Interaction of Angiotensin and Noradrenaline in Rat’s Hindlimb Preparation.
A Possible Mechanism of the Experimental Renal Hypertension

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The concept that the experimental acute renal hypertension results primarily from the release of renin from the affected kidney and subsequently increased formation of angiotensin appeared to be generally accepted, however, the lack of detectable amounts of vasoconstrictor substances in the chronic hypertension involves indirectly something other than a humoral mechanism. A number of recent reports indicated that angiotensin interact with sympathetic nervous system in the various sites and proposed the possibilities that the renin-angiotensin and sympathetic nervous system may interact in maintaining the high blood pressure in the chronic phase of hypertensive state, regarded as an indirect action of angiotensin to the vasoconstriction.

On the other hand, the vasoconstrictor activity of angiotensin was originally considered to be due to a direct effect on vascular smooth muscle, that is, so far, the major factor of blood pressure increases in the acute phase, however, the phenomenon of angiotensin tachycardia, the fact proved in vivo and also in the isolated system, is a potential drawback to its vasoconstricting activity. We had observed significantly augmented vascular responses to noradrenaline in both aortic strips and isolated hindlimb preparations of rats with chronic renal hypertensive groups produced by the Goldblatt’ method comparing that of normotensive control one and also somewhat augmentation of noradrenaline responses in the group of rats daily administered subcutaneously a suppressor amount of angiotensin for several weeks1. The results suggested the maintenance of high blood pressure in chronic stage might be dependent in part upon the hyperreactivity of the vessels to noradrenaline from sympathetic nerve endings in the presence of a small amount of angiotensin in the circulating system. The present works were undertaken to observe the continuous variation of vascular responsiveness to noradrenaline under the infusion of different concentration of angiotensin, considered to be its indirect action, and also to assess the effect of noradrenaline on direct vasoconstriction by angiotensin using isolated hindlimb preparations i.e. the interaction of the vasoactive substances on the peripheral vascular beds of which increased resistance is thought to be the most important factor in both production and maintenance of hypertension.

**Materials and Methods**
Untreated white female rats weighing about 200 gm with sodium pentobarbital (4 mg/kg, i.p.) and heparin (300 i.u.) as an anticoagulant. After laparotomy and excision of major visceral organs, plastic cannula was inserted through abdominal aorta and its tip was positioned at right common iliac artery. The unilateral hindlimb preparation could prevent such leakage of perfusate from the lumbar arteries as frequently seen previous experiments in bilateral

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hindlimb perfusion. In the abdominal section at the level of renal arteries, the vena cava was cut open. The cannula in the isolated preparation was immediately perfused with Tyrode solution, kept at 37°C, oxygen saturated, by connecting to the constant rotary pump. Flow rate was maintained at 1.2 ml/min. and change of perfusion pressure, representing resistance of vascular beds of the preparation, was recorded on smoked drum. The solution of test substances, 0.05-0.1 ml, were injected into circuit close by the preparation.

In infusion, perfusate was replaced with another Tyrode solution contain in the appropriate concentration of test substances. Angiotensin used; Hypertesin II (CIBA).

RESULTS
A) Effect of angiotensin infusion on noradrenaline responses.
1) The blood components of preparation were washed out within twenty minutes when perfusion pressure were almost settled at 10-20 mmHg, and about a half an hour was

Fig. 1. Effect of angiotensin on noradrenaline responses.

Fig. 2. Effect of angiotensin infusion on noradrenaline responses.
response of angiotensin occurred, recognized as its tachyphylaxis. Approximately 30 minutes time lag from infusion, successive noradrenaline responses increased markedly with time as shown in Fig. 1.

2) Effect of angiotensin infusion, 2 ng/min., the dose uninfluenced perfusion pressure, on noradrenaline responses were observed under same mode of experiment in seven untreated animals. Also in these cases, followed by twenty minutes time lag, marked increase of noradrenaline responses continued almost constantly during the infusion. Fig. 2. Results summarized in the Fig 3. showed augmented responses statistically analysed.

B) Effect of noradrenaline on angiotensin responses.

1) Repeated administration of the same dose, 0.5γ, of angiotensin diminished rapidly vasoconstrictor activities in the hindlimb preperation, Fig. 4.

2) However, just as tachyphylaxis to angiotensin appeared its reversal promptly occurred followed by a single application of noradrenaline. Alternate injections of noradrenaline and angiotensin produced no angiotensin tachyphylaxis, Fig. 4a.

C) Angiotensin tachyphylaxis and its reversal in different conditions.

1) Angiotensin tachyphylaxis was observed as well during perfusion flow and pressure were increased.

Fig. 3. Noradrenaline responses (Untreated).

Fig. 4. Effect of noradrenaline on angiotensin responses.
2) Angiotensin tachyphylaxis tended to be inhibited during noradrenaline infusion, (0.5γ/min.) which produced sustained elevation of perfusion pressure, Fig. 5.

3) Complete inhibition of vasoconstrictor activity of noradrenaline for about 20 minutes followed by a single application of phentolamine, 1 to 5γ, was verified previously and it was then proved that the effect of noradrenaline to reverse angiotensin tachyphylaxis seen as before, was not affected in the alpha-adrenergic receptors being blocked by phentolamine, Fig. 6.

**DISCUSSION**

In a possible mechanisms of experimental renal hypertension, the interaction of renin-angiotensin and sympathetic nervous system has been suggested by many workers. The present study has been investigated interaction between both systems in the peripheral site within the confines of isolated preparation denervated and perfused with artificial fluid. From results obtained, it might be assumed that in an acute phase, direct vasoconstriction with increase in its amount of angiotensin in the peripheral circulation will bring about rise of blood pressure. Then, participation of interaction between angiotensin and noradrenaline.
may take place in the peripheral sites. Using isolated cat carotid strips Khairallah et al. reported that no reversal of angiotensin tachyphylaxis occurred when they were kept in contact with noradrenaline in the bath chamber\(^1\) but, we obtained prompt reversal of tachyphylaxis in the hindlimb preparation in which angiotensin receptors are quite easily occupied by angiotensin molecules. So it is supposed that noradrenaline has activity to remove angiotensin molecules from its receptor sites besides its primary alpha-adrenergic stimulating activity, because this effect of noradrenaline was not inhibited under condition of alpha-receptors were being blocked by phentolamine. This may cause maintaining biological activity of angiotensin in the acute phase of the renal hypertension. The augmented responses by noradrenaline in the period of angiotensin tachyphylaxis could also increase the blood pressure.

In the chronic stage, on the other hand, the peripheral vessels perfused by a very small amount, non-pressor dose of angiotensin will produce the hyper-vascular-reactivity to noradrenaline, and it might play a role of sustaining high blood pressure\(^5\,\!^6\).

**Summary**

Interaction of angiotensin and noradrenaline on vascular responsiveness were investigated in the isolated rat's hindlimb preparations perfused with Tyrode solution in low perfusion pressure. Highly significant enhancement of responses to noradrenaline occurred in angiotensin infusion with concentration influenced or uninfluenced to the perfusion pressure. Diminished responses of repeated application of angiotensin, tachyphylaxis, easily produced in the preparation, were promptly reversed by an application of noradrenaline and no tachyphylaxis was made as far as both substances were alternately administered. In the possible mechanism of the experimental renal hypertension, the participation of interaction in renin-angiotensin and sympathetic neurohumoral agent on the peripheral sites of the circulating system were suggested.

**REFERENCES**


