Potentiating Effects of Angiotensin II on Norepinephrine-Induced Vasoconstriction in Isolated and Perfused Dog Mesenteric Arteries

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Abstract—Effects of angiotensin II on norepinephrine- and KCl-induced vasoconstrictions were investigated in isolated canine mesenteric arteries which were perfused with Krebs' solution at 37°C. Angiotensin II at 0.03–0.3 μg showed tachyphylaxis in the injection intervals used (10, 20 or 30 min intervals). After treatment with angiotensin II (0.18–1.8 μg), norepinephrine- and KCl-induced constrictions were consistently potentiated. Imipramine which blocked tyramine-induced action did not significantly influence norepinephrine-induced constriction. Pretreatments with both angiotensin II and indomethacin enhanced norepinephrine-induced constriction much greater than that with angiotensin II alone. It is suggested that angiotensin II may modify intracellular Ca movements and prostaglandin production induced by norepinephrine.

The role of angiotensin II in the maintenance of systemic blood pressure and the involvement of angiotensin II in several kinds of hypertension have been widely investigated. It seems that angiotensin II has an important role in the regulation of vascular reactivity, since it has been confirmed that converting enzyme inhibitors exert its anti-hypertensive action in many patients with essential hypertension. In 1982, Ito (1) reported that pretreatment with angiotensin II significantly augmented norepinephrine-induced constriction in the rat aortic strips. Previously, Hongo and Chiba (2) developed a new method for isolated and perfused vessel preparations, and it was modified by Tsuji and Chiba (3). By use of this cannula inserting method, we tried to confirm the potentiating effects of angiotensin II on norepinephrine-induced vasoconstriction, and we made an attempt to clarify a participation of the mechanism of norepinephrine uptake into the adrenergic nerve elements, because Khairallah (4) considered that these might be due to the inhibitory action of angiotensin II on norepinephrine uptake by adrenergic nerve endings.

Twenty-three mongrel dogs weighing from 8 to 20 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After treatment with sodium heparin (200 units/kg, i.v.), dogs were sacrificed by rapid exsanguination from the right common carotid artery. Median branches of the cranial mesenteric artery which supplied the large middle portion of the small intestine were carefully isolated. Isolated arteries selected for study were 10–15 mm in length and 0.8–1.2 mm in outer diameter; they were cannulated as described previously (2, 3). The isolated, cannulated artery was placed in a bath maintained at 37°C and perfused with Krebs' solution by means of a peristaltic pump. The perfusion solution was bubbled with 95% O₂ and 5% CO₂ which maintained the pH of the solution between 7.2–7.4. The flow rate (0.5–2.0 ml/min) was initially adjusted so that the perfusion pressure was between 50–100 mmHg, subsequently the flow rate was kept constant throughout the experiments. The constrictor response was, therefore, observed as an increase in perfusion pressure. Drugs used in this study were d/-norepinephrine hydrochloride (San-kyo), angiotensin II (Hypertensin, Ciba-Geigy), indomethacin (Sigma), imipramine
hydrochloride (Fujisawa), and tyramine hydrochloride (Tokyo Kasei). The drug solution was administered into the rubber tubing close to the cannula in a volume of 0.01-0.03 ml. Indomethacin was dissolved in 2% sodium bicarbonate solution before the experiment, which was slowly infused intraluminally for 5–10 min by use of a 5 ml injector. The data are presented as the means ±S.E.M. in the text and illustration.

When norepinephrine was injected into the cannulated mesenteric artery, immediate increases in perfusion pressure were obtained in a dose-related manner as reported before (5). Norepinephrine at a dose of 0.5 μg usually induced strong vasoconstriction of over 100 mmHg. Repeated injections of norepinephrine induced the same degree of vasoconstriction without tachyphylaxis. Norepinephrine-induced vasoconstriction was not potentiated by pretreatment with imipramine which significantly blocked tyramine induced vasoconstriction. Tyramine at 10, 30 and 100 μg induced dose-dependent increases in perfusion pressure of 11±4 (mean±S.E., n=9), 16±3 (n=8) and 21±10 (n=3) mmHg, respectively. After treatment with 1 μg of imipramine, they were suppressed to 2±2 (n=4), 4±3 (n=4) and 4±1 (n=3) mmHg, respectively. Norepinephrine at 0.3 and 1 μg induced increases in 67±19 (n=9) and 212±25 (n=9) mmHg (mean±S.E.), respectively. They were not potentiated but suppressed to 4±3 (n=7) and 148±35 (n=7) mmHg, respectively, by 1 μg of imipramine. Imipramine (1 and 10 μg) did not modify vasoconstrictor responses to 1 and 3 mg of KCl (n=8–13).

Bolus injections of angiotensin II (0.03–0.3 μg) consistently induced tachyphylaxis at 10, 20 and 30 min intervals. Thus, we could not obtain the dose response curve for angiotensin II (0.03–0.3 μg) in this study. Initial injection of angiotensin II induced a temporary strong vasoconstriction such as 200 mmHg in the majority of cases which continued for 2–3 min. The vasoconstrictor responses to norepinephrine and KCl were significantly enhanced by pretreatment with angiotensin II. Figure 1 shows an example tracing of effects of pretreatment with total doses of 0.6 μg of angiotensin II on vasoconstrictor responses to 0.5 μg of norepinephrine and 2 mg of KCl. Angiotensin II, 0.1 μg, was repeatedly administered 6 times at 10 min intervals. The initial injection of 0.1 μg of angiotensin II produced strong vasoconstriction, but the second much smaller.

Fig. 1. Potentiating effects of angiotensin II on norepinephrine- and KCl-induced vasoconstrictions. Before and after treatment with total doses of 0.6 μg of angiotensin II (which were repeatedly administered 6 times at 10 min intervals), vasoconstrictor responses to 0.5 μg of norepinephrine (NE) and 2 mg of KCl are shown in an isolated and perfused canine mesenteric arterial preparation.
Fig. 2. Potentiating effects of angiotensin II on norepinephrine- and KCI-induced vasoconstrictions. Angiotensin II enhances vasoconstrictor responses to 0.5 µg of norepinephrine and 2 mg of KCl, and pretreatments with indomethacin and angiotensin II induces much larger vasoconstriction in isolated and perfused canine mesenteric arteries. Angiotensin II was given six times by repeated injections at a total dose range of 0.18 to 1.8 µg (0.9±0.5 µg, mean±S.E., n=16). Vertical lines represent S.E.M. *P<0.05; **P<0.01.

and the last 4 injections did not produce any vasoconstriction at all. Effects of norepinephrine and KCl before and after angiotensin II treatment are summarized in Fig. 2. In seven preparations treated with 5 mg of indomethacin, norepinephrine-induced vasoconstrictions were much more markedly potentiated by pretreatment with angiotensin II. Summarized data are also shown in Fig. 2.

Ito (1) demonstrated that in normal Tyrode's solution, pretreatment with angiotensin II did not modify the contraction due to high potassium ions, whereas it significantly augmented the contraction due to norepinephrine in rat aortic strips. In this study, we showed that in normal Krebs' solution, pretreatment with angiotensin II significantly enhanced both norepinephrine- and KCl-induced vasoconstrictions. Moreover, an indirect sympathomimetic amine, tyramine, produced only a slight vasoconstriction as reported previously (6), and a potent uptake blocking agent, imipramine, in doses which blocked the tyramine-induced vasoconstriction did not show significant enhancement of norepinephrine-induced vasoconstriction in this arterial preparation. Therefore, it is concluded that the potentiating effects of angiotensin II on norepinephrine action are not mediated via adrenergic mechanisms.

It is suggested that angiotensin II might increase the passive permeability of vascular smooth muscles to sodium ions (1). An increase of intracellular sodium concentration may bring on an increase in intracellular Ca ions and a development of tension in vascular smooth muscle. However, it seemed that the change of the passive permeability by angiotensin II appeared after prolonged treatment. In 1970, Villamil et al. (7) performed their experiments by using dogs treated with a continuous infusion of angiotensin II at 35–100 ng/kg per min for 5–6 weeks. They suggested that angiotensin II increases the permeability of the vascular muscle to sodium ions and probably to calcium ions. In the experiments in which angiotensin was applied acutely, Turker et al. (8) and Guinard and Friedman (9) reported the enhancement of the sodium pump activity by angiotensin II. Therefore, it seems that effects of angiotensin II on sodium permeability are different between acute and chronic experiments. In this study, we demonstrated that norepinephrine-induced constriction was potentiated by it. Thus, it may be difficult to explain the potentiating effects by changing sodium ion permeability. Anderson et al. (10) reported that angiotensin II stimulates phosphorylation of the myosin light chain in cultured vascular smooth muscle cells from rat superior mesenteric arteries, indicating the possibility of another mechanism for the potentiating effects of angiotensin II.

Although the potentiating mechanism of norepinephrine action by angiotensin II is not clear yet, intracellular calcium ion move-
ment and prostaglandin production may be involved in part of the mechanism.

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