Effect of Salt Loading and Calcium Antagonist on the Pressor Response to Angiotensin Infusion in the Rat

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IN the pathogenesis of human and experimental hypertension, the mechanisms of sustained high blood pressure are involved cardiac function, sympathetic tone, peripheral resistance, levels of circulating humoral agents, electrolyte of plasma and tissue, variation in vascular reactivity to circulating pressor substances and so on, however, the mechanism has not been able to explained by the sole factor. Among several critical factors, the evidence of close relationship between amount of salt intake and development of hypertension is widely recognized. In the uncultivated people who take almost no dietary salt no hypertensives were found by epidemiologic study;¹⁴ on the contrary, excess salt ingestion proved to produce high incidence of hypertension in the human¹² and experimental animals.¹⁷⁻¹⁹ Despite of widely recognized correlation between Na metabolism and hypertension, the underlying mechanisms has been remained unknown. Serial studies were reported in our laboratory effects of interaction of angiotensin and noradrenaline on the vascular reactivity with isolated rats hindlimb preparations in the possible mechanism of experimental renal hypertension,^{1,2,5} and the augmented effect of salt loading on the vascular action of noradrenaline in the isolated system.⁴ Recently, hypertension in pathophysiologically classified into two groups; volume and vaso-

constricting hypertension?²¹ Hypertension in the latter seems to be maintained mainly by direct vasoconstricting action of angiotensin II, the end-product of renin-angiotensin system. From the point of view about vascular smooth muscle contraction of angiotensin, vascular reactivity to angiotensin in its receptor sites is important as well as the concentration level of circulating blood. Angiotensin displays no significant potency in state of Na depletion which induced high plasma renin activity (PRA) but augmented pressor response despite of low PRA.¹³ Angiotensin also shows tachyphylaxis as characteristic phenomenon. So that it will be necessary for searching the factors to maintain the mechanisms of vasoconstricting or renin-dependent hypertension.

The Ca ions are immediate trigger for contraction in the vascular smooth muscle. Recent review of Blaustein¹¹ suggested that Na ions play a critical role in the maintenance of Ca balance in the vascular smooth muscle. The present work was aimed to observe the effects of salt loading and the Ca ion antagonist on the pressor responses and tachyphylaxis of angiotensin infusion.

MATERIALS AND METHOD

Experiments were divided to three sections.

Section 1: Two groups of 6, weighing about 200 g, Wistar female rats were used. Both groups were feeded with standard rat chow. The one is provided as drinking water and the other is 1% saline for 4-6 weeks. Each rat was anesthetized with sodium pentobarbital (4 mg/kg, ip), tracheotomized and vagotomized. Mean arterial

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pressure through cannulation into right carotid artery was recorded on the smoked drum by mercury manometry. Pressor responses were obtained by infusing angiotensin (Hypertensin, CIBA), 20 ng/min (infusion rate: 0.001 ml/min) with chronofusor in 5 min intermittent, repeated 8 times followed by 30 min continuously in both groups.

Section 2:

A. Isolated rats hindlimb preparations were used. Methods for setting preparation was described previously in details.¹ Untreated Wistar female rats weighing about 200 g were anesthetized with sodium pentobarbital and given heparin, (300 iu) intraperitoneally. After laparotomy and excision of major visceral organs, plastic cannula was inserted through the abdominal aorta and its tip was positioned at right common iliac artery. In abdominal section at the level of renal arteries, the vana cava was cut open. Isolated hindlimb preparation was perfused with standard Tyrode's solution, kept at 37°C, saturated with oxygen, by connecting to constant rotary pump and flow rate was maintained at 1.0 ml/min. Change in perfusion pressure, representing the total resistance of vascular beds of preparation, was recorded on smoked drum. The test substances dissolved in physiological saline were injected as a bolus, 0.025 ~ 0.1 ml, in the circuit adjacent to the preparation and were infused continuously by chronofusor at the rate 0.001 ml/min. Drugs used were angiotensin, noradrenaline, Diltiazem hydrochloride (CRD-401)²⁰ presented by Tanabe Pharmaceutical Company, Japan and angiotensin-antagonist, (1-Sar, 8-Ileu) angiotensin II (The Peptide Institute, Protein Research Foundation, Osaka, Japan)

B. Untreated 200 ~ 250 g of female Wistar rats were used and they were prepared in the same methods as described in Section 1. As the experiments in Section 2 were pilot study, results were not statistically analysed.

Section 3:

Twelve Wistar female rats, weighing about 200 g were divided into two groups. Group A received tap water and standard laboratory chow ad libitum as the control. Group B received 1% saline as drinking water and regular laboratory chow. They were feeded 4 weeks. Method of anesthetizing and recording of the carotid arterial pressure was same as that of Section 1, and bilateral femoral veins and left jugular vein were used for single injection and continuous infusion of test substances.

All groups of animals in Section 1 and Section 3 gained weight during 4–6 weeks period. Blood pressures were measured in the first day of the trial and the last day of the experiment with tail cuff method. No significant differences in the body weight and blood pressure between two groups of drinking water and 1% saline. In Section 2, every three rats with acute and chronic hypertension, blood pressures were measured at weekly interval.

RESULTS

Section 1:

A. Effect of angiotensin infusion on pressor response in the group of normal diet with tap water. (Fig. 1) Response in the first, 20 ng/min, 5 min duration of angiotensin infusion was about 40 mmHg, however, the responses declined gradually with repeated application of angiotensin and blood pressure returned to the level prior to the infusion after 30 min continuous infusion.

B. In contrast, 1% saline group showed augmented responses in 5 min intermittent and following 30 min continuous infusion. No tachyphylaxis to angiotensin was observed in all cases. (Fig. 2)

C. Changes in the mean arterial pressures in two groups were statistically analysed. Significant differences (mean $\pm$ S.D.) were obtained. (Fig. 3)

Section 2:

The works in Section 2 were aimed to observe the general pharmacological properties of calcium antagonist, diltiazem, (CRD) as the pilot study.

A. Change in response in perfusion pressure in the hindlimb preparation.

1) Direct effect of diltiazem on the perfusion pressure:

After response to noradrenaline became stable, diltiazem was applied in increasingly graded dose, 10–100 μ g in bolus. No direct effect to vascular beds were observed in low and higher perfusion pressure. (Fig. 4)

2) Effect of diltiazem, single injection and infusion, on the responses to noradrenaline:

Comparing the response in the control period, responses to noradrenaline, 0.25 and 0.5 μ g were reduced about 60% by prior application of diltiazem, 200–500 μ g as a bolus. Large dose of diltiazem, 1.0 mg, could not completely inhibit the response to 0.25 μ g of noradrenaline, and showed itself vasoconstriction of which mechanism was unknown. (Fig. 5) In constant infusion of diltiazem in 30 μ g/min, response to noradrenaline was diminished about 50% as shown in Fig. 5.

Fig.1. Pressor response to angiotensin infusion.

Fig.2. Pressor response to angiotensin infusion.

3) Effect of diltiazem on response to angiotensin: As previously reported¹ responses to repeated applications of angiotensin were decreased rapidly and no response was occurred after several administrations in this preparation. (tachy-

phylaxis)¹ However, the second application of angiotensin lost its activity immediately after a bolus of diltiazem. (Fig. 6) In another preparation when a bolus of diltiazem was given prior to the first application of angiotensin, the response

Pressor Responses to Angiotensin Infusion

Fig.3. Pressor responses to angiotensin infusion.

Direct Effect of CRD on Perfusion Pressure in Hindlimb Preparation

Fig.4. Direct effect of diltiazem on perfusion pressure in hindlimb preparation.

was markedly reduced as shown in Fig. 6.

B. Change in pressor responses to noradrenaline and angiotensin in the whole animal:

1) Effect of diltiazem on pressor response to noradrenaline and angiotensin:

After the equipressor dose of noradrenaline (0.05, 0.1 μ g) and angiotensin (5, 10 ng) was determined, the effect of diltiazem on these substances was observed. In the continuous

infusion of diltiazem, 3 μ g/min, responses were not affected in spite of decreasing in the mean carotid arterial pressure, however, increasing dose of infused diltiazem to 10 μ g/min, diminished responses to both substances equally about 50% and returned promptly to prior level after the infusion was ended. (Fig. 7)

2) Effect of diltiazem on pressor response to continuous angiotensin infusion:

Fig.7. Effect of diltiazem on pressor response to noradrenaline and angiotensin.

Fig.8. Effect of diltiazem on pressor response to angiotensin infusion.

hypertensive rats. (1-Sar, 8-Ileu) angiotensin II, angiotensin antagonist showed biphasic response in acute phase (3 W) and agonistic only in chronic phase (6 W) of hypertension respectively. However, admini-

stered bolus of diltiazem produced greater depressor responses in the same degree in both acute and chronic hypertensive rats? (Fig. 9)

Section 3:

Effect of diltiazem on pressor responses to

Fig.9. Effect of diltiazem and angiotensin-antagonist, (1-Sar, 8-Ileu)-angiotensin II, on blood pressure in goldblatt hypertensive rats.

Fig.10. Effect of diltiazem on pressor response to angiotensin infusion.

angiotensin infusion were compared in both groups of drinking water and 1% saline. After the basal blood pressure became stable, angiotensin, 20 ng/min, was infused as the first infusion, for 20 min. When the blood pressure was decreased to the prior level after cessation of the

first trial of angiotensin infusion, diltiazem was given with continuous infusion, 10 μ g/min. After the blood pressure lowered and kept constant, angiotensin, 20 ng/min was re-infused, as the second infusion, for 20 min. In the water drinking group the response to the second infu-

Effect of CRD on Pressor Response to Angiotensin Infusion

Fig.11. Effect of diltiazem on pressor response to angiotensin infusion.

Pressor Responses to Angiotensin Infusion before and during CRD Administration

Fig.12. Pressor responses to angiotensin infusion before and during diltiazem administration.

sion of angiotensin was significantly lower than that of the first infusion. (Fig. 10) However, in the saline drinking rats the pressor response to the first infusion of angiotensin was significantly greater than that of the water drinking group and

that the response to the second angiotensin infusion was not only augmented as that of the first infusion but also produced no tachyphylaxis in spite of continuous infusion of diltiazem dose enough to suppress the response to angiotensin in

the water drinking rats (Fig. 11) Comparison of pressor responses to the first and second angiotensin infusion in water and 1% saline drinking groups were statistically analysed. (Fig. 12)

DISCUSSION

Angiotensin is one of the most potent substance capable of producing contraction of the vascular smooth muscle. However repeated application of relatively large dose or continuous infusion of the substance induce tachyphylaxis phenomenon. In various mechanisms proposing about this phenomenon, the angiotensin receptor occupation theory is considered to be most satisfactorily, however, this theory still awaits further elucidation.¹⁰ In 1972, Meyer et al²⁰ proposed that in the angiotensin receptors on the rat aortic strip using ³H-labeled angiotensin, specific angiotensin binding sites exist in the cell membrane and this binding would be correlated to the smooth muscle contraction of the aorta. Paiva et al⁸ in 1977, also proposed that angiotensin exerts its agonistic action by placing Ca ions from the binding sites in the cell membrane and tachyphylaxis was induced by tightly binding of angiotensin molecules to its specific binding sites and decrease intracellular Ca ions necessary for subsequent angiotensin initiated response. However, the finding that tachyphylaxis was not inhibited by increasing the Ca ions concentration in the medium indicated that proposed interaction of angiotensin with Ca ions binding sites might be more complex than a simple competitive phenomenon. On the other hand, Blaustein et al¹¹ suggested that hypertension may be developed in the results of disorder of Na balance and Na-Ca exchange system which result in the increase of peripheral vascular resistance. In the salt loading animal, Na-pump was partially inhibited with certain substances (hormones) and a little increase of intracellular Na ions concentration will bring about abnormality in Na-Ca exchange system followed by the increase of intracellular Ca ion concentration which result in change in tension of smooth muscle and influence to the peripheral resistance. The results obtained in our series of experiments were not observation in the cellular levels. From the phenomenon recognized in the untreated animal given tap water, response to angiotensin was thought to be reduced contractility of the vascular smooth muscle by inhibiting the inward current of Ca ions with Ca-antagonist in the cell membrane.²⁰ In the salt loading group the angio-

tensin pressor response was augmented and no tachyphylaxis phenomenon was produced in spite of administration of Ca-antagonist. In rats of high sodium intake, vascular receptor affinity for angiotensin was increased by proving increase amount of angiotensin II antibody required to block the angiotensin response.¹⁵ In our previous work, using rats hindlimb preparation, angiotensin tachyphylaxis reversal by noradrenaline was observed in the group of water drinking and not observed in the group of drinking 1% saline suggested that angiotensin binding power to its receptor sites (affinity) was more tightly than water drinking group.⁴ We postulated that high sodium intake might increase affinity of angiotensin molecule to its receptor sites and by influencing the change in the gradient of between intra- and extracellular Na ions concentration increase permeability of Ca ion in the cell membrane and/or increase utilization of intracellular free Ca ions necessary for maintaining the continuous contraction with angiotensin in the circulating system. Dose of Ca ion antagonist (diltiazem) influenced a little to the blood pressure of normal control rats, showed greater and long-lasting depressor effect to the hypertensive animal induced by angiotensin infusion and produced by Goldblatt's methods indicated that Ca ion play not so important role for the regulation of blood pressure of normal control animals but remarkably important for the high blood pressure maintaining by the pressor substances and/or vascular hyperreactivity in the chronic stage of the experimental hypertension.

SUMMARY

Elucidation of mechanisms of angiotensin tachyphylaxis will be necessary for explaining the continuous vasoconstricting action of angiotensin in the vascular smooth muscle, which might be closely correlated to the high blood pressure in renin-dependent or vasoconstricting hypertension. From the results obtained sodium loading rats showed not only augmented pressor response to angiotensin but also inhibiting the appearance of its tachyphylaxis under the infusion of Ca-antagonist dose enough to suppress the response to angiotensin in the rats of water drinking. Speculated mechanisms about this phenomenon has been discussed.

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