The Release of Endogenous Norepinephrine from the Coccygeal Artery of Spontaneously Hypertensive and Wistar-Kyoto Rats

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SUMMARY. The potassium (K⁺)-induced release of endogenous norepinephrine from the coccygeal artery of spontaneously hypertensive rats has been studied as a function of the development of hypertension. The absolute amount of norepinephrine released by potassium was greater in spontaneously hypertensive rat than the normotensive Wistar-Kyoto rat, regardless of age or blood pressure. However, the % fractional release was elevated only in the rats with chronic hypertension. Preincubation of tissues with the α₂-antagonist, yohimbine, significantly enhanced norepinephrine overflow in all tissues studied. Young hypertensive animals demonstrated an enhancement equal to the Wistar-Kyoto rat controls. In the adult spontaneously hypertensive rat, however, there was a significantly lesser enhancement produced by yohimbine. Levels of norepinephrine in the nerves supplying the artery were greater in the prehypertensive spontaneously hypertensive rat than the age-matched Wistar-Kyoto rat. The norepinephrine content in arteries from adult animals was equivalent. The explanation for the attenuation of the yohimbine effect of chronic hypertensive animals is unclear. Although several explanations are possible, the data are consistent with the hypothesis that spontaneously hypertensive rats with chronic hypertension have subsensitive prejunctional α₂-receptors as evidenced by an increased % fractional release of norepinephrine and a decreased enhancement of overflow in the presence of yohimbine. Clearly, further studies are needed to answer this provocative question and to understand the complex interactions of adrenergic neurotransmission in hypertensive animals. (Circ Res 51:225-232, 1982)

There is increasing evidence that the nervous system plays a primary role in the development and maintenance of several forms of experimental hypertension. In the spontaneously hypertensive rat, evidence is available which supports the contribution of both central catecholamines and the peripheral sympathetic nervous system in the development and, possibly, the maintenance of the hypertension. For instance, in pre- and young hypertensive animals, there is an increase in urinary catecholamine excretion (DeChamplain et al., 1969), an increase in plasma catecholamines and dopamine β-hydroxylase (Reid et al., 1975; Grobecker et al., 1975; DeChamplain et al., 1976; Nakaoka and Lovenberg, 1976; Nagatsu et al., 1976), as well as increased nerve traffic over sympathetic neurons (Okamoto et al., 1967; Nosaka, 1973; Coote and Sato, 1977). Depletion of central stores of catecholamines prevents (Haeusler, 1976; Kubo and Hashimoto, 1978), whereas peripheral sympathectomy markedly attenuates, the hypertension (Folkow et al., 1972; Yamori et al., 1972; Provoost and De Jong, 1978). Changes in brain catecholamine turnover and enzymatic activity also have been reported, especially in pre- or young hypertensive rats (Saavedra et al., 1978; Renaud et al., 1978; Petty and Reid, 1979; Lew et al., 1979). Exaggerated sympathetic discharge to various types of stress or stimuli have also been reported (Yamori et al., 1969; Hallback and Folkow, 1974; Yen et al., 1978; Kvetnansky et al., 1979). Although controversial, increased vascular responsiveness to vasoconstriction stimuli and nerve stimulation also have been observed (Folkow et al., 1979; Haeusler and Haeufely, 1970; Kubo, 1978; Lais and Brody, 1978).

The release of labeled or endogenous norepinephrine in isolated or perfused organs of SH rats as well as normotensive controls has been examined. Vanhoutte et al., have provided evidence of increased release of norepinephrine from the perfused kidney to periarterial nerve stimulation in young SH rats, with normal release being observed in adult animals (Vanhoutte 1980, 1981). In contrast, Eikenburg et al., using ¹H as a marker of transmitter release from the perfused mesenteric artery, observed enhanced release to field stimulation in adult SH rats as compared to the Wistar-Kyoto control (Eikenburg et al., 1981).

In an attempt to define further the role of peripheral noradrenergic systems in hypertension, we have initiated a developmental study of the release of norepinephrine in vitro from the coccygeal (caudal) artery of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Arteries were isolated, incubated in Krebs-Ringer buffer (with uptake blockers) and then depolarized under hyperkalemic conditions in the absence or presence of drugs. The media then was subjected to high-pressure liquid chromatography.
(HPLC) to resolve norepinephrine and the amount of endogenous transmitter quantitated by electrochemical (amperometric) detection. In addition to determining the absolute amount and the %-fractional release of norepinephrine, we calculated the levels of transmitter in neurons innervating the artery. These parameters were compared in SHR and WKY rats before and after the development of hypertension. The effects of yohimbine on norepinephrine release in SHR are compatible with the notion of prejunctional α2-desensitization during the course of hypertension.

Methods

Male SHR and normotensive WKY rats were purchased from Taconic Farms, and were maintained under standard vivarium conditions with food and water available ad libitum. One or 2 days prior to sacrifice, systolic blood pressures were measured by tail-cuff plethysmography with an electrophysymomanometer and a pneumatic pulse transducer (Narco Biosystems) connected to a chart recorder. The average of five measurements per animal was taken as the blood pressure. The average systolic blood pressures of the animals used in the study are shown in Figure 1. The genetically hypertensive rats of 6 weeks of age were pre-hypertensive but became clearly hypertensive by 10 weeks of age.

Isolation of the Coccygeal Artery

Rats were weighed and then killed by decapitation. A circular incision was made at the base of the tail, then, after a lateral longitudinal incision and removal of the epithelium, the tail was severed at the base. The coccygeal artery was exposed by cutting the fascia surrounding it and removed by gently lifting the artery with a probe. The proximal 3-inch portion of the artery was incubated in 0.5 ml of continuously oxygenated (95% O2/5% CO2) Krebs-Ringer buffer at 37°C with agitation. The Krebs-Ringer buffer contained the following reagent grade chemicals (mm): NaCl, 118; KCl, 4.85; CaCl2, 2.5; MgSO4, 1.15; KH2PO4, 1.13; NaHCO3, 25; and d-glucose, 11.1. After 30 minutes, the tissue was transferred to 0.5 ml of Krebs-Ringer buffer containing 1 μM desmethylimipramine and 80 μM corticosterone and incubated an additional 15 minutes. All subsequent incubations were carried out in the presence of uptake inhibitors. Tissues were then placed in Krebs-Ringer buffer containing 56 mM KCl osmotically substituted for NaCl (S2). After 2 minutes in high K+, the artery was washed with 0.1 ml of buffer and then incubated for 5 minutes in 0.5 ml of buffer containing yohimbine, or no added drug. A second potassium exposure (S2) was carried out as described above and the tissue was incubated for 5 minutes in Krebs-Ringer buffer. After this incubation, tissues were blotted dry, weighed, and then homogenized in 0.3 ml of 0.4 M HClO4 containing 1% EDTA and 1% Na2S2O5. The homogenate was centrifuged in a Beckman microfuge and an aliquot (5 μl) of the supernatant injected directly onto the HPLC (see below). After the tissue was removed from each media, 0.05 ml of 0.4 M HClO4 with EDTA and Na2S2O5 was added to stabilize released norepinephrine. In addition, the 0.1 ml-wash following each stimulation was added to the medium containing potassium.

Measurement of Norepinephrine

Norepinephrine released under basal or high potassium conditions was measured by injecting a 20-μl aliquot of the incubation media directly into the HPLC. Norepinephrine was quantitated with an electrochemical detector maintained at 0.740 V from Bioanalytical Systems (model LC-4A, glassy carbon electrode). The HPLC unit is equipped with a M-45 solvent delivery system (Waters Assoc.), a Rheodyne injector (model 7125), and 5-μm reversed-phase octadecylsilane column (Bioanalytical Systems). The routine solvent for the separation of norepinephrine was 12% methanol, 175 mM acetic acid, 1 mM EDTA, 2 mM heptanesulfonic acid, and the pH was adjusted to 3.8 with 10 mM NaOH. The norepinephrine peak in media samples co-migrated with authentic norepinephrine in two solvent systems, could be manipulated by pharmacological treatments of the tissue, and could be adsorbed and eluted from aluminum oxide. The release of norepinephrine has been expressed in two ways. First, as the absolute amount (i.e., nanograms of norepinephrine liberated per artery of equal length), and second as the %-fractional release. The %-fractional release is the amount of norepinephrine released into the medium, divided by the amount of norepinephrine present in the tissue prior to the exposure to potassium, times 100. The amount of amine in the tissue at the start of any time interval was calculated by adding cumulatively the amount of norepinephrine released into the medium to the amount of norepinephrine in the coccygeal artery at the end of the experiment.

Statistics and Chemicals

The number of animals used to generate the following data is indicated in each figure. Data presented are from one group of animals; each experiment has been performed...
Results

Release of Endogenous Norepinephrine from the Caudal Artery

An HPLC-EC analysis of high-K+ media after a 2-minute incubation of a SHR or WKY caudal artery is shown in Figure 2. Also depicted is the effect of a preincubation with yohimbine (10^{-6} M) on the K+ induced release of norepinephrine (see below). The rate of K+-induced norepinephrine release was increased approximately 50-fold, compared to basal release in Krebs-Ringer buffer during the 15-minute period prior to stimulation. Furthermore, preliminary incubations of caudal arteries revealed that the K+-induced release of norepinephrine was linear for at least 5 minutes ($r^2 = 0.99$). In the absence of Ca++ and the presence of 0.5 mM EGTA, the amount of norepinephrine released by K+ was reduced by about 95% (Table 1). When tissues were incubated in various concentrations of veratradine, norepinephrine was released in a dose-dependent fashion (Table 2). Thus, the K+-induced release of endogenous norepinephrine from the caudal artery is a convenient model for the study of peripheral noradrenergic function.

Potassium-Stimulated Release from Caudal Artery of SHR’s and WKY’s

Compared to vessels from normotensive WKY rats, SHR caudal arteries exposed to high potassium resulted in a significantly greater release of norepinephrine when expressed as the absolute amount (ng) of norepinephrine liberated by an artery of equal length. This increase was evident, regardless of age or blood pressure (Table 3). When the data were expressed as the %-fractional release (see Methods), there was no difference between young SHR and WKY rats. The chronic hypertensive rats, however, released a significantly greater fraction, compared to WKY rats (Table 3). Levels of norepinephrine in the neurons innervating the proximal 3 inches of the caudal arteries were greater in all age groups of SHR than in weight-matched WKY rats (Fig. 3) and were correlated with the elevated amount of norepinephrine released from the SHR artery (Table 3). As a function of tissue weight, however, only the prehypertensive SHR had elevated levels of norepinephrine compared ...
TABLE 3

<table>
<thead>
<tr>
<th>Animals</th>
<th>Norepinephrine released</th>
<th>%-fractional†</th>
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</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>6 wk old WKY</td>
<td>1.9 ± 0.11*</td>
<td>6.9 ± 0.55</td>
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<tr>
<td></td>
<td>3.4 ± 0.18†</td>
<td>8.1 ± 0.43</td>
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<tr>
<td>10 wk old WKY</td>
<td>2.1 ± 0.18</td>
<td>5.7 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>4.2 ± 0.31†</td>
<td>6.2 ± 0.19</td>
</tr>
<tr>
<td>Adult WKY</td>
<td>3.2 ± 0.14</td>
<td>3.9 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>6.2 ± 0.45‡</td>
<td>5.7 ± 0.29‡</td>
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* Data are expressed as ng norepinephrine released/2 min per 3-inch strip of caudal artery.
† Data are expressed as the %-fractional release (ng norepinephrine released/ng norepinephrine in the tissue prior to stimulation \( \times 100 \); see Methods for further details).
‡ \( P < 0.001 \), compared to corresponding WKY.

Effect of Yohimbine on the Potassium-Induced Release of Norepinephrine from SHR's and WKY's

Since the %-fractional release of norepinephrine was equivalent in young SHR and WKY rats (Table 3), the elevated absolute amount of norepinephrine released by the SHR artery may be a consequence of increased tissue levels of norepinephrine (Fig. 3). In contrast, the increased absolute and %-fractional release observed in the adult SHR do not correlate with equal tissue levels of norepinephrine at this age. To test the possibility that the enhanced release in chronic hypertensives may be a result of subsensitive prejunctional \( \alpha \)-adrenoceptors, arteries were incubated in the absence (\( S_1 \)) or presence (\( S_2 \)) of the \( \alpha \)-antagonist, yohimbine. Ratios (\( S_2/S_1 \)) of the %-fractional release then were compared between SHR and WKY rats, and are shown in Figure 4. In all cases, preincubation with yohimbine (\( 10^{-6} \) M) significantly enhanced the K+-induced overflow of norepinephrine, indicative of functional prejunctional \( \alpha_2 \)-receptors. The magnitude of enhancement was similar in young animals (SHR vs. WKY). However, the chronic hypertensives demonstrated a lesser enhancement of release (i.e., a decreased \( S_2/S_1 \) ratio) after incubation with yohimbine. In addition, this phenomenon was also evident if the \( S_2/S_1 \) ratio was expressed in terms of the absolute (ng) amount released.

Discussion

Four major differences in noradrenergic dynamics of the isolated caudal artery of the SHR compared to WKY control have been observed in the present study. First, the absolute amount of norepinephrine released from the caudal artery in response to potassium stimulation was greater in the SHR at all ages studied (i.e., prehypertensive, 6-weeks of age; young hypertensive, 10 weeks old; and adult or chronic hypertensive, 28 weeks of age); second, the tissue concentration of norepinephrine was significantly greater in pre- and young hypertensive animals but similar in adults; third, the %-fractional release of...
norepinephrine to potassium stimulation was similar in pre- and young hypertensive rats but greater in chronic hypertensive animals; and fourth, prejunctional α2 activity was similar in pre- and young hypertensive animals, but was significantly attenuated in chronic hypertensive rats.

The result of increased release of norepinephrine that we obtained is consistent with that reported for the perfused kidney of 6-week-old SHR compared to the WKY, and the release of 3H from the superfused mesenteric artery of 14- to 18-week-old SHR (Vanhoutte, 1981; Eikenburg et al., 1981). Moreover, we have carried out parallel studies in which the field stimulation induced release of 3H-norepinephrine was measured in the portal vein obtained from the same hypertensive animals. Low frequencies of nerve stimulation resulted in a greater release of 3H-norepinephrine from vessels of hypertensive SHR compared to the WKY (Westfall et al., 1982).

These results are consistent with the idea that there is an increase in the exocytotic release of norepinephrine from several sympathetically innervated tissues in the SHR, as compared to genetically matched normotensive controls. In the kidney, it has been suggested that exocytotic release of norepinephrine is enhanced primarily early in the genesis of hypertension, and may be an important factor in increasing peripheral resistance and, thus, in causing the hypertension (Vanhoutte, 1980, 1981). In contrast, nerve stimulation of the perfused kidney of the adult SHR (6 months old) was reported to result in a smaller increase in the overflow of norepinephrine (Vanhoutte, 1981). These investigators also reported that the vasoconstrictor response to exogenous norepinephrine, but not sympathetic nerve stimulation, was enhanced in the kidneys of adult animals (Collis et al., 1980; Vanhoutte, 1980, 1981). These results prompted the hypothesis that, since there was a normal vasoconstrictor response to nerve stimulation despite an enhanced response to exogenous norepinephrine, there was, in fact, a reduced exocytotic release of norepinephrine in the adult SHR (Vanhoutte, 1981).

Our results in the caudal artery do not support this hypothesis, since there was a greater release of norepinephrine when expressed as total nanograms or as fractional release of norepinephrine in chronic hypertensive animals. One explanation for the apparent difference in results could be due to the manner of inducing release of the transmitter. Although we have not examined the effect of field stimulation as a means of transmitter release in the present study, potassium has been shown by numerous investigators to be a valuable secretagogue that mimics, in many respects, depolarization of nerve terminals (Kirpekar and Wakade, 1968). In the present study, the potassium-induced release of norepinephrine was dependent upon extracellular calcium, and was subject to prejunctional α2 modulation in a manner similar to nerve stimulation. Therefore, we feel results obtained with this secretagogue are applicable to the physiological situation. Further studies are necessary to verify that similar results can be obtained with field stimulation. Similar results have been obtained, however, when the field stimulation-induced release of 3H-norepinephrine from the superfused portal vein obtained from the same animals was examined (Westfall et al., 1982). Differences in adrenergic transmission of the vasculature of the kidney compared to the caudal artery during hypertension could also exist, although, as mentioned, we have observed a similar effect on transmitter release from another blood vessel, the portal vein.

Critical to the hypothesis that there is a decreased transmitter release in adult SHR is the fact that there must be a similar vascular responsiveness to nerve stimulation while, at the same time, there is an increased responsiveness to exogenously administered norepinephrine. The responsiveness of the vasculature to nerve stimulation of SHR compared to normotensive controls is variable, however, and both increased responsiveness (Folkow et al., 1970; Haessler and Hafely, 1970; Kubo, 1978; Lais and Brody, 1978), and normal responsiveness (Collis and Vanhoutte, 1976; Webb and Vanhoutte, 1979; Vanhoutte, 1981) have been reported. Since our studies on release were carried out in the presence of norepinephrine uptake blockers, and since we did not measure the responsiveness of the caudal artery to potassium stimulation, we cannot rule out the possibility that the effect of increased release is balanced by increased reuptake. Such a situation could result in a lack of effect on vascular reactivity. However, parallel studies that examined the response of the portal vein to field stimulation from the same animals showed that veins obtained from adult SHR's had a greater responsiveness than did WKY's (Westfall et al., 1982). These results, therefore, are inconsistent with the hypothesis that there is a decrease in the exocytotic release of norepinephrine in the adult hypertensive animals.

The higher absolute levels of norepinephrine per unit length of artery observed in the pre- and young hypertensive animals could be due to several factors, such as a more dense innervation, an accelerated biosynthesis of norepinephrine, more storage vesicles per varicosity, more norepinephrine per vesicle, or increased reuptake of norepinephrine. Our data do not differentiate among these possibilities, but it is interesting to note that differences in catecholamine biosynthetic enzymes have been reported in SHR's. Tarver et al. (1971) noted a decreased activity of tyrosine-3-monoxygenase in blood vessels from young (10 week old) SHR's, whereas Nagatsu et al. (1976) observed elevated dopamine-β-hydroxylase activity in peripheral blood vessels from 3-week-old SHR's. Also, Graham et al. (1970) reported an increased number of adrenergic vesicles per varicosity in renal hypertension. Although Whall et al. (1980) showed that norepinephrine uptake is enhanced in SHR mesenteric arteries, this phenomenon would not interfere with our results, since all studies were carried out in the presence of uptake blockers. Moreover,
Eikenburg et al. (1981) have obtained results inconsistent with there being an enhanced uptake of norepinephrine in the mesenteric artery of 14- to 18-week-old SHR's. Whether the increased norepinephrine content is a result of hypertension or a contributing factor in the development of hypertension is unknown. It is of interest that others have also reported greater-than-normal norepinephrine content in several vascular beds in the SHR, including the mesenteric artery (Head and Berkowitz, 1979). Moreover, it has been observed that adrenergic nerve function (i.e., norepinephrine content, norepinephrine uptake, and neurogenic contractions) increased in blood vessels after partial ligation of the rabbit aorta (Bevan et al., 1975). Our data suggest that, in the prehypertensive SHR, elevated levels of norepinephrine precede the manifestation of genetic hypertension (Figure 3).

Although the amount of norepinephrine released was greater in the SHR, the %-fractional release did not differ from WKY in young animals. Thus, prehypertensive and young hypertensive animals have control over the fraction released per stimulus. This indicates a lack of an aberration in prejunctional control of release (Westfall, 1977, 1980) and, coupled with elevated norepinephrine levels, may be indicative of a more dense innervation, rather than more vesicles per neuron. In contrast to the prehypertensive and young hypertensive animals, those with chronic hypertension (28 weeks) apparently have lost a great deal of the capacity to modulate norepinephrine release, evidenced by an increased %-fractional release (Table 3). This notion is supported by the finding that preincubation of the tissues with the α2-antagonist, yohimbine, did not enhance the overflow of norepinephrine in the chronic hypertensive to the same extent as the WKY. The explanation for the attenuation of the yohimbine effect in chronic hypertensive animals is unclear. It is of interest that Crews and Smith observed that inhibition of norepinephrine uptake by chronic tricyclic antidepressant treatment resulted in an attenuation of the phenoxybenzamine-induced enhancement of neurogenic contraction of the rat atria (Crews and Smith, 1980). These authors suggested that a refractiveness to the prejunctional activity of norepinephrine on α2-adrenoceptors took place as a result of increased transmitter in the neuroeffector phase. A similar phenomenon may have taken place in our experiments whereby the constant increase in norepinephrine release due to sympathetic nerve stimulation during hypertension resulted in an alteration in the functional activity of prejunctional α2-adrenoceptors. Further experiments are clearly needed to answer this provocative question, especially whether or not chronic hypertension results in a selective or non-specific change in prejunctional α2-adrenoceptor activity.

Numerous studies now point to increased sympathetic nerve activity in the development and/or maintenance of hypertension in the SHR (Okamoto et al., 1967; Volier et al., 1968; Iriuchijima, 1973; Grobecker et al., 1975; Roizen et al., 1975, Judy et al., 1976; Nakaoka and Lovenberg, 1976; Provoost and Delong, 1976, to name but a few). Our results directly demonstrate elevated sympathetic activity at the neurovascular smooth muscle junction. Furthermore, our data indicate decreased responsiveness of the prejunctional α2-response and a possible loss of control over neurotransmitter release. Thus, the increased release of norepinephrine by neurons innervating vascular smooth muscle during early hypertension may lead to a refractory desensitization of the prejunctional adrenergic receptor during the development of hypertension, and thereby contribute to the maintenance of increased vasoconstriction.

There are also data that suggest that there may be alterations in other prejunctional receptor activity in the vasculature of the SHR. We have observed that the facilitatory effect of angiotensin on adrenergic neurotransmission in the portal vein is enhanced in the SHR compared to WKY (Westfall et al., 1981, 1982). This effect was seen in young (10-week-old) and adult (28-week-old) hypertensive animals but not in 6-week-old prehypertensive animals. In contrast, the facilitatory effect of isoproterenol to field stimulation of the portal vein of the SHR and WKY was not different. Similar results for angiotensin have also been reported in the perfused mesenteric bed of the SHR (Su et al., 1982; Kowasaki et al., 1982). In this preparation, the facilitatory effect of angiotensin on adrenergic transmission has also been reported to be enhanced. A decrease in the inhibitory effect of purines on adrenergic transmission in SHR's also has been reported (Kamikawa et al., 1980). Moreover, several investigators have reported that there are alterations in central and peripheral adrenergic receptors in hypertensive animals (Limas and Limas, 1978; Yamada et al., 1980; Chiu, 1981; Cantor et al., 1981).

The cause of the elevated norepinephrine levels in peripheral neurons of pre- and young hypertensive SHR's and whether this is the reason for the increase in stimulation-induced release remains to be determined. It is tempting to speculate that increased sympathetic nerve traffic and enhanced facilitory activity of angiotensin may be quite important in producing the initial increase in adrenergic transmission which may contribute to the development of hypertension. The increased release of norepinephrine may subsequently lead to subsensitivity in prejunctional α2-adrenoceptors in chronic hypertensive animals. In chronic hypertensive animals, the decrease in the activity of prejunctional α2-adrenoceptors also results in enhanced release of norepinephrine during adrenergic neurotransmission which now may be important in the maintenance of hypertension. Further studies clearly are needed to answer these intriguing questions and complex interactions.

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