Participation of Angiotensin II in Pressor Response and Norepinephrine Release to Spinal Nerve Stimulation in Pithed Rats

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Experiments were carried out to examine whether endogenous angiotensin II (A-II) is involved in the regulation of release of norepinephrine (NE) elicited by the stimulation of spinal sympathetic nerves in pithed rats. It was assessed in terms of the alterations in concentrations of arterial blood plasma A-II and NE elicited by nerve stimulation (5 Hz, 50 V, 1 msec for 45 s) in pithed rats under vehicle or captopril (3 mg/kg, i.v.) treatment. Comparative study with pentobarbital anesthetized rats showed that pithing rats have the characteristics of lower basal blood pressure and lower NE level, whereas they have higher basal A-II level. In pithed rats treated with vehicle, pressor response to nerve stimulation was accompanied by increases in both A-II and NE level. In rats treated with captopril, the nerve stimulation caused about 40% lower increases in pressor response and NE level than those observed in rats treated with vehicle. These results suggest that the sympathetic nerve-induced NE release is facilitated by endogenous A-II in pithed rats, and that captopril exerts its inhibitory effect on the pressor response to nerve stimulation through the suppression of this interaction.

Key words angiotensin II; norepinephrine release; sympathetic nerve; captopril

A well-documented interaction between the renin-angiotensin system and sympathetic nervous system is the facilitation of adrenergic function at various sites in the autonomic nervous system.1-3 Exogenously administered angiotensin II (A-II) is known to enhance the vascular response to electrical sympathetic nerve stimulation in various vascular beds.3-6 The norepinephrine (NE) release into the arterial blood during stimulation of the spinal sympathetic outflow has been shown to be potentiated by A-II in nephrectomized pithed rabbits.5 These results have led to a hypothesis that exogenous A-II is capable of potentiating the vascular responses to sympathetic nerve stimulation by enhancing the release of NE from the nerve endings.5,6 Therefore, an endogenous A-II would be expected to interfere with the sympathetic nervous system through peripheral mechanisms. In fact, angiotensin converting enzyme (ACE) inhibitors have been shown to attenuate the pressor response to sympathetic nerve stimulation in pithed rats7-10 and the authors suggested the facilitative interaction of A-II with sympathetic function. Although the pithed rat model is appropriate for the assessment of the interaction between A-II and the peripheral sympathetic nervous system, the preceding conclusions are based on the possible assumption that a certain extent of reduction in the plasma A-II concentration by ACE inhibitor treatment and its effect of NE release resulted in the final attenuation of the pressor response. To confirm this, in the present study we simultaneously measured arterial blood plasma A-II, NE and pressor response to the stimulation of spinal sympathetic outflow and investigated the effect of captopril on these parameters in pithed rats.

MATERIALS AND METHODS

Animal Preparation Male Wistar-Kyoto rats (9 to 11 weeks old, 240-280 g body weight) were maintained on a normal rat chow and tap water freely. Each rat was anesthetized with 40 mg/kg of sodium pentobarbital (i.p.), and a tracheal cannula was inserted for artificial respiration. The right and left jugular veins were cannulated for drug administration and bilateral vagotomy was performed at the midcervical level. The left carotid artery was cannulated for the measurement of arterial blood pressure with a pressure transducer (MPU-0.5; Nihon Kohden, Tokyo, Japan). The right carotid artery was ligated with silk thread. For arterial blood samplings, the left femoral artery was cannulated with polyethylene tube filled with heparin to prevent the blood clotting. Arterial respiration with room air at 50 strokes/min and a tidal volume of 10 ml/kg was started and then a stainless steel pithing rod was passed into the spinal column for electrical stimulation of the sympathetic outflow. A stainless steel rod was inserted subcutaneously from the left shoulder to the left hindlimb and served as an indifferent electrode. Somatic motor effects were blocked with d-tubocurarine chloride (1 mg/kg, i.v.). The rat rectal temperature was maintained at 37°C using a heating lamp. Stimulation of the spinal sympathetic outflow was performed with rectangular 1-ms pulses of 5 Hz, at 50 V for 45 s. After completion of the pithing procedure, 40-60 min was allowed for stabilization.

Experimental Protocol The effects of phosphate buffer vehicle (Group 1, n = 8) or captopril (Group 2, n = 8) on the pressor response and on the increase in arterial plasma NE and A-II concentrations elicited by spinal sympathetic nerve stimulation were tested. Ten minutes after the injection of vehicle or captopril (3 mg/kg, i.v.), the first 0.8 ml blood sample was taken through the left femoral arterial cannula as a basal sample for the estimation of NE and A-II. Thirty min elapsed, then the second blood sampling was initiated 30 s after the start of sympathetic nerve stimulation. After each blood sampling, rats received an equivalent volume of blood taken from the pentobarbital anesthetized rats which had been bilaterally nephrectomized 18-24 h earlier in which arterial plasma A-II concentration was assayed as 21.7±4.8 pg/ml (n=5). In another group of anesthetized rats (Group 3, n = 7), measurements of arterial plasma NE and A-II concentrations were performed before and after the pithing to assess any possible effect of pithing procedure on these variables.

Measurements of Arterial Plasma A-II and NE Concentrations
centrations Blood samples were transferred to chilled tubes containing peptatin A (100 μg/ml final conc.), o-
phenanthroline (0.44 mM final conc.) and EDTA-2NH₄ (25 mM final conc.), and then centrifuged at 4°C to obtain plasma samples and to determine hematocrit values. Plasma samples were kept frozen at -80°C until analyses of A-II and NE concentration. A-II was extracted, separated and measured by methods described previously. The overall recovery of the known amount of A-II, which is the amount added to 1 ml of rat plasma (run through the entire extraction and HPLC processes) was 73.1 ± 2.0% (n = 6) in the preliminary study. Values in this paper were not adjusted based on this percentage of recovery. Catecholamines were extracted from plasma by the alumina absorption method, and plasma NE concentration was determined by HPLC with an amperometric detector (LC-304, Bioanalytical System, West Lafayette, IN), as described previously.

Drug and Data Analysis Captopril, kindly provided by Sankyo Co., Ltd. (Tokyo), was dissolved in 67 mM phosphate buffer (pH 7.4). All values are expressed as means ± S.E.M. Student's paired t-test was performed to compare the values of anesthetized rats to that of pithed rats in Group 3. Other data were analyzed by two-way analysis of variance with repeated measures and multiple comparison. Differences were considered statistically significant at values of p < 0.05.

RESULTS AND DISCUSSION

The main finding of this study is that the ACE inhibitor, captopril, attenuated the peripheral sympathetic nerve-mediated pressor response which was accompanied by the partial reduction of the increases in plasma NE and almost total inhibition of the plasma A-II in pithed rats. All results are schematically represented in Fig. 1. A-II is one of the candidates to enhance adrenergic activity by increasing NE release at peripheral sympathetic nerve endings. As far as we know, the present study is the first to try to assess the issue of the facilitative interaction between A-II and NE release in adrenergic nerve stimulation from the viewpoints of possible interrelations among functional vascular responsiveness and plasma NE and A-II levels in pithed rats. Assessments of the present issue in pithed rats have been made by many investigators, but very few of them have included the data concerning plasma A-II and NE levels that identify the mechanisms involved in the regulation of vascular responsiveness.

The pithed rat, in which the central and afferent aspects of neural control are effectively eliminated by spinal cord destruction, is a useful animal model for analysis of the facilitative interaction of A-II with the peripheral sympathetic nervous system. Compared with the value obtained in pentobarbital rats, the pithed rats showed about 7.8 times lower basal NE level (44.8 ± 10.2 vs. 347.3 ± 34.8 pg/ml, p < 0.01) but about 2.6 times higher basal A-II level (142.6 ± 35.9 vs. 55.1 ± 17.6 pg/ml, p < 0.01) both of which were accompanied by lower diastolic blood pressure (38 ± 3 vs. 101 ± 6 mmHg, p < 0.01) (Group 3). These observations suggest that tonic sympathetic outflow from the central nervous system is eliminated and the A-II level is increased along with the pithing procedure. Because the plasma renin activity of pithed rats has been reported to be 1.9—2.6 times higher than pentobarbital anesthetized rats, together with the high A-II level observed in the present study, it is noted that the activated renin-angiotensin system, possibly because of low blood pressure, may emphasize the effects of captopril in this model.

The A-II is known to facilitate the sympathetic nerve-induced pressor response through two mechanisms: the facilitation of NE release from nerve endings and the facilitation of NE response by the post synaptic mechanism. We previously demonstrated in the pithed rat that the pressor response to injected NE tended to be attenuated by ACE inhibitor, but the extent of inhibition did not reach a significant level. The pressor response to sympathetic nerve stimulation (1—10 Hz) was attenuated by ACE inhibitor treatment and this effect of inhibitor was prevented by subsequent infusion of A-II. Based on these results, we have hypothesized that the ACE inhibitor attenuates the pressor response by sympathetic nerve stimulation mainly through the inhibition of endogeneous A-II formation and its facilitation of NE release in pithed rats. The measurement of NE level in the plasma in the present study confirmed this possibility; upon nerve stimulation in pithed rats treated with captopril, the extent of increases in blood pressure and NE were approximately 40% smaller than the corresponding values in pithed rats treated with vehicle (increase in diastolic blood pressure: 39 ± 8 vs. 65 ± 8 mmHg, NE: 700 ± 119.6 vs. 1080 ± 153.2 pg/ml). Although we cannot guess the role of A-II on the post-synaptic response to released NE from the present results, the diminished NE release by captopril treatment may suggest the
facilitative effect of endogenous A-II on the NE release from sympathetic nerve endings in the pithed rat.

The measurement of plasma A-II level confirmed the adequate inhibition of ACE by captopril and the role of A-II in the facilitative effects on sympathetic nerve function. The stimulation of spinal sympathetic outflow increased the concentrations of arterial plasma NE (from 68.1 ± 25.0 to 1148.4 ± 155.6 pg/ml, p < 0.01) and A-II (from 175.3 ± 44.7 to 221.0 ± 41.9 pg/ml, p < 0.05). The extent of the increase in A-II was somewhat smaller than the pressor response to nerve stimulation, whereas the increases in NE appeared to be closely related to the pressor response. The pressor effect of nerve stimulation per se reduces renin release through the intrarenal baroreceptor mechanism in juxtaglomerular cells, and may partially offset the sympathetic increase in renin release, thus, overall increase in the A-II level appeared to be small in the present study. Captopril treatment reduced the basal A-II level to 33.5 ± 6.8 pg/ml and completely inhibited the sympathetic nerve-stimulated increase in A-II level. The reduction of NE release and pressor response to sympathetic nerve stimulation is thought to reflect the effect of captopril on basal and/or stimulation-induced increase in A-II level. We and other investigators have previously showed that ACE inhibitor-induced attenuation of pressor responses to sympathetic stimulation was essentially prevented by bilateral nephrectomy. Taken together, the data would also suggest that the renal renin-angiotensin system, the activity of which is increased under a basal condition and further increased upon renal sympathetic nerve stimulation, is involved in the pressor response to stimulation of spinal sympathetic outflow in pithed rats.

Because it is well known that the ACE inhibitor induces the increase in intrinsic bradykinin level as well as the decrease in A-II level, the changes in bradykinin level are thought to affect the sympathetic nerve-stimulated pressor response. However, recent studies have shown that the bradykinin stimulates the NE release and its effect is completely abolished by the B2-receptor antagonist HOE 140 in pithed rats. Based on these results, it is hard to postulate that the captopril-induced reduction of NE release and pressor response to nerve stimulation is the result of the accumulation of bradykinin by ACE inhibitor. Because the inhibitory effect of ACE inhibitor on the pressor response to nerve stimulation has been reported to be abolished by subsequent infusion of A-II in our preparation, it is possible that the reduction of A-II level mediates this effect of captopril.

In conclusion, the NE release and pressor response to nerve stimulation was diminished by the treatment of captopril with concomitant reduction of A-II in pithed rats. This result further emphasizes the possible facilitative effect of endogenous A-II on the sympathetic nerve function in pithed rats proposed by earlier experiments.

REFERENCES