned of connective tissue and cut into rings (2 mm in length). In some rings, the endothelium was removed by gently rubbing the intimal surface with small forceps. Care was taken not to touch the inner surface of the remaining rings. Rings were then suspended between two L-shaped stainless steel hooks and placed in 10 ml water-jacketed tissue bath filled with physiological salt solution (PSS) of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 5.5. The solution was maintained at 37°C, aerated with 95% O_2, 5% CO_2 to give a pH of 7.4 (Laurant *et al*, 1995). The upper hook was connected to a force displacement transducer (F-60 myograph, Narco Biosystem). Aortic rings were stretched to obtain a passive force of 2 g (determined to be optimal in preliminary length-tension experiments) and then allowed to equilibrate for 2 h. During this incubation period, the medium was changed every 15 min. The rings were then exposed to noradrenaline (1 μM). The integrity of the endothelium was checked by testing the vasorelaxant response to acetylcholine (1 μM) of aortic rings pre-contracted with noradrenaline (0.1 μM).

Experimental protocol

Rings with and without endothelium were incubated in PSS containing normal (1.2 mM), high (4.8 mM), or no (0 mM) extracellular magnesium concentrations for 60 min. A cumulative concentration-response curve to endothelin-1 was then generated. Some rings without endothelium were incubated in PSS containing either 0, 1.2, 4.8 mM magnesium for 45 min. They were then exposed to calcium-free PSS for 15 min, with the same magnesium concentrations, prior to establishing cumulative concentration-response curves to endothelin-1. Calcium-free PSS was made by removing $CaCl_2$ from PSS and adding ethyleneglycol *bis*(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA) (0.5 mM). Some rings without endothelium were incubated in PSS containing 0, 1.2, 4.8 mM magnesium plus ryanodine (1 μM, which inhibits intracellular calcium release without affecting extracellular calcium influx (Ito *et al.*, 1986), added 30 min prior to establishing cumulative concentration-response curves to endothelin-1.

Drugs

The following drugs were used: noradrenaline bitartrate (arterenol), acetylcholine chloride, endothelin-1, ryanodine (all from Sigma Chemical Co). All drugs were prepared in Krebs-Henseleit solution immediately before use.

Data and statistical analysis

Values are presented as mean ± s.e.mean. The concentration of the agonists causing half-maximal contraction was calculated for each experiment and expressed as negative log molar (pD_2 value). The pD_2 values and E_{max} values (values of maximal contraction) were determined by fitting the original dose-response curves using the Graphad Inplot program (Graphad Software Inc., version 1992). Statistical evaluation of the data was performed by two-way analysis of variance (2-way ANOVA) for repeated-measures to compare the concentration-response curves to endothelin-1. Two-way ANOVA and a *post-hoc* Student-Newman-Keuls test were used to compare pD_2 values and E_{max} values. A P value less than 0.05 was considered statistically significant. In all experiments, n equals the number of rats.

Results

Blood pressure

Eight weeks after the operative intervention the systolic blood pressures were 141 ± 6 mmHg and 224 ± 8 mmHg for normotensive and DOCA-salt hypertensive rats, respectively ($P<0.001$).

Effect of DOCA-salt hypertension on endothelin-1-induced contractions

After the equilibration period, the aortic rings were contracted with 1 μM noradrenaline. Contractile responses to noradrenaline were similar in normotensive and DOCA-salt hypertensive rats (1.76 ± 0.11 vs 1.68 ± 0.09 g) as it has been previously shown (Nguyen *et al.*, 1992; Laurant *et al.*, 1995).

Cumulative addition of endothelin-1 caused concentration-dependent contractile responses of the aortic rings which developed slowly. When endothelium was present, the contractile responses to endothelin-1 were significantly less pronounced in the aortic rings from DOCA-salt hypertensive rats ($P<0.001$) (Figure 1a). The maximal response (E_{max} value) to endothelin-1 was significantly depressed (1.49 ± 0.13 vs 2.38 ± 0.10 g; $P<0.001$) whereas the sensitivity (pD_2 value) to endothelin-1 was significantly increased (8.65 ± 0.06 vs 8.27 ± 0.04; $P<0.05$) in aortic rings from DOCA-salt hypertensive rats than in those from normotensive rats. Removal of endothelium significantly

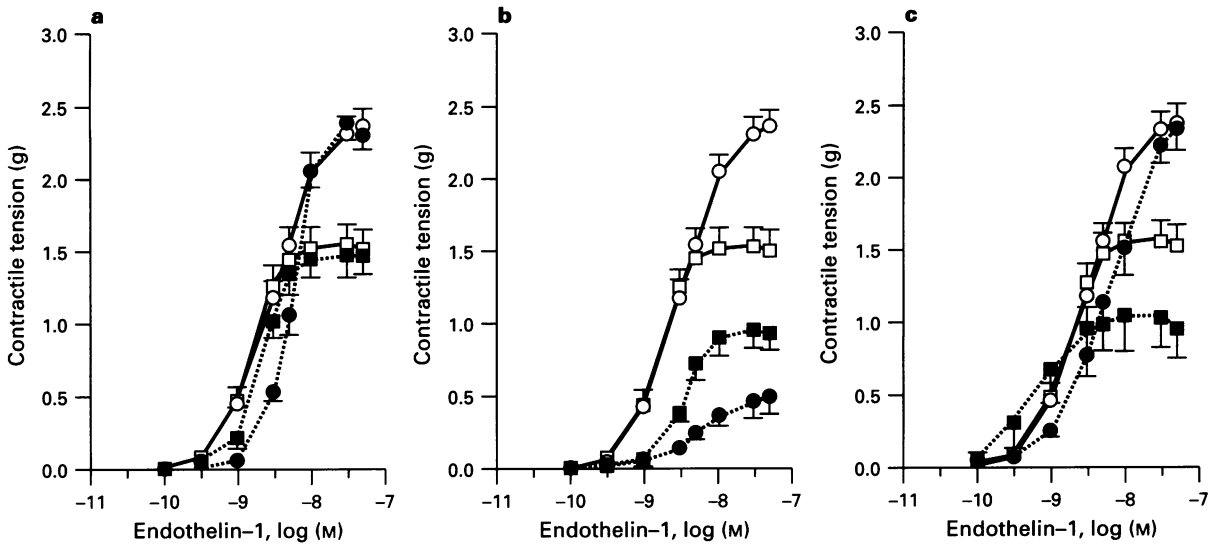

Figure 1 Concentration-response curves to endothelin-1 (a) in isolated aortae without (○) or with (●) endothelium from normotensive rats and in isolated aortae without (□) or with (■) endothelium from DOCA-salt hypertensive rats, (b) in isolated aortae with disrupted endothelium from normotensive rats in the absence (○) or presence of calcium-free PSS (●) and from DOCA-salt hypertensive rats in the absence (□) or presence of calcium-free PSS (■); (c) in isolated aortae with disrupted endothelium from normotensive rats in the absence (○) or presence of ryanodine (●) and from DOCA-salt hypertensive rats in the absence (□) or presence of ryanodine (■). $n=9$ rats for each group.

increased the pD_2 values in aortic rings from normotensive rats but not in those from hypertensive rats (8.68 ± 0.05 vs 8.27 ± 0.04, $P<0.05$ in normotensive; 8.82 ± 0.05 vs 8.65 ± 0.06 in DOCA-salt). Removal of endothelium did not modify the magnitude of endothelin-1-induced contractions in tissues from either strain. The contractile responses to endothelin-1 remained significantly less pronounced in aortae with disrupted endothelium from hypertensive rats than in those from normotensive rats ($P<0.05$) (Figure 1a).

Calcium-free PSS significantly depressed endothelin-1-induced contractions in endothelium-denuded aortae from both normotensive and hypertensive rats (Figure 1b). The contractile responses to endothelin-1 in calcium-free PSS were, however, greater in aortae from DOCA-salt hypertensive rats than in tissues from normotensive rats ($P<0.05$). Calcium-free PSS significantly interfered with the effect of DOCA-salt hypertension on endothelin-1-induced contraction (two-way ANOVA, $P<0.05$). Ryanodine significantly decreased the endothelin-1-induced contractions in endothelium-denuded aortae from both normotensive and DOCA-salt hypertensive rats ($P<0.05$) (Figure 1c). Ryanodine significantly decreased the pD_2 values (8.26 ± 0.08 vs 8.68 ± 0.05; $P<0.05$) without affecting the E_{max} values to endothelin-1 in aortae from normotensive rats. Ryanodine significantly decreased the E_{max} values to endothelin-1 in aortae from DOCA-salt hypertensive rats (1.06 ± 0.15 vs 1.54 ± 0.12 g; $P<0.05$). In the presence of ryanodine, endothelin-1-induced contractions were significantly more decreased in aortae from hypertensive rats than in those from normotensive rats (2-way-ANOVA; $P<0.001$) (Figure 1c).

Effects of magnesium on endothelin-1-induced contractions of isolated endothelium-denuded aortae

High magnesium did not modify endothelin-1-induced contractions in aortae from normotensive rats, whereas it significantly attenuated those in aortae from DOCA-salt hypertensive rats ($P<0.05$) (Figure 2a). High magnesium significantly decreased the pD_2 values in hypertensive rats (8.46 ± 0.04 vs 8.82 ± 0.05, $P<0.01$) whereas the E_{max} values were not modified. Thus, DOCA-salt treatment significantly interfered with the effect of high magnesium on endothelin-1-induced contractions (two-way ANOVA, $P<0.05$). In the presence of calcium-free PSS, high magnesium significantly depressed endothelin-1-induced contractions in aortae from both normotensive and hypertensive rats ($P<0.05$) (Figure 2b). In the presence of ryanodine, high magnesium did not modify endothelin-1-induced contractions in either normotensive or DOCA-salt hypertensive rats (Figure 2c).

Magnesium withdrawal from the medium significantly inhibited endothelin-1-induced contractions in aortae from both normotensive and hypertensive rats ($P<0.01$) (Figure 3a). Magnesium withdrawal significantly decreased the E_{max} values to endothelin-1 (1.36 ± 0.21 vs 2.45 ± 0.16 g in normotensive, $P<0.01$; 1.10 ± 0.15 vs 1.54 ± 0.15 g in DOCA-salt, $P<0.05$), whereas it significantly increased the pD_2 values only in normotensive rats (8.90 ± 0.04 vs 8.68 ± 0.05, $P<0.05$). In the presence of calcium-free PSS, magnesium withdrawal totally inhibited the contractile responses to endothelin-1 in aortae from normotensive rats, but had no effect on the responses of aortae from DOCA-salt hypertensive rats (Figure 3b). In the presence of ryanodine, magnesium withdrawal strongly depressed endothelin-1-induced contractions in aortic rings from DOCA-salt hypertensive rats ($P<0.05$) whereas it significantly increased the pD_2 values (8.65 ± 0.08 vs 8.27 ± 0.07, $P<0.05$) and attenuated the E_{max} values to endothelin-1 of rings from normotensive rats (1.89 ± 0.09 vs 2.30 ± 0.14 g, $P<0.05$) (Figure 3c)

Effects of magnesium on endothelin-1-induced contractions of isolated aortae with endothelium

Endothelin-1-induced contractions remained attenuated in the presence of high magnesium in aortae from DOCA-salt hypertensive rats when endothelium was present (Figure 4). High magnesium significantly decreased the pD_2 values in aortic rings from DOCA-salt hypertensive rats (8.44 ± 0.04 vs 8.74 ± 0.06, $P<0.05$), whereas it did not modify the E_{max} values. The presence of endothelium did not significantly interfere with the effect of high magnesium on sensitivity to endothelin-1 in rings from DOCA-salt hypertensive rats. In aortae from normotensive rats, endothelin-1-induced contractions were unaffected by high magnesium even when the endothelium was present (Figure 4).

When the endothelium was present, the attenuation of the endothelin-1 response caused by magnesium withdrawal in

aortae from both normotensive and DOCA-salt hypertensive rats was abolished, and the pD_2 and E_{max} values to endothelin-1 were not significantly modified. The presence of endothelium significantly interfered with the effect of magnesium withdrawal on endothelin-1-induced contractions in tissues from both groups of rats (two-way ANOVA, $P<0.05$) (Figure 4).

Discussion

The results of the present study show that the aortae from DOCA-salt hypertensive rats exhibited reduced contractions in response to endothelin-1, independently of the presence of endothelium. This reduction in response appears related to diminished extracellular calcium flux across the vascular membrane. Changes in magnesium concentration differentially altered contractions to endothelin-1 in isolated aortae from normotensive and DOCA-salt hypertensive rats. This study also showed that magnesium may interfere with calcium utilization during endothelin-1-induced contractions.

Endothelin-1 induces contractile responses in vascular smooth muscle cells through specific receptors linked to G protein-coupled phospholipase C activation. The activation of phospholipase C and the breakdown of membrane phosphoinositides (leading to the release of inositol triphosphate and diacylglycerol) results in an increase in intracellular free calcium due to the release of stored calcium from the sarcoplasmic reticulum, and in the activation of protein kinase C (Griendling *et al.*, 1989; Marsden *et al.*, 1989). Endothelin-1 induces contraction also by promoting calcium influx in response to a membrane potential change, by increasing the probability of the opening of voltage-operated calcium chan-

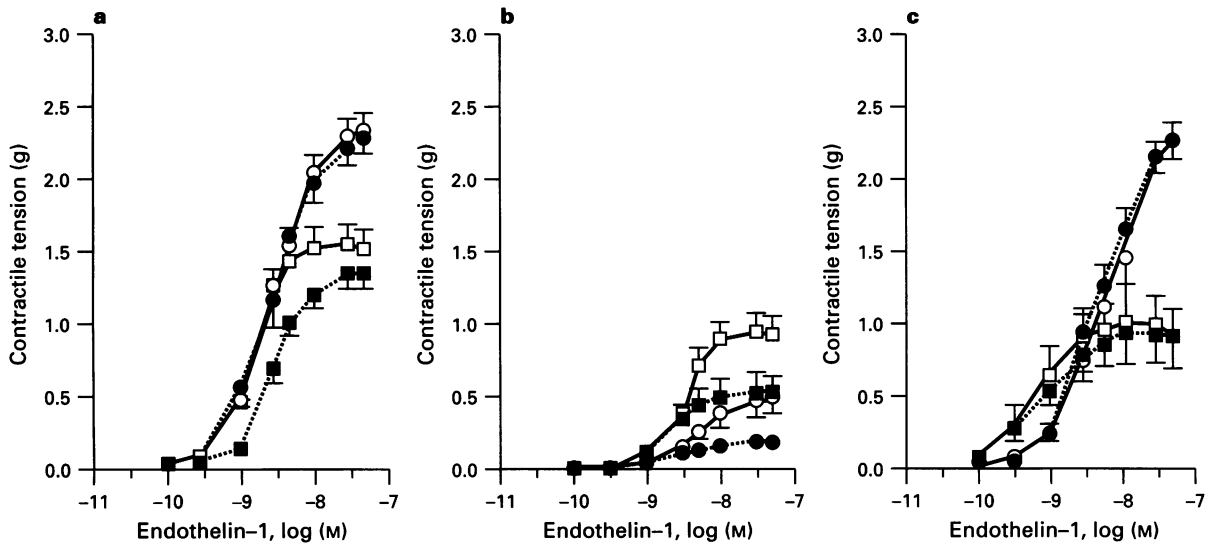

Figure 2 Concentration-response curves to endothelin-1 (a) in resting condition, (b) in calcium-free PSS and, (c) in presence of ryanodine, in isolated aortae with disrupted endothelium from normotensive rats bathed in PSS containing 1.2 mM (○) or 4.8 mM (●) magnesium and from DOCA-salt hypertensive rats bathed in PSS containing 1.2 mM (□) or 4.8 mM (■) magnesium. $n=9$ rats for each group.

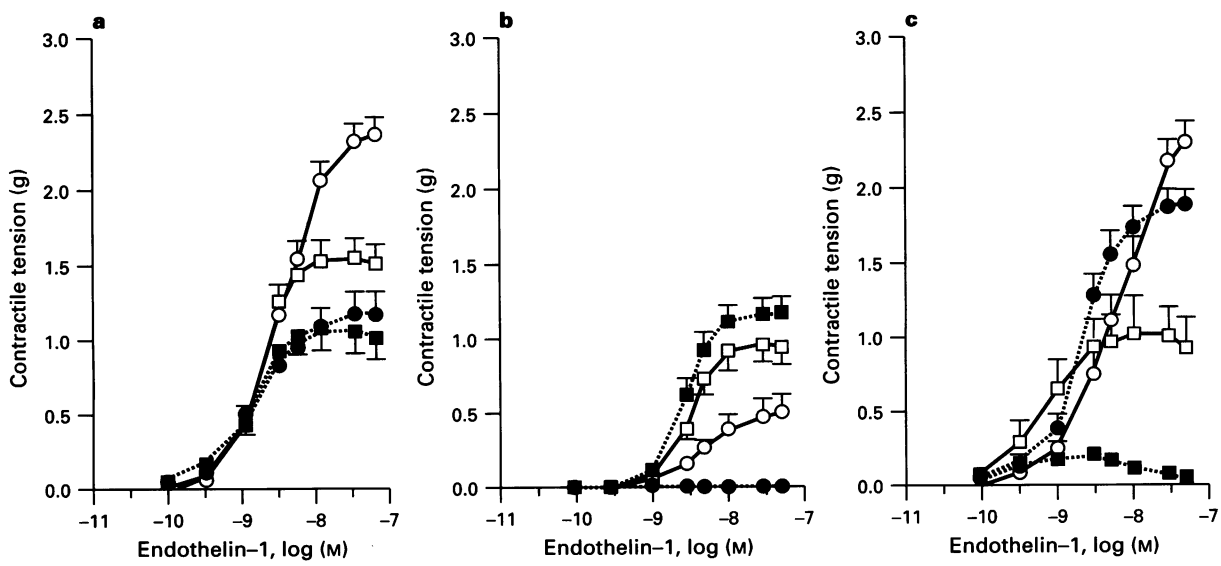

Figure 3 Concentration-response curves to endothelin-1, (a) in resting condition, (b) in calcium-free PSS and, (c) in presence of ryanodine, in isolated aortae with disrupted endothelium from normotensive rats bathed in PSS containing 1.2 mM (○) or 0 mM (●) magnesium and from DOCA-salt hypertensive rats bathed in PSS containing 1.2 mM (□) or 0 mM (■) magnesium. $n=9$ rats for each group. Symbols indicate means ± s.e.mean.

nels (Goto *et al.*, 1989). The present findings in aortae from normotensive rats support this concept, since contractions to endothelin-1 were attenuated in calcium-free PSS or in the presence or ryanodine. Ryanodine blocks the release of the intracellular calcium from calcium stores induced by inositol triphosphate without affecting the influx of extracellular calcium into vascular smooth muscle cells (Ito *et al.*, 1986). Therefore, ryanodine is a selective tool to eliminate the contribution of intracellular calcium release. Although the sensitivity to endothelin-1 was decreased in the presence of ryanodine, the maximal contraction of aortae from normotensive rats was not affected. These findings are in agreement with previous reports indicating that ryanodine inhibits contractions when agonist concentrations are low, whereas contractions elicited by high concentrations of the agonist are not affected (Kanmura *et al.*, 1988).

The finding that contractions induced by endothelin-1 are blunted in DOCA-salt hypertensive rats has been previously shown in aortae and mesenteric arteries (Deng & Schiffrin, 1992; Nguyen *et al.*, 1992). Decreased endothelin-1 binding

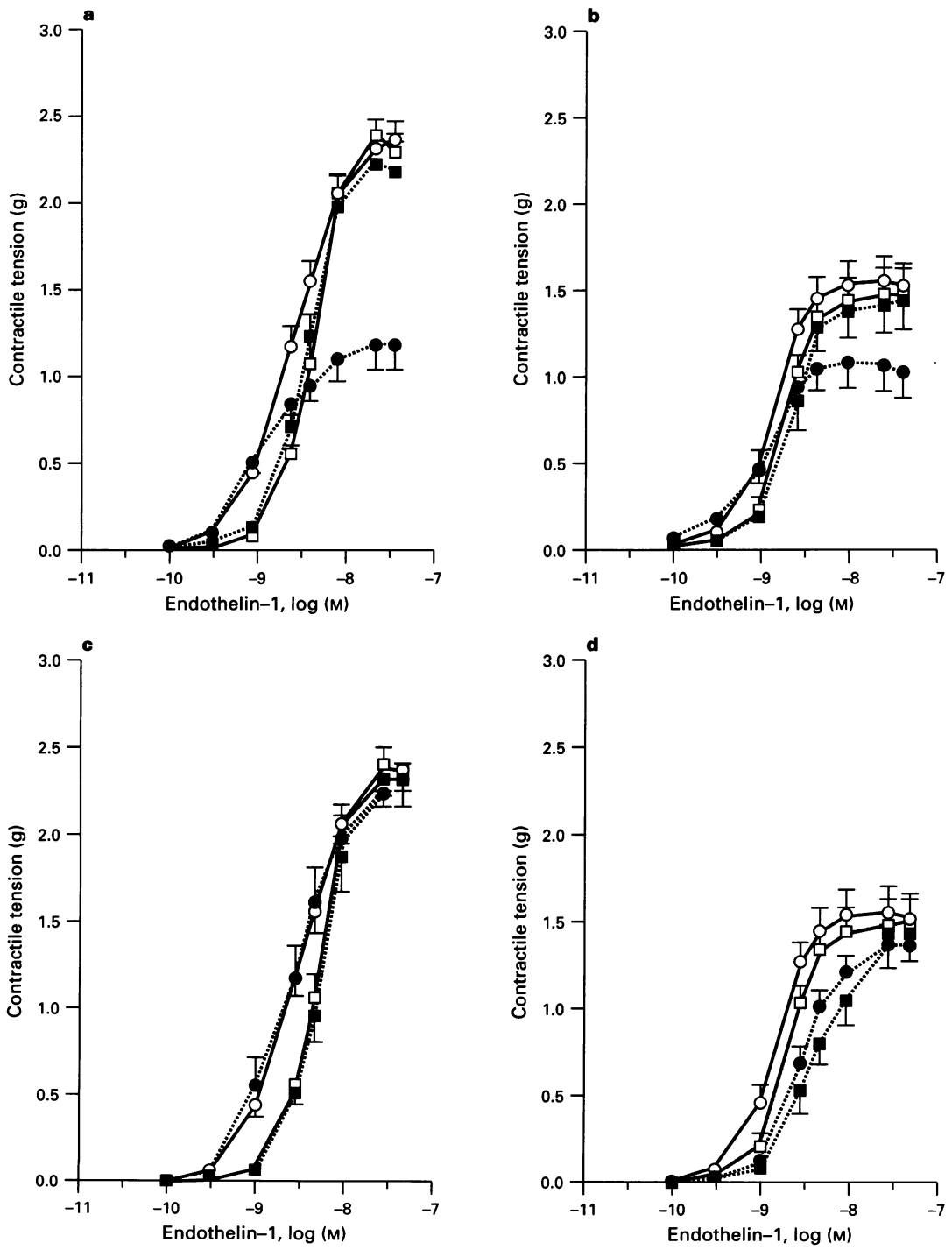

Figure 4 Concentration-response curves to endothelin-1 in isolated aortae from normotensive rats (a, c) and DOCA-salt hypertensive rats (b, d). (a, b) Concentration-response curves to endothelin-1 in isolated aortae without endothelium bathed in PSS containing 1.2 mM (○) or 0 mM (●) magnesium and in isolated aortae with endothelium bathed in PSS containing 1.2 mM (□) or 0 mM (■) magnesium. (c, d) Concentration-response curves to endothelin-1 in isolated aortae without endothelium bathed in PSS containing 1.2 mM (○) or 4.8 mM (●) magnesium and in isolated aortae with endothelium bathed in PSS containing 1.2 mM (□) or 4.8 mM (■) magnesium. $n=9$ for each group. Symbols indicate means ± s.e.mean.

sites and decreased activation of phospholipase C, with decreased production of inositol phosphates may explain the blunted contraction in tissues from DOCA-salt hypertensive rats with 2–3 weeks of developing hypertension (Fukuda *et al.*, 1988; Nguyen *et al.*, 1992). In other studies, however, phosphoinositide breakdown has been shown to be enhanced in response to endothelin-1 in mesenteric arteries and atria from DOCA-salt hypertensive rats (De Champlain *et al.*, 1989). In the present study, although the contractile response to endothelin-1 was reduced in hypertensive rats, contraction was increased in calcium-free PSS. These findings suggest that endothelin-1-induced calcium release from intracellular calcium sites storage is greater in aortae from DOCA-salt hypertensive rats than in those from normotensive rats. In agreement with our results, recent studies report that the contraction elicited by calcium release from the sarcoplasmic reticulum using 5-hydroxytryptamine or caffeine was greater in aortae from DOCA-salt hypertensive rats than in normotensive rats, suggesting an enlargement of intracellular calcium stores in DOCA-salt hypertension (Tostes *et al.*, 1995). However, in the presence of ryanodine endothelin-1-induced contractions were significantly depressed in aortae from DOCA-salt hypertensive rats. Thus, the blunted response to endothelin-1 seen in aortae from DOCA-salt hypertensive rats may be related to an alteration of endothelin-1-induced calcium entry into smooth muscle cells. Recently, it has been reported that the relative increase cytosolic free calcium concentration induced by endothelin-1 is lower in mesenteric arteries from DOCA-salt hypertensive rats than in normotensive rats (Flückiger *et al.*, 1992). Although the reasons for this are unclear, they could be related to changes in intracellular calcium metabolism. DOCA-salt hypertension is characterized by increased intracellular total and free calcium concentrations (Fukuda *et al.*, 1988; Flückiger *et al.*, 1992) and altered activity of membrane calcium-ATPase (Touyz *et al.*, 1991). It has been shown that calcium-induced contraction is attenuated in endothelium-denuded mesenteric arteries from DOCA-salt hypertensive rats, suggesting that the calcium handling capability of the vascular smooth muscle membrane is perturbed in DOCA-salt hypertensive rats (Mäkynen *et al.*, 1994). The reduced response to endothelin-1 in DOCA-salt hypertensive rats may also be explained by the fact that increased intracellular calcium levels exert a negative feedback on endothelin-1-induced calcium entry (Muldoon *et al.*, 1991).

Increasing magnesium concentration attenuates the responses *in vitro* to many contractile agents by decreasing agonist-receptor affinity and reducing calcium influx, binding, and translocation in vascular smooth muscle (Altura *et al.*, 1987; Altura & Altura, 1990). In the present study, however, high magnesium concentration did not change the response to endothelin-1 in aortae (with or without endothelium) prepared from normotensive rats. These findings are in agreement with those of previous reports (Torregrosa *et al.*, 1994). It is not clear why magnesium does not inhibit the contractile response to endothelin-1 of vessels from normotensive rats if magnesium possesses natural calcium antagonistic properties. Magnesium is able to block both voltage- and receptor-operated calcium channels, to inhibit, in a dose-dependent manner, inositol triphosphate-induced calcium release from the sarcoplasmic reticulum, and to stimulate calcium sequestration into the sarcoplasmic reticulum (Altura *et al.*, 1987; Meissner & Henderson, 1987; Volpe *et al.*, 1990). In our study, a high concentration of magnesium inhibited endothelin-1-induced contractions in aortae from normotensive rats incubated in calcium-free PSS, but did not change them when stored intracellular calcium release was inhibited by ryanodine. Although magnesium inhibits endothelin-1-induced intracellular calcium release, an attenuated contraction did not occur in tissues from normotensive rats. Although further studies are needed to clarify the effects of high magnesium on the events leading to endothelin-1-induced contraction in aortae from normotensive rats, our findings suggest that magnesium attenuates signalling and this inhibiting effect would be suppressed by calcium influx.

In the present study, a high concentration of magnesium significantly attenuated endothelin-1-induced contractions, in the presence or absence of endothelium, in aortae from DOCA-salt hypertensive rats. Furthermore, the inhibitory effect of high magnesium on contraction may be explained by magnesium inhibiting endothelin-1-induced intracellular calcium release from calcium stores. It is not clear why a high concentration of magnesium affects contractile responses to endothelin-1 in aortae from DOCA-salt hypertensive rats but not in tissues from normotensive rats. These findings may be related to altered cellular events which result in abnormal contractile function in DOCA-salt hypertension (Fukuda *et al.*, 1988; Deng & Schiffrin, 1992; Nguyen *et al.*, 1992). Although the mechanisms by which a high magnesium concentration attenuates endothelin-1-induced contraction in aortae from DOCA-salt hypertensive rats remain unclear, magnesium-linked changes in intracellular signalling pathways and/or altered cellular magnesium/calcium interactions in vascular smooth muscle cells may play a role.

Although contractions induced by endothelin-1 were blunted in aortae from DOCA-salt hypertensive rats, the sensitivity to endothelin-1 was enhanced. Previous studies have shown that endothelin-1 may act to elevate blood pressure via action at both ET_A and ET_B receptors (Schiffrin, 1995). Endothelin-1 exerts mitogenic and hypertrophic effects on vascular smooth muscle cells (Hirata *et al.*, 1989; Bobik *et al.*, 1990). Recently, it has been shown that oral treatment with the combined ET_A/ET_B antagonist, bosentan, attenuated blood pressure elevation, and abolished vascular hypertrophy of arteries in DOCA-salt hypertensive rats, suggesting that endothelin-1 is involved in the pathogenesis of hypertension and vascular hypertrophy in this hypertensive model (Li *et al.*, 1994). In the present study, a high concentration of magnesium decreased the sensitivity to endothelin-1 of aortae (with and without endothelium) from DOCA-salt hypertensive rats, indicating that high magnesium reduces the affinity of endothelin-1 for its specific receptors. Since there is no evidence for the presence of ET_B receptors in rat aorta (Sumner *et al.*, 1992; Warner *et al.*, 1993), it is speculated that high magnesium reduces the affinity of endothelin-1 for ET_A receptors. These findings could be of pathophysiological importance in the development and maintenance of high blood pressure in DOCA-salt hypertension. Dietary magnesium supplementation inhibits the development of DOCA-salt hypertension in rats, at least in part by a vascular action (Laurant *et al*, 1995). The precise mechanisms underlying this effect are not known but the present findings suggest that increased extracellular magnesium, by reducing the affinity of endothelin-1 for its receptors, may decrease its vasoconstrictor and hypertrophic effects in DOCA-salt hypertensive rats.

In the present study, magnesium withdrawal attenuated contractions induced by endothelin-1 in endothelium-denuded aortae from both normotensive and DOCA-salt hypertensive rats. It is generally reported that decreasing the magnesium concentration in PSS enhances the contractions induced by many agonists (Altura *et al.*, 1987; Altura & Altura, 1990). However, some studies such as the present one, demonstrate that complete absence of magnesium inhibits the vascular response to several agonist such as acetylcholine, adrenaline or vasopressin (Altura & Altura, 1971; 1987; Altura, 1974), suggesting that magnesium may be important in the expression of the vascular response to these agonists. The present findings suggest that attenuated responses to endothelin-1 induced by magnesium withdrawal are related to inhibition of endothelin-1-induced intracellular calcium release in aortae from normotensive rats. Thus, the events leading to endothelin-1-induced intracellular calcium release in aortae from normotensive rats require the presence of magnesium. However, in DOCA-salt hypertensive rats magnesium was not necessary for endothelin-1-induced intracellular calcium release, whereas it was required for endothelin-1-induced calcium entry into smooth muscle cells. The reasons for this alteration in endothelin-1-induced contractions in aortae from DOCA-salt hypertensive rats in

the absence of magnesium are not clear. Although pathophysiological changes in vascular events leading to endothelin-1 responses in DOCA-salt hypertension may be involved, further studies are needed to understand the cellular mechanisms whereby magnesium withdrawal alters endothelin-1-induced contraction in the vasculature of DOCA-salt hypertensive rats.

The present study also demonstrated that the inhibitory effect of magnesium withdrawal on endothelin-1 contractions was absent when endothelium was present in aortae from both normotensive and hypertensive rats, indicating that endothelium may generate and release endothelial-derived contracting factors in response to magnesium withdrawal. These, in turn, may improve the attenuated contractile response to endothelin-1. Endothelium synthesizes and releases endothelium-derived relaxant factors such as nitric oxide and prostacyclin, and endothelial-derived contracting factors such as endothelins and cyclo-oxygenase metabolites (Vane *et al.*, 1990). Some studies have described relationships between magnesium and endothelium. Calcium antagonistic properties of magnesium have been reported in the formation and/or release of endothelium-derived relaxing factor in various arteries (Ku & Ann, 1987; Gold *et al.*, 1990). The endothelium may modulate the effects of magnesium on vascular tone and reactivity (Farago *et al.*, 1991; Ku & Ann, 1991; Laurant & Berthelot, 1994). Furthermore, magnesium deficiency has been shown to increase the production and release of endothelin and cyclo-oxygenase metabolites in rats (Nigam *et al.*, 1986; Soma *et al*, 1988; Weglicki *et al.*, 1992).

In conclusion, the blunted response to endothelin-1 in aortae from DOCA-salt hypertensive rats was related to altered calcium utilization during contraction. Varying the extracellular magnesium concentration affected differentially contractions induced by endothelin-1 in aortae from normotensive and DOCA-salt hypertensive rats. Whereas magnesium may be required for the vascular responsiveness to endothelin-1, our findings suggest that increasing magnesium may be beneficial in limiting the vascular effects of endothelin-1 in blood vessels in DOCA-salt hypertension.

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