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

RIHEI SATO, YOSHIKI MASUYAMA,
SHO TANAKA AND ICHIRO NISHIO

The various types of experimental hypertensive phenomena have been devised to elucidate the mechanisms of essential hypertension. SHR are now being available for detecting the role of a genetic factor of the hypertension. On the other hand, renal hypertensive animals produced by the Goldblatt's method are also widely used because of the simple surgical procedure; sustained stable high blood pressure and of similarity in the hemodynamic pattern of essential hypertension. In general, in acute phase of various types of experimental hypertension, the mechanism of high blood pressure appears to be closely related to their original methods, however, that of chronic phase are much more complex as being suggested by many investigators, during which various related factors might contribute to maintain high blood pressure changing their properties quantitatively and qualitatively with time.

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, 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 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, 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, but the phenomenon of angiotensin tachyphylaxis, 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 made by the Goldblatt's 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 weeks. The results suggested the maintenance of high blood pressure in chronic stage might be dependent in part upon the hyper-reactivity 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 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.

Key Word: Renal Hypertension

1st Department of Internal Medicine, University of Tokyo, Tokyo
* This paper was presented at "The 1st Conference of Pathogenesis of Hypertension", Tokyo, November 7, 1971.

Japanese Circulation Journal Vol. 36, June 1972 595
MATERIALS AND METHODS

Untreated white female rats weighing about 200 gm were anesthetized with sodium pentobarbital and heparin as an anticoagulant given intraperitoneally. 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 lumbal arteries as frequently seen previous experiments in bilateral 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, saturated with oxygen, by connecting to the constant flow rotary pump. Flow rate was maintained at 1.2 ml/min and change of perfusion pressure, representing total resistance of the vascular beds of preparation, was recorded on smoked drum. The solution of test substances, 0.025–0.1 ml, were injected into circuit close to the preparation. In infusion perfusate was replaced with another Tyrode solution containing the appropriate concentration of test substances. Angiotensin used; Hypertensin II (CIBA)

RESULTS

A) Effect of angiotensin infusion on noradrenaline responses

1) The blood components of preparation were washed out within 20 minutes when perfusion pressure was almost settled at 10–20 mmHg, and about a half an hour was generally needed until responses of noradrenaline successively injected became stable. Angiotensin infusion, then started, 20 ng/min, brought about significant elevation of perfusion pressure, followed by the peak, it returned to the level prior to the infusion within 15 min and after that no response of angiotensin occurred, recognized as its tachyphylaxis. Approximately 30 minutes time lag from infusion, successive noradrenaline responses increased markedly with time during angiotensin infusion.

2) Effect of angiotensin infusion, 2 ng/min, the dose which did not influence perfusion pressure, on noradrenaline responses were observed under same mode of experiment in seven untreated animals. Also in these cases, followed by 20 minutes time lag, marked increase of noradrenaline responses continued almost constantly during the infusion. Significantly augmented responses were statistically analysed.

B) Effect of noradrenaline on angiotensin responses

1) The same dose, 0.5 µg, of angiotensin given repeatedly diminished rapidly vasoconstrictor activities in the hindlimb preparation.

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.

C) Angiotensin tachyphylaxis and its reversal in the different conditions

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

2) Angiotensin tachyphylaxis tended to be inhibited during noradrenaline infusion, 0.5 µg/min, which produced sustained elevation of perfusion pressure.

3) Complete inhibition of vasoconstrictor activity of noradrenaline for about 20 minutes followed by a single application of phentolamine, 1 to 5 µg, and also no effect of phentolamine on angiotensin tachyphylaxis were 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 were being blocked by phentolamine.

4) Response of isoproterenol: No vasodilation occurred during both low and high perfusion pressure which might prove to be no existence of beta-receptors in the preparation.

5) Effects of phentolamine and propranolol on noradrenaline responses: Adrenaline that has alpha- and beta-stimulating effects showed only pressor one in the preparation like noradrenaline. Vasoconstricting actions of both substances were completely inhibited by phentolamine, however, they were not influenced by propranolol. In the vascular beds of this preparation, alpha receptors seemed to be predominant.

6) Tyramine response and tachyphylaxis of angiotensin and tyramine: Several application of 0.025 mg of tyramine produced its tachyphylaxis. In this state, no tyramine response was observed followed by the single injection of exogenous noradrenaline, showing that the uptake of single injected noradrenaline in the hindlimb preparation might be negligible. After tyramine lost its activity, angiotensin still had its pressor activity, namely, no cross tachyphylaxis between angio-

Japanese Circulation Journal Vol. 36, June 1972
tensin and tyramine was confirmed. Response of released endogenous noradrenaline by application of tyramine was completely inhibited like exogenous noradrenaline by phentolamine.

7) Effects of tyramine and adrenaline on angiotensin response: Potency of angiotensin tachyphylaxis reversal of tyramine was not recognized significantly, that is due to released noradrenaline may be small in its amount. On the other hand, adrenaline has effect of the reversal, however, not so markedly as that of noradrenaline.

**SUMMARY AND CONCLUSION**

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 the results obtained, it might be assumed that in an acute phase, direct vasoconstriction of angiotensin with increase in its amount 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 aortic strip Kharallah et al. reported that no reversal of angiotensin tachyphylaxis occurred when they were kept in contact with noradrenaline in the bath chamber, but, we obtained prompt reversal of the 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, nonpressor dose of angiotensin will produce the vascular hyperreactivity to noradrenaline, and it might play a role of sustained high blood pressure.

**REFERENCES**


**CONCLUDING REMARKS**

Chairman: HIROFUMI SOKABE, Toho Univ.

Dr. T. Sakurai, Department of Urology, Faculty of Medicine, Osaka University also presented his data on the interaction of angiotensin II (Ang) and norepinephrine (NE), using perfused mesenteric artery preparations in the dog or cat. Ang increased the vasoconstrictor responses caused by both exogenous NE and electrical stimulation of the sympathetic nerve. This effect of Ang was not inhibited by pretreatment with desmethylimipramine. The augmenting effect of Ang was not specific to NE, but Ang also increased the vasoconstrictor responses of serotonin and KCl. Tachyphylaxis of Ang pressor response was recovered by exogenous NE, and electrical stimulation of the sympathetic nerve. The recovery was accelerated by cocaine pretreatment. Phentolamine did not inhibit the development of Ang tachyphylaxis, but inhibited the recovery from Ang tachyphylaxis by exogenous NE or the nerve stimulation. The results suggest that the vasoconstrictor action of Ang is partly due to the adrenergic mechanism. But the exact mechanism of this interaction is unknown. (Reported with J. W. McCubbin and R. D. Bunag at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, 1967, and the Annual Meeting of the Japanese Urological Society, 1969).

Interaction between Ang and NE exists as it has long been recognized by many investigators! The exact mechanism of this interaction is not fully elucidated. Ang enhances the effect of NE. Theoretically, the site of interaction could be either post- or presynaptic. Postsynaptically, NE receptor might be sensitized by Ang. Presynap-
tically, two possibilities exist: either re-uptake of endo- or exogenous NE might be blocked by Ang, or release of endogenous NE by the nerve stimulation of drugs might be accelerated. Recent reports favored the last mechanism\textsuperscript{2,3} NE may also affect on the action of Ang. Theoretically, NE might sensitize Ang receptor, or NE might replenish its stores at the nerve endings from which Ang releases NE. Neither mechanism could be elucidated from the results reported.

Although it is important to consider the interaction of the renin-angiotensin system and the sympathetic nervous system for the possible mechanism of hypertension, no definite conclusion can be drawn from the above results.

REFERENCES

