Effect of Isoproterenol on Vascular Adrenergic Neurotransmission in Prehypertensive and Hypertensive Spontaneously Hypertensive Rats

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It is generally accepted that the release of noradrenaline from adrenergic nerves is influenced via various presynaptic receptors (1, 2). Some of these modulation mechanisms may be involved in the development and maintenance of hypertension. Our previous reports in fact have demonstrated that the adenosine- and 5-hydroxytryptamine-mediated inhibitory modulation is suppressed in the perfused mesenteric vasculature of adult SHR (3, 4).

The presynaptic beta-adrenoceptor is thought to mediate an augmentation of sympathetic neurotransmission (5, 6). Some workers have reported that the presynaptic beta-adrenoceptor-mediated facilitation is enhanced in the isolated mesenteric vasculature from adult SHR (7). However, other studies with the isolated perfused kidney (8) and the portal vein (9) have failed to show such changes in the sensitivity of presynaptic beta-adrenoceptors in adult SHR. The sensitivity of beta-adrenoceptors has been shown to change under various conditions including chronic hypertension (10). In the present study, therefore, the presynaptic beta-adrenoceptor-mediated facilitatory modulation was evaluated in isolated blood vessels from prehypertensive young SHR in addition to hypertensive adult SHR, as compared with age-matched normotensive Wistar-Kyoto rats (WKY).

Male SHR (5- and 15-week-old) and age-matched male WKY supplied by Charles River Japan, Inc. were used in this study. Before experiments, the systolic blood pressure was measured in the conscious state by the tail-cuff method. The splenic, renal and superior mesenteric arteries adjacent to the aortic joint were excised and short ring segments were prepared. The splenic artery was cut longitudinally into narrow strips. They were incubated at 37°C for 60 min with Krebs solution containing 1-[7,8-3H]-noradrenaline, 10^-7 M (5 µCi/ml, specific activity 36.8 Ci/mmol, Amersham International plc, Amersham, U.K.), ascorbic acid (100 mg/l) and EDTA (1.5 mg/ml) as described previously (5). The Krebs solution had the following composition (in mM): NaCl, 118.4; KCl, 4.7; CaCl2, 2.5; MgCl2, 1.18; NaHCO3, 25; KH2PO4, 1.2; glucose, 11.1.

After rinsing for 10 min with fresh Krebs solution, the preparations were mounted vertically between a pair of platinum-stimulating electrodes and superfused with Krebs solution at a constant flow rate of 1 ml/min using microtube pumps. A 90-min equilibration period was allowed before the first transmural field stimulation. Forty-sec trains of field stimulation, 2 msec square wave pulse of supramaximal voltage of 10 V at 5 Hz, were applied 2 times (S1, S2) at 15 min intervals. Perfusion of 10 nM 1-isoproterenol hydrochloride (Sigma) was begun 10 min before and performed through the 2nd stimulation. The perfusate was collected into a tube every 1 min and 6 ml of ACS-II solution (Amersham/Searle Corporation, Des Plaines, Illinois) was added. Total 3H activities expressed as counts per minute were determined using a liquid scintillation spectrometer (Packard 3330). Impulse-evoked net 3H-efflux was calculated as the difference between basal efflux before stimulation and total efflux evoked by stimulation. The S2 efflux in the presence or absence of
isoproterenol was expressed as a percentage of the \( S_1 \) efflux. Student's \( t \)-test was used for statistical analysis of the results.

The mean systolic blood pressure did not significantly differ between 5-week-old WKY and SHR, whereas considerably higher blood pressure developed in 15-week-old SHR. The net \(^{3}H\)-effluxes from \(^{3}H\)noradrenaline-treated arteries during the 1st field stimulation did not significantly differ between WKY and SHR at either age, except that in renal arteries from 15-week-old SHR, the efflux was greater than in those from age-matched WKY (Table 1).

Table 1. Systolic blood pressure and stimulus-evoked \(^{3}H\)-efflux

<table>
<thead>
<tr>
<th>Arterial pressure (mm Hg)</th>
<th>(^{3}H)-Efflux (cpm/mg wet tissue weight)</th>
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<tbody>
<tr>
<td></td>
<td>Splenic arteries</td>
</tr>
<tr>
<td>WKY (5)</td>
<td>96±4 (17)</td>
</tr>
<tr>
<td>SHR (5)</td>
<td>97±3 (18)</td>
</tr>
<tr>
<td>WKY (15)</td>
<td>131±4 (14)</td>
</tr>
<tr>
<td>SHR (15)</td>
<td>178±3 (14)</td>
</tr>
</tbody>
</table>

Values are given as the mean±S.E.M. (n). \(^{3}H\)-Efflux during the 1st period of stimulation. \( *P<0.05 \), compared to WKY.

As shown in Fig. 1, the net \(^{3}H\)-effluxes during the 2nd stimulation were comparable to the effluxes during the 1st stimulation in all the arteries from both ages of WKY and SHR: their \( S_2/S_1 \) ratios were around 100%. Isoproterenol significantly increased the nerve stimulation-induced \(^{3}H\)-effluxes in splenic arteries from 5- and 15-week-old SHR and from 15-week-old WKY, in renal arteries from 5-week-old SHR, and in mesenteric arteries from 5- and 15-week-old SHR and from 15-week-old WKY. The facilitatory effect of isoproterenol was clearly greater in splenic arteries from 5-week-old SHR and

Fig. 1. Effects of isoproterenol (10 nM) on impulse-evoked net \(^{3}H\)-efflux from isolated splenic, renal and mesenteric arteries of 5-week-old (A) and 15-week-old (B) WKY and SHR. The blood vessels were incubated for 60 min in \( 10^{-4} \) M \(^{3}H\)noradrenaline, rinsed, set up and superfused with Krebs solution. Isoproterenol was simultaneously superfused 5 min after the 1st transmural field stimulation at 5 Hz for 40 sec, 90 min after setting up the preparation. Abscissas show \(^{3}H\)-efflux expressed as a percentage of the 1st stimulation-induced \(^{3}H\)-efflux. Horizontal bars show S.E.M. and parentheses show numbers of estimations. \( *P<0.05 \), compared with no drug. \( +P<0.05 \), compared with WKY.
tended to be greater in renal and mesenteric arteries from 5-week-old SHR than in the corresponding arteries from age-matched WKY. In contrast, there was no significant difference between 15-week-old WKY and SHR in the facilitatory action of isoproterenol.

The present study demonstrates that isoproterenol produces an increase in the impulse-evoked \(^3\)H-efflux from rat blood vessels, suggesting that there exists a considerable number of presynaptic beta-adrenoceptors in the rat vasculature system. The facilitatory action of isoproterenol was significantly greater or had a tendency to be greater in the arteries from 5-week-old SHR as compared with age-matched WKY, whereas there was no significant difference in arterial pressure between 5-week-old WKY and SHR. These suggest that the facilitation of the neurotransmission mediated by the presynaptic beta-adrenoceptor is genetically enhanced in young SHR.

In contrast, the facilitatory effect of isoproterenol was within the normal limit in 15-week-old SHR. This is in agreement with the results of the previous workers showing that in the isolated portal vein (9) and isolated perfused kidney (8), facilitation of adrenergic neurotransmission mediated by the presynaptic beta-adrenoceptors is similar in both adult WKY and SHR.

Numerous investigators have demonstrated that chronic elevation of blood pressure due to various forms of experimental hypertension in rats leads to a lessened sensitivity of the beta-adrenoceptor to agonist stimulation in the heart and blood vessels (10). Such a decrease in sensitivity of presynaptic beta-adrenoceptor may occur following chronic hypertension in 15-week-old SHR, which may extinguish the genetically occurring enhancement of the isoproterenol-mediated facilitation of vascular adrenergic neurotransmission.

There is evidence that the presynaptic beta-adrenoceptor can be activated by circulating adrenaline, adrenaline taken up and released as a co-transmitter or, in part, noradrenaline released from sympathetic nerve endings (5, 11). The supersensitivity of the presynaptic beta-adrenoceptor observed in prehypertensive 5-week-old SHR may contribute to the development of hypertension in SHR.

In summary, the presynaptic beta-adrenoceptor-mediated facilitation of adrenergic transmission is likely to be genetically enhanced in SHR.

References