Alpha-Adrenoceptor- and Prostaglandin-Mediated Modulation of Vascular Adrenergic Neurotransmission in Spontaneously Hypertensive Rats

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Abstract—The modulation of vascular adrenergic neurotransmission in the spontaneously hypertensive rat (SHR) and Wistar-Kyoto rat (WKY) mediated by alpha-adrenoceptor and prostaglandin E2 (PGE2) were evaluated. The pressor responses of the perfused mesenteric vascular bed to perivascular adrenergic nerve stimulation (NS) and infusion of norepinephrine (NE) and the NS-induced 3H-efflux in preparations pretreated with 3H-norepinephrine were determined. In both SHR and WKY, a selective alpha2 agonist, B-HT 920 (30-300 nM), inhibited neurogenic vasoconstriction, and B-HT 920 (100-300 nM) potentiated the pressor response to NE in a dose-dependent manner. The effects of B-HT 920 did not significantly differ in SHR and WKY. Another alpha2 agonist, clonidine (100 nM), decreased the 3H-efflux approximately by 30% both in SHR and WKY. A selective alpha2 blocking agent, yohimbine (3-300 nM), potentiated the neurogenic vasoconstriction and inhibited the pressor response to NE equally in SHR and WKY. No difference in enhancement of 3H-efflux by yohimbine (30-300 nM) was seen between SHR and WKY either in the absence or presence of cocaine (10 μM) and metanephrine (20 nM). PGE2 (0.1-1 μM) potentiated pressor responses to NS and NE in SHR and WKY, but this compound (1 μM) did not affect the NS-induced 3H-efflux in either group of rats. It appears that adrenergic neurotransmission is inhibited presynaptically via an alpha2 receptor mechanism and facilitated postsynthetically by PGE2 in the rat mesenteric vascular bed. The hypertensive state in SHR can not be accounted for by alteration of these modulator mechanisms.

The spontaneously hypertensive rat (SHR), along with the normotensive control Wistar Kyoto (WKY) (1), has been widely used as a model for the study of human essential hypertension. A body of evidence indicates that the hypertensive state is maintained by increased adrenergic nervous vasoconstrictor influences in this model (2-4) and in human hypertension (5). Since transmitter release from noradrenergic neurons can be influenced via presynaptic receptors (6), changes in sensitivity of these receptors may well contribute to excessive neurogenic vasoconstrictor activity. In fact, our previous studies have demonstrated that the adenosine- or serotonin-mediated inhibition of adrenergic transmitter release in mesenteric arteries is suppressed in SHR (7-10), whereas the angiotensin- or beta-adrenoceptor-mediated facilitation of the release is enhanced in SHR (11, 12). Our results also suggest some basic difference between SHR and the two kidney-one clip renal hypertensive rats with regard to adenosine subsensitivity (8). This raises the question whether all presynaptic receptor-mediated modulatory mechanisms are altered in favor of elevated blood pressure in SHR, and
secondly, whether such alterations are the consequence of hypertension rather than its cause in this hypertension model.

The transmitter norepinephrine (NE) is generally thought to partake in an important feedback inhibition via presynaptic alpha2-adrenoceptors. Ekas and Lokhandwala (13, 14) reported that phentolamine, a non-selective alpha1-alpha2 antagonist, substantially augmented the 3H-NE release elicited by periarterial sympathetic nerve stimulation in the isolated perfused rat mesenteric vasculature. Since phentolamine equally increased the release in WKY and SHR in one series and in normotensive and DOCA-salt hypertensive rats in another, they suggested that the presynaptic alpha-adrenoceptor mechanism was unaltered in these hypertensive rats. Gallaway and Westfall (15) also found no difference between the tail arteries of young SHR and WKY in the enhancement of KCl-induced NE overflow by yohimbine, an alpha2 antagonist. In adult (28 weeks old) SHR, however, significantly diminished effect of yohimbine and hence subsensitivity of presynaptic alpha2-receptors was noted.

In view of the apparent importance of alpha2-receptor mediated modulation, its potential relationship to hypertension of the SHR model was reexamined in the perfused mesenteric preparation using selective alpha2 agonists and antagonist. The modulator effect of prostaglandin E2 (PGE2) was considered together because of its close association with adrenergic neurotransmission and reported increase of transmitter release by this PG in the rat mesenteric vasculature (16). Our preliminary results have been presented (17).

Materials and Methods

Fourteen-week-old male SHR and WKY rats were obtained from Taconic Farms (Germantown, NY). The animals were housed in a room maintained on a 12 hr light-dark cycle at 25°C. Experiments were performed on the animals when they reached 15–17 weeks of age. Body weights of WKY and SHR were 309.4±2.0 g and 302.0±1.9 g (mean±S.E.M., n=77 each), respectively, and their systolic blood pressures obtained by tail-cuff plethysmography (Narco Biosystem, Houston, TX) were 128.3±1.0 mm Hg and 192.2±1.3 mm Hg, respectively.

The mesenteric vasculature was isolated under pentobarbital sodium anesthesia (50 mg/kg, i.p.) and prepared by a modification of the method of McGregor (18, 19), as described previously (7). Four main branches from the superior mesenteric artery trunk running to the terminal ileum were perfused, all other branches being tied off. The medium, a modified Krebs buffer (pH 7.4, 37°C), was continuously bubbled with a mixture of 95% O2–5% CO2. It had the following composition (mM): NaCl, 122; KCl, 5.2; CaCl2·2H2O, 2.4; MgSO4·7H2O, 1.2; K2HPO4, 1.2; NaHCO3, 25.6; EDTA·2Na, 0.03 and dextrose, 11. The perfusion flow rate was maintained at 5 ml/min using a polystaltic perfusion pump (Buchler Instruments). The outer surface of the mesenteric vasculature was superfused at the rate of 1 ml/min to keep it moist. Changes in perfusion pressure were measured with a pressure transducer (Statham P23DC) and recorded on a Grass polygraph (Model 79D). Thirty to forty min were allowed for the perfusion pressure to stabilize prior to experiments.

Pressor response studies: Postganglionic nerve fibers were stimulated by platinum electrodes placed around the proximal end of the superior mesenteric artery. Square wave pulses of 2 msec duration and supramaximal voltage delivered by a Grass S44 stimulator were applied for 30 sec at the frequency of 8 Hz and 5 min intervals. I-Norepinephrine was diluted with Krebs solution and infused into a section of rubber tubing immediately above the mesenteric cannula. Each dose (0.2 nmol) was given in a volume of 50 μl in 10 sec at 5 min intervals by an infusion pump (Harvard Apparatus model 975). Krebs solution containing yohimbine, B-HT 920 or PGE2 was perfused 3 min before and through two successive periods of nerve stimulation or norepinephrine infusion.

3H-Efflux studies: The isolated mesenteric arcade was preincubated for 60 min with modified Krebs bicarbonate solution containing 0.1 μM 3H-I-NE (7.8·3H-NE; specific activity, 25.4 Ci/mmol; New England Nuclear, Boston, MA) in a 10 ml organ bath
maintained at 37°C. After 60 min perfusion with fresh Krebs solution, the perfusate was collected every 2 min. Trains of 1 min periarterial nerve stimulation at the frequency of 8 Hz were applied 5 times (S₁–S₅) at 16 min intervals, except for the clonidine experiments in which trains of 2 min stimulation at 2 Hz were applied. Aliquots (0.5 ml) of the perfusate were mixed with 4.0 ml of Ready-Solv HP (Beckman) and counted using a Beckman Scintillation Counter (LS-3133P). The stimulation-induced net ³H-efflux was derived from the overall increase above the prestimulation level. Yohimbine or PGE₂ was administered 10 min before and during S₄, and clonidine was administered 10 min before and through S₃ (10 μM clonidine) and S₄ (100 μM). Control preparations were subjected to the same protocol without drug administration. Data were expressed as the ratio between stimulation-induced net ³H-effluxes in the presence and absence of a drug, i.e., S₄/S₃, S₃/S₂ and S₄/S₂, respectively.

**Drugs and statistics:** I-Norepinephrine bitartrate, yohimbine hydrochloride, prostaglandin E₂, metanephrine hydrochloride and clonidine hydrochloride from Sigma Chemical Co. and cocaine hydrochloride from Ciba Pharmaceutical Co. were used. B-HT 920 was kindly donated by Ernst Boehringer Institute, Austria. All values are expressed as the mean±S.E.M. Statistical levels of less than 0.05 by Student's t-test were considered to be significant.

**Results**

The base-line perfusion pressure was slightly but significantly (P<0.001) higher in SHR (25.9±0.2 mm Hg, n=77) than in WKY (23.1±0.4 mm Hg, n=77). Nerve stimulation (NS) produced perfusion pressure increases of 24.8±2.1 mm Hg and 8.3±0.7 mm Hg in SHR (n=19) and WKY (n=19), respectively. They were comparable to the increases in response to norepinephrine (NE, 0.2 nmol) in SHR (27.4±2.5 mm Hg, n=17) and WKY (9.4±0.9 mm Hg, n=17).

As shown in Fig. 1, a selective alpha₂ agonist, B-HT 920 (30–300 nM), produced a dose-dependent decrease in the NS-induced pressor response and in higher concentrations (100–300 nM), produced a dose-dependent increase in the NE-induced response. There was no difference in response to B-HT 920 in SHR and WKY in the effects of clonidine. B-HT 920 alone did not affect the basal perfusion pressure at the concentrations used.

Since the presynaptic alpha adrenoceptor-mediated inhibition may be more prominent at lower frequencies, the effect of clonidine, a selective alpha₂ agonist, was tested at the frequency of 2 Hz. Both in SHR and WKY, 100 nM but not 10 nM clonidine significantly reduced the NS-induced ³H-efflux (P<0.02). No significant difference between SHR and WKY resulted (Table 1).

As shown in Fig. 2, yohimbine (3–300 nM) produced a concentration-dependent increase in the response to NS and decrease in the NE response. No significant difference was observed between SHR and WKY, except that the potentiation of response to NS by 3 nM yohimbine was greater in SHR.
and inhibition of response to NE by 100 nM yohimbine was greater in WKY. Yohimbine alone did not affect the basal perfusion pressure at the concentration used. Figure 3 shows a representative experiment on 3H-efflux elicited by NS in the mesentery pretreated with 3H-NE. Yohimbine (100 and 300 nM) produced significant increases (P<0.05) in the NS-induced 3H-efflux, in the presence or absence of cocaine (10 μM) and metanephrine (20 μM) (Table 1). There was no difference between SHR and WKY in the NS-induced 3H-efflux.

Figure 4 depicts the effect of PGE2 (0.1–1 nM) on the NS- and NE-induced pressor responses. This prostaglandin equally potentiated the responses to NS and NE in SHR and WKY. At the higher level (1 nM) of PGE2, the enhancement of vasoconstrictor responses to NS or NE was significantly smaller in SHR (P<0.05). PGE2 alone did not affect the basal perfusion pressure at the concentrations used. As shown in Table 1, PGE2 (1 nM) produced no significant change in the NS-induced 3H-efflux in SHR or WKY.

Discussion

Earlier, the adrenergic transmitter release was shown to be greater in the mesenteric vasculature (13) and tail artery (15) of SHR than those of WKY. From studies using alpha2 antagonists, it was suggested that the greater transmitter release in SHR did not result from alterations in the presynaptic alpha-adrenoceptor mechanism (13, 15).

Table 1. Nerve stimulation-induced 3H-efflux in rat mesentery pretreated with 3H-norepinephrine

| Drugs (μM)                  | WKY          | SHR          |
|-----------------------------|--------------|--------------|
| Control                     | 0.75±0.03 (5)* | 0.75±0.04 (5) |
| Yohimbine, 0.1              | 0.98±0.07 (5)* | 0.94±0.03 (5)* |
| Yohimbine, 0.3              | 1.05±0.08 (5)** | 1.09±0.08 (5)** |
| Cocaine, 10+Metanephrine, 20| 0.75±0.02 (5) | 0.74±0.03 (5) |
| Cocaine, 10+Metanephrine, 20+ Yohimbine, 0.3 | 1.25±0.10 (5)** | 1.22±0.09 (5)** |
| Control                     | 0.94±0.02 (4) | 0.96±0.03 (4) |
| Clonidine, 0.01             | 0.90±0.06 (4) | 0.89±0.05 (4) |
| Control                     | 0.90±0.03 (4) | 0.86±0.05 (4) |
| Clonidine, 0.1              | 0.63±0.05 (4)** | 0.57±0.05 (4)** |
| Control                     | 0.79±0.02 (4) | 0.79±0.04 (4) |
| PGE2, 1                     | 0.80±0.01 (4) | 0.79±0.07 (4) |

*aRatio between effuxes in presence and absence of drug, mean±S.E.M. (number of experiments). **P<0.05, ***P<0.02 compared with the control. There was no significant difference between WKY and SHR in all experiments.
This view has been reexamined in the present study using not only an alpha2 antagonist, yohimbine, but also selective alpha2 agonists, B-HT 920 and clonidine.

In SHR and WKY, B-HT 920 and clonidine inhibited the NS-induced vasoconstriction or $^3$H-efflux, whereas yohimbine enhanced them, suggesting the occurrence of considerable alpha2-mediated autoinhibition in both SHR and WKY. No significant difference
between SHR and WKY was seen in the inhibitory effect of B-HT 920 and clonidine. In addition, there was no significant difference between SHR and WKY in the enhancement of NS-induced vasoconstriction and $^3$H-efflux by yohimbine. It has been reported that neuronal uptake of NE is greater in SHR than WKY (20). However, the effect of this factor on the NS-induced $^3$H-efflux can be ruled out since there was no difference between SHR and WKY in the yohimbine-induced enhancement of $^3$H-efflux also in the presence of cocaine and metanephrine, inhibitors of uptake$_1$ and uptake$_2$. These results suggest that the mesenteric vasculature from SHR or WKY has comparable alpha$_2$-mediated presynaptic autoinhibition.

Our results are in agreement with an earlier report (16, 21) that PGE$_2$ potentiates vasoconstrictor responses of the rat mesenteric vasculature to NS or NE infusion, although it inhibits these responses of the rabbit (16) and guinea-pig (22) mesenteric artery. The enhancement can be attributed solely to a postsynaptic effect since PGE$_2$ did not augment the response to NS any more than that to NE, and it failed to increase the NS-induced $^3$H-efflux. This is contrary to the view that PGE$_2$ enhances release of the adrenergic transmitter in this rat vasculature (16). Only at a higher dose (1 $\mu$M) of PGE$_2$, the enhancement of vasoconstrictor responses was smaller in SHR. This may possibly be due to a ceiling effect of the greater vasoconstriction in SHR rather than receptor subsensitivity.

Findings in one vasculature can not be necessarily extrapolated to another since isoproterenol produced a similar degree of enhancement in the portal veins of SHR and WKY at all ages (23), unlike in our studies of the mesenteric arteries (12). Nevertheless, the present results in the mesenteric vasculature may be valuable since the vasculature is within the splanchnic area which greatly contributes to the total vascular resistance (24).

Our previous studies demonstrated that in SHR compared to WKY, the inhibitory modulation by adenosine or serotonin is diminished (7–10), and the facilitation via angiotensin II or beta-receptors is enhanced (11, 12). By contrast, the present study shows no significant change in the presynaptic alpha-receptor-mediated inhibition or postsynaptic PGE$_2$-mediated enhancement of adrenergic transmission in the mesenteric arteries of SHR. Thus, alterations favorable to excessive vasoconstriction occur only in selected modulator systems of SHR, not as a generalized SHR-related phenomenon nor as a consequence of elevated arterial blood pressure. It may also be noted that of all the modulator systems we have studied, none appears to be changed in the direction of compensating for the elevated pressure. Some of the alterations may possibly be causally related to hypertension in SHR.

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