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Role of Endothelium on the Effects of Neuropeptide Y in Mesenteric Resistance Arteries of Spontaneously Hypertensive and Wistar-Kyoto Normotensive Rats[1]

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ABSTRACT

The role of the endotheliumn in the effects of neuropeptide Y (NPY) and norepinephrine was investigated in mesenteric resistance arteries of the spontaneously hypertensive rat (SHR) and of the normotensive Wistar-Kyoto rat (WKY). Endothelium-dependent relaxation to acetylcholine (1 μM) was reduced in arteries of SHR compared with WKY. In the presence of the endothelium, the vessels of the two strains responded similarly to norepinephrine and NPY (100 nM) produced only a slight contraction. After removal of the endothelium, the response to norepinephrine was greater in WKY than in SHR. Furthermore, endothelium denudation enhanced markedly contraction elicited by NPY in WKY (up to 40% of the maximal effect of norepinephrine), but not in SHR. NPY potentiated the contractile response to low concentrations of norepinephrine (less than 300 nM) in both strains regardless whether the endothelium was intact or not. These results indicate that the contractile responses to NPY and to norepinephrine are inhibited by the endothelium in vessels of WKY, but not in those of the SHR. Furthermore, the potentiating effect of NPY occurs *via* an endothelium-independent mechanism in mesenteric arteries of both SHR and WKY. It is proposed that the differential responses between the two strains are related to abnormal function of the endothelium and to decreased responsiveness of smooth muscle cells in mesenteric resistance arteries of SHR compared to WKY.

NPY is colocalized and coreleased with norepinephrine from adrenergic nerve terminals in the perivascular sympathetic nervous system (Lundberg *et al.*, 1982; Edvinsson *et al.*, 1984; and substitute, Westfall *et al.*, 1987). Intravenous administration of NPY induces an increase in blood pressure secondary to an elevation in peripheral resistance (Dahlof *et al.* 1985; Zukoska-Grojec *et al.*, 1986; Pegram and Hunter, 1988). The main action of the peptide, *in vitro*, is its ability to potentiate responses elicited either by nerve stimulation or by various agonists, including norepinephrine (Wahlestedt *et al.*, 1985; Zukoska-Grojec *et al.* 1986; Neild, 1987; Andriantsitohaina and Stoclet, 1988, 1990). In addition, NPY has a direct vasoconstrictor action which varies markedly with the anatomical origin of the vessels (Edvinsson *et al.*, 1983; Thorpe *et al.*, 1987; Suzuki *et al.* 1988; Pernow *et al.*, 1987).

It is now well established that the vascular endothelium is an important modulator of blood vessel wall function (Furchgott, 1984). The role of the endothelium as an effector of the effects of NPY is not understood completely. For example, it has been reported that the ability of NPY to potentiate the stimulation-induced vasoconstriction in rabbit ear artery and in canine saphenous vein depends on the presence of an intact vascular endothelium (Daly and Hieble, 1987; Hieble *et al.*, 1989). In contrast to these results, Budai *et al.* (1989) reported that removal of endothelium does not affect potentiation by NPY of stimulation-evoked contraction in perfused rabbit ear artery. In addition, the direct contractile effect of NPY on arteries from human skeletal muscle and pig spleen has been reported to occur through an endothelium-independent mechanism (Pernow and Lundberg, 1988). The aim of the present work therefore was to better understand the possible role of the endothelium as a mediator of the effects of NPY.

It has also been reported that endothelial cell function is disturbed in the SHR (Luscher *et al.*, 1987; Tesfamariam and Halpern, 1988; Watt and Thurston, 1989). In addition, changes in NPY-containing perivascular nerves of the major cerebral vessels (Dhital *et al.*, 1988) used in the plasma concentration of the peptide (Howe *et al.*, 1986) have been described. We therefore also tested the hypothesis that vascular smooth mus-

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ABREVIATIONS: NPY, neuropeptide Y; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; PSS, physiological salt solution.

cle of the SHR is hyper-responsive to NPY and shows an altered endothelial-dependent component.

The results demonstrate that the potentiating effect of NPY occurs *via* an endothelium-independent mechanism in mesenteric resistance arteries of both SHR and WKY. In contrast, the contractile response to NPY appears to be modulated by the endothelium in WKY but not SHR. Whereas the vessels of WKY and the SHR respond similarly to NPY and to norepinephrine in the presence of endothelium, after removal of the endothelium, the vessels of WKY exhibit enhanced responses to NPY and norepinephrine compared to those from SHR. These differences may be related to abnormal function of the SHR endothelium and to an altered responsiveness of smooth muscle cells in resistance vessels of SHR, or both.

Materials and Methods

Animals. The rats used in these studies were 12- to 14-week-old male Aoki-Okamoto SHR and the genetic WKY control and were both obtained from Charles River Laboratories (Wilmington, MA). Systolic blood pressure was measured using a pneumatic tail-cuff device (Narco-Bio Systems, Houston, TX) as described previously (Bukoski and McCarron, 1986). Blood pressure values obtained from four consecutive measurements were averaged and recorded as the pressure of a given rat. All rats were maintained in a colony room with fixed dark:light cycles and constant humidity and temperature and provided with Purina rodent chow and tap water *ad libitum*.

Arterial preparation and mounting. The animals were sacrificed by decapitation using a guillotine and exsanguinated. The viscera were exposed, and a proximal segment of the small bowel was removed and pinned in a dissecting dish containing PSS of the following composition (in millimolar): NaCl, 130; KCl, 4.7; KH_2PO_4, 1.4; Na_2PO_4, 1.15; $NaHCO_4$, 15; $MgSO_4 \cdot 7H_2O$, 1.18; $CaCl_2$, 2.5; and glucose, 5.0. Branch II or III resistance arteries, as classified by Owens *et al.* (1988), were cleaned of fat and connective tissue, and a segment 1.5 to 2.0 mm in length was removed. In experiments testing the effect of removal of the endothelium, the endothelial layer was disrupted immediately after the dissection by passing a human hair through the lumen several times. The segment was then mounted on a previously described myograph (Mulvany and Halpern, 1977; Julou and Freslon, 1986, Bukoski *et al*, 1989) using two tungsten wires (20 μm in diameter) inserted through the lumen of the vessel. Mechanical activity was recorded isometrically by a force transducer (Kistler-Morse, Redmond, WA). After mounting, the vessel was placed in PSS, kept at 37°C and gassed continuously with 95% O_2 plus 5% CO_2 (pH 7.4). After an equilibration period of 30 min, the vessel was stretched 3 times to minimize subsequent hysteresis effects, then passively stretched as described by Mulvany and Halpern (1977) and Mulvany *et al.* (1978), to a length that yields a circumference equivalent to 90% of that which the vessel would have had with an intramural pressure of 100 mm Hg (L_{90}). In the method described by Mulvany *et al.* (1978), the vessel was stretched initially to a resting wall tension of 0.1 mN/mm, whereas in our study, the initial stretch (which was essentially zero) was achieved when the two tungsten wires just touched the inner walls of the vessel, as observed with a Labophot microscope (Nikon) using a 40× salt-water objective (Nikon). The initial axial length, radius and media thickness of the vessel were then measured. After setting the vessel to L_{90} the new radius was measured and the media thickness at L_{90} was calculated assuming a constant volume of media.

After a 30-min equilibration period, each vessel was then given five "wake-up" challenges to elicit reproducible contractile responses. Three challenges were made with an elevated potassium (high-KCl) solution of the following composition (in millimolar): NaCl, 34.7; KCl, 100; KH_2PO_4, 1.4; Na_2PO_4, 1.15; $NaHCO_3$, 15; $MgSO_4 \cdot 7H_2O$, 1.18; $CaCl_2$, 2.5; and glucose, 5.0. The fourth challenge was made with the high-KCl solution also containing 10 μM norepinephrine to maximally activate the vessels. The fifth challenge was made with 10 μM norepinephrine in normal PSS and, when the contraction reached a plateau, acetylcholine (1 μM) was added. The absence of a relaxation response to acetylcholine was taken as evidence that the vessel segments were functionally denuded of endothelium.

Experimental protocol. Thirty minutes after the final wake-up challenge, responses to cumulative addition of norepinephrine were elicited and concentration-response curves constructed. After a wash-out period of 30 min, NPY (100 nM) was applied 5 min before repeating cumulative additions of norepinephrine. Cocaine (3 μM) was present in the bath at all times during the experiment to inhibit the neuronal uptake of norepinephrine.

Expression of results and statistical analysis. The contractile force responses were normalized to cross-sectional area of the vessel wall (product of axial length and medial thickness) and are reported as active stress (mN/mm^2). The sensitivity to norepinephrine is expressed as pD2 value, where $pD_2 = -\log EC_{50}$, EC_{50} being the concentration of norepinephrine required to give a half-maximal contractile response. EC_{50} values were obtained by logit/log regression analysis. All results are expressed as means ± S.E.M. of number (n) of experiments. Comparisons between groups or repeated measures were carried out using one or two-way analysis of variance.

Drugs. Norepinephrine, acetylcholine and porcine NPY (whole peptide) were purchased from Sigma Chemical Co. (St. Louis, MO). Pharmaceutical grade cocaine was purchased from the Oregon Health Sciences University Pharmacy.

Results

Blood pressure, media thickness and normalized lumen diameters. As expected, systolic blood pressures of SHR were significantly higher than those of WKY being 174 ± 2.6 (n = 12) for SHR and 131 ± 2.3 mm Hg (n = 16) for WKY, respectively ($P < .001$). Also as expected, the media thickness of the mesenteric resistance arteries was significantly increased ($P < .001$) by about 20% in SHR (in micromoles: 35 ± 1.9 (n = 7) and 39 ± 1.2 (n = 7) in intact and endothelium-rubbed vessels, respectively) as compared to WKY (in μm: 31 ± 1.1 (n = 8) and 27 ± 1.2 (n = 8) in intact and endothelium-rubbed vessels, respectively). These media thickness values were larger than those reported previously by other authors (Mulvany *et al.*, 1978; Lee *et al.*, 1983) and may be due to the use of a different method as indicated above (see "Materials and Methods"). The lumen diameters, normalized at L_{90}, of the mesenteric arteries of the two strains in the presence and in the absence of the endothelium were respectively in micromoles: 206 ± 11 (n = 8) and 208 ± 10 (n = 8) for WKY and 188 ± 10 (n = 7) and 213 ± 10 (n = 7) for SHR. No significant differences were observed between normalized lumen diameters of vessels of the two strains either in the presence or absence of the endothelium.

Endothelium-dependent relaxation. In both strains, acetylcholine (1 μM) induced an endothelium-dependent relaxation of vessels that were precontracted with 10 μM norepinephrine (fig. 1). The relaxation response to acetylcholine was significantly reduced in vessels of SHR compared with WKY ($P < .001$). Figure 1 also illustrates that by passing a human hair several times through the lumen of the vessel, the relaxation induced by acetylcholine was abolished essentially in both strains, indicating that the endothelium was functionally removed.

Effect of endothelium removal on norepinephrine-induced contractile response. Figure 2a illustrates the effect of endothelium removal on the concentration response of WKY vessels to norepinephrine. The maximal active stress elicited

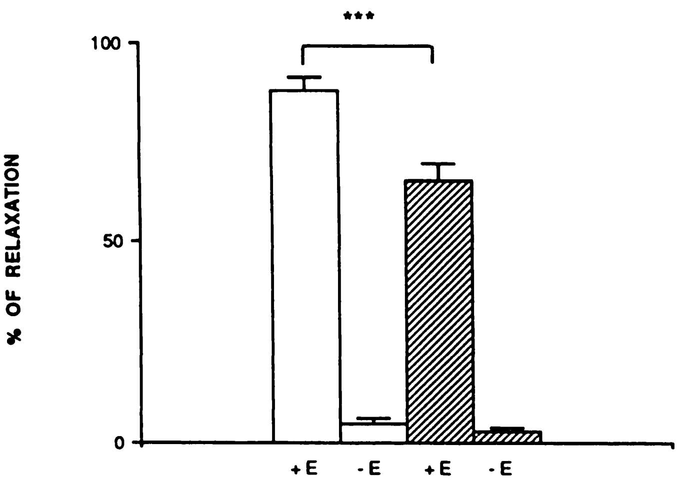

Fig. 1. Relaxation induced by acetylcholine on rat mesenteric resistance arteries with endothelium (+E) or without endothelium (−E) of WKY (open columns, n = 8) and SHR (hatched columns, n = 7). Responses are expressed as percentage of relaxation induced by 1 μM acetylcholine on vessels precontracted with 10 μM norepinephrine. Each column represents the mean ± S.E.M. ★★★P < .001, significantly different between WKY and SHR.

by norepinephrine was significantly increased by removal of the endothelium in WKY segments (P < .05). Denuded vessels also showed a significant leftward shift of the norepinephrine concentration-response curve, *i.e.*, pD_2 values increased from 6.6 ± 0.06 to 7.1 ± 0.09, in the presence and in the absence of endothelium, respectively (P < .001). In contrast, removal of endothelium resulted in a significant fall in the active stress response to norepinephrine in resistance arteries of SHR (P < .001) (fig. 2b). Furthermore, no significant leftward shift of the norepinephrine concentration-response curve was observed in SHR vessel segments, i.e., pD_2 values were 6.6 ± 0.07 and 6.8 ± 0.06, respectively, in the presence and in the absence of endothelium. No difference in the sensitivity of the mesenteric resistance arteries to norepinephrine or in maximal active stress elicited by the catecholamine were detected between strains in the presence of functional endothelium. However, after removal of the endothelium, the maximal active stress elicited by norepinephrine and the sensitivity to the amine were both lower in SHR than in WKY vessels. Expressing the results in active tension (mN/mm) did not reveal any statistically significant influence of the endothelium on the maximal response to norepinephrine in WKY vessels (3.60 ± 0.41 and 4.07 ± 0.37, n = 8, with and without endothelium, respectively). However it showed a statistically significant decrease (P < .01) in maximal response to norepinephrine when the endothelium was removed in SHR vessels (from 4.67 ± 0.41 to 3.26 ± 0.20, n = 7). Thus, the strain difference in maximal response to norepinephrine of endothelium-denuded vessels which was revealed when active stress was estimated was no more apparent when active tension was measured, in spite of an increased media thickness in the mesenteric arterioles from SHR compared to WKY.

Effect of endothelium on NPY-induced contractile response. Representative traces and the corresponding mean of the contractile responses induced by NPY expressed as active stress (mN/mm^2) and percentage of the maximal contractile response elicited by norepinephrine (10 μM) are illustrated in figures 3 and 4, respectively. NPY (100 nM) induced a weak and sustained increase in tone of mesenteric resistance arteries of both SHR and WKY (fig. 3). In the presence of functional endothelium, there was no statistically significant difference between contractile responses elicited by NPY in the arterioles of the two strains (fig. 4). In WKY, the increase in tone was enhanced by removal of the endothelium, whereas in SHR, removal of the endothelium resulted in a small, nonsignificant increase in the NPY response. Furthermore, whereas the increase in tone induced by NPY was not different between SHR and WKY for vessels with endothelium, the response was significantly greater in WKY compared to SHR after removal of the endothelium (fig. 4).

Effect of NPY on norepinephrine-induced contractile response. The effect of NPY on norepinephrine-induced contractile response, expressed as percentage of maximal contraction is illustrated in figures 5 and 6. The responses to lower concentrations of norepinephrine (from 10–100 nM) were larger in the presence than in the absence of NPY (100 nM) in mesenteric arteries of both strains, in the presence or absence of endothelium. At higher concentrations of norepinephrine, no significant effect of NPY was detected. NPY (100 nM) had no effect on the maximal norepinephrine-induced response of mesenteric resistance arteries of either the WKY (fig. 5) or SHR (fig. 6) in the presence (fig. 6a) or absence (fig. 6b) of endothelium. The maximal active tension to norepinephrine in the presence of NPY were (in mN/mm: 3.78 ± 0.44 in WKY with endothelium (n = 8); 4.32 ± 0.48 in WKY without endothelium (n = 8); 4.97 ± 0.43 in SHR with endothelium (n = 8); and 3.31 ± 0.21 in SHR without endothelium (n = 8), respec-

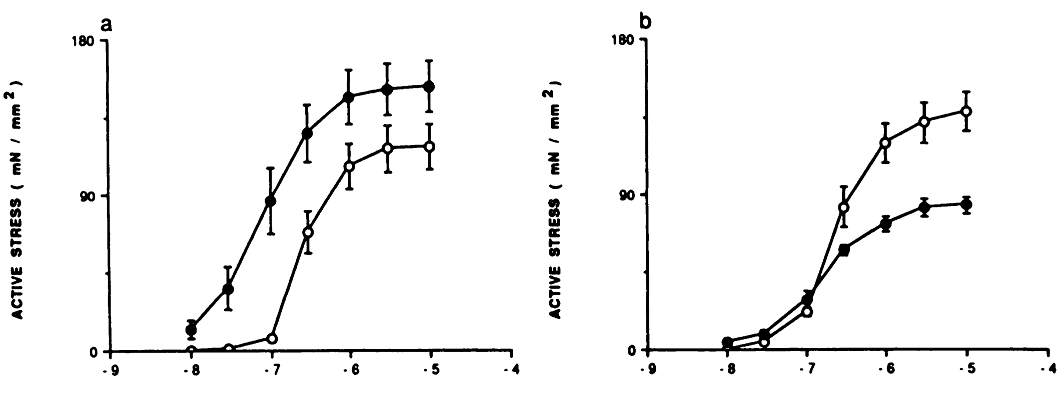

Fig. 2. Concentration-response curves to norepinephrine in rat mesenteric resistance arteries with (○) or without (●) endothelium taken from WKY (a, n = 8) or SHR (b, n = 7). The values are mean ± S.E.M.

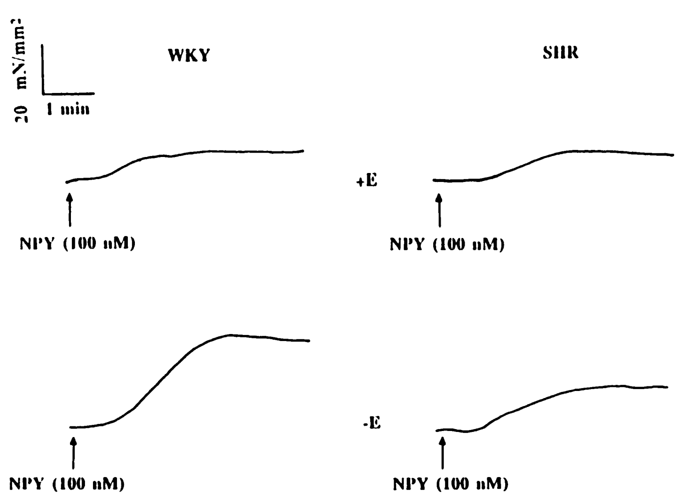

Fig. 3. Representative traces illustrating the contractions elicited by NPY on rat mesenteric resistance arteries from WKY (left) and from SHR (right) in the presence of endothelium (+E, upper panel) or in the absence of endothelium (−E, lower panel).

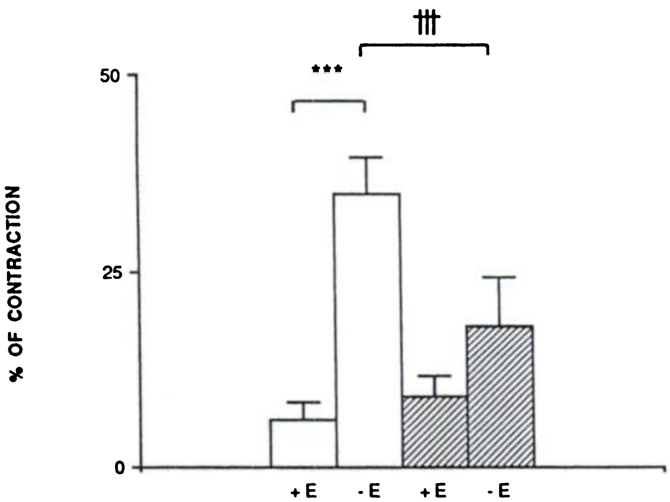

Fig. 4. Contractions elicited by NPY (100 nM) on rat mesenteric resistance arteries from WKY (open columns, $n = 8$) and from SHR (hatched columns, $n = 7$), in the presence of endothelium (+E) or in the absence of endothelium (−E). Contractions are expressed as percentage of the maximal contractile responses induced by 10 μM norepinephrine. Each column represents the mean with the bars representing S.E.M. ★★★P < .001, significantly different between arteries with and without endothelium taken from WKY and †††P < .001, significantly different between arteries without endothelium taken from WKY and SHR.

tively. These values were not significantly different from the above indicated values obtained in the same vessels in the absence of NPY.

Discussion

The aim of this study was to investigate the role of the endothelium on the potentiating and contractile effects of NPY in rat mesenteric resistance arteries. The results demonstrate that the direct contractile response to NPY is modulated by the endothelium, whereas the potentiation by the peptide of norepinephrine-induced contraction does not appear to involve an endothelium-dependent mechanism.

In vessels of normotensive rats, NPY only produced a slight increase in contraction in the presence of endothelium and induced contraction that was 40% of the maximal response to norepinephrine in the absence of endothelium. This result indicates that NPY can elicit contraction by acting directly on arterial vascular smooth muscle of the WKY. In addition, the endothelium can inhibit the contractile effect of NPY in WKY. This observation contrasts with the results reported by Pernow and Lundberg (1988) using arteries from human gluteus maximus muscle and pig spleen in which the direct contractile effect of NPY occurs through an endothelium-independent mechanism. Recently, MacLean and McGrath (1990) reported that the pressor response to NPY was endothelium-dependent in the isolated vascular bed of the rat tail. One potential explanation for the present results is that endothelial cells of WKY mesenteric resistance arteries may release a relaxing factor(s) (Furchgott and Zawadski, 1980) that impairs the contractile response to NPY. Also, it is possible that NPY can promote by itself the release of relaxing factor(s) in mesenteric resistance arteries of WKY rats. Perhaps the endothelium-dependent mechanism varies with the anatomical origin of the vessel because NPY is a potent vasoconstrictor of cerebral (Edvinsson *et al.*, 1983, 1984; Hanko *et al.*, 1986; Thorpe *et al.*, 1987) and skeletal muscle arteries (Pernow *et al.*, 1987; Pernow and Lundberg, 1988), but has only a weak effect on peripheral arteries (Edvinsson *et al.*, 1983; Glover, 1985; Pernow *et al.*, 1987; Thorpe *et al.*, 1987).

The effect of removal of the endothelium on the potentiation by NPY of norepinephrine-induced contraction was also investigated. NPY was observed to enhance the response to norepinephrine in mesenteric arteries of WKY, both in the presence or absence of endothelium. This result is consistent with that obtained by Budai *et al.* (1989) on perfused rabbit ear artery. In contrast, this finding does not agree with the results of Hieble *et al.* (1988, 1989) who reported that an intact endothelium is required for the potentiation by NPY of the constrictor responses to field stimulation and to exogenous norepinephrine in superfused segments of both rabbit ear artery and canine saphenous vein. It should be noted that Hieble *et al.* (1989) found NPY to have no effect on endothelium-derived relaxing factor release from cultured endothelial cells and suggested that NPY might release an endothelial-derived vasoconstrictor substance. Possible reasons for the differences in endothelial influence might be attributed to differences in species, tissues and experimental protocols. We currently cannot distinguish among these possibilities. However, because after removal of the endothelium the enhancement by NPY of norepinephrine response remained, it is more likely that in rat mesenteric resistance arteries the potentiating effect of NPY occurs *via* an endothelium-independent mechanism.

Differences in NPY content and responsiveness have been reported previously in the SHR model of essential hypertension (Howe *et al.*, 1986; Daly *et al.*, 1988; Dhital *et al.*, 1988). We therefore carried out experiments using mesenteric resistance arteries of SHR to test the hypothesis that vessels of hypertensive rats display differential responses to the potentiating and contractile effects of NPY.

No differences in the potentiating effect of NPY were observed in mesenteric resistance arteries of SHR and WKY. In vessels of SHR, NPY produced a slight increase in tone that was not different in the presence or in the absence of endothelium. However, whereas the increase in tone induced by NPY was not different between SHR and WKY with endothelium, the response was significantly greater in WKY than SHR after

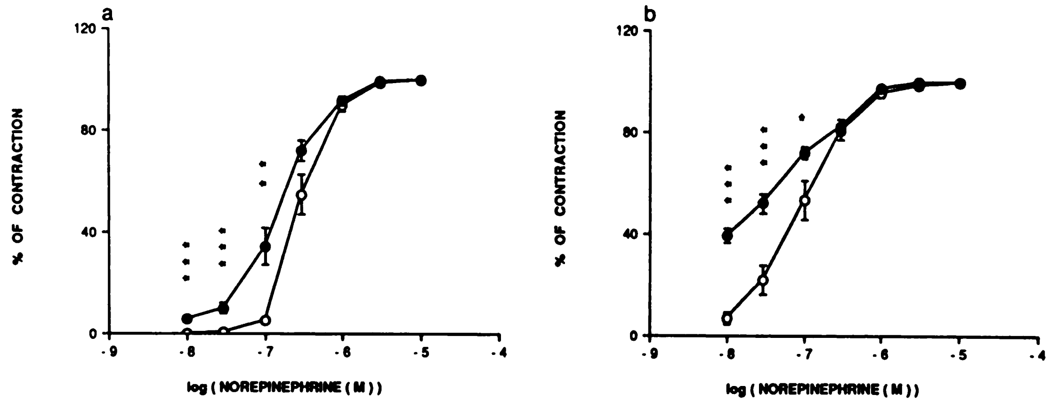

Fig. 5. Contractions induced by norepinephrine in the absence (O) or in the presence (●) of 100 nM NPY on rat mesenteric resistance arteries with (a) or without (b) endothelium taken from WKY. Contractions are expressed as percentage of the maximal contractile responses induced by 10 μM norepinephrine. The points show the mean with the bars representing the S.E.M. *P < .05; **P < .01; and ***P < .001, significantly different with NPY compared to without. The maximal active stresses to norepinephrine in the absence and in the presence of NPY were (in mN/mm^2): 118 ± 13 and 123 ± 14 in WKY with endothelium (n = 8); 154 ± 15 and 163 ± 19 in WKY without endothelium (n = 8), respectively.

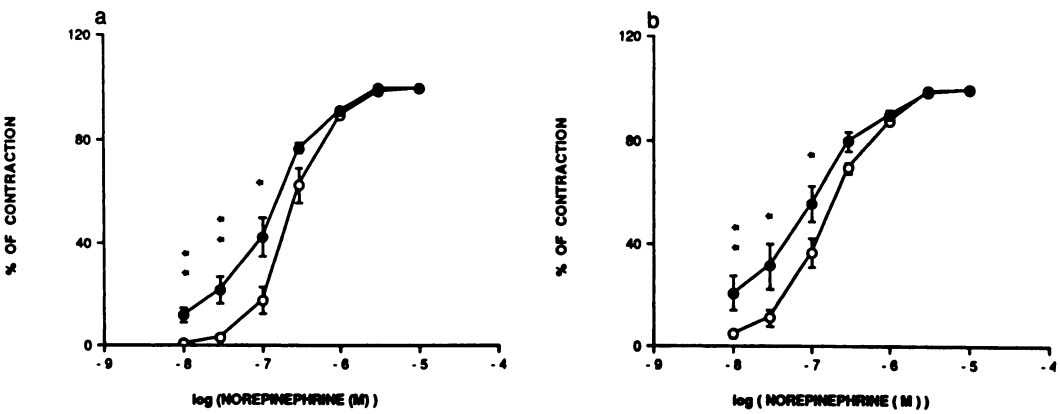

Fig. 6. Contractions induced by norepinephrine in the absence (O) or in the presence (●) of 100 nM NPY on rat mesenteric resistance arteries with (a) or without (b) endothelium taken from SHR. Contractions are expressed as percentages of the maximal contractile responses induced by 10 μM norepinephrine. The points show the mean with the bars representing the S.E.M. *P < .05 and **P < .01, significantly different with NPY compared to without. The maximal active stresses to norepinephrine in the absence and in the presence of NPY were (in mN/mm^2): 138 ± 11 and 143 ± 13 in SHR with endothelium (n = 7); 84 ± 5 and 85 ± 5 in SHR without endothelium (n = 7), respectively.

removal of endothelium. Thus, an enhanced sensitivity to NPY cannot be a causal factor for elevated peripheral resistance.

This strain difference in response to NPY was of interest because of functional alterations of vascular endothelial function that have been reported in hypertensive animals (Lüscher *et al.*, 1987; Tesfamariam and Halpern, 1988; Watt and Thurston, 1989; Diederich *et al.*, 1990). For example, Diederich *et al.* (1990) reported recently that endothelium-dependent relaxations to acetylcholine were impaired in mesenteric resistance arteries of stroke-prone SHR because of a cyclooxygenase-dependent substance interfering with the release and/or action of endothelium-derived relaxing factor.

In concordance with the above reports, the present study reveals a decreased endothelium-dependent relaxation to acetylcholine in mesenteric resistance arteries of SHR compared with WKY. In addition, the sensitivity and the maximal response to norepinephrine in mesenteric resistance arteries of WKY were increased after removal of the endothelium, whereas no change in sensitivity and a decrease in the maximal response to norepinephrine were observed in mesenteric arteries of SHR without endothelium. It is possible that the decreased maximal response observed in the SHR is a result of removal of an endothelium constricting factor. Moreover, the contractile responses of the mesenteric resistance arteries of WKY and SHR to norepinephrine and to NPY were similar in the presence of the endothelium. However, mesenteric arteries from WKY exhibited enhanced responses to norepinephrine and to NPY *vs.* SHR after removal of the endothelium. In addition to the decreased inhibitory effect of the endothelium, it is possible that an alteration of calcium handling and/or an impairment of the excitation-contraction process occurred in vessels of SHR. Recently, Bukoski (1990) reported that calcium handling, as monitored by the calcium-sensitive dye FURA 2, is similar in intact mesenteric resistance arteries of SHR and WKY. Thus, a depressed excitation-contraction coupling is the most likely hypothesis. Additional studies are required to understand more completely the underlying mechanism.

In conclusion, the present study demonstrates that the potentiating effect of NPY on mesenteric resistance arteries from SHR and WKY does not require the presence of functional endothelium. Differential contractile responses to NPY and to norepinephrine between the two strains were only observed after removal of endothelium. The results show that, in the SHR the response to NPY of vascular smooth muscle cells is reduced, whereas the inhibitory effect of the endothelium is enhanced compared the WKY. Finally, inasmuch as vessels of

the WKY and SHR exhibit similar responses to NPY in the presence of the endothelium, an increased contractile response of resistance arteries to NPY is not a causal factor for elevated peripheral resistance.

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