Role of cholinergic receptors in adrenal catecholamine secretion in spontaneously hypertensive rats

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Nagayama, Takahiro, Takayuki Matsumoto, Makoto Yoshida, Mizue Suzuki-Kusaba, Hiroaki Hisa, Tomohiko Kimura, and Susumu Satoh. Role of cholinergic receptors in adrenal catecholamine secretion in spontaneously hypertensive rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1057–R1062, 1999.—We investigated the role of nicotinic and muscarinic receptors in secretion of catecholamines induced by transmural electrical stimulation (ES) from isolated perfused adrenal glands of spontaneously hypertensive rats (SHRs) and normotensive Wistar-Kyoto (WKY) rats. ES (1–10 Hz) produced frequency-dependent increases in epinephrine (Epi) and norepinephrine (NE) output as measured in perfusate. The ES-induced increases in NE output, but not Epi output, were significantly greater in adrenal glands of SHRs than in those of WKY rats. Hexamethonium (10–100 µM) markedly inhibited the ES-induced increases in Epi and NE output from adrenal glands of SHRs and WKY rats. Atropine (0.3–3 µM) inhibited the ES-induced increases in Epi and NE output from adrenal glands of SHRs, but not from those of WKY rats. These results suggest that endogenous acetylcholine-induced secretion of adrenal catecholamines is predominantly mediated by nicotinic receptors in SHRs and WKY rats and that the contribution of muscarinic receptors may be different between these two strains.

There is increasing evidence suggesting that the sympathetic nervous system plays an important role in the development and maintenance of hypertension in spontaneously hypertensive rats (SHRs; Refs. 10, 30). Of particular interest is the importance of the adrenal medulla in hypertension. It has been reported that the reduction in plasma catecholamine levels by surgical adrenal demedullation attenuates hypertension development in young SHRs (3, 4) and that epinephrine (Epi) supplementation induces hypertension in normotensive rats (23). These findings suggest that the secretion of adrenal catecholamines may be partially involved in the pathogenesis of hypertension. A vasoconstrictor hyperresponsiveness to sympathetic activation has been observed in the isolated organs of SHRs (9, 11, 19), which is thought to be due to enhanced norepinephrine release from the sympathetic adrenergic nerve terminals (7, 8). However, there has been no information on the difference between neural control of adrenal catecholamine secretion in hypertensive rats and normotensive rats.

Adrenal medullary chromaffin cells secrete catecholamines into the bloodstream as a physiological response to stress. This response is mediated by splanchnic nerve activity. Activation of the splanchnic nerve causes the release of acetylcholine from its terminal into the intrasynaptic cleft, and released acetylcholine subsequently stimulates nicotinic and muscarinic receptors present on the surface of chromaffin cells. The contribution of muscarinic receptors to catecholamine secretion in response to splanchnic nerve stimulation is still unclear and probably varies from one species to another (32). It was reported that the endogenous acetylcholine-induced catecholamine secretion was barely affected by atropine and largely reduced by hexamethonium in the perfused rat adrenal gland (33). However, whether muscarinic receptors contribute functionally to catecholamine secretion from adrenal glands of SHRs has not been explored.

In the present study, we investigated the effects of hexamethonium and atropine on the secretion of catecholamines induced by transmural electrical stimulation (ES) from isolated perfused adrenal glands of SHRs and normotensive Wistar-Kyoto (WKY) rats to elucidate the functional role of nicotinic and muscarinic receptors in neural control of the secretion of adrenal catecholamines in this model of hypertension.

MATERIALS AND METHODS

Animals. Male SHRs (246 ± 6 g; n = 31) and WKY rats (252 ± 7 g; n = 29) of 9 wk of age were obtained from SLC (Shizuoka, Jpn.). Rats were maintained in the animal care facility at an ambient temperature of 23 ± 1°C and humidity of 55%. Animals were fed a standard diet and had free access to tap water. Animals were chosen at random for control or test experiments. All procedures for handling animals were approved by the Animal Experimentation Committee of Tohoku University Graduate School of Pharmaceutical Sciences. On the day of the experiment, tail-cuff recordings were used to ensure that the SHRs were clearly hypertensive.

Surgical preparation. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The left adrenal gland was exposed by a midline incision of abdomen. The stomach, intestines, and portions of the liver were pulled over to the right side and covered by saline-soaked gauze pads to obtain enough working space for tying blood vessels and for cannulation. A polyethylene cannula, used for perfusion of the adrenal gland, was inserted into the adrenal vein through the renal vein after all the branches of the adrenal vein, the renal artery, and the renal vein were ligated. Then the adrenal gland; nicotinic receptors; muscarinic receptors; hexamethonium; atropine.

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gland, along with the tied blood vessels and the cannula, was carefully removed from the animal and a small slit was made into the adrenal cortex just opposite the entrance of the adrenal vein. Perfusion of the adrenal gland was started to ensure that no leak was present, and the perfusate escaped only from the slit of the adrenal gland. The adrenal gland was placed on a bipolar platinum electrode used for ES. The adrenal gland together with an electrode was placed in a water-jacketed chamber, the temperature of which was maintained at 37°C by a thermostatically controlled water circulator (NTT-1200, Tokyo Rikakikai, Tokyo, Japan). Perfusion of the adrenal gland was started to ensure that no leak was present, and the perfusate escaped along the tied blood vessels and the cannula, was weighed after removing the adrenal vein. Perfusion of the adrenal gland was performed by means of a peristaltic pump (MP-3A, Tokyo Rikakikai) at a rate of 0.2 ml/min. The perfusion was carried out with Krebs-Henseleit solution of the following composition (in mM): 118 NaCl, 4.7 KCl, 1.2 MgSO4, 2.6 CaCl2, 1.2 KH2PO4, 24.9 NaHCO3, and 11.1 glucose. Krebs solution was maintained at 37°C by the thermostat bath and bubbled with a mixture of 95% O2 and 5% CO2. Perfusate samples were collected in chilled tubes containing 50 µl of 0.1 M perchloric acid to prevent oxidation of catecholamines. Before starting an experiment, the adrenal gland was initially perfused for 60 min with Krebs solution. ES (1 Hz; duration, 1 ms; supramaximal voltage, 50 V) was applied by a bipolar platinum electrode with an electronic stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan) and an isolation unit (SS-302J, Nihon Kohden). Stimulus frequency was raised stepwise from 1 to 2, 5, and 10 Hz at 5-min intervals, stimulation at each frequency being applied with 100-pulse trains. Experimental protocol. The rats were divided into six groups. In groups 1 (n = 13) and 2 (n = 11), the effects of repeated ES on increases in catecholamine (Epi and NE) output from adrenal glands of SHRs and WKY rats were examined, respectively, without drug treatment. A set of ES was repeated four times at 30-min intervals. In groups 3 (n = 8) and 4 (n = 10), the Effects of hexamethonium on the ES-induced increases in catecholamine output from adrenal glands of SHRs and WKY rats were examined, respectively. The first set of ES trials was regarded as a control. Perfusion with 10, 30, and 100 µM hexamethonium-Krebs solutions was started 10 min before the start of the second, third, and fourth set of ES, respectively. In groups 5 (n = 10) and 6 (n = 8), the effects of atropine (0.3, 1, and 3 µM) on the ES-induced increases in catecholamine output from adrenal glands of SHRs and WKY rats were examined with the same protocol as used in groups 3 and 4, respectively. In all groups, the adrenal gland was weighed after removing the adrenal vein at the end of the experiment. Perfusion sampling. Perfusate was sampled before and during each frequency of ES to determine catecholamine output. The sampling during the basal state was performed for 60 s just before ES. In preliminary experiments, it was found that the catecholamine responses to various frequencies of ES returned to prestimulation level within ~50 s after stopping the stimulation. Thus the samplings during ES at 1, 2, 5, and 10 Hz were performed for 150, 100, 70, and 60 s, respectively. Determination of adrenal catecholamine output. Catecholamines in perfusate sample were measured by high-performance liquid chromatography with electrochemical detection (LC-4C, Bioanalytical Systems, West Lafayette, IN), as described previously (15). Adrenal Epi and NE output (ng·min⁻¹·g⁻¹) were calculated by perfusate catecholamine concentration (ng/ml), perfusion rate (0.2 ml/min), and adrenal gland weight (g). The basal catecholamine output was determined from samples collected just before each ES. The ES-induced increases in catecholamine output were calculated by subtracting basal catecholamine output from that obtained during the stimulus state.

Analysis of data. The results are expressed as means ± SE. Student’s unpaired t-tests or two-factor ANOVA with Dunnett’s test was used for statistical analysis of data. P values <0.05 were considered to be statistically significant.

Drugs. The drugs used were hexamethonium chloride and atropine sulfate (Sigma Chemical, St. Louis, MO). Both drugs were dissolved in Krebs-Henseleit solution.

RESULTS

Increases in catecholamine output in response to ES. The adrenal gland weights of SHRs (groups 1, 3, and 5; n = 31) and WKY rats (groups 2, 4, and 6; n = 29) were 19.2 ± 0.4 and 22.6 ± 0.7 g, respectively, and this value in SHRs was significantly smaller than that in WKY rats (P < 0.01). Basal catecholamine output from adrenal glands of SHRs (groups 1, 3, and 5; n = 31) and WKY rats (groups 2, 4, and 6; n = 29) at 60 min after initial perfusion were 772 ± 99 and 396 ± 56 ng·min⁻¹·g⁻¹ in Epi and 191 ± 27 and 87 ± 12 ng·min⁻¹·g⁻¹ in NE, respectively. These values in SHRs were significantly greater than those in WKY rats (Epi output, P < 0.01; NE output, P < 0.01). ES (1, 2, 5, and 10 Hz) produced frequency-dependent increases in Epi and NE output from adrenal glands of SHRs and WKY rats. The increases in Epi and NE output induced by ES during the four stimulation periods are shown in Tables 1 and 2, respectively. The increases in catecholamine output induced by ES did not vary during the time course of the experiment in adrenal glands of SHRs or WKY rats. The increase in NE output, but not Epi output, induced by ES (5 and 10 Hz) in SHRs was significantly greater than that in WKY rats (Tables 1 and 2).

Effects of hexamethonium and atropine on the ES-induced increases in catecholamine output. Hexamethonium (10, 30, and 100 µM) inhibited the ES-induced increases in Epi and NE output from adrenal glands of SHRs and WKY rats (Tables 1 and 2). The ES-induced increases in catecholamine output were calculated by subtracting basal catecholamine output from that obtained during the stimulus state.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Epi Output, ng·min⁻¹·g⁻¹</th>
<th>1st Trial</th>
<th>2nd Trial</th>
<th>3rd Trial</th>
<th>4th Trial</th>
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<tbody>
<tr>
<td>SHR Group 1</td>
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<td></td>
<td></td>
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<tr>
<td>n = 13</td>
<td></td>
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<tr>
<td>ES 1 Hz</td>
<td>258 ± 62</td>
<td>246 ± 47</td>
<td>213 ± 38</td>
<td>212 ± 38</td>
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<tr>
<td>ES 2 Hz</td>
<td>517 ± 98</td>
<td>472 ± 95</td>
<td>434 ± 83</td>
<td>395 ± 83</td>
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<tr>
<td>ES 5 Hz</td>
<td>1077 ± 172</td>
<td>1015 ± 182</td>
<td>947 ± 166</td>
<td>913 ± 159</td>
<td></td>
</tr>
<tr>
<td>ES 10 Hz</td>
<td>1418 ± 229</td>
<td>1295 ± 223</td>
<td>1223 ± 216</td>
<td>1117 ± 222</td>
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<tr>
<td>WKY Group 2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>n = 11</td>
<td></td>
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<td></td>
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<tr>
<td>ES 1 Hz</td>
<td>329 ± 138</td>
<td>296 ± 97</td>
<td>244 ± 74</td>
<td>231 ± 61</td>
<td></td>
</tr>
<tr>
<td>ES 2 Hz</td>
<td>491 ± 76</td>
<td>494 ± 116</td>
<td>435 ± 86</td>
<td>446 ± 99</td>
<td></td>
</tr>
<tr>
<td>ES 5 Hz</td>
<td>966 ± 109</td>
<td>975 ± 117</td>
<td>877 ± 99</td>
<td>790 ± 86</td>
<td></td>
</tr>
<tr>
<td>ES 10 Hz</td>
<td>1242 ± 145</td>
<td>1219 ± 132</td>
<td>1101 ± 101</td>
<td>1025 ± 85</td>
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</tr>
</tbody>
</table>

Table 1. Epi output from perfused adrenal glands of SHRs and WKY rats in response to ES

Values represent means ± SE. There were no significant differences (P > 0.05) between 2 strain or between values during 1st trial and those during 2nd, 3rd, or 4th trial. Epi, epinephrine; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto; ES, electrical stimulation.
SHRs and WKY rats (Figs. 1 and 2, respectively). Atropine (0.3, 1, and 3 µM) significantly attenuated the ES-induced increases in Epi and NE output from adrenal glands of SHRs (Fig. 3). The ES-induced increases in Epi and NE output from adrenal glands of WKY rats were not affected even by the highest concentration (3 µM) of atropine (Fig. 4). Neither hexamethonium nor atropine affected basal Epi and NE output from adrenal glands of SHRs and WKY rats (data not shown).

**DISCUSSION**

The aims of this study were to investigate whether SHRs are different from normotensive WKY rats in the degree of secretion of catecholamines induced by ES and in the functional contributions of nicotinic and muscarinic receptors to the secretion of catecholamines. ES produced frequency-dependent increases in Epi and NE output from adrenal glands of SHRs and WKY rats. Although the ES-induced Epi secretion was almost to the same degree between the two strains, the NE response was greater in adrenal glands of SHRs than in those of WKY rats. It has been reported that NE but not Epi content is higher in adrenal glands of SHRs than in those of WKY rats (6, 20, 21, 26). Thus the facilitated NE secretion in SHRs may be due to higher NE content in the adrenal gland. Furthermore, NE release induced by ES has been reported to be greater in the kidney (5) and the perfused mesenteric arteries (13, 25, 28, 31) of SHRs than in those of WKY rats. These results and ours have provided evidence suggesting that the exocytosis of NE from sympathetic nerves in various organs and from the adrenal gland may be

<table>
<thead>
<tr>
<th>Table 2. NE output from perfused adrenal glands of SHRs and WKY rats in response to ES</th>
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<tr>
<td>Experiment</td>
</tr>
<tr>
<td>SHR Group 1 (n = 13)</td>
</tr>
<tr>
<td>ES 1 Hz</td>
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<tr>
<td>ES 2 Hz</td>
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<tr>
<td>ES 5 Hz</td>
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<td>ES 10 Hz</td>
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<tr>
<td>WKY Group 2 (n = 11)</td>
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<tr>
<td>ES 1 Hz</td>
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<tr>
<td>ES 2 Hz</td>
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<tr>
<td>ES 5 Hz</td>
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<tr>
<td>ES 10 Hz</td>
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</tbody>
</table>

Values represent means ± SE. NE, norepinephrine. *P < 0.05, †P < 0.01 compared with corresponding values in WKY rats. There were no significant differences (P > 0.05) between values during 1st trial and those during 2nd, 3rd, or 4th trial.

Fig. 1. Effects of hexamethonium (Hexa) on epinephrine (Epi; A) and norepinephrine (NE; B) output from perfused adrenal glands of spontaneously hypertensive rats (SHRs) in response to ES. Points and vertical bars represent means ± SE. *P < 0.05, **P < 0.01 compared with corresponding control response obtained before hexamethonium treatment.

Fig. 2. Effects of hexamethionium on Epi (A) and NE (B) output from perfused adrenal glands of Wistar-Kyoto (WKY) rats in response to ES. Points and vertical bars represent means ± SE. *P < 0.05, **P < 0.01 compared with corresponding control response obtained before hexamethionium treatment.
facilitated in the developmental stage of hypertension. It is thought that SHRs have heightened sympathetic drive from regions of the brain such as the rostral ventral lateral medulla and hypothalamic paraventricular nucleus. Our results obtained with the isolated perfused adrenal gland preparation clearly indicate that NE secretion due to peripheral neuronal excitation was facilitated at the adrenal glands of SHRs. Therefore it would seem that the augmented central sympathetic drive combined with augmented stimulated secretion of NE at the adrenal gland would be a powerful stimulus to support blood pressure in the SHR.

Hexamethonium markedly inhibited increases in Epi and NE output in response to ES from adrenal glands of WKY rats. These results are consistent with the observations that hexamethonium largely reduced catecholamine secretion evoked by ES in the perfused rat adrenal gland (33) and that another nicotinic receptor antagonist, mecamylamine, inhibited the secretion of catecholamines induced by ES in the perfused cat adrenal gland (1). In adrenal glands of SHRs, hexamethonium also inhibited the ES-induced increases in Epi and NE output. This is the first study to suggest that neuronally evoked secretion of catecholamines is mediated by not only nicotinic but muscarinic receptors in SHRs.

Atropine did not affect increases in Epi and NE output in response to ES from adrenal glands of WKY rats. This result indicates that muscarinic receptors do not contribute functionally to neuronally evoked secretion of catecholamines in normotensive rats. These findings agree with the results that atropine has no effect on the splanchnic nerve stimulation-induced secretion of catecholamines in the rat adrenal gland (33) and that another muscarinic antagonist, pirenzipine, does not modify the catecholamine secretion induced by splanchnic nerve stimulation in the dog adrenal gland (16). On the other hand, atropine inhibited the ES-induced increases in Epi and NE output from adrenal glands of SHRs. This is the first study to suggest that neuronally evoked secretion of catecholamines is mediated by not only nicotinic but muscarinic receptors in SHRs.

It has been suggested that an augmentation of various receptor populations may be involved in the development and maintenance of hypertension in the

neuronal activity (0.5–1 Hz) in the isolated perfused rat adrenal gland (24). However, in the present study, secretion of catecholamines from adrenal glands of SHRs or WKY rats induced by ES at 1 Hz was inhibited by hexamethonium by >80%. These results suggest that neuronally evoked secretion of adrenal catecholamines is predominately mediated by nicotinic receptors in SHRs as well as WKY rats.
SHR, such as cholecystokinin receptors in the nucleus accumbens (18), D1 dopamine receptors in the brain (17), α2-adrenergic receptors in the locus ceruleus (22), and glomerular endothelin B receptors in the kidney (14). Therefore, the inhibitory effect of atropine on the ES-induced secretion of catecholamines from adrenal glands of SHRs might be explained by the augmentation of muscarinic receptor populations located on the surface of adrenal medullary chromaffin cells.

In the present study, the basal Epi and NE output were greater in adrenal glands of SHRs than in those of WKY rats. These results are not consistent with the observation that there is no significant difference in basal NE overflow from the perfused mesenteric arteries (13, 28, 31) and from the isolated synaptosomes of hypothalamus and brain stem (12) between the two strains. These different results may be due to differences in the examined organs or tissues. Plasma concentrations of Epi and NE have been reported to be high in SHRs, but not from those of WKY rats. These results are not consistent with the hypothesis that endogenous acetylcholine-induced secretions of Epi and NE have been reported to be high in SHR (2, 27, 29). Our results suggest that spontaneous Epi and NE secretion from the adrenal gland may be facilitated in the stage of pathogenesis of hypertension, and this facilitation may partially contribute to the elevated plasma concentrations of Epi and NE in the SHR.

In conclusion, this study demonstrates that the ES-induced increases in NE output, but not Epi output, were significantly greater in adrenal glands of SHRs than in those of WKY rats. We also found that hexamethonium markedly inhibited the ES-induced increases in Epi and NE output from adrenal glands of SHRs and WKY rats and that atropine inhibited the ES-induced increases in Epi and NE output from adrenal glands of SHRs, but not from those of WKY rats. These results suggest that endogenous acetylcholine-induced secretion of adrenal catecholamines is predominantly mediated by nicotinic receptors in SHRs and WKY rats and that the contribution of muscarinic receptors may be different between these two strains.

Perspectives

In this study, an augmentation of functional contribution of muscarinic receptors to catecholamine secretion from adrenal glands of SHRs was observed. However, it is not known whether this result is due to an augmentation of muscarinic receptor populations or a greater affinity of atropine for muscarinic receptors. This issue should be clarified in further studies.

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REFERENCES


