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Reactivity and sensitivity of mesenteric vascular beds and aortic rings of spontaneously hypertensive rats to endothelin: effects of calcium entry blockers

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1 The vasoconstrictor effects of endothelin-1 were studied in perfused mesenteric vascular beds (MVB) and aortic rings of 14–16 week-old spontaneously hypertensive rats (SHR) and age-matched Wistar Kyoto rats (WKY).

2 Reactivity to endothelin-1 was increased in MVBs of SHR, as indicated by the maximum perfusion pressure obtained (264 ± 8 and 141 ± 9 mmHg respectively) ($P < 0.001$), whereas sensitivity was not significantly different between the two strains (EC_{50} 171 ± 21 and 102 ± 19, respectively).

3 In aortic rings, in constrast, reactivity to endothelin-1 was reduced in SHR as compared to WKY, whereas sensitivity was similar (EC_{50} 0.78 ± 0.08 and 0.87 ± 0.09 nM).

4 As with endothelin-1, reactivity to noradrenaline and potassium chloride was increased in MVBs, but not in aortic rings of SHR. Endothelin-1 was 30 times more potent than noradrenaline in MVBs of SHR, and 15 times more potent than noradrenaline in aortic rings.

5 In both strains, nifedipine and nitrendipine almost completely blocked potassium-induced contractions in MVB and aortic rings, respectively, whereas contractions induced by endothelin-1 or noradrenaline were only partially inhibited.

6 It is concluded that calcium influx via the voltage-operated calcium channel is only partially responsible for the vasoconstrictor action of endothelin-1 in MVBs and aortic rings of SHR and WKY rats. The increased reactivity of the MVB of SHR to endothelin-1 at this stage of the hypertensive process is most likely to be the result of a change in vascular structure rather than due to a primary hypertensive mechanism.

Introduction

Since the original demonstration of the obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine (Furchgott & Zawadzki, 1980), the vascular endothelium has been recognized as playing an important role in vascular homeostasis (Huttner & Gabbiani, 1983; Gryglewski *et al.*, 1988; Luescher, 1988). It has also been shown that bovine cultured aortic and pulmonary endothelial cells release vasoconstrictor peptide(s), in addition to relaxant agents, into culture medium (Hickey *et al.*, 1985; Gillespie *et al.*, 1986). Yanagisawa *et al.* (1988) isolated a peptide, which they termed endothelin, from the supernatant of porcine cultured endothelial cells and determined its amino acid sequence. Porcine endothelin (endothelin-1) has been characterized as a very potent vasoconstrictor in a variety of vessels of different species including man (Yanagisawa *et al.*, 1988). *In vitro*, the vasoconstriction induced by endothelin-1 is long-lasting, difficult to wash out (Yanagisawa *et al.*, 1988; Tomobe *et al.*, 1988) and strongly dependent on extracellular calcium.

The observation that nicardipine, a dihydropyridine-type calcium entry blocker, attenuated the vasoconstriction induced by this peptide, led to the suggestion that endothelin may be an endogenous agonist of the dihydropyridine-sensitive calcium channels (Yanagisawa *et al.*, 1988). However, subsequent studies have shown the existence of specific endothelin binding sites, independent of the nicardipine binding sites (Hirata *et al.*, 1988), indicating that the site of action of endothelin may be different from that originally proposed by Yanagisawa *et al.* (1988).

The role of endothelin in the induction and/or maintenance of high blood pressure is not well understood (Yanagisawa & Masaki, 1989). Increased sensitivity to endothelin-1 has been shown to occur in renal arteries of 12 week-old spontaneously hypertensive rats (SHR) (Tomobe *et al.*, 1988), suggesting that endothelin could contribute to the maintenance of high blood pressure in this animal model. However, other authors did not find any augmented sensitivity to endothelin-1 in the same vessel of SHR (Auch-Schwelk & Vanhoutte, 1989).

The objectives of the present study were: (1) to investigate the reactivity and sensitivity to endothelin-1 in two different vessels, namely the isolated perfused mesenteric vascular bed (MVB) and rings from the thoracic aorta, taken from spontaneously hypertensive and age-matched Wistar Kyoto rats (WKY); (2) to study the effects of nifedipine and nitrendipine, two classical blockers of voltage-operated calcium channels on endothelin-1-induced vasoconstriction. For comparison, vasoconstriction was also induced with noradrenaline and potassium chloride.

Methods

All experiments were performed on 14–16 week-old male spontaneously hypertensive rats and age-matched Wistar Kyoto rats, supplied by IFFA CREDO, L'Arbresle, France. Arterial blood pressure was measured by the tail-cuff method. The mean body weights of the SHR and WKY rats were 317 ± 3.2 g ($n = 49$) and 320 ± 2.4 g ($n = 52$), respectively. The animals were anaesthetized with ether and exsanguinated by cutting both the carotid arteries. The preparation of the mesenteric vascular beds and aortic rings was performed as described below.

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Isolated perfused mesenteric vascular beds

MVBs were prepared according to a modification of the method of McGregor (1965). A cannula (PP 50) was inserted into the superior mesenteric artery at its junction with the aorta, just rostral to the left renal artery. The caecal part of the superior mesenteric artery was tied, and the MVB dissected free from the intestine. The MVBs were mounted on a perfusion system, and perfused at a constant rate of 5 ml min^{-1} with a peristaltic pump (Ismatec MP 13 GJ-4) with physiological salt solution (PSS) of the following composition (mM): NaCl 136, KCl 2.5, $NaHCO_3$ 11.9, $CaCl_2$ 1.36, $MgCl_2$ 0.5, NaH_2PO_4 0.42. The solution was aerated with 95% O_2, 5% CO_2 to give a pH of 7.4, and maintained at room temperature (22°C). Perfusion pressure, which under constant flow conditions is proportional to vascular resistance, was measured by a Gould P25 1D pressure transducer and recorded continuously (Hellige recorder).

Experimental protocol The MVBs were left for approximately 2.5 h to stabilize before the experiments were begun. To examine the effect of nifedipine on the pressor actions of KCl, noradrenaline (NA) and endothelin-1, dose-response curves were constructed in the presence of nifedipine (3, 30, 300 nM); in the control an appropriate dilution of solvent was used. Nifedipine was infused at a rate of 0.1 ml min^{-1} with a peristaltic pump (Ismatec MP 25 GJ-4) 30 min before the first dose-response curve was determined. Dose-response curves were then recorded in the following order: KCl, NA and endothelin-1; a 5 min interval was allowed between consecutive curves. KCl and NA were added as a 0.5 ml bolus injection directly into the perfusion system by a peristaltic pump (Ismatec JPN-12) at intervals of 2.5 and 5 min, respectively. Endothelin-1 was added as a 0.1 ml bolus with a syringe, at 5 min intervals. Only one MVB per concentration was used.

Thoracic aortic rings

The thoracic aorta of SHR and WKY were removed and cleaned of all loosely adherent connective tissue. Four rings from each aorta, about 2.5 mm wide, were cut close to the aortic arch. The rings were suspended under a tension of 1.5 g between two parallel hooks in a 20 ml organ bath containing a modified Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.52, $NaHCO_3$ 24.8, KH_2PO_4 1.2, glucose 10; at 37°C, gassed with 95% O_2 and 5% CO_2. Each preparation was allowed to equilibrate for at least one hour. Isometric responses were measured with a force transducer (K30, Hugo Sachs Electronics, Freiburg, F.R.G.) coupled to a tissue bath data acquisition system (Buxco Electronics, Inc., Sharon, CT, U.S.A.).

Experimental protocol After 30 min, the tension on the rings was readjusted to 1.5 g and after a further 30 min equilibration period, the experiments were begun. To examine the effects of nitrendipine on the contractions induced by endothelin-1, noradrenaline and potassium chloride, dose-response curves were constructed in the presence of nitrendipine 10, 100, 1000 nM; in the control an appropriate dilution of solvent was used. The rings were incubated for 30 min with nitrendipine or solvent before addition of the agonists.

Drugs and solutions

The following drugs were used: porcine endothelin (endothelin-1), nifedipine and nitrendipine (CIBA-GEIGY, Switzerland), potassium chloride (Merck, F.R.G.), noradrenaline hydrochloride (Fluka, Switzerland). Endothelin-1 was dissolved in a bicarbonate buffer solution (pH 7.4) and diluted in physiological salt solution (PSS). Albumin 1 mg ml^{-1} (Fluka, Switzerland) was added to the solutions to prevent adsorption of endothelin-1 onto the glassware. Noradrenaline was dissolved in distilled water and further diluted in PSS and 0.1 mM ascorbic acid. Stock solutions of nitrendipine (50% DMSO) and nifedipine (50% ethanol) were further diluted in PSS and protected from light.

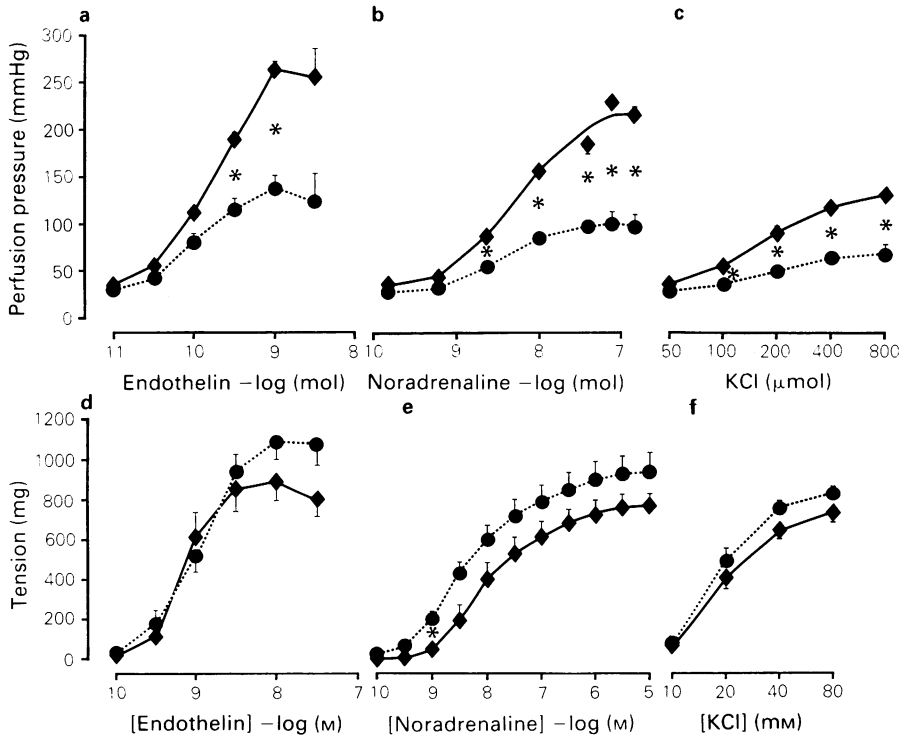

Figure 1 Vasoconstrictor effect of (a,d) endothelin-1, (b,e) noradrenaline, and (c,f) potassium chloride (KCl) in mesenteric vascular beds (a–c) and aortic rings (d–f) of spontaneously hypertensive rats (◆) and Wistar Kyoto rats (●). Results are the means of 6–12 preparations; vertical lines indicate s.e.mean. Significant differences ($P < 0.05$) are indicated by the asterisks.

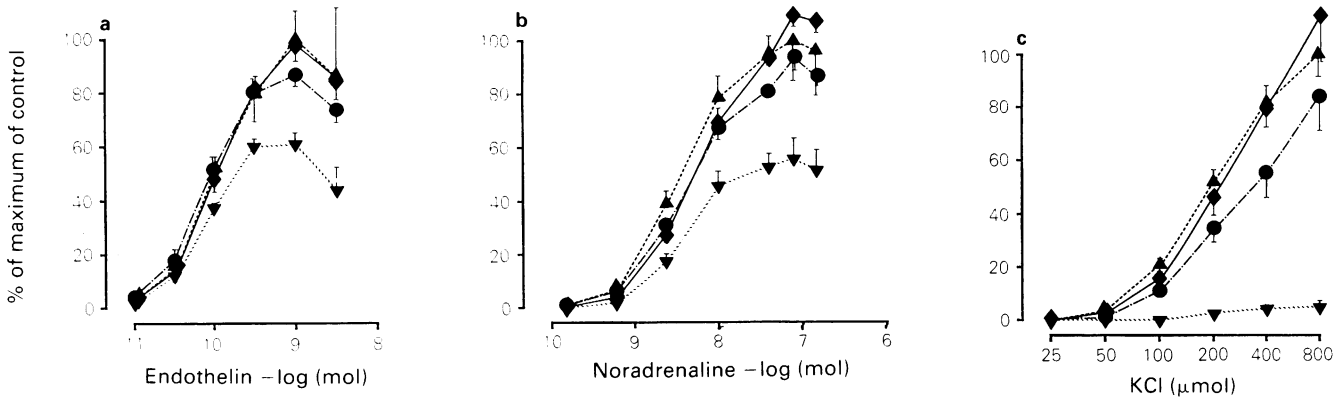

Figure 2 Inhibitory effects of nifedipine (◆ 3; ● 30; ▼ 300 nM) on the vasoconstrictor action of (a) endothelin-1, (b) noradrenaline and (c) potassium chloride (KCl) in mesenteric vascular beds of Wistar Kyoto rats. Results are expressed as percentage of the maximum vasoconstrictor response obtained with each agent in the preparations treated with appropriate concentrations of the solvent (control, ▲). Values are means of 10–12 preparations; vertical lines indicate s.e.mean.

Data presentation and analysis.

All values represent means ± s.e.mean. Data were analysed by Student's unpaired *t* test or one-way ANOVA, followed by Bonferroni's procedure (Wallestein *et al.*, 1980), as appropriate. Differences were considered statistically significant when $P < 0.05$.

Results

Effects of endothelin-1, noradrenaline and potassium on mesenteric arteries and aortic rings

In SHR the initial systolic blood pressures were significantly higher than those of the WKY rats: 199 ± 3.6 ($n = 49$) vs 150 ± 2.2 mmHg ($n = 52$); $P < 0.001$. After the stabilization period of 2.5 h, baseline perfusion pressure in MVB of SHR was significantly higher than in MVB of WKY (33 ± 0.3 mmHg and 27 ± 0.4 mmHg, respectively, $P < 0.05$). Endothelin-1 produced a concentration-dependent increase in perfusion pressure (i.e. vasoconstriction) in the MVBs of both strains (Figure 1a). MVBs of SHR were, however, more reactive to endothelin-1 than MVBs of WKY rats. The maximum increase in perfusion pressure obtained was 264 ± 8 mmHg in MVBs of SHR and 141 ± 9 mmHg in the MVBs of WKY rats. However, sensitivity to endothelin-1 was not significantly different between the two strains. In fact, the EC_{50} values were 171 ± 21 pmol for SHR and 102 ± 19 pmol for WKY. A similar trend was observed after noradrenaline and potassium chloride (Figure 1b,c). In both strains the maximum perfusion pressure obtained after noradrenaline and potassium were significantly ($P < 0.05$) lower than that induced by endothelin (Figure 1a,b,c). In SHR endothelin was 30 times more potent than noradrenaline.

In contrast to the mesenteric vascular beds, in aortic rings of SHR and WKY rats endothelin-induced contractions were similar. EC_{50}s were 0.78 ± 0.08 and 0.87 ± 0.09 nM, respectively (Figure 1d). In rings of SHR rats maximum developed tension was less than in WKY (Figure 1d). There was no significant difference in developed tension after noradrenaline and potassium chloride in rings of either strain (Figure 1e,f).

Effects of nifedipine on endothelin-induced vasoconstriction in the mesenteric vascular beds

In the MVBs of both strains, nifedipine at concentrations of 30 and 300 nM inhibited potassium-induced vasoconstriction. The concentration of 300 nM almost completely blocked the effects of potassium (Figures 2c and 3c). The same concentration of nifedipine only partially inhibited endothelin- and noradrenaline-induced vasoconstriction in a non-competitive manner (Figures 2 and 3, a and b). In MVB of SHR nifedipine was more effective than in WKY in inhibiting endothelin- and noradrenaline-induced vasoconstriction. However, only the values for 30 nM nifedipine were significantly different; $P < 0.05$.

Effects of nitrendipine on endothelin-induced contractions in thoracic aortic rings

Similar to the MVBs, in aortic rings of both strains, potassium-induced contractions were inhibited after 10, 100 and 1000 nM nitrendipine, and the latter concentration exerted an almost complete inhibition (Figures 4c and 5c). However,

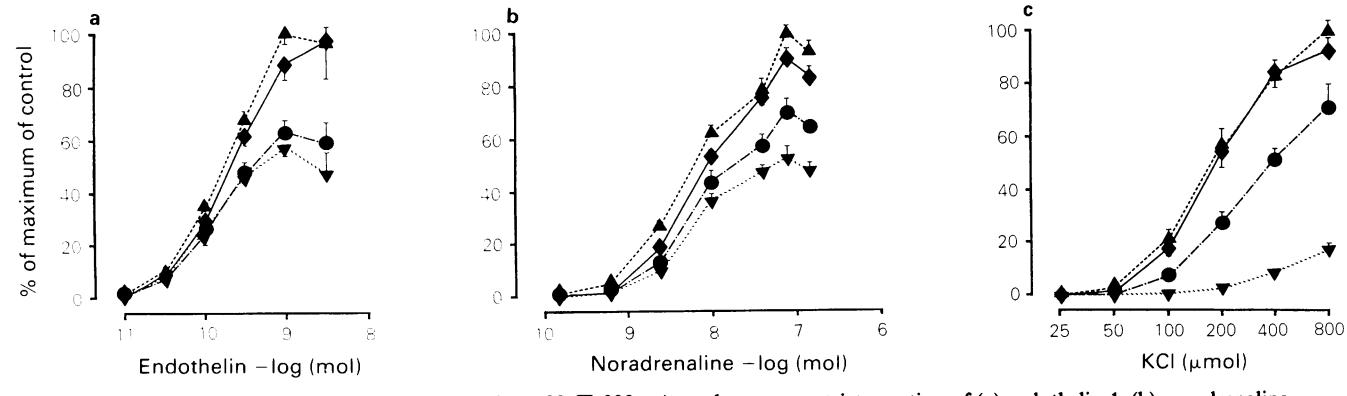

Figure 3 Inhibitory effects of nifedipine (◆ 3, ● 30, ▼ 300 nM) on the vasoconstrictor action of (a) endothelin-1, (b) noradrenaline and (c) potassium chloride (KCl) in mesenteric vascular beds of spontaneously hypertensive rats. Results are expressed as percentage of the maximum vasoconstrictor response obtained with each agent in the preparations treated with appropriate concentrations of the solvent (control, ▲). Values are means of 10–12 preparations; vertical lines indicate s.e.mean.

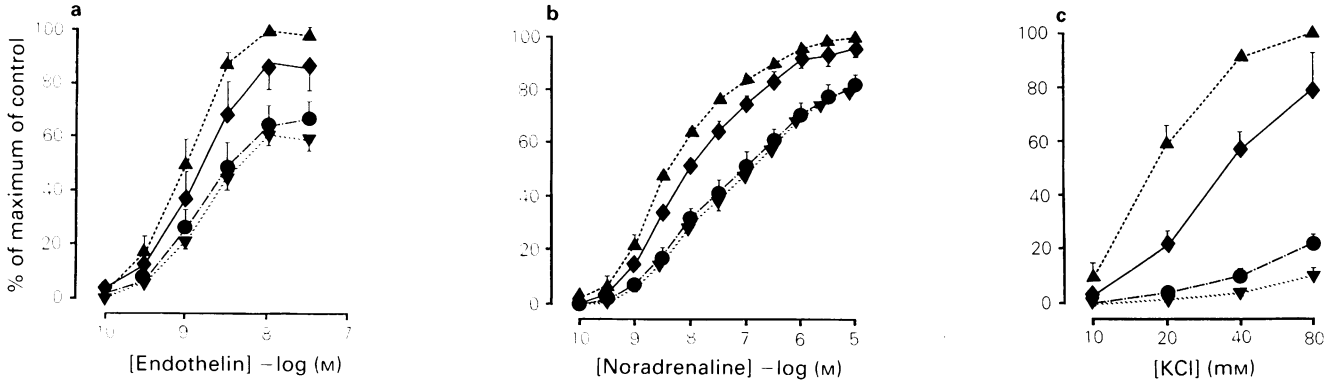

Figure 4 Inhibitory effects of nitrendipine (◆ 10, ● 100, ▼ 1000 nM) on contractions induced by (a) endothelin-1, (b) noradrenaline and (c) potassium chloride (KCl) in aortic rings of Wistar Kyoto rats. Results are expressed as percentage of the maximum vasoconstrictor response obtained with each agent in the preparations treated with appropriate concentrations of the solvent (control, ▲). Values are means of 4–6 preparations; vertical lines indicate s.e.mean.

endothelin- and noradrenaline-induced contractions were only partially inhibited by nitrendipine in the same concentration range (Figures 4 and 5, a and b).

Discussion

The present experiments demonstrate that the reactivity to endothelin-1 of perfused mesenteric vascular beds, but not aortic rings is greater in spontaneously hypertensive than in Wistar Kyoto rats. However, sensitivity to endothelin-1 is similar in the two strains and both types of vessel. Since an increase in sensitivity would indicate a role of endothelin-1 in the genesis of high blood pressure, these data suggest that endothelin-1 is not a primary hypertensive mechanism at this stage of hypertension in SHR. Our findings also show that calcium influx via 1,4-dihydropyridine-sensitive calcium channels is only partly responsible for the vasoconstrictive action of endothelin-1. The same is true of noradrenaline, but not of potassium chloride.

The greater reactivity of mesenteric vascular beds of SHR to endothelin-1 is most likely the result of structural changes (i.e. increases in wall-to-lumen ratio) and is not selective for endothelin-1, since it was also found with the other two vasoconstrictors used, i.e. potassium chloride and noradrenaline. Increased reactivity to noradrenaline in perfused vascular preparations of SHR has also been obtained by other authors (see Triggle & Laher, 1985, for review). Folkow (1978) has suggested that increased vascular resistance and exaggerated vascular reactivity in hypertension are attributable to thickening of the vessel walls. They demonstrated that the vasoconstrictor response of the perfused hindquarters of SHR, or of rats with renal hypertension, closely corresponds to that of a theoretical model based solely on circumferential shortening of the media and its encroachment on the lumen. According to Folkow (1978), the characteristic features of this response are (1) elevated resistance at maximal dilatation, (2) unchanged threshold, (3) supranormal maximal response, and (4) proportionally steeper vasoconstrictor response. All these criteria are met by the data presented here for the perfused mesenteric vascular beds of SHR. In fact, at the time of maximal dilatation, perfusion pressure in the vascular beds of SHR was significantly higher than that of WKY rats. Since, under conditions of constant flow, perfusion pressure is proportional to vascular resistance, these data indicate an elevated vascular resistance in the mesenteric vascular beds of the SHR. The differences in the perfusion pressure values in the experiments on mesenteric beds described here are very close to those obtained for perfused hindquarters of SHR (Folkow, 1978) and for mesenteric arteries (Triggle & Laher, 1985). In our study, baseline perfusion pressures at maximal dilatation were 33 mmHg in SHR and 27 mmHg in WKY rats. According to Folkow's model (1978), this difference in resistance corresponds to an increase in the thickness of the media of SHR vessels by about 30% as compared to WKY vessels. This means that the influence of structural adaptations can be taken into account to explain the abnormalities obtained in perfused vascular preparations from hypertensive animals.

However, in contrast to the mesenteric beds, aortic rings of SHR showed a decreased reactivity to both of these vasoconstrictors by comparison with those of WKY rats. Other investigators have also noted a lower reactivity of aortic rings to noradrenaline and potassium (Spector *et al.*, 1969). Similar to

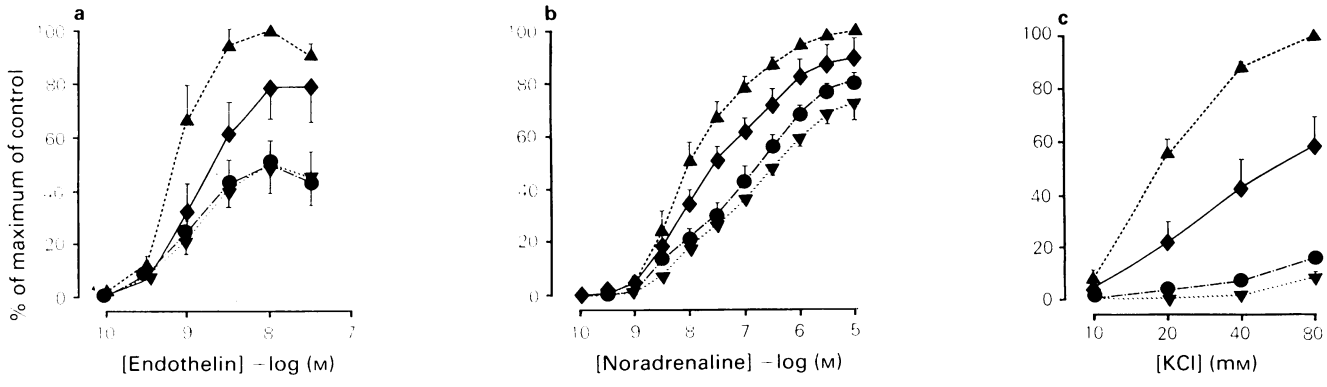

Figure 5 Inhibitory effects of nitrendipine (◆ 10, ● 100, ▼ 1000 nM) on contractions induced by (a) endothelin-1, (b) noradrenaline and (c) potassium chloride (KCl) in aortic rings of spontaneously hypertensive rats. Results are expressed as percentage of the maximum vasoconstrictor response obtained with each agent in the preparations treated with appropriate concentrations of the solvent (control, ▲). Values are means of 4–6 preparations; vertical lines indicate s.e.mean.

the results presented here, Auch-Schwelk & Vanhoutte (1989) found a reduced contraction to endothelin-1, by a similar extent, in rat aorta and renal arteries from SHR, as compared to those of WKY. The absence of increased sensitivity to endothelin-1 in the aortic preparations, where geometric factors play a minor role (Webb & Bohr, 1981), indicates that there is no alteration in smooth muscle function in response to endothelin-1 at this stage of hypertension in SHR. However, increased sensitivity of renal arteries of SHR to endothelin-1 has been demonstrated (Tomobe *et al.*, 1988). These authors came to a different conclusion, namely that endothelin-1 contributes to the maintenance of high blood pressure in SHR. The reasons for this discrepancy are at present unclear. Further studies, especially in blood vessels of prehypertensive SHR, are needed to clarify the exact role of endothelin-1 in hypertension.

Originally, endothelin-1 was represented as 'an endogenous agonist of the dihydropyridine-sensitive Ca^{2+} channels' (Yaganisawa *et al.*, 1988). Several lines of experimental evidence, however, indicate that the substance has no affinity for dihydropyridine binding sites (Hirata *et al.*, 1988; Van Renterghem *et al.*, 1988). The first specific endothelin-1 binding sites were detected in vascular smooth muscle. Endothelin-1 and [^{3}H]-nitrendipine do not displace each other from their respective binding sites (Hirata *et al.*, 1988).

The finding that high concentrations of nifedipine, or nitrendipine, only partially inhibited endothelin-induced vasoconstriction indicates that the calcium needed for this effect is not primarily derived from the extracellular medium via voltage-operated calcium channels. The dependency of endothelin-1 contractions on extracellular calcium (Yanagisawa *et al.*, 1988) has also been observed in experiments with other vasoconstrictors, such as noradrenaline (Karaki, 1987). Thus the absence of contractions after removal of extracellular calcium, or after inhibition by calcium-entry blockers, is not necessarily indicative of a direct effect of a vasoconstrictor on the voltage-dependent calcium channels, particularly with regard to the coronary arteries, originally used by Yanagisawa *et al.* (1988). In fact, the activation of contraction of the coronaries is more dependent on extracellular calcium than that of other arteries (Van Breemen & Siegel, 1980; Sato *et al.*, 1982). In addition, coronary and cerebral vessels are particularly susceptible to the effects of calcium-entry blockers (Nakayama *et al.*, 1983). More recent studies have indicated that endothelin-1, at a concentration of 10 nM, is capable of contracting porcine coronary arteries independently of the presence of extracellular calcium (Kodama *et al.*, 1989). The inhibitory action of calcium-entry blockers on endothelin-induced contractions is quantitatively very similar to their effects on contractions due to other vasoconstrictors, such as noradrenaline (Godfraind, 1985). Thus the effects of endothelin-1 resemble those of an activator of receptor-operated calcium channels (Godfraind, 1985). According to Godfraind (1985), activation of receptor-operated channels is typically only incompletely inhibited by calcium-entry blockers.

Recently, it has been shown that endothelin-1 stimulates the metabolism of inositol phosphates, leading to mobilization of intracellular free calcium stores (Resink *et al.*, 1988 Van Renterghem *et al.*, 1988; Marsden *et al.*, 1989). In addition, it has been shown that by transiently activating calcium-sensitive K-channels, endothelin-1 initially provokes hyperpolarization of the membrane, followed by sustained depolarization due to the opening of a non-specific cation channel permeable to Ca^{2+} and Mg^{2+}. It is this depolarization that then activates L-type Ca^{2+}-channels (Van Renterghem *et al.*, 1988). Hence, the effect of endothelin-1 on L-type Ca^{2+}-channels is only a part of its total effect, and, only an indirect one. This mode of action, which is very likely shared by other vasoconstrictors, is supported by the finding that calcium-channel blockers only partially inhibited endothelin-induced vasoconstriction. As suggested by Van Renterghem *et al.* (1988), the remaining component of endothelin-induced contraction in the presence of calcium-entry blockers is probably due to influx of calcium via non-selective cation channels and to calcium released from intracellular stores.

In conclusion, reactivity to endothelin-induced vasoconstriction was found to be greater in isolated perfused mesenteric vascular beds, but not aortic rings, of SHR than in those of WKY rats. Similar patterns of activity were observed with the other vasoconstrictor agents used, noradrenaline and potassium chloride. These data indicate that in SHR with fully developed hypertension the augmented reactivity to endothelin-1 is due to a structural rather than a functional change. They do not rule out the involvement of endothelin-1 in some primary hypertensive mechanism operating at a prehypertensive age.

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References

AUCH-SCHWELK, W. & VANHOUTTE, P.M. (1989). Contractions to endothelin in isolated arteries of SHR and WKY rats. *FASEB*, **3**, A1008.

FOLKOW, B. (1978). Cardiovascular structural adaptation; its role in the initiation and maintenance of primary hypertension. *Clin. Sci.*, **55**, 3–22s.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

GILLESPIE, M.N., OWASOYO, J.O., McMURTRY, I.F. & O-BRIEN, R.F. (1986). Sustained coronary vasoconstriction provoked by a peptidergic substance released from endothelial cells in culture. *J. Pharmacol. Exp. Ther.*, **236**, 339–343.

GODFRAIND, T. (1985). Transmembrane movements of calcium in vascular smooth muscle: Ca channels, Ca pumps, Na-Ca exchange. In *Vascular Neuroeffector Mechanisms*, ed. Bevan, J.A., pp. 37–43. Amsterdam: Elsevier Science Publishers B.V.

GRYGLEWSKI, R.J., BOTTING, M.R. & VANE, J.R. (1988). Mediators produced by endothelial cell. *Hypertension*, **12**, 530–548.

HICKEY, K.A., RUBANYI, G., PAUL, R.J. & HIGHSMITH, R.F. (1985). Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. *Am. J. Physiol.*, **248**, C550–556.

HIRATA, Y., YOSHIMI, H., TAKATA, S., WATANABE, T.X., KUMAGAI, S., NAKAJIMA, K. & SAKAKIBARA, S. (1988). Cellular mechanism of action by a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **154**, 868–875.

HUTTNER, I. & GABBIANI, G. (1983). Vascular endothelium in hypertension. In *Hypertension* ed. Genest, J., pp. 473–487. New York: McGraw-Hill Book Company.

KARAKI, H. (1987). Use of measurements to delineate the mode of action of vasodilators. *J. Pharmacol. Methods*, **18**, 1–21.

KODAMA, M., KANAIDE, H., ABE, S., HIRANO, K., KAI, H. & NAKAMURA, M. (1989). Endothelin-induced Ca-independent contraction of the porcine coronary artery. *Biochem. Biophys. Res. Commun.*, **160**, 1302–1308.

LUESCHER, T.F. (1988). Endothelial Vasoactive Substances and Cardiovascular Diseases. Basel, Switzerland: Karger.

MARSDEN, P.A., DANTHULURI, N.R., BRENNER, B.M., BALLERMANN, B.J. & BROCK, T.A. (1989). Endothelin action on vascular smooth muscle involves inositol trisphosphate and calcium mobilization. *Biochem. Biophys. Res. Commun.*, **158**, 86–91.

McGREGOR, D.D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessel of the rat. *J. Physiol.*, **177**, 21–30.

NAKAYAMA, K., ISHII, K. & KARO, H. (1983). Effect of Ca-antagonists on the contraction of cerebral and peripheral arteries produced by electrical and mechanical stimuli. *Gen. Pharmacol.*, **14**, 111–113.

RESINK, T.J., SCOTT-BURDEN, T. & BÜHLER, F.R. (1988). Endothelin stimulates phospholipase C in cultured vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **157**, 1360–1368.

SATO, M., OHASHI, M., METZ, M.Z. & BING, R.J. (1982). Inhibitory effect of a calcium antagonist (diltiazem) on aortic and coronary contractions in rabbit. *J. Mol. Cell Cardiol.*, **14**, 741–744.

SPECTOR, S., FLEISCH, J.H., MALING, M.H. & BRODIE, B.B. (1969). Vascular smooth muscle reactivity in normotensive and hypertensive rats. *Science*, **166**, 1300–1301.

TOMOBE, Y., MIYAUCHI, T., SAITO, A., YANAGISAWA, M., KIMURA, S., GOTO, K. & MASAKI, T. (1988). Effects of endothelin on the renal artery from spontaneously hypertensive and Wistar Kyoto rats. *Eur. J. Pharmacol.*, **152**, 373–374.

TRIGGLE, C.R. & LAHER, I. (1985). A review of changes in vascular smooth muscle function in hypertension: isolated tissue versus *in vivo* studies. *Can. J. Physiol. Pharmacol.*, **63**, 355–365.

VAN BREEMEN, C. & SIEGEL, B. (1980). The mechanism of alpha-adrenergic activation in the dog coronary artery. *Circ. Res.*, **46**, 426–429.

VAN-RENTERGHEM, C., VIGNE, P., BARHANIN, J., SCHMID-ALLIANA, A., FRELIN, C. & LAZDUNSKI, M. (1988). Molecular mechanism of action of vasoconstrictor peptide endothelin. *Biochem. Biophys. Res. Commun.*, **157**, 977–985.

WALLESTEIN, S., ZUCKER, C.L. & FLEIS, J.L. (1980). Some statistical methods useful in circulation research. *Circ. Res.*, **47**, 1–9.

WEBB, R.C. & BOHR, D.F. (1981). Recent advances in the pathogenesis of hypertension: consideration of structural, functional and metabolic vascular abnormalities resulting in elevated arterial resistance. *Am. Heart J.*, **102**, 251–264.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332**, 411–415.

YANAGISAWA, M. & MASAKI, T. (1989). Endothelin, a novel endothelium derived peptide. *Biochem. Pharmacol.*, **38**, 1877–1883.

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