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Characterization of the *Alpha*-1 Adrenoceptors in the Mesenteric Vasculature from Deoxycorticosterone-Salt Hypertensive Rats: Studies on Vasoconstriction, Radioligand Binding and Postreceptor Events

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ABSTRACT

The vasoconstrictor responses to *alpha*-1 adrenoceptor agonists, binding behavior of *alpha*-1 adrenoceptors and postreceptor events in the mesenteric vasculature from deoxycorticosterone acetate-salt hypertensive rats were studied. The reactivity of perfused mesenteric artery to norepinephrine (NE) and phenylephrine, but not KCl, was enhanced significantly in the hypertensive rats compared with control rats. Prazosin antagonized the pressor response to NE more effectively in the hypertension than in the control. [^{3}H]Prazosin binding was saturable and a single class of specific sites. Scatchard analysis revealed that the dissociation constant for [^{3}H]prazosin was lower and the maximum binding capacity was greater in the hypertensive rats than in the control rats. The NE-stimulated phosphatidylinositol hydrolysis, estimated by measuring inositol 1,4,5-triphosphate (IP_3) accumulation, was greater in the hypertensive rat artery compared with the control one. The IP_3-induced contraction in the β-escin-treated mesenteric large resistance vessel was smaller in the hypertensive rats. The vasoconstrictor response to phorbol 12,13-dibutyrate of the perfused mesenteric artery was larger in the hypertensive animals than in the control. Staurosporine antagonized the phorbol 12,13-dibutyrate-induced vasoconstriction in preparations from both rats. These results suggest that the increases in the number and affinity of *alpha*-1 adrenoceptors may result in an enhanced phosphatidylinositol hydrolysis accounting for an increased vascular reactivity to *alpha*-1 adrenoceptor agonists in deoxycorticosterone acetate-salt hypertensive rats. Furthermore, the enhancement of vasoconstrictor mechanism mediated by protein kinase *C* pathway may also contribute to vascular hyper-reactivity to NE, whereas the decreased IP_3-induced contraction may function to minimize the hyper-reactivity observed in this model of experimental hypertension.

Many studies have demonstrated an augmented vascular sensitivity to catecholamines in DHR compared with normotensive rats (Berecek *et al.*, 1980; Ekas and Lokhandwala, 1980; Hagen and Webb, 1984; Soltis and Field, 1986; Tsuda *et al.*, 1986; Perry and Webb, 1988). This catecholamine supersensitivity precedes the development of hypertension (Berecek *et al.*, 1980; Katovich *et al.*, 1984). Thus, one of the pathogenic factors of DOCA-salt hypertension is thought to be an enhanced vascular responsiveness to catecholamines, leading to an elevation of the total peripheral resistance. Perry and Webb (1988) have speculated that an alteration in *alpha* adrenoceptor density or postreceptor events may account for increased vascular sensitivity to catecholamines. There are, however, few reports that have been performed in an attempt to correlate enhanced vascular sensitivity to catecholamines with changes in *alpha* adrenoceptor properties and postreceptor events.

Radioligand binding studies represented that *alpha*-1 adrenoceptor density in the mesenteric artery (Meggs *et al.*, 1988) and in some regions of the brain (Yamada *et al.*, 1980) of DHR is greater than that of normotensive rats. On the other hand, the binding capacity for *alpha*-1 antagonists in the aorta from aldosterone hypertensive rats was not altered in spite of the observed increase in vascular sensitivity to NE (Smith *et al.*, 1987). An *in vitro* autoradiographic study indicated that there was significant reduction in binding of aortic and mesenteric vascular *alpha*-1 adrenoceptors of DHR (Wilson, 1991). Thus, whether the increased vascular sensitivity to NE is related to changes in *alpha*-1 adrenoceptor density remains unclear.

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ABBREVIATIONS: DHR, deoxycorticosterone acetate-salt hypertensive rats; DOCA, deoxycorticosterone acetate; NE, norepinephrine; PI, phosphatidylinositol; IP_3, inositol 1,4,5-triphosphate; DAG, 1,2-diacylglycerol; PKC, protein kinase *C*; IP, inositol monophosphate; PDBu, phorbol 12,13-dibutyrate; CMC, carboxymethyl cellulose; KBS, Krebs-bicarbonate solution; Suc-MOPS, 0.25 M sucrose and 10 mM morpholinopropanesulfonic acid; B_{max}, maximum binding capacity; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid; DR, dose ratio; PLC, phospholipase C; MLC, myosin light chain.

Postreceptor events closely associated with *alpha*-1 adrenoceptor-mediated vascular contraction involve PI hydrolysis (Abdel-Latif, 1986). PI hydrolysis results in a generation of two second messengers, IP_3 and DAG (Abdel-Latif, 1986). IP_3 releases Ca^{++} from intracellular store sites of smooth muscle cells (Somlyo *et al.*, 1985) and DAG as well as tumor-promoting phorbol esters activates PKC (Nishizuka, 1986), both of which lead to vascular contraction. Activation of PI hydrolysis by NE can be estimated by measuring IP accumulation in the presence of lithium, because this is a simple method for monitoring changes in PI hydrolysis in response to *alpha*-1 adrenoceptor stimulation (Fox *et al.*, 1985). Eid and de Champlain (1988) and Takata *et al.* (1989) showed that arterial IP formation stimulated by NE was greater in DHR than in normotensive rats. However, recent studies demonstrated that an enhanced pressor response to NE of the tail artery from spontaneously hypertensive rats was followed by an increased accumulation of IP_3, but not IP (Muir and Wardle, 1989; Guild *et al.*, 1992). These results suggest that IP accumulation is not necessarily taken as a measure of PI hydrolysis.

In the present study we found an increased responsiveness to NE and phenylephrine and a more effective antagonistic action of prazosin against NE-induced constriction in the mesenteric vascular bed from DHR. So, we investigated further whether the enhanced responsiveness to NE in hypertension is accompanied by increased density and affinity of *alpha*-1 adrenoceptors and increased PI hydrolysis which was estimated by measuring IP_3 formation. In addition, we also studied whether the vascular constrictor responses to both IP_3 and the tumor-promoting phorbol ester, PDBu, are altered in this model of experimental hypertension. All the experiments were conducted by using the mesenteric vascular bed or mesenteric large resistance vessels (0.2–0.3 mm in lumen diameter), because the large resistance vessels as well as the vascular bed have been shown to play an important functional role in an increased peripheral resistance characteristic of hypertension (Mulvany, 1985).

Methods

Hypertensive animals. Male Wistar rats (7 weeks old) were anesthetized with sodium pentobarbital, 50 mg/kg i.p., and the left kidney was removed. After surgery, DOCA (10 mg/kg s.c.) suspended in 0.5% CMC was administered twice a week for 4 to 6 weeks until systolic blood pressure measured in the conscious state by tail-cuff plethysmography (Narco Bio-systems, PE-300, Houston, TX) reached 180 mm Hg. The DOCA-treated rats were given free access to 1% NaCl drinking solution. Normotensive control rats were uninephrectomized, administered 0.5% CMC instead of DOCA and received normal tap water for drinking (water control). Part of the control rats uninephrectomized were given 1% NaCl as drinking water (salt control). All rats were maintained on standard laboratory chow. Under additional anesthesia with sodium pentobarbital (50 mg/kg i.p.), either the mesenteric vascular bed or the mesenteric large resistance vessels were isolated from the rat as described below.

Vasoconstrictor responses of the mesenteric vascular bed to agonists. The mesenteric vascular bed was prepared according to the method of McGregor (1965) with minor modifications. The abdominal cavity was opened and the superior mesenteric artery was exposed. A polyethylene cannula was inserted distally into the superior mesenteric artery from its origin and the mesenteric vascular bed was flushed gently with KBS. The KBS had the following composition (in millimolar): NaCl, 118.2; KCl, 4.6; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 24.8; and dextrose, 10.0. Six main branches from the superior mesenteric trunk were used for the experiment. The mesenteric vasculature was connected to the perfusion apparatus and then perfused at a rate of 5 ml/min by a peristaltic pump with the KBS containing 1 μM propranolol to block *beta* adrenoceptors. The KBS was maintained at 37°C and bubbled with a gas mixture of 95% O_2-5% CO_2 to obtain a pH of 7.4. Changes in perfusion pressure were measured with a pressure transducer (Nihon Kohden, MPU-0.5, Tokyo, Japan) and recorded on a Nihon Kohden polygraph (WT-645G). After a 60-min equilibration period, the experiment was started.

In the first series of experiments, noncumulative dose-response curves for four drugs were constructed in the following order: KCl, NE, phenylephrine and xylazine. Each drug at stepwise increasing doses was injected into the perfusion stream at 15-min intervals. The injection volume was 0.1 ml. Thirty-minute intervals were allowed between the dose-response curves for each drug. In some experiments, dose-response curves for KCl were obtained in the absence or presence of 1 μM phentolamine.

In the second series of experiments, antagonistic effects of prazosin against the pressor responses to NE were examined. Pilot experiments in which, after reproducible responses to 11.9 nmol of NE were obtained, five consecutive dose-response curves for NE were recorded showed that there was no significant difference among NE responses in the first to third dose-response curves in DHR, whereas no difference was observed among the third to fifth curves in normotensive rats. Therefore, prazosin at increasing concentrations was perfused after the first dose-response curve in hypertensive rats and after the third curve in normotensive rats. The prazosin pretreatment period was 10 min.

In the third series of experiments, the pressor responses to PDBu were examined in the absence or presence of staurosporine, a potent PKC inhibitor (Tamaoki *et al.*, 1986). In the experiments without staurosporine, the mesenteric vasculature was activated twice with 12.5 nmol of PDBu in hypertensive rats and with 50.0 nmol of PDBu in water control rats. Then, a noncumulative dose-response curve for PDBu was determined by using the same technique as the first series of experiments except that subsequent doses were applied when the response had returned to base line. In the experiments in which staurosporine effects against the pressor response to PDBu were examined, after the activation procedure, the dose of PDBu required to cause about a 50-mm Hg elevation in perfusion pressure was injected twice every 60 min. In pilot experiments we confirmed reproducible responses to this dose of PDBu in both DHR and control rats. Staurosporine (5 nM) was treated from 20 min before the second injection of PDBu.

Radioligand binding experiments. A particulate fraction of the mesenteric artery was prepared by modifications of the method of Kwan *et al.* (1979), but the fraction was not subjected to a discontinuous sucrose gradient centrifugation. The entire mesenteric arcade was immersed in ice-cold Suc-MOPS buffer (pH 7.4) and cleaned over an ice-cold plate to remove the mesenteric vein, fat, lymph nodes and mesenteries with nerves. The cleaned mesenteric vascular bed was frozen rapidly and stored at −85°C up to 1 month. On the day of the experiment, the vascular bed was thawed, finely minced and homogenized in 10 volumes of ice-cold Suc-MOPS buffer by using a Biotron BT 20 homogenizer (setting 6 for 15 sec). The homogenate was centrifuged at 900 × *g* for 10 min at 4°C. The supernatant was then centrifuged at 105,000 × *g* for 60 min at 4°C in a Hitachi ultracentrifuge. The pellet was washed in 7 volumes of binding assay buffer (pH 7.5, 5 mM $MgCl_2$ and 50 mM Tris) and recentrifuged at 105,000 × *g* for 60 min at 4°C. The final pellet was resuspended in the assay buffer. The particulate fraction from 36 rats was utilized for each experiment, resulting in a protein concentration of 1 mg/ml. The protein content was determined according to the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

[^{3}H]Prazosin was used to label *alpha*-1 adrenoceptors in the particulate fraction of mesenteric vasculature of the rat. The binding assay mixture contained 100 μl of various concentrations of [^{3}H]prazosin and 100 μl of the assay buffer with (for nonspecific binding) or without (for total binding) 1 μM unlabeled prazosin. The binding reaction was

started by adding 100 μl of particulate suspension (containing 100 μg of membrane protein). The assay mixture was incubated at 25°C for 30 min. Assays were conducted in duplicate. The reaction was terminated by filtration under reduced pressure through Whatman GF/C glass-fiber filters. The tubes and filters were rinsed quickly 4 times with 5 ml of buffer which was subsequently filtered. Filters were left overnight at room temperature to equilibrate in 10 ml of scintillation mixture and then were counted by using a liquid scintillation counter (Aloka, LSC-3500, Tokyo, Japan). Specific binding was calculated as the difference between total and nonspecific binding. Saturation binding curves for [^{3}H]prazosin were analyzed by the method of Scatchard (1949) to determine the K_d and the B_{max}.

IP_3 measurement. The mesenteric vascular bed was cleaned of the mesenteric vein, fat, lymph nodes and mesenteries with nerves as described above. The vascular bed of each rat was divided into two parts. One was treated with NE and the other received an equivalent volume of distilled water to measure unstimulated basal IP_3 formation. After a 60-min preincubation at 37°C in KBS (bubbled with 95% O_2-5% CO_2) containing 10 mM LiCl, the vasculature was incubated at 37°C in 200 μl of KBS with or without various concentrations of NE. The incubation was carried out for 12 sec in hypertensive rats and for 15 sec in normotensive rats. These different incubation periods were selected, because preliminary experiments showed that IP_3 accumulation elicited by 0.3 μM NE reached peak level at these incubation periods. The reaction was terminated by adding 50 μl of ice-cold 10% perchloric acid. The mixture was frozen rapidly and stored at −85°C. On the day of study, the mixture was thawed and centrifuged at 2,000 × g for 15 min at 4°C. The pH of the supernatant was adjusted to 7.5 with 75 mM HEPES containing 1.53 M KOH in the presence of Universal Indicator (BDH Chemicals Ltd., Poole, UK). The solution was kept on ice for 60 min and recentrifuged at 2,000 × g for 15 min at 4°C. The amount of IP_3 in the final supernatant was measured in duplicate with an IP_3 assay system (Amersham-Japan, TRK 1000, Tokyo, Japan).

Solutions used in skinned muscle experiments. The composition of the modified KBS was as follows (in millimolar): NaCl, 121.9; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 15.5; and dextrose, 11.5. The Ca^{++}-free modified KBS was obtained by omitting Ca^{++} from the modified KBS. High KCl (80 mM) solution was prepared by substituting NaCl with equimolar KCl. The skinning solution contained the following (in millimolar): CH_3COOK, 140.0; $(CH_3COO)_2$ Mg, 0.5; EGTA, 5.0; dithiothreitol, 1.0; imidazole, 20.0; and β-escin, 0.095. The relaxing solution was composed of the following (in millimolar): CH_3COOK, 140.0; $(CH_3COO)_2$ Mg, 0.5; EGTA, 1.0; ATP-Na_2, 4.0; creatine phosphate, 5.0; NaN_3, 5.0; dithiothreitol, 1.0; and imidazole, 20.0. The Ca^{++}-loading solution was prepared by adding $(CH_3COO)_2Ca$ [0.3 μM free Ca^{++} (pCa 6.5)] to the relaxing solution. The activating solution was obtained by adding various free Ca^{++} concentrations to the relaxing solution and by elevating EGTA concentration to 4 mM. These solutions used for skinned fiber experiments were much the same as those described by Wong and Rahwan (1988).

IP_3-induced contraction. Ring preparation of mesenteric large resistance vessels (about 0.3 mm in outer diameter, 0.4 mm in length) was mounted under the optimum resting tension of 10 mg in a 0.3-ml organ bath and superfused at a rate of 3 ml/min with the modified KBS bubbled with 95% O_2-5% CO_2 at 25°C. Developed tension was measured isometrically with a strain gauge force transducer (U-gauge, Shinko Co., Tokyo, Japan). After a 60-min equilibration period, the artery was contracted with 80 mM KCl and washed with the Ca^{++}-free modified KBS for 10 min. The skinning solution was introduced for 60 min at 5°C. Aeration of each solution was stopped during and after skinning. Each solution was maintained at 20°C after skinning. The artery was exposed to the relaxing solution for 30 min and then bathed in the Ca^{++}-loading solution for 2 min. After re-exposure to the relaxing solution for 2 min, the preparation was kept for 7 min in the relaxing solution containing 50 μM EGTA. IP_3-induced contraction, taken as the amount of Ca^{++} released from store sites, was produced by replacing the relaxing solution containing 50 μM EGTA in the organ bath with the IP_3-containing solution (pCa 6.5) buffered with 50 μM EGTA. Only two concentrations of IP_3 were applied in each preparation, using a randomized order. After the concentration-response curve for IP_3, the muscle was allowed to contract with the activating solution of pCa 5.0. The IP_3-induced contraction was normalized to a percentage of the maximum contraction elicited by pCa 5.0. The efficiency of the skinning procedure was confirmed by the loss of the contractile response to 80 mM KCl.

pCa-tension relationship. After skinning the artery was exposed to the relaxing solution containing 4 mM EGTA for 30 min. Then the activating solutions (pCa 7.0–5.0) containing 4 mM EGTA were superfused cumulatively to the artery to obtain pCa-tension relationship. These experiments were conducted at 20°C.

Drugs. The following drugs used were purchased: DOCA (Wako Pure Chemical Industries, Osaka, Japan); *dl*-propranolol HCl, NE (*l*-arterenol bitartrate), *l*-phenylephrine HCl, prazosin HCl, PDBu, 4α-phorbol 12,13-didecanoate, β-escin and morpholinopropanesulfonic acid (Sigma Chemical Co., St. Louis, MO); staurosporine (Boehringer, Mannheim, W. Germany); xylazine HCl (Bayer Yakuhin, Osaka, Japan); phentolamine mesylate [CIBA-GEIGY (Japan), Takarazuka, Japan]; IP_3 tripotassium salt, HEPES and EGTA (Dojindo Laboratories, Kumamoto, Japan); and [^{3}H]prazosin (25.0 Ci/mmol, DuPont-New England Nuclear, Boston, MA).

Statistical analysis. All data are expressed as the mean ± S.E. Statistical analyses were performed by using an unpaired Student's t test for two-sample comparison and one-way analysis of variance followed by Dunnett's test for multiple comparisons. In each case, P values less than .05 were considered significant.

Results

Systolic blood pressure in conscious rats. The systolic blood pressures of water control, salt control rats and DHR used in the present study were 128.5 ± 0.5 (n = 202), 132.2 ± 1.7 (n = 26) and 205.0 ± 0.7 mm Hg (n = 202), respectively. The systolic blood pressure in the hypertensive rats was significantly ($P < .01$) higher than those in the controls, whereas no difference was found between the two control groups.

Vasoconstrictor responses to NE, phenylephrine and KCl. Unstimulated basal perfusion pressure (25.8 ± 1.2 mm Hg, n = 59) in the hypertensive rats was significantly ($P < .01$) greater than those in water (20.4 ± 0.8 mm Hg, n = 59) and salt (21.3 ± 1.7 mm Hg, n = 6) control rats. Figure 1 shows dose-response curves for NE (0.19–12 nmol), phenylephrine (0.63–40 nmol) and KCl (25–400 μmol) in the hypertensive and normotensive control rats. The vasoconstrictor responses to the three agonists in water control rats were similar to those in salt control rats. The dose-response curves for NE and phenylephrine were shifted to the left over the entire dose range tested in the hypertensive rats. The dose ($ED_{100\ mm\ Hg}$, 1.9 ± 0.5 nmol) of NE to produce a 100 mm Hg increase in perfusion pressure in DHR was significantly ($P < .01$) smaller than that in water control (6.4 ± 1.0 nmol) or salt control (5.0 ± 1.0 nmol) rats. The increased vascular sensitivity to phenylephrine was also observed, the $ED_{100\ mm\ Hg}$ value being 8.0 ± 2.2, 18.9 ± 2.7 and 20.2 ± 2.5 nmol in the DOCA-salt, water and salt controls, respectively. In contrast, the dose-response curve for KCl in the hypertensive rats was similar to that in the water control rats. The pressor responses to KCl (25–400 μmol) in both groups were not affected by 1 μM phentolamine treated from 10 min before the first dose of KCl, whereas the responses to NE (0.19–12 nmol) were almost abolished completely by phentolamine (data not shown). Xylazine (0.1–30 nmol), a selective *alpha*-2 adrenoceptor agonist (Hsu, 1981; Docherty

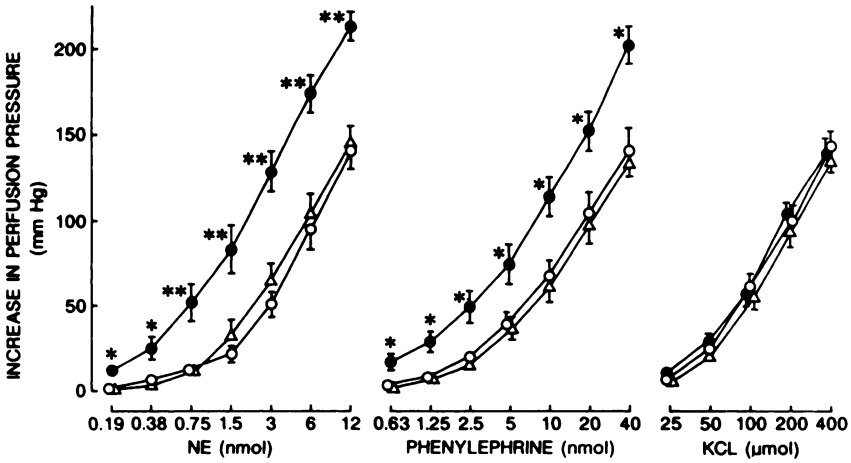

Fig. 1. Dose-response curves for NE (left panel), phenylephrine (center panel) and KCl (right panel) in perfused mesenteric vasculatures from water control rats (○), salt control rats (△) and DHR (●). Values are expressed as the mean from six to seven experiments with S.E. shown by vertical bars. Asterisks indicate significant difference from water control group: *P < .05; **P < .01.

and Starke, 1982), failed to increase perfusion pressure in the hypertension and water control.

Antagonistic effects of prazosin against NE-induced vasoconstriction. As shown in figure 2, prazosin (10 and 30 pM) shifted concentration-dependently the NE dose-response curve to the right in a parallel manner. When the negative logarithm (DR_2) of the molar concentration of prazosin required to cause a 2-fold shift to the right in $ED_{100\ mm\ Hg}$ of NE was calculated according to the method of Arunlakshana and Schild (1959), the DR_2 value in the hypertension was significantly greater than that in the water control (11.04 ± 0.08 *vs.* 10.75 ± 0.06, P < .05). The slope of the regression line from Schild plots was not significantly different from unity (1.03 ± 0.12 in hypertension and 1.27 ± 0.17 in control), suggesting competitive antagonism by prazosin.

[^{3}H]Prazosin binding. Figure 3 shows a typical result for specific binding of [^{3}H]prazosin to the particulate fraction prepared from the mesenteric vascular bed of DHR and water control rats. Specific binding to [^{3}H]prazosin was saturable in both groups.

The linearity of Scatchard plots revealed that the binding was a single class of specific sites. As shown in table 1, the K_d value for [^{3}H]prazosin was significantly lower in the hypertensive rats than in the control rats. The B_{max} in the hypertension was approximately 2.7-fold greater than that in the control.

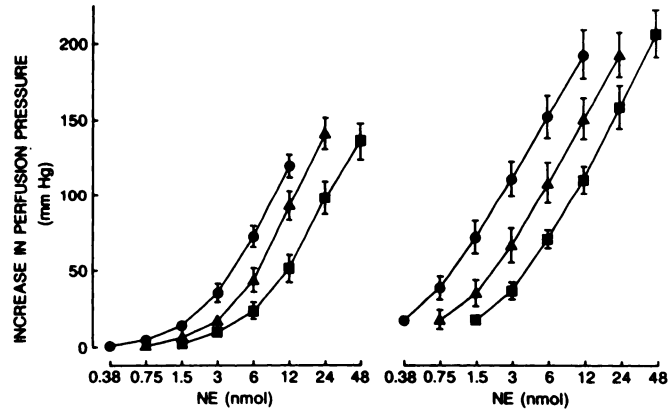

Fig. 2. Effects of prazosin on dose-response curves for NE in perfused mesenteric vasculatures from water control rats (left panel) and DHR (right panel). Values are expressed as the mean from eight experiments with S.E. shown by vertical bars. ●, NE alone; ▲, NE with 10 pM prazosin; ■, NE with 30 pM prazosin.

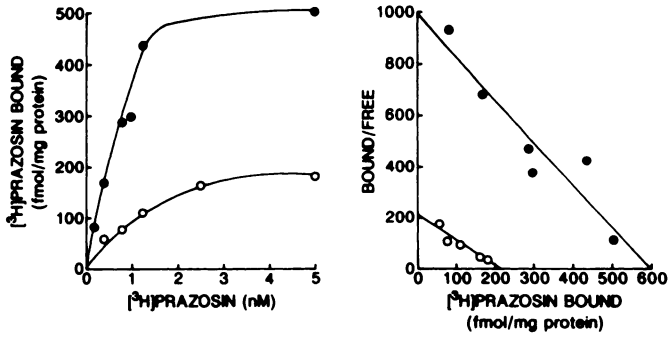

Fig. 3. Saturation curves (left panel) and Scatchard analyses (right panel) demonstrating specific binding of [^{3}H]prazosin to a particulate fraction of mesenteric vasculatures from water control rats (○) and DHR (●). Values are expressed as the mean from duplicate determinations in one typical experiment. Scatchard plots yielded a straight line in the control (r = −0.94, P < .05) and DHR (r = −0.93, P < .01). The K_d values were 1.01 and 0.60 nM in the control and DHR, respectively. The B_{max} values were 211.5 and 594.6 fmol/mg of particulate fraction protein in the control and DHR, respectively.

TABLE 1

Binding characteristics of [^{3}H]prazosin in a particulate fraction of mesenteric vasculatures from water control rats and DHR

Values are expressed as the mean ± S.E. from three separate experiments performed in duplicate.

	K_d	B_{max}
	nM	*fmol/mg protein*
Control	0.99 ± 0.03	200.0 ± 5.8
DHR	0.59 ± 0.06**	536.9 ± 46.9**

Asterisks indicate significant difference from the control: ** P < .01.

IP_3 accumulation. The basal levels (femtomoles per milligram of wet weight) of IP_3 in the absence of NE in DHR (3.88 ± 0.72, n = 20) did not differ significantly from that in water control (3.38 ± 0.31, n = 20) or salt control (3.29 ± 0.28, n = 20) rats. Net IP_3 accumulation induced by NE was determined by subtracting basal IP_3 level from IP_3 level in the presence of NE. As shown in figure 4, NE (0.03–30 μM) increased net IP_3 accumulation in a concentration-dependent manner. The NE-stimulated IP_3 accumulation above basal levels was significantly greater in the hypertensive rats than in the water and salt control rats. There was no significant difference between the water and salt control groups in the NE-stimulated IP_3 generation.

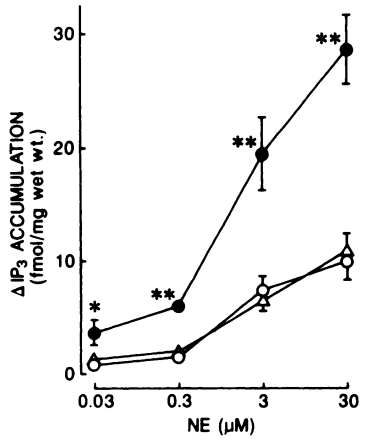

Fig. 4. Concentration-response curves for NE-stimulated IP_3 accumulation above basal levels in mesenteric vasculatures from water control rats (○), salt control rats (△) and DHR (●). The mesenteric vasculature of each rat was divided into two parts. One was treated with NE and the other was used to measure basal IP_3 level. Values are expressed as the mean from five separate experiments performed in duplicate with S.E. shown by vertical bars. Asterisks indicate significant difference from water control group: $^*P < .05$; $^{**}P < .01$.

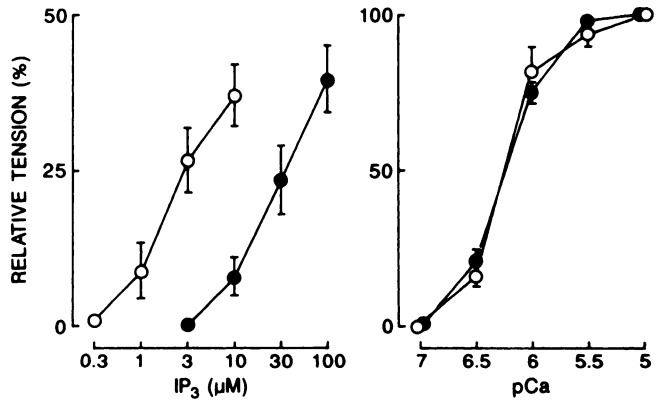

Fig. 5. Concentration-response curves for IP_3 (left panel) and pCa-tension curves (right panel) in β-escin-skinned mesenteric arteries from water control rats (○) and DHR (●). In IP_3 experiments, only two IP_3 concentrations were applied in each artery. Tensions developed by IP_3 and Ca^{++} were normalized to a percentage of the force obtained at pCa 5.0 in each artery. The tension (28.9 ± 3.3 mg, $n = 15$) developed at pCa 5.0 in DHR did not differ significantly from the tension (39.1 ± 3.9 mg, $n = 15$) in control rats. Values are expressed as the mean from five experiments with S.E. shown by vertical bars.

IP_3-induced contraction and pCa-tension curve in skinned muscles. As shown in figure 5, IP_3 produced a concentration-dependent contraction in β-escin-skinned mesenteric arteries from DHR and water control rats. The concentration-response curve for IP_3 in the mesenteric artery from DHR was characterized by a 10-fold shift to the right of the control.

Cumulative pCa-tension curve was constructed by using separate skinned mesenteric arteries to assess the Ca^{++} sensitivity of the contractile system itself. The curve for DHR was not significantly different from the control group (fig. 5), indicating that the Ca^{++} sensitivity of the contractile system is similar in the two groups.

PDBu-induced vasoconstriction. As shown in figure 6, the dose-response curve for PDBu was shifted to the left in DHR. This shift became progressively greater with increasing PDBu dose. 4α-Phorbol 12,13-didecanoate, an inactive phorbol ester, produced no elevation in perfusion pressure in both DHR

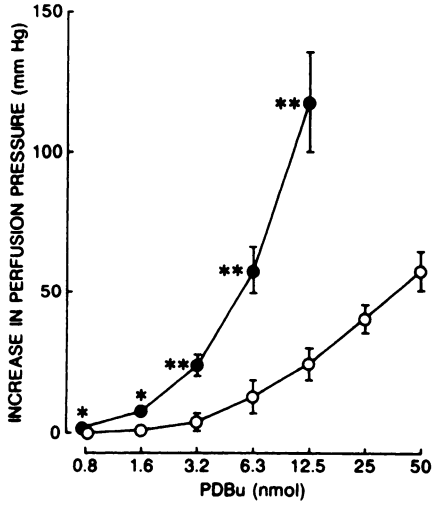

Fig. 6. Dose-response curves for PDBu in perfused mesenteric vasculatures from water control rats (○) and DHR (●). Values are expressed as the mean from five experiments with S.E. shown by vertical bars. Asterisks indicate significant difference from water control group: $^*P < .05$; $^{**}P < .01$.

and water control rats. Staurosporine (5 nM) inhibited the vasoconstriction induced by the dose (6.3 nmol in DHR and 36 nmol in control) of PDBu required for about a 50-mm Hg elevation in perfusion pressure by 80.5 ± 7.1% ($n = 5$) in DHR and 81.0 ± 5.3% ($n = 5$) in the control. Thus, the inhibitions by staurosporine were similar in both rats.

Discussion

In the present study the vasoconstrictor responses of the mesenteric vascular bed to NE and phenylephrine, but not KCl, were significantly greater in DHR than in water and salt control rats. The increased reactivity was observed over the entire dose range of NE and phenylephrine tested. The mesenteric vasculature in DHR was more sensitive to the two *alpha* adrenoceptor agonists in comparison with normotensive control rats, as evidenced by a smaller $ED_{100\ mm\ Hg}$ value. These results confirmed an enhanced vascular responsiveness to *alpha* adrenoceptor agonists which is considered as a primary characteristic of DHR. Xylazine, a selective *alpha*-2 adrenoceptor agonist (Hsu, 1981; Docherty and Starke, 1982), caused no increase in perfusion pressure in both hypertensive and water control rats. This observation is in agreement with the results of Meggs *et al.* (1988) and Kong *et al.* (1991) for clonidine, another *alpha*-2 adrenoceptor agonist, and suggests that the vasoconstrictor responses to NE and phenylephrine observed in the present study could be mediated entirely by *alpha*-1 adrenoceptors.

In the blood vessels with rich adrenergic innervation, the NE-releasing action of KCl combines with its depolarizing effect on the smooth muscle cell to produce contraction. However, the participation of endogenously released NE in the vasoconstrictor response to KCl can be ruled out, because phentolamine did not affect the response. This view was supported additionally by the fact that the vasoconstriction induced by KCl was not enhanced in the hypertensive rats. Ekas and Lokhandwala (1980) have reported that the reactivity to NE in the perfused mesenteric vasculature from DHR was increased compared with control rats, whereas that to $BaCl_2$ was identical. The present study with KCl and the result of Ekas and Lokhandwala (1980) suggest that the increased re-

sponsiveness to NE and phenylephrine in the hypertensive rats is not due to a structural alteration (increased medial thickness) of the vasculature or a change in the contractile machinery, *per se*.

The vasoconstrictor response to NE was significantly more sensitive to prazosin blockade in DHR than in water control rats, as indicated by a greater DR_2 value. This finding suggests that the affinity of prazosin for *alpha*-1 adrenoceptors, which was estimated from mechanical response, was increased in the mesenteric vasculature from the hypertensive rats. Hicks *et al.* (1983) have also shown that prazosin was significantly more effective as an antagonist of NE-induced vasoconstriction in pithed DHR compared with normotensive rats. The increased effectiveness of *alpha* adrenoceptor agonists and prazosin observed in the present study suggests initially that the properties of *alpha*-1 adrenoceptors may be changed in the hypertension. However, the literatures on the density and affinity of cardiovascular *alpha*-1 adrenoceptors in DHR are conflicting (for review see Rosendorff *et al.*, 1985); the experiments with ventricular tissue from DHR have demonstrated either a decrease in [^{3}H]prazosin affinity (Eid and de Champlain, 1988) or a decrease in *alpha*-1 adrenoceptor number (Meggs *et al.*, 1992). On the other hand, Smith *et al.* (1987) have reported no changes in the number and affinity of aortic *alpha*-1 adrenoceptors in aldosterone hypertensive rats. In an *in vitro* autoradiographic study, DHR showed significant reductions in aortic and mesenteric arterial *alpha*-1 adrenoceptor binding compared with normotensive control rats (Wilson, 1991), although it is unclear whether the reduced receptor binding is related to a decrease in the number or affinity of the receptors. In the present study, the B_{max} value for [^{3}H]prazosin was significantly greater in the hypertensive rats, whereas its K_d value was lower, suggesting increases in both density and affinity of *alpha*-1 adrenoceptors in the mesenteric vasculature from the hypertensive rats. These differences in the binding properties of cardiovascular *alpha*-1 adrenoceptors may occur because of differences in preparations or conditions used. However, previous experiments with the mesenteric vasculature from DHR showed a decreased affinity and an increased density of *alpha*-1 adrenoceptors (Meggs *et al.*, 1988). Meggs *et al.* (1988) have also reported that the vasoconstrictor response to phenylephrine of perfused mesenteric vascular bed in DHR was not significantly different from that in normotensive rats and that the response to its maximum dose (10 μg) tested did not exceed 30 mm Hg even in DHR. On the other hand, the present study in which a similar dose range was used demonstrated that the vasoconstriction induced by phenylephrine in DHR was much greater than that of Meggs *et al.* (1988) and was also significantly greater than that in the control rats (fig. 1). With respect to both the amplitude of the vasoconstriction and the increased reactivity in DHR, the present results are in agreement with the findings reported by Ekas and Lokhandwala (1980) and Longhurst *et al.* (1988) by using the perfused mesenteric vasculature. Although the discrepancy between the observations made by Meggs *et al.* (1988) and those of us is difficult to explain, the duration of hypertensive state was longer in the former report than in our study (3 *vs.* 1 week), judging from the period exposed to systolic blood pressure of more than 180 mm Hg. Thus, it is tempting to speculate that the severity of vascular injury associated with a longer duration of hypertension may alter *alpha*-1 adrenoceptor conformation, resulting in a decreased receptor affinity. The findings that no enhanced vascular response to phenylephrine was observed in DHR (Meggs *et al.*, 1988) might be due to the countervailing changes (increased density and decreased affinity) in binding properties of mesenteric *alpha*-1 adrenoceptors. The animals used by us were younger than those employed by Meggs *et al.* (1988). The older age of the animals might, in part, account for the reduced amplitude of the *alpha*-1 adrenoceptor-mediated vasoconstriction, because contractile response to *alpha*-1 adrenoceptor agonists of vascular smooth muscle of the rat is decreased with increasing age (McAdams and Waterfall, 1986).

The present observations that both density and affinity of *alpha*-1 adrenoceptors were increased in the mesenteric vasculature from DHR may account for the increased effectiveness of *alpha* adrenoceptor agonists and prazosin. The precise mechanism of these alterations in increases in *alpha*-1 adrenoceptors in the hypertensive rats remains unclear. However, it is of interest to note that NE content in the mesenteric artery from DHR was lowered significantly by about 35% compared with control rats (Meggs *et al.*, 1988), because catecholamine depletion with reserpine results in an increase in *alpha*-1 adrenoceptor affinity in the rat mesenteric artery (Colucci *et al.*, 1981). The increased affinity occurs at 1 hr after reserpine administration (Colucci *et al.*, 1981), at which time vascular NE content seems to be only partially depleted. Therefore, it is speculated that an increase in vascular *alpha*-1 adrenoceptor affinity in the hypertension might be, at least in part, related to reduced vascular NE content. However, the increased *alpha*-1 adrenoceptor density could not be explained by a reduced NE content, because the density was unchanged after reserpine treatment (Colucci *et al.*, 1981). The DOCA-salt rats are potassium-depleted (Rascher *et al.*, 1981). Because potassium depletion results in an increase in binding capacity for other pressor substances such as angiotensin II in the rat mesenteric artery but the binding affinity is unchanged or decreased (Paller *et al.*, 1984; Linas *et al.*, 1990), the possibility that hypokalemia caused by DOCA treatment may be, in part, responsible for an increased density of vascular *alpha*-1 adrenoceptors cannot be excluded.

We also investigated whether alterations in vascular *alpha*-1 adrenoceptor properties in mechanical response and radioligand binding experiments are accompanied by changes in post-receptor events. Postreceptor events after *alpha*-1 adrenoceptor occupancy are linked to PI hydrolysis resulting in productions of IP_3 and DAG (Abdel-Latif, 1986). PI hydrolysis can be estimated by measuring IP_3, DAG, their metabolite levels, PLC activity and so on. Of these parameters the measurements of IP, an IP_3 metabolite, level has been frequently used to monitor PI hydrolysis. In mineralocorticoid-salt hypertensive rats, vascular IP formation stimulated by NE is significantly greater compared with control rats (Eid and de Champlain, 1988; Jones *et al.*, 1988; Takata *et al.*, 1989). However, IP can be produced not only from IP_3 breakdown but also from PI and phosphatidylinositol monophosphate without IP_3 formation (Williamson, 1986). In addition, it has been shown recently that enhanced pressor response to NE of the tail artery from spontaneously hypertensive rats was followed by increased accumulation of IP_3, but not IP (Muir and Wardle, 1989; Guild *et al.*, 1992). Therefore, we measured the formation of IP_3, but not IP, as an index of PI hydrolysis. The results showed that IP_3 accumulation in response to NE at the entire concentration range was significantly greater in the mesenteric vasculature from DHR than in that from control rats. The augmented IP_3 generation

causes a greater release of Ca^{++} from intracellular store sites, leading to vascular hyper-reactivity to NE. Indeed, Storm and Webb (1992) have shown by using $^{45}Ca^{++}$ efflux technique that NE-induced release of intracellular Ca^{++} was increased in the mesenteric artery from DHR. It has also been reported that phasic NE contraction in Ca^{++}-free medium resulting from Ca^{++} release was enhanced in the mesenteric artery from DHR (Perry and Webb, 1991).

As PLC is a key enzyme involved in PI hydrolysis, an enhanced PLC activity would facilitate PI hydrolysis. However, Uehara *et al.* (1987) have reported that arterial PLC activity was reduced by 20% in DHR in comparison with control rats. Therefore, the enhanced PI hydrolysis observed in the present study may be not due to altered PLC activity, but rather due to increases in the density and affinity of *alpha*-1 adrenoceptors. A reduced PLC activity might operate as a negative feedback mechanism against increased PI hydrolysis.

Whether the amount of Ca^{++} released by IP_3 itself from intracellular store sites is altered in DHR is unknown. In the present study the Ca^{++} amount released by IP_3 was estimated from the amplitude of IP_3-induced contraction in β-escin-skinned mesenteric artery. The concentration-response curve for IP_3 was shifted to the right in DHR, indicating that the mesenteric artery from DHR is less sensitive to the contraction induced by IP_3 than that from control rats. This reduced sensitivity was not due to altered Ca^{++} sensitivity of the contractile system itself, because the pCa-tension curve was similar in the skinned preparations from the hypertensive and control rats. No changes in Ca^{++} sensitivity have also been shown in the superior mesenteric artery (Storm and Webb, 1992) and in the aorta (McMahon and Paul, 1985) from mineralocorticoid-salt hypertensive rats. Perry and Webb (1991) have demonstrated that the size of intracellular Ca^{++} store sites does not change in the mesenteric artery from DHR. None of calmodulin level, actomyosin content and actin-to-myosin ratio are altered in the vascular muscle of DHR (Moreland *et al.*, 1982; Higaki *et al.*, 1985). From these results, it is most likely that a decreased amplitude of IP_3-induced contraction observed in the skinned artery from the hypertensive rats may reflect a reduced amount of Ca^{++} released by IP_3 itself, although a direct measurement of IP_3-evoked Ca^{++} release is further required. The decreased Ca^{++} release might be compensatory to an increased production of IP_3 in this model of experimental hypertension. Ferris and Snyder (1992) have reported that IP_3 receptors are phosphorylated by both PKC and cyclic AMP-dependent protein kinase. The receptor phosphorylation by the latter kinase in the rat cerebellar membrane results in a diminished potency of IP_3 in releasing Ca^{++} (Supattapone *et al.*, 1988). If IP_3 receptor phosphorylation by PKC impairs IP_3-induced Ca^{++} release, as did cyclic AMP-dependent phosphorylation of the IP_3 receptor, then it would contribute to a less potency of IP_3 to release Ca^{++} in DHR, because enhanced PI hydrolysis in DHR leads to a greater extent of IP_3 receptor phosphorylation by PKC.

Tumor promoting phorbol esters mimic the action of DAG, another product of PI hydrolysis, in activating PKC to cause vascular contraction (Nishizuka, 1984; Rasmussen and Barrett, 1984). The present observations demonstrate that PDBu-induced vasoconstriction was significantly greater in DHR than in control rats, whereas 4α-phorbol 12,13-didecanoate, an inactive phorbol ester, failed to increase perfusion pressure. Staurosporine, a PKC inhibitor (Tamaoki *et al.*, 1986), caused a similar antagonism against the vasoconstriction induced by concentration of PDBu which constricted to a similar extent in both DHR and normotensive rats. Several mechanisms of the increased vascular responsiveness to PDBu in DHR are possible; enhanced Ca^{++} sensitivity of PKC to PDBu, increased PKC amount, increased affinity of PKC for PDBu and enhanced vascular contractile processes after PKC activation. In the rat mesenteric artery, PKC activation with PDBu causes an opening of nifedipine-sensitive voltage-dependent Ca^{++} channels (Abebe and MacLeod, 1990), which results in activation of MLC kinase and subsequent increased MLC phosphorylation (Rembold and Murphy, 1988). PDBu also increases the Ca^{++} sensitivity of contractile system in the rat mesenteric artery (Nishimura and van Breemen, 1989). This Ca^{++} sensitization is produced by increased Ca^{++} sensitivity of MLC phosphorylation (Rembold and Murphy, 1988; Sato *et al.*, 1992). In addition, phorbol esters including PDBu contract vascular smooth muscle by a mechanism which is not proportional to MLC phosphorylation (Chatterjee and Tejada, 1986; Singer and Baker, 1987; Sato *et al.*, 1992). Which of the mechanisms mentioned above is responsible for the enhanced vascular reactivity to PDBu in DHR remains to be resolved.

In conclusion we have shown an increased reactivity to NE and phenylephrine, but not KCl, in mesenteric vasculatures from DHR. The *alpha*-1 adrenoceptor affinity for prazosin, estimated from antagonism against vasoconstrictor response as well as radioligand binding studies, was increased in mesenteric vasculatures from DHR. The maximum binding capacity of *alpha*-1 adrenoceptors was also increased in DHR. The NE-stimulated PI hydrolysis and the PDBu-induced vasoconstriction were greater in DHR than in control rats, whereas the IP_3-induced contraction was smaller in chemically skinned mesenteric arteries from DHR. These results suggest that the increases in the number and affinity of vascular *alpha*-1 adrenoceptors may lead to enhanced PI hydrolysis accounting for hyper-reactivity to *alpha*-1 adrenoceptor agonists in DHR. In addition, the enhanced vasoconstrictor mechanism mediated through PKC pathway may also contribute to increased vascular responsiveness to the agonists, whereas the decreased IP_3-induced contraction probably due to the less Ca^{++} release may operate to minimize the vascular hyper-reactivity to the agonists.

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