Effect of Angiotensin II on Renal Hemodynamics and Urinary Excretion of Sodium and Potassium in the Rat

HIROYASU YAMAZOE, KENJIRO YAMAMOTO AND JURO UEDA*

Effect of angiotensin II on the renal hemodynamics and electrolytes excretion were studied with a clearance method in rats.

Intraperitoneal administration of angiotensin II reduced RPF and GFR. The hemodynamic effect of angiotensin II was disappeared following adrenalectomy, and partially restored DOC or cortisone.

Angiotensin II increased a urine flow and an excretion of Na with slight decrease in an excretion of K. But, it showed no such effect in adrenalectomized rats. DOC or cortisone restored the action of angiotensin II on the excretion of electrolytes in adrenalectomized rats.

Adrenal cortex hormone may play an important role on the action of angiotensin II on the renal hemodynamics and renal tubular cells in rats.

IN THE NORMAL dog and man under moderate diuresis, angiotensin II mainly acts as antidiuretic and antinatriuretic possibly because of its direct action on renal vessels^{3, 4, 24}. But the substance has an obvious diuretic and natriuretic effect in patients with various hypertension or liver cirrhosis with ascites^{1, 11, 17}. It also causes diuresis and natriuresis in normal rats with possible influence on tubular cells^{2, 5, 12, 18}. Its different behaviours have been reported depending upon animal species, dose and rout of administration as well as upon water or saline loading and functioning of adrenal glands.

Some authors have reported that the renin-angiotensin system mediates aldosterone secretion in the dog and man^{7, 8, 15, 20}, but in the rat a role of the renin-angiotensin system on the aldosterone secretion has been left unclarified^{9, 13, 21}. In the present experiments, effects of angiotensin on the renal hemodynamics and urinary excretion of Na and K were studied simultaneously in rats under various experimental conditions, in an attempt to evaluate a possible role of adrenal hormones on the action of angiotensin II.

MATERIALS AND METHODS

Male rats of the weighing from 100 to 250 g were employed. They were fed on an Oriental stock diet MF in which NaCl content ranged from 0.15 per cent (low Na diet), 0.63 per cent (normal NaCl diet) to 3.3 per cent (high NaCl diet) with water ad lib. for 4 to 5 weeks. Those except the adrenalectomized were fasted 18 hrs. before experiments. Adrenalectomy and postoperative treatment were performed as described by NAKAO¹⁶. Adrenalectomized rats were fed on cube sugar and water until the experiment, which was made about 40 hrs. after operation. A daily intake of sugar and water in adrenalectomized rats was 2 to 4.1 g and 3 to 6.4 ml per 100 g of body weight respectively.

The renal clearance test was performed following the author's method²⁵ in which an oral load of 5 ml of warm water per 100 g of body weight was given followed by subcutaneous injection of inulin and para-aminohippuric acid (PAH). Thirty minutes later the urine remaining in the bladder was excreted by pressing the pubic area. The rats were placed in carefully washed metabolic cages, which were rinsed with 10 ml of distilled water again after experimental period. Urine samples were collected during a 40-minute period two times successively and blood samples were taken twice in the middle of a 40-minute period from the orbital sinus with a glass capillary wetted with heparin solution. The

(Received for Publication, November 29, 1966)

* The Department of Pharmacology, Osaka City University, Medical School, Osaka

blood and urine samples thus obtained were made a pair and used for the determination of inulin and PAH. The rats could tolerate this procedure at least once a week for 5 to 7 weeks. Inulin and PAH were measured by the diphenylamine method and BRATTON-MARSHALL'S method respectively, and glomerular filtration rate (GFR) and renal plasma flow (RPF) were routinely calculated²⁵. Angiotensin II (Hypertensin Cica) was administered just after sampling of the first urine specimen. Urinary excretion of Na and K examined with a procedure as like as that in renal clearance test with an additional water load of 2 per cent of body weight given between the first and the second 40-minute period. In some cases, an oral water load was replaced by 0.45 per cent saline solution. Effects of angiotensin II on Na and K of blood plasma were studied in another group of rats. Blood samples were obtained through a polyethylene tube which was inserted into the carotid artery under pentobarbital anesthesia (35 mg/Kg). Since daily variations were marked in urinary Na and K excretion, the same number of treated and non-treated rats in pair was used in each experiment. Na and K in plasma and urine were determined by flame photometry. The carotid arterial blood pressure was recorded on a smoked drum with a mercury monometer under pentobarbital anesthesia. A granulation index of juxtaglomerular cells (JGI) of rats was calculated with HARTROFT'S method slightly modified by the present authors following Zenkel-formol fixation and MASSON'S trichrome staining of juxtaglomerular cells¹⁰.

RESULTS

A. General condition and JGI of rats fed on a low or high salt diet.

Rats weighing about 120 g fed on a low or a high salt diet for 4 to 5 weeks being apparently healthy, showed a growth similar to that on a normal salt diet. Daily food intake of the three groups of rats was almost equal, 7.3 to 8.9 g/100 g BW, but daily water intake of rats fed on a low or normal salt diet was 15.1 to 17 ml/100 g BW and that of those fed on a high salt diet was 20.6 to 21.4 ml/100 g BW. On average the normal diet group revealed its JGI to be 15, and the low salt diet group to be 28 with a significant increase in granularity.

B. Effects of vasoactive agents on systemic blood pressure.

Among the three groups of rats, there were no differences in their blood pressure or in

responses to intravenous angiotensin II, adrenaline, noradrenaline, vasopressin or acetylcholine. In rats fed on a normal salt diet, intraperitoneal injection of angiotensin II in doses less than 20 μ g/Kg produced no elevation of systemic blood pressure, but that in a dose of 100 μ g/Kg caused a slight rise in blood pressure of 10 mmHg with one minute after injection. The elevation wore off, returning to a control level in about 30 minutes. Neither the duration nor magnitude to the pressure response to angiotensin II was modified by adrenalectomy.

C. Effects of angiotensin II on renal hemodynamics.

Fig. 1. Effect of angiotensin II on GFR, RPF and FF of rats fed on normal salt diet.

Upper curves ... 20 μ g/Kg of angiotensin II (30 rats).

Under curves ... 100 μ g/Kg of angiotensin II (45 rats).

Fig. 2. Effect of angiotensin II in a dose of 20 $\mu\text{g}/\text{Kg}$ on GFR, RPF and FF in adrenalectomized rats with or without adrenal hormones.

No significant differences in the three groups of rats were observed either in GFR or RPF. Intraperitoneal injection of angiotensin II in a subpressor dose of 20 $\mu\text{g}/\text{Kg}$ produced no changes in GFR, but slight decrease in RPF. The substance in a dose of 100 $\mu\text{g}/\text{Kg}$ reduced both, especially in RPF, causing an increase in filtration fraction (FF) (Fig. 1). Although not so significant, the effects of angiotensin II were more marked in the low salt diet group than in the normal or the high salt diet group. On the other hand, adrenalectomized rats maintained on saline showed a decrease in the renal hemodynamic response to angiotensin II. Supportive treatment with cortisone (5 mg/Kg) or DOC (5mg/Kg), which was injected one hour

Fig. 3. Effect of angiotensin II on Na and K excretion and urine volume in normal rats.

Upper curves ... 20 $\mu\text{g}/\text{Kg}$ of angiotensin II (22 rats).

Under curves ... 100 $\mu\text{g}/\text{Kg}$ of angiotensin II (18 rats).

before experiments, restored partially the responsiveness to angiotensin II, but d-aldosterone (3 $\mu\text{g}/\text{Kg}$) showed little or no effect (Fig. 2).

D. Effects of angiotensin II on urinary excretion of Na and K.

1) Experiments in normal rats.

Amounts of both Na and K excreted into urine showed variations in two successive 40-minute periods, but average values of 53 control rats remained almost constant; Na and K in the first periods were 0.42 ± 0.05 $\mu\text{Eq}/\text{min}/100\text{g BW}$ and 0.51 ± 0.04 $\mu\text{Eq}/\text{min}/100\text{g BW}$ and those in the second period were 0.46 ± 0.06 $\mu\text{Eq}/\text{min}/100\text{g BW}$ and 0.50 ± 0.04 $\mu\text{Eq}/\text{min}/100\text{g BW}$, respectively. Intraperitoneal

injection of angiotensin II in a dose of $1\mu\text{g}/\text{Kg}$ to rats caused no change in water and electrolytes excretion, but the substance in a dose of $20\mu\text{g}/\text{Kg}$ increased urine flow by about 20 per cent, accompanied by an increase in Na excretion by about 60 per cent and slight decrease in K excretion. Thus there was a marked increase in urinary Na/K. No further increase in urine flow or Na excretion was observed following administration of $100\mu\text{g}/\text{Kg}$ of angiotensin II (Fig. 3).

It was observed that the diuretic and natriuretic action of angiotensin II was well reflected on the amount of Na excretion before administration of angiotensin II. Rats excreting Na less than $0.6\mu\text{Eq}/\text{min}/100\text{g BW}$ responded to angiotensin II more markedly than rats with higher excretion (Fig. 4). On the other hand, rats fed on 0.45 per cent NaCl instead of water resulted in high Na excretion, over $2\mu\text{Eq}/\text{min}/100\text{g BW}$, they showed a diuresis and natriuresis following administration of angiotensin II.

Fig. 4. Effect of angiotensin II on excretion in reference to different Na excretion levels before administration of angiotensin II.

2) Experiments in adrenalectomized rats.

Following adrenalectomy, the action of angiotensin II, a decrease of RPF and an increase of Na excretion, were almost disappeared, although its pressor action remained unaffected. Such disappearance was partially restored by the administration of DOC (1.5 to

Fig. 5. Effects of angiotensin II in a dose of $20\mu\text{g}/\text{Kg}$ on electrolytes excretion and urine volume in adrenalectomized rats loading 0.45 per cent saline with or without adrenal hormones.

10 mg/Kg), d-alldosterone (3 to $20\mu\text{g}/\text{Kg}$) or cortisone (5mg/Kg) 1 to 2 hours before experiment. Cortisone combinedly used with DOC or d-alldosterone corrected the deficiency in the diuretic and natriuretic response to angiotensin II, but not completely. The recovery effect of these hormones was more obvious in saline loaded rats than in water loaded rats (Fig. 5).

3) Experiments in normal rats treated with adrenal hormones.

DOC (10mg/Kg) or d-alldosterone ($3\mu\text{g}/\text{Kg}$), given subcutaneously 1 hour before experiment, failed to affect the response of urine flow and Na excretion to angiotensin II, so did cortisone (1 to 10 mg/Kg).

E. Effects of angiotensin II on electrolyte level of plasma.

Twenty minutes after intraperitoneal injection of angiotensin II in a dose of 100 $\mu\text{g}/\text{Kg}$, the Na level in plasma decreased slightly, without significant change in the K level in rat fed on a normal salt diet. Adrenalectomy resulted in a slight decrease in Na content and an increase in K content. Angiotensin II administered to adrenalectomized rats did not affect the Na level but reduced K level to normal. In adrenalectomized rats, a treatment with DOC (5 mg/Kg), d-aldosterone (3 $\mu\text{g}/\text{Kg}$) or d-aldosterone plus cortisone (5mg/Kg) increased Na level without significant effect on K level. Cortisone (5 mg/Kg) alone influenced neither Na nor K level. In all adrenalectomized rats, angiotensin II showed no influence on Na level but various effect on K level.

DISCUSSION

A lot of data have been reported to assert that the amount of Na in daily diet is an important factor in the regulatory mechanism of blood pressure and in the response of blood vessels to vasoconstrictive agents. A high Na intake tends to cause hypertension and to accelerate the vasopressor effect of angiotensin II^{19,20}. MENEELY et al.¹⁴ fed rats with a diet containing various amount of NaCl for twelve or more months. The rats which consumed either 2.6 or 5.6 per cent NaCl developed a mild hypertension, while those with 7 or 10 per cent NaCl often developed a moderate or a severe hypertension. DAHL et al.⁶ found that many rats developed a permanent hypertension 6 weeks after they had taken a high salt diet.

In the present experiments, rats were fed on a low (0.15 per cent NaCl), medium (0.63 per cent NaCl) or high (3.3 per cent NaCl) salt diet for 4 to 5 weeks. Irrespective of the amount of NaCl consumed, there were no significant differences in rats' weight gain, blood pressure, renal hemodynamics and vasopressor responses to adrenaline, noradrenaline, angiotensin II or vasopressin. The discrepancy between the present results and the aforesaid reports^{6,14} may be explained by the animal's maturity at which salt feeding was

begun, by the duration of feeding as well as by amount of dialy salt intake. In the present experiments, however, granularity of juxtaglomerular cells was increased in rats fed on a low salt diet, but it remained within a normal range in rats with high salt intake in agreement with the results reported by other investigators.

Intraperitoneal injection of angiotensin II in a subpressor dose produced no marked influence in GFR, but a slight decrease in RPF. With an increase in dosage RPF showed a marked decrease associated with a slight decrease in GFR, indicating a rise in FF and a constriction of afferent arteriole. The results obtained in rats fed on a low or a high salt diet were almost similar to those fed on a normal salt diet. Following adrenalectomy rats failed to respond to angiotensin II with a reduction in RPF. Pretreatment with d-aldosterone had little effect on the response to angiotensin II in adrenalectomized rats. However, DOC or cortisone partially restored the response. These data suggest that the effect of angiotensin II on renal hemodynamics has a close relation with the presence of certain adrenal hormones.

Intraperitoneal administration of angiotensin II in a subpressor dose produced a marked diuresis and natriuresis, but its action was not further increased with a dose of 100 $\mu\text{g}/\text{Kg}$. Similar results in rats have been reported by several investigators who, however, adopted an intravenous route. The natriuretic action of angiotensin II was not closely related with Na intake but with the amount of Na excreted into urine before administration of angiotensin II. It has been reported that angiotensin II inhibits Na reabsorption on the distal tubules in dogs²³ and on the proximal tubules in rats¹². The present results may be explained by a direct action of angiotensin II on tubular cells, although the agent's participation on local circulation cannot be neglected. Following adrenalectomy the natriuretic action and diuretic action of angiotensin II disappeared as like as its action on renal hemodynamics. Either cortisone or DOC partially corrected the action of angiotensin II on renal tubules. The present data seem to strongly advocate effects of angiotensin II on renal hemodynamics and tubular

cells are in need of the presence of adrenal hormones.

SUMMARY

A study was made of effects of angiotensin II on renal hemodynamics and electrolytes excretion in normal and adrenalectomized rats.

1) Intraperitoneal administration of angiotensin II in a dose of 20 $\mu\text{g}/\text{Kg}$ reduced renal plasma flow, but not glomerular filtration rate. Angiotensin II in a dose of 100 $\mu\text{g}/\text{Kg}$ showed a marked reduction in both renal plasma flow and glomerular filtration rate, but no effect on renal hemodynamics in adrenalectomized rats. Disappearance of effects in adrenalectomized rats was partially restored by the administration of DOC or cortisone.

2) Angiotensin II increased a urine flow and excretion of Na with a slight decrease in an excretion of K. But, it showed no such effects in adrenalectomized rats.

3) DOC or cortisone restored the action of angiotensin II on the excretion of electrolytes in adrenalectomized rats.

4) Adrenal cortex hormones may play an important role on the action of angiotensin II on the renal hemodynamics and renal tubular cells in rats.

REFERENCES

1. AMES, R.P. AND BORKOWSKI, A.J.: Prolonged infusion of angiotensin II and noradrenaline on blood pressure electrolyte balance and aldosterone and cortisol secretion in normal man and cirrhosis with ascites, *J. Clin. Invest.* **44**: 1171, 1965.
2. BORKOWSKI, A.J. AND HOWARDS, S.S.: Angiotensin and electrolyte excretion in renovascular hypertension, *Am. J. Physiol.* **208**: 1087, 1965.
3. BOCK, K.D. AND DENGLE, H. et al.: Untersuchung über die Wirkung von Synthetischen Hypertensin II auf Electrolythaushalt, Nierenfunktion und Kreislauf beim Menschen, *Klin. Wochenschr.* **36**: 808, 1958.
4. BROWN, J.J.: Actions of angiotensin on the circulation, Hormones and the kidney, Academic Press, 303, 1963.
5. CROXATTO, H. AND BARNAFI, L. et al.: Some endocrine factors in the polyuric action of renin, *Endocrinology* **54**: 239, 1954.
6. DAHL, L.K. AND HEINE, M. et al.: Effects of chronic excess salt ingestion, *J. Exptl. Med.* **115**: 1173, 1962.
7. DAVIES, J.G.A.B. AND COX, J.R.: The Effect of intravenous angiotensin on urinary aldosterone excretion, body fluid compartments and intra- and extracellular sodium content, *Clin. Sci.* **28**: 591, 591, 1965.
8. DAVIS, J.O. AND HARTROFT, P. M. et al.: The role of renin-angiotensin system in the control of aldosterone secretion, *J. Clin. Invest.* **41**: 378, 1962.
9. EILERS, E.A. AND PETERSON, R.E.: Aldosterone secretion in the rat, Aldosterone, Blackwell Scientific Publications, Oxford, 251, 1954.
10. HARTROFT, P.M. AND HARTROFT, W.S.: Studies on renal juxtaglomerular cells, *J. Exptl. Med.* **102**, 205, 1955.
11. LARAGH, J.H.: Interrelationships between angiotensin, norepinephrine, epinephrine, aldosterone and electrolyte metabolism in man, *Circulation* **25**: 203, 1962.
12. LEYSSAC, P.P.: The in vivo effect of angiotensin on the proximal tubular reabsorption of salts in rat kidney. *Acta Physiol. Scand.* **62**: 436, 1964.
13. MARIEB, N.J. AND MULROW, P.J.: Role of the renin-angiotensin system in the regulation of aldosterone secretion in the rat, *Endocrinology* **76**: 657, 1965.
14. MENEELY, G.R. AND TUCKER, R.G. et al.: Chronic sodium chloride toxicity in the albino rat, *J. Exptl. Med.* **98**: 71, 1953.
15. MULROW, P.J. AND GANONG, W.F.: The role of the renin-angiotensin system in the regulation of aldosterone secretion in the dog and man, Aldosterone, Blackwell Scientific Publications, Oxford, 265, 1964.
16. NAKAO, K., Steroids and electrolyte metabolism, Proceedings of the 16th general assembly of the Japan medical congress, 493, 1962.
17. PEART, W.S. AND BROWN, J.J., The effect of angiotensin on urine flow and the electrolytes excretion in hypertensive patients, *Lancet* **1**: 28, 1961.
18. PETERS, G., Renal tubular effect of val⁵-angiotensin II amide in rats, *Proc. Soc. Exptl. Biol. Med.* **112**: 771, 1963.
19. REID, W.D. AND LARAGH, J.H., Sodium and potassium intake, blood pressure and pressor response to angiotensin, *Proc. Soc. Exptl. Biol. Med.* **120**, 26, 1965.
20. SLATER, J.D.H. AND BARBOUR, B.H. et al.: Influence of the pituitary and the renin-angiotensin system on the secretion of aldosterone, cortisole, and cortisone, *J. Clin. Invest.* **42**, 1504, 1963.
21. SPAT, A. AND STURCZ, J. et al., New observation on the function of the angiotensin system, *Acta Physiol. Hung.* **27**: 199, 1965.
22. TAKAGI, H.: Effects of sodium and blood volume on arterial pressure and vascular reactivity, *Japanese Circulation J.* **30**, 474, 1966.
23. VANDER, A.J.: Inhibition of distal tubular sodium reabsorption by angiotensin II, *Am. J. Physiol.* **205**: 122, 1963.
24. WOOD, J.E.: Cardiovascular effects of angiotensin, Angiotensin systems and experimental renal diseases, Little, Brown and company 71, 1963.
25. YAMAMOTO, K. AND YAMAZOE, H. et al.: Studies on renal clearance in rats, *Osaka City Med. J.* **8**: 171, 1962.