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Phosphodiesterase Isozyme Inhibition and the Potentiation by Zaprinast of Endothelium-Derived Relaxing Factor and Guanylate Cyclase Stimulating Agents in Vascular Smooth Muscle

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ABSTRACT

We have examined the interaction of zaprinast with mediators of guanylate cyclase on the relaxation of aortic smooth muscle. Zaprinast, a selective inhibitor of the low K_m-cyclic GMP (cGMP) phosphodiesterase [low K_m cGMP phosphodiesterase (PDE)], was equally effective in relaxing phenylephrine-contracted aortas from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) with an intact endothelium [EC_{50} = 7.6 (3.5–16.6) μM *vs.* 9.3 (4.1–21.3) μM, respectively]. In contrast, the vasorelaxant activity of zaprinast in intact and denuded phenylephrine-contracted guinea pig aortas, as well as denuded (SHR and WKY) aortas was minimal. Sodium nitroprusside and atriopeptin II were significantly ($P < .05$) more potent as vasorelaxants in denuded SHR aortas when compared with denuded aortas from WKY. Pretreatment with zaprinast potentiated the vasorelaxant potency of sodium nitroprusside in both SHR and WKY aortas whereas atriopeptin II responses were potentiated only in WKY aortas. In studies with the low K_m cGMP PDE, isolated *via* DEAE column chromatography, the apparent K_m for cGMP and potency of zaprinast were approximately 2-fold greater ($P < .05$) in WKY when compared with the same PDE isozyme isolated from SHR aortic preparations. However, the V_{max} (picomoles per milligram per minute) for cGMP hydrolysis was greater in SHR than in WKY. In conclusion, these data show that, although there are no apparent differences in the influence of spontaneously released endothelium-derived relaxing factor from SHR and WKY aortas, reactivity differences to other agents known to stimulate guanylate cyclase activity exist between SHR and WKY denuded aortas. The low K_m cGMP PDE isozyme is apparently not responsible for these difference in relaxation responsiveness in intact aortic smooth muscle.

The endothelium of the blood vessel wall has been shown to play an important role in the mechanism of action of a number of vasodilators. Furchgott and Zawadzki (1980) have demonstrated that the vasorelaxation produced by acetylcholine in the rabbit aorta is mediated by an unidentified, nonprostanoid substance released from the endothelium cells that has been termed EDRF. Subsequent studies have shown that a large number of agents, including substance P, histamine and bradykinin, induce relaxation in blood vessels by releasing EDRF. The mechanism by which EDRF produces vasorelaxation is related to the formation of cGMP *via* stimulation of the soluble fraction of guanylate cyclase (Forstermann *et al.*, 1986). Removal of the endothelium, or treatment with an inhibitor of guanylate cyclase activity such as methylene blue, inhibits the effect of endothelium-dependent vasodilators. Treatment with hemoglobin has also been shown to produce an endothelium-dependent augmentation of tone in rat and rabbit aortas (Martin *et al.*, 1985, 1986). It has been proposed that EDRF is spontaneously released from these tissues (Martin *et al.*, 1986a). The tonic influence of EDRF appears to be much greater in the rat than in the rabbit (Martin *et al.*, 1986a), and this probably accounts for the greater endothelium-dependent inhibition of contractile responses in the rat when compared to the rabbit (Allen *et al.*, 1983).

An altered endothelium in arterial walls has been implicated in the pathophysiology of a number of cardiovascular diseases including hypertension and atherosclerosis (Bossaller *et al.*, 1987). Reduced vasorelaxant responses to the endothelium-dependent vasodilator, acetylcholine, have been demonstrated in aortas from SHR and New Zealand hypertensive rats compared to their normotensive controls (Konishi and Su, 1093; Winquist *et al.*, 1984a; Sim and Singh, 1987). In the present study, the influence of the spontaneous release of EDRF on contraction of aortas from SHR and age/sex matched normotensive WKY rats was compared. The responses in the rat were also compared with another species, the guinea pig. We have

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ABBREVIATIONS: EDRF, endothelium-derived relaxing factor; cGMP, cyclic GMP; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto; PDE, phosphodiesterase; cAMP, cyclic AMP: DTT, dithiothreitol; CI, confidence intervals; ATPase, adenosine triphosphatase.

used the selective low K_m cGMP PDE inhibitor, zaprinast (also known as M & B 22948) to assess some of these properties, as it has been shown that vasorelaxation produced by zaprinast is dependent upon the spontaneous release of EDRF (Martin *et al.*, 1986b). This study has also compared the ability of this PDE inhibitor to potentiate vasorelaxation induced by sodium nitroprusside (which stimulates soluble guanylate cyclase) and atriopeptin II (which stimulates particulate guanylate cyclase) in SHR, WKY and guinea pig aortas (Kukovetz *et al.*, 1979; Winquist *et al.*, 1984b). The inhibitory effects of zaprinast on the activity of PDE isozymes isolated from SHR, WKY and guinea pig aortas as well as the apparent K_m and V_{max} for cGMP hydrolysis were also assessed.

Materials and Methods

Rat aortic ring preparations. Male SHR and WKY rats (Charles River Breeding Laboratories, Inc., Wilmington, MA; 13–17 weeks old), weighing 275 to 350 g were sacrificed by cervical dislocation. The abdomen and thorax were opened and the thoracic aorta was dissected out carefully and placed in a modified Krebs' solution of the following composition (in millimolar: NaCl, 118; KCl, 4.7; $CaCl_2$, 1.6; $MgCl_2$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 24.9; dextrose, 11.1; and disodium Ca EDTA, 0.026; pH = 7.3 to 7.4. The aortas were cleaned of adjoining fatty and connective tissue and cut into rings 3 to 4 mm long. The aortic rings were placed on ringholders *via* two stainless steel wires (40-gauge) threaded through the lumen. One wire was fastened to a holder which was connected to a Grass FT03 force displacement transducer; the other was attached to a fixed holder. The assembly was immersed in a 10-ml jacketed organ bath filled with modified Krebs' solution, maintained at 37.5, and bubbled with 95% O_2–5% CO_2.

Endothelial cells were removed from the aortas by gently rubbing the intimal surface of the aortas with a slightly abraided polyethlyene tubing of varied diameter (depending on the size of the aortas), as described by Furchgott and Zawadzki (1980). Successful removal of the endothelium was confirmed at the end of the experiment by the inability of carbachol (1 μM), a stable acetylcholine analog, to induce relaxation of phenylephrine-contracted aortas (Martin *et al.*, 1986b).

Paired blood vessels (±endothelium) were placed under an optimal passive tension of 2 g (determined in pilot studies) and were allowed to equilibrate for 90 min, during which time the modified Krebs' solution was changed every 15 to 20 min. Changes in force were recorded on a Grass model 7D polygraph. After equilibration, tissues were either used for an ascending phenylephrine concentration-response study, in which cumulative additions of phenylephrine, in 0.5 log units, were added to the tissue bath or tissues were contracted by addition of 1 μM phenylephrine. The amount of force produced by 1 μM phenylephrine, used in the relaxation/potentiation studies, was greater in denuded aortas from WKY than from SHR (2.2 ± 0.1 g *vs.* 1.5 ± 0.1 g, respectively). However, this concentration produced approximately 90% of maximum attainable contraction in both SHR and WKY aortas (fig. 1). When a stable contractile response was obtained (10–15 min), the effect of the cumulative addition of increasing concentrations of either zaprinast, sodium nitroprusside or atriopeptin II was assessed. In the potentiation-interaction studies, zaprinast (30 or 100 μM) or vehicle was added to the blood vessels 15 min before the addition of phenylephrine. These concentrations of zaprinast were chosen to ensure maximum inhibition of low K_m cGMP PDE (fig. 5) and were similar to previous studies utilizing zaprinast to selectively potentiate cGMP-induced and not cAMP-induced vasorelaxation (Martin *et al.*, 1986b).

Guinea pig aortic ring preparation. Male and female guinea pigs (550–800 g) were sacrificed by a blow to the head and subsequent exanguination. The aortas were dissected out, placed on ring holders in modified Krebs' solution identical to that described for rat aortas. After 45 min of equilibration, tissues were contracted by the addition of 3 μM phenylephrine for 5 min, after which the tissues were washed thoroughly and allowed to equilibrate for 45 min. Blood vessels were then contracted with 3 μM phenylephrine and tested for the presence or absence of endothelium by the addition of 1 μM carbachol. Successful removal of endothelial cells was confirmed by the inability of carbachol (1 μM) to induce relaxation, as described by Martin *et al.* (1986b).

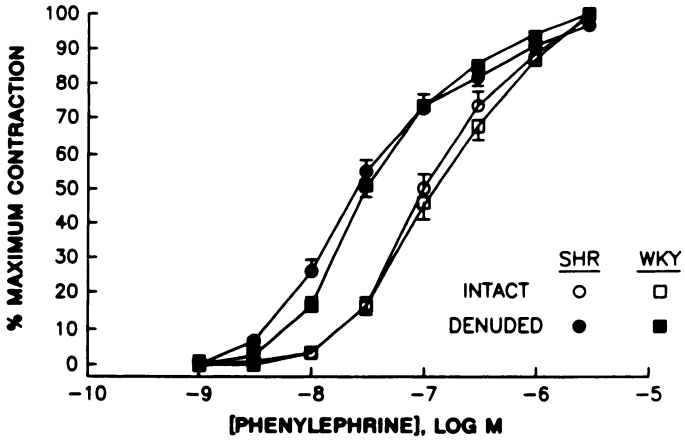

Fig. 1. Concentration-response curves to phenylephrine in SHR and WKY aortic rings in the presence and the absence of endothelium. Each point is the mean of 6 to 12 determinations and vertical bars indicate the S.E. Statistical differences between endothelium-containing aortic rings and endothelium-denuded rings as measured by potency to phenylephrine (EC_{50}) occur for both SHR and WKY rats ($P < .05$). There were no differences between responses from SHR and WKY aortas. Maximum contractile force for SHR aortas was 1.73 ± 0.1 g with endothelium and 1.94 ± 0.1 g without endothelium; maximal force for WKY aortas was 1.99 ± 0.2 g with endothelium and 1.94 ± 0.1 g without endothelium.

PDE isozymes. Slight modifications of the methods of Weishaar *et al.* (1986) were used to separate the isozymes of PDE. Aortic smooth muscle from both guinea pigs and rats was used as the vascular smooth muscle source of PDE isozymes. Thoracic aortas were obtained from either species and cleaned of adhering connective tissue and endothelium. Typically, 50 to 60 aortas were used for each preparation. The tissues were minced with fine scissors and homogenized immediately in 10 volumes of a PDE extraction buffer containing 10 μM Tris acetate, pH 7.5, 2 mM $MgCl_2$, 1 mM DTT and 2000 U/ml of aprotinin. This and subsequent procedures were performed at 0–4°C. Homogenization was accomplished using a Brinkman PT-20 polytron (3–6 bursts at medium setting; 20 sec/burst). The homogenate was then sonicated (20 sec; 6 times at 80% of maximum) in a Heat Systems Ultrasonics sonicator. The resultant homogenate was centrifuged at 48,000 × *g* for 30 min; the resultant supernatant fraction was applied to a DEAE cellulose column (30 × 1.6 cm; 30 ml bed volume) that had been equilibrated with 35 or 70 mM sodium acetate.

Application of the supernatant fractions were standardized so that approximately 70 to 80 ml were applied to each column. After application of the sample, the columns were washed with 2 to 3 bed volumes of 35 or 70 mM sodium acetate-1 mM DTT (pH = 6.5). PDE isozymes were eluted from the columns using a continuous 70 to 1000 mM sodium acetate gradient (containing 1 mM DTT, pH = 6.5, total volume = 400 ml). Fractions (4–6 ml) were collected and assayed for cAMP and cGMP PDE activity at substrate concentrations of 1 μM. In addition, calmodulin stimulation of cGMP hydrolysis was assessed by the addition of 2 μM calmodulin plus 10 μM $CaCl_2$. The low K_m cGMP PDE fraction used in these studies was sensitive to inhibition by zaprinast and insensitive to calmodulin-stimulation (Lugnier *et al.*, 1986). Appropriate fractions corresponding to the low K_m cGMP PDE and low K_m cAMP peak III PDE (Weishaar *et al.*, 1986) were pooled separately for each preparation and dialyzed against 70 mM sodium acetate-0.5 mM DTT (pH = 6.5) for at least 20 hr. After dialysis PDE fractions were concentrated to 14% of original volume using an Amicon ultrafiltration cell system (8050) with a YJM 10 membrane under 25 psi of nitrogen. The concentrated fractions were diluted with ethylene

glycol to 50% and stored at −20°C. No significant changes in hydrolysis or sensitivity to inhibitors was noted with storage up to at least 2 months.

PDE activity was measured as described previously (Weishaar *et al.*, 1986) in reaction mixtures containing 40 mM Tris (pH 8.0), 5 mM $MgCl_2$ and 1 mM DTT. The concentration of substrate (0.2 μM), either [^{3}H]cAMP or [^{3}H]cGMP, was at or slightly below the K_m for all isozymes (Hofstee, 1952). Each assay was performed in triplicate for each concentration of zaprinast. The concentration of inhibitor which produced 50% inhibition of hydrolysis (IC_{50}) was calculated from concentration-response curves. At least four concentration-response curves from between one to four enzymes preparations were generated for each agent with each tissue source. IC_{50} values and 95% CL were calculated as described by Tallarida and Murray (1986).

Materials. The sources of the compounds were as follows: phenylephrine (base; Sterling-Winthrop Research Institute, Rensselaer, NY), carbamylcholine chloride, sodium nitroprusside and papaverine HCl (Sigma Chemical Co., St. Louis, MO), atriopeptin II and calmodulin (Calbiochem, San Diego, CA) and zaprinast (May and Baker, Dagenham, Essex, UK).

[^{3}H]cAMP (30–50 Ci/mmol) and [^{3}H]cGMP (5–10 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Aprotinin was obtained from Sigma Chemical Co., DTT from Calbiochem (LaJolla, Ca) and DEAE from Bio-Rad (Richmond, CA)

Statistical analysis. Percentage of relaxation or contraction was expressed as the mean ± S.E. Percentage of relaxation was defined as the percentage of relaxation of the phenylephrine-induced contraction. The concentration of drug that causes 50% relaxation (EC_{50}) was calculated, where appropriate, by the probit method. EC_{50} values were expressed as geometric means and their 95% CI. Comparisons for significant differences ($P < .05$) were made by an analysis of variance and the Duncans multiple range test.

Results

Phenylephrine reactivity in rat aorta. In age/sex matched SHR and WKY rat aortic ring preparations, removal of the vascular endothelium eliminated the vasorelaxant response to 1 μM carbachol. The effect of 1 μM carbachol on intact aortas was significantly greater ($P < .05$) in WKY than in SHR aortas (87 ± 2% *vs.* 68 ± 3% relaxation, respectively). Subsequent phenylephrine concentration-response studies, in both SHR and WKY rats, showed that endothelium-denuded aortic rings were significantly ($P < .05$) more sensitive to the vasoconstrictor effects of phenylephrine than were the endothelium-containing rings (fig. 1). EC_{50} values for phenylephrine-contractions and their corresponding 95% CI for denuded and intact aortic rings, respectively, were: SHR, 33 nM (24–45); 125 nM (91–171); WKY, 41 nM (30–56); 140 nM (93–213). Endothelium-containing SHR aortas pretreated with 10 μM hemoglobin, which blocks the vasodilator action of EDRF (Martin *et al.*, 1985), significantly ($P < .05$) increased the sensitivity to phenylephrine (EC_{50} = 6.1 nM), compared to either intact (EC_{50} = 125 nM) or denuded aortas (EC_{50} = 33 nM).

Effects of zaprinast upon phenylephrine-induced contractions of the rat and guinea pig aortas. The selective low K_m cGMP PDE inhibitor, zaprinast, produced concentration-dependent relaxation of 1 μM phenylephrine-induced contractions of SHR and WKY aortic rings that contained an intact endothelium (fig. 2). EC_{50} values for zaprinast in SHR and WKY aortas, respectively, were 7.6 μM (3.5–16.6), n = 8 and 9.3 μM (4.1–21.3), n = 11. Relaxation of denuded aortic smooth muscle in response to cumulative concentrations of zaprinast in both strains of rat was greatly reduced (34 ± 1%

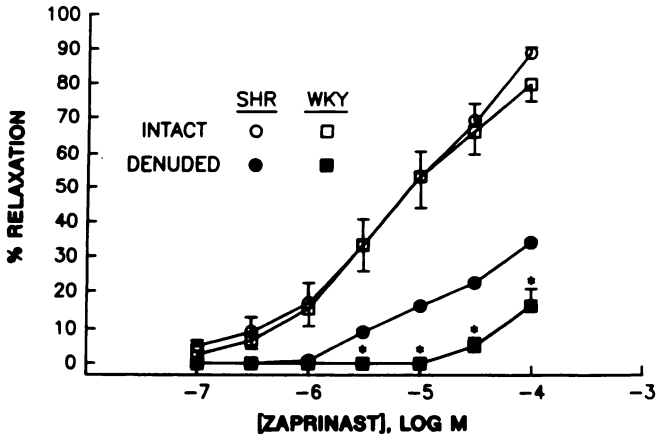

Fig. 2. Concentration-response curves demonstrating the ability of zaprinast to relax near-maximal phenylephrine-induced tone in SHR and WKY aortic rings in the presence and absence of endothelium. The response to zaprinast was significantly ($P < .05$) attenuated in denuded aortas from both SHR and WKY rats. There were no differences between SHR and WKY rats in the response to zaprinast in the presence of endothelium. There were, however, significant differences (*$P < .05$) between SHR and WKY rat responses to zaprinast in endothelium-denuded aortic rings. Each point is the mean of 9 to 15 determinations and vertical bars indicate S.E. Maximal phenylephrine-induced force before zaprinast addition was: 1.5 ± 0.1 g with endothelium and 1.5 ± 0.1 g without endothelium for SHR; and 1.3 ± 0.1 g with endothelium and 1.6 ± 0.2 g without endothelium for WKY rats.

relaxation at 100 μM in the SHR aortas and 16 ± 5% relaxation at 100 μM in the WKY aortas; fig. 2). The effect of zaprinast on denuded rat aortas was significantly greater ($P < .05$) in SHR aortas than in WKY aortas at concentrations of 3 to 100 μM. Relaxation of denuded aortas by high concentrations of zaprinast may be related to low K_m cAMP PDE inhibition by this agent.

Zaprinast was a relatively weak vasorelaxant of either intact or denuded guinea pig aortic rings contracted by 3 μM phenylephrine. In intact aortas, zaprinast caused 29 ± 3% relaxation at 300 μM, whereas in denuded aortas it produced only 20 ± 2% relaxation of phenylephrine-induced tone (data not shown). Guinea pig aortic rings with an intact endothelium also had a lessened response to 1 μM carbachol (44 ± 5% relaxation), compared to that obtained with carbachol in rat aortas (68–87%).

Interaction of zaprinast with sodium nitroprusside and atriopeptin II in rat and guinea pig aortic preparations. Sodium nitroprusside and atriopeptin II both produced effective and potent vasorelaxation of endothelium-denuded, phenylephrine-contracted SHR and WKY rat (figs. 3–4; table 1) and guinea pig aortas (table 1). In both SHR and WKY aortas, pretreatment with single concentrations of 30 or 100 μM zaprinast (which, unlike cumulative concentrations, did not significantly affect isometric force relative to vehicle), significantly ($P < .05$) potentiated the vasorelaxant potency of sodium nitroprusside. The vasorelaxant potency (EC_{50}) of sodium nitroprusside was significantly greater ($P < .05$) in SHR aortas than in WKY aortas both in the absence and the presence of zaprinast (table 1). The same concentrations of zaprinast did not potentiate the vasorelaxant effects of atriopeptin II in SHR aortas, but did potentiate the effects of atriopeptin II in WKY aortas. In the absence of zaprinast, the potency (EC_{50}) of atriopeptin II was significantly greater in SHR aortas than that in WKY aortas (table 1). Zaprinast (30

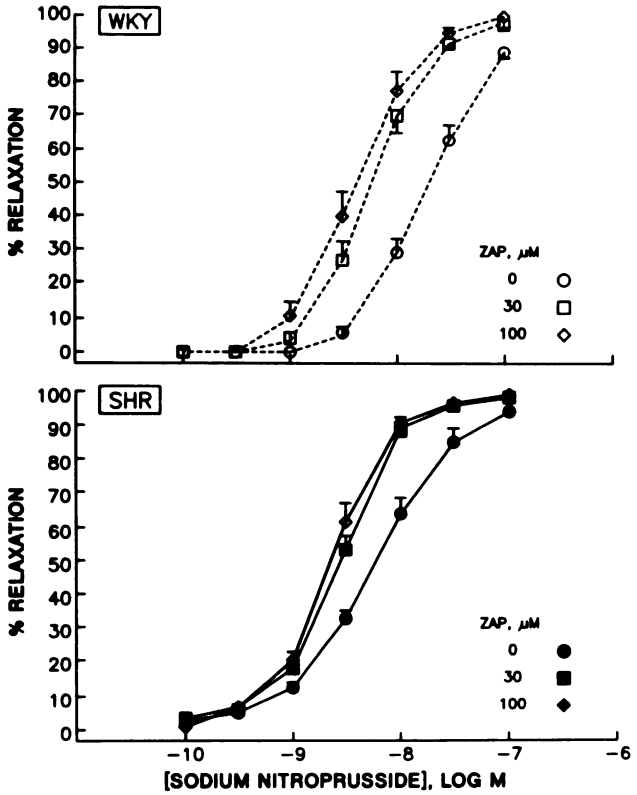

Fig. 3. Concentration-response curves demonstrating the potentiating effect of zaprinast (ZAP) on sodium nitroprusside-induced relaxation in endothelium-denuded, phenylephrine (1 mM) contracted WKY (top panel) and SHR (bottom panel) aortic ring preparations. Each point is the mean of 8 to 14 determinations and vertical bars indicate S.E. Phenylephrine-induced force before addition of sodium nitroprusside was 2.0 ± 0.1 g for WKY rats and 1.4 ± 0.05 g for SHR.

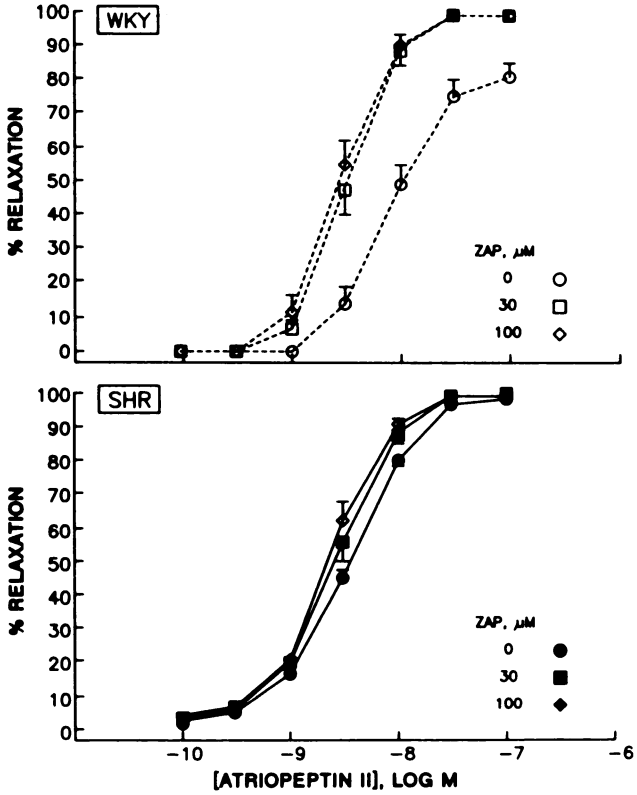

Fig. 4. Concentration-response curves examining the potentiation by zaprinast (ZAP) of atriopeptin II-induced relaxation in endothelium-denuded, phenylephrine (1 mM) contracted WKY (top panel) and SHR (bottom panel) aortic ring preparations. Each point is the mean of 8 to 14 determinations and vertical bars indicate S.E. Phenylephrine-induced force before addition of atriopeptin II was 2.26 ± 0.1 g for WKY rats and 1.65 ± 0.045 g for SHR.

and 100 μM) did not significantly potentiate the vasorelaxing potency (EC_{50}) of either sodium nitroprusside or atriopeptin II in phenylephrine-contracted guinea pig aortic smooth muscle (table 1).

PDE isozyme characteristics and inhibition by zaprinast of PDE isozyme from SHR, WKY and guinea pig aortic smooth muscle. Comparisons of the isozymes from the three vascular smooth muscle sources showed that the apparent K_m for cAMP for the low K_m cAMP PDE isozyme is similar for SHR and WKY rats, but significantly different ($P < .05$) from that of the guinea pig (table 2). The apparent K_m for cGMP for the low K_m cGMP PDE isozyme is significantly different ($P < .05$) between SHR and WKY aortas, whereas the apparent K_m for cGMP in guinea pig aortas is similar to that from WKY and different from that of SHR (table 2). The V_{max} for cGMP for this PDE isozyme from SHR (500 ± 50 pmol of cGMP hydrolyzed per mg of protein per min) was significantly greater ($P < .05$) than the V_{max} for WKY aortic smooth muscle (320 ± 50 pmol/cGMP per mg/min.

Concentration-dependent inhibition of both PDE isozymes from all three vascular sources was evident with zaprinast (fig. 5). In all cases zaprinast was markedly more potent as an inhibitor of the low K_m cGMP PDE than the low K_m cAMP PDE. The degree of selectivity and the potency for low K_m cAMP PDE was very similar for zaprinast for all three aortic smooth muscle sources. However, zaprinast was most potent in inhibiting the low K_m cGMP PDE from WKY aortic smooth muscle, followed by SHR aortic smooth muscle and least potent in inhibiting this PDE isozyme from guinea pig aortic smooth muscle (table 2). Inhibition by zaprinast of the low K_m cGMP PDE from all three sources was competitive with respect to cGMP when analyzed with a Lineweaver-Burk plot (data not shown).

Discussion

The present experiments demonstrate that the influence of the spontaneous release of EDRF on vascular tone from SHR aortas appears to be similar to that in WKY aortas. This conclusion is based on the observations that the leftward shift in the phenylephrine concentration-response curve, caused by endothelium removal (fig. 1), as well as the zaprinast concentration-relaxation curves (fig. 2), are similar in SHR and WKY aortas. Hence, the tonic influence of EDRF, measured by two different procedures (*i.e.*, removal of endothelium with subsequent attenuation of the relaxing influence of EDRF, or potentiation of a tonic EDRF-effect with a selective low K_m cGMP PDE inhibitor) were similar in SHR and WKY aortas.

In the present study it was also shown that the vasorelaxant response to exogenously added carbachol, which causes a concentration-dependent release of EDRF from vascular endothelium (Furchgott and Zawadzki, 1980), is less in aortas from SHR compared to that from WKY aortas. These results are similar to those reported previously by Konishi and Su (1983), and as well as by Sim and Singh (1987). Alternatively, it has been shown that the endothelium of SHR aortas releases a

TABLE 1

Effect of zaprinast pretreatment on sodium nitroprusside and atriopeptin-II vasorelaxation in phenylephrine-contracted SHR and WKY rat and guinea pig aortas

Animal/Agent	EC_{50} (95% CI) in nM[a]					
	Vehicle[a]	n	Zaprinast (30 μM)[a]	n	Zaprinast (100 μM)[a]	n
SHR:						
Sodium nitroprusside	6.0 (4.4–8.1)[b]	9	2.6 (2.1–3.1)[b, c]	9	2.3 (1.8–3.1)[b, c]	9
Atriopeptin II	3.5 (3.0–4.1)[b]	10	2.5 (1.8–3.5)	8	2.3 (1.8–3.0)	8
WKY rats:						
Sodium nitroprusside	19.9 (15–26)	12	5.9 (4.4–7.9)[c]	9	4.2 (2.8–6.3)[c]	8
Atriopeptin II	13.2 (9–20)	12	3.6 (2.5–5.0)[c]	8	2.9 (2.1–4.2)[c]	8
Guinea pig:						
Sodium nitroprusside	33 (19–57)	7	20 (13–32)	8	17 (9–32)	8
Atriopeptin II	8.0 (6–11)	4	9.0 (7–11)	4	6.0 (5–8)	4

[a] EC_{50} is defined as the concentration of vasorelaxant that produced 50% relaxation of phenylephrine-contracted rat (1 μM) or guinea pig (3 μM) aortas. Vehicle groups were pretreated with zaprinast vehicle (0.025 M NaOH) 15 min before phenylephrine addition, whereas zaprinast groups were pretreated with 30 or 100 μM zaprinast 15 min before phenylephrine addition. 95% CI is the 95% CI of the geometric means. Significant differences were determined with an analysis of variance and Duncan's multiple range test. *n*, number of tissues (one tissue/animal) tested.

[b] EC_{50} values are significantly different when compared with similarly-treated WKY aortas.

[c] EC_{50} values are significantly different from vehicle-pretreated aortas.

TABLE 2

Comparisons of DEAE-cellulose separated PDE isozymes from WKY, SHR and guinea pig aortic smooth muscle

	Source of Aortic Smooth Muscle		
	WKY	SHR	Guinea pig
Low K_m cGMP PDE			
K_m cGMP (μM)[a]	0.66 ± 0.1	1.1 ± 0.16	0.49 ± 0.03
V_{max}[b]	320 ± 50	500 ± 50	N.D.
Inhibition by zaprinast			
IC_{50} (μM)[c]	0.52	0.80	2.0
(95% CI)	(0.44–0.62)	(0.67–0.97)	(2.6–3.3)
Low K_m cAMP (Peak III) PDE			
K_m cAMP (μM)[a]	0.15 ± 0.02	0.15 ± 0.04	0.35 ± 0.01
Inhibition by zaprinast			
IC_{50} (μM)[c]	80	60	70
(95% CI)	(72–88)	(44–82)	(57–85)

[a] Values are the mean ± S.E. for at least four experiments from two to four enzyme preparations. Each enzyme preparation comprised 50 to 60 aortas. The K_m for cGMP and cAMP was determined by the method of Hofstee (1952).

[b] V_{max} is expressed as picomoles of cGMP hydrolyzed per minute per milligram of protein. V_{max} was not determined for low K_m cAMP.

[c] IC_{50} values are the concentration of zaprinast that produced 50% inhibition and were determined as described in the text. Values in parentheses are the 95% CI for the IC_{50} value. N.D., not determined.

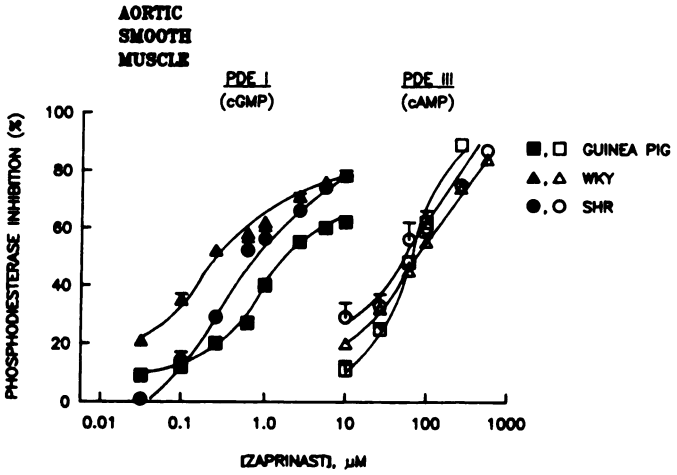

Fig. 5. Inhibition of SHR, WKY and guinea pig aortic PDE isozymes by zaprinast. The effect of graded concentrations of zaprinast on inhibition of cyclic nucleotide hydrolysis by DEAE-isolated isozymes of PDE are shown. Isolation of the two peak fractions (peaks I, low K_m cGMP and III, low K_m cAMP) and the enzyme assay are described under "Materials and Methods." The concentration of substrate was 0.2 μM for SHR, WKY and guinea pig aortic smooth muscle. Inhibition of peak I was determined with cGMP as substrate; cAMP was substrate for peak III PDE. Results are the mean ± S.E. from four experiments from two to four different enzyme preparations. Each enzyme preparation comprised 50 to 60 aortas.

contracting factor (in addition to EDRF) in response to carbachol whereas in the WKY aortas only EDRF is released (Lüscher and Vanhoutte, 1986). It has been suggested (Lüscher and Vanhoutte, 1986) that the diminished responsiveness to carbachol in SHR rats is related to this release of a contracting factor in response to carbachol that offsets some of the relaxant activities of EDRF in SHR. This explanation would be consistent with the results of the present study in which the responsiveness to the spontaneous release of EDRF is similar in SHR and WKY aortas, suggesting that EDRF is not different in SHR and WKY aortas. It is also possible that vascular smooth muscle from SHR is more sensitive to contraction induced by carbachol.

There were a number of other differences in reactivity observed between SHR and WKY aortas in this study. Both atriopeptin II and sodium nitroprusside were significantly more potent in SHR aortas than in WKY aortas. This is in contrast to results reported with methoxamine-contracted aortas from New Zealand hypertensive rats, which were less sensitive to the vasorelaxing effects of sodium nitroprusside than aortas from their normotensive controls (Winquist *et al.*, 1984a). One possible reason for this difference may be related to the different sources of hypertensive animals, Japanese SHR (this study) *vs.* New Zealand genetically hypertensive rats. A recent study in another model of hypertension has shown that sodium nitroprusside has a greater vasodilator response in deoxycorticosterone-induced hypertensive pigs than in normotensive pigs (Webb *et al.*, 1987).

These differences in sensitivity to sodium nitroprusside and atriopeptin II between SHR and WKY rats may be attributable to different reasons. For example, the amount of force of the 1 μM phenylephrine-induced contraction was greater in WKY aortas than that in SHR aortas (2.2 *vs.* 1.5 g, respectively). Hence, it may take a greater concentration of vasorelaxant to reduce tone in WKY aortas. The concentrations of phenyleph-

rine used, however, were those that produced approximately 90% of the maximum attainable contraction in both preparations. Therefore, inasmuch as the amount of precontraction used in the vasorelaxation studies was from a similar portion of the phenylephrine concentration-response relationship for both preparations, this explanation seems unlikely. Another possible explanation may rise from the cGMP-mediated mechanism of vasorelaxation. cGMP-induced vasorelaxation has been causally related to a reduction in the intracellular calcium concentration in vascular smooth muscle cells (see Hardman, 1984 for review). In this regard, cGMP-stimulation of the sarcolemmal Ca^{++} ATPase extrusion pump has been proposed (Popescu *et al.*, 1985). Defects in calcium handling have been well-documented in SHR blood vessels and isolated membrane preparations, and may be linked to sarcolemmal Ca^{++}-ATPase activity (Devynck *et al.*, 1981; Pedersen, 1979; Noon *et al.*, 1978). It is therefore possible that the increased sensitivity to sodium nitroprusside and atriopeptin II in this study in SHR aortic smooth muscle may be related to a more general cGMP-related alteration in calcium transport in the SHR aortas.

Another explanation for these differences may have been related to the low K_m cGMP PDE isozyme. In the present study the concentration-relaxation relationships to sodium nitroprusside were significantly potentiated by pretreatment with the selective low K_m cGMP PDE inhibitor, zaprinast, in both SHR and WKY rats. However, the vasorelaxant response of sodium nitroprusside in the presence of zaprinast in WKY was equivalent to the effect of sodium nitroprusside in the absence of zaprinast in SHR aortas. The concentration-relaxation relationship to atriopeptin II was potentiated by zaprinast pretreatment in WKY aortas, only. Once again, however, the atriopeptin II response in WKY rats with zaprinast was equivalent to the vasorelaxant response of atriopeptin II without zaprinast pretreatment in SHR aortas. These differences may be possibly explained by the 2-fold lessened sensitivity for cGMP hydrolysis by the low K_m cGMP PDE from SHR aortas when compared with this PDE isozyme from WKY aortas (table 2). Thus, the vasorelaxant agents that increase cGMP (sodium nitroprusside and atriopeptin II) would seem to be more potent in SHR compared to WKY aortas by virtue of a lessened cGMP hydrolysis by the low K_m cGMP PDE. Removal of this site with the selective inhibitor, zaprinast, obviates this difference, resulting in equal vasorelaxant effects of these agents in SHR and WKY aortas. However, this explanation is likely not responsible for these differences in intact aortic smooth muscle, as the V_{max} for SHR low K_m cGMP PDE was higher than the V_{max} for WKY low K_m cGMP PDE. Thus the 2-fold higher K_m for cGMP in SHR may offset by the approximate 2-fold increase in V_{max} in SHR.

Although there were not differences in the influence of spontaneously released EDRF in aortas from SHR and WKY aortas, this study has shown that the effect and/or release of EDRF is substantially less in guinea pig aortas. This was demonstrated in muscles that were all precontracted with phenylephrine, so that the method of inducing tone was similar. Evidence supporting this decreased responsiveness to EDRF in guinea pig aortas was demonstrated by a lessened vasorelaxant response to zaprinast, as well as a reduced vasorelaxant response to 1 μM carbachol. However, zaprinast was also less potent in inhibiting low K_m cGMP isolated from guinea pig aortas, compared to that isolated from rat (SHR and WKY). Moreover, the maximum efficacy of PDE inhibition by zaprinast was greater in the rat (80% inhibition) than in the guinea pig (65%). These differences may be related to possible differences in the isozyme(s) present in the low K_m cGMP fraction isolated from DEAE columns. Although these differences are significant, it is unlikely, however, that these differences between rat and guinea pig with the isolated isozymes would solely account for the 2 to 3 log unit difference in activity of zaprinast in intact guinea pig and rat aortic smooth muscle. However, *in toto*, these data would indicate that the zaprinast-sensitive form of low K_m cGMP PDE is important in modulating force in rat aortic smooth muscle and may not be as important in guinea pig aortic smooth muscle.

In summary, these results suggest that the magnitude of spontaneous release of EDRF, or the expression of EDRF-mediated vasorelaxation, is similar in aortas isolated from SHR and WKY rats. These results also show that denuded aortas from SHR are more sensitive to vasorelaxation induced by sodium nitroprusside and atriopeptin II than aortas from WKY rats. Moreover, this study has shown that EDRF is either not spontaneously released, or its expression as a vasorelaxant is less, in guinea pig aortas when compared with rat aortas. This suggests further that species differences exist in EDRF-modulation of vascular tone, as well as in responsiveness to selective inhibitors of low K_m cGMP PDE.

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