

# **Changes of Plasma Renin Activity Following Intracerebroventricular Administration of Biologically Active Peptides in Two-Kidney, One Clip Hypertensive Rats**

Takeru IWATA, M.D., Kunio HIWADA, M.D.,  
and Tatsuo KOKUBU, M.D.

## **SUMMARY**

We studied the effects of intracerebroventricular (ICV) administration of angiotensin II (ANG II), bradykinin (BK), leucine-enkephalin (Leu-ENK) and neurotensin (NT) on plasma renin activity (PRA), blood pressure and heart rate in acute (less than 4 weeks) and chronic (more than 12 weeks) two-kidney, one clip (2K-1C) hypertensive rats. These four peptides all produced pressor responses. The pressor responses caused by ICV injection of ANG II, BK, Leu-ENK and NT in hypertensive rats did not differ significantly from the response in normal rats. In both acute and chronic 2K-1C hypertensive rats, ANG II and BK significantly suppressed PRA, NT did not affect PRA, and Leu-ENK produced a significant increase in PRA followed by a significant decrease in PRA. As compared to normal rats, suppression of PRA by ANG II and NT was attenuated or abolished but BK and Leu-ENK produced significant reductions in PRA in 2K-1C hypertensive rats. The results indicate that the effects of these four centrally administered peptides on blood pressure, heart rate and PRA in acute and chronic 2K-1C hypertensive rats were not essentially different from those in normal rats.

## **Additional Indexing Words:**

Angiotensin II      Bradykinin      Leucine-enkephalin      Neurotensin

**T**HE role of the central nervous system in hemodynamic regulation is complex. Recently, a number of biologically active peptides such as angiotensin, kinins, enkephalins and neurotensin, have been demonstrated

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From the Second Department of Internal Medicine, Ehime University School of Medicine, Ehime, Japan.

Address for reprint: Tatsuo Kokubu, M.D., Second Department of Internal Medicine, Ehime University School of Medicine, Shigenobu-cho, Onsen-gun, Ehime 791-02, Japan.

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to be present in the brain.<sup>1)-4)</sup> These peptides in the brain are involved in blood pressure control.<sup>6)</sup> Central peptidergic stimulation is important for the elevation and maintenance of high blood pressure in experimental animals.<sup>6)</sup> Although the renin-angiotensin system plays a primary pathogenic role in two-kidney, one clip (2K-1C) hypertension in rats,<sup>7)</sup> the central nervous system has been shown to be involved in the development and maintenance of 2K-1C hypertension.<sup>8)-13)</sup> On the other hand, the central mechanism of renin release has been suggested from the results of electrical stimulation of the central nervous system.<sup>14),15)</sup> We<sup>16)</sup> and the other investigators<sup>17)-20)</sup> have reported that central peptidergic stimulation influences PRA and blood pressure in normal rats.

In this report, we studied the effects of ICV injection of ANG II, BK, Leu-ENK and NT on PRA, blood pressure and heart rate in acute and chronic 2K-1C hypertensive rats with elevated PRA.

#### MATERIALS AND METHODS

Male Wistar rats weighing 350–500 Gm at the time of the ICV experiment were used. Rats were divided into 3 groups: (1) normal rats, (2) 2K-1C hypertensive rats in the acute phase and (3) 2K-1C hypertensive rats in the chronic phase.

Surgical procedures for the preparation of the hypertensive rats were as follows. Under pentobarbital anesthesia (50 mg/Kg, i.p.), the left kidney was exposed via a flank incision and a silver clip having an internal diameter of 0.21 mm was placed on the left renal artery. The right kidney was left untouched. Rats were maintained in a room at constant temperature ( $24 \pm 1^\circ\text{C}$ ) and humidity ( $60 \pm 10\%$ ) with a daily light cycle of 12 hours. Tap water and a rat diet containing 11 mEq of sodium and 19 mEq of potassium per 100 Gm were offered ad libitum. Indirect blood pressure measurements were carried out by the tail-cuff method.<sup>21)</sup> Rats with systolic blood pressures in excess of 160 mmHg were used in the ICV experiment within 4 weeks (acute phase) or after 12 weeks (chronic phase) of the renal artery stenosis.

Three days before the ICV experiment, a stainless steel cannula was stereotactically implanted into the lateral cerebral ventricle and a polyethylene catheter was placed in the femoral artery under pentobarbital anesthesia as described previously.<sup>16)</sup>

The ICV experiment was performed in conscious and unrestrained rats as described previously.<sup>16)</sup> Four biologically active peptides (purchased from Protein Research Foundation, Osaka, Japan), ANG II, BK, Leu-ENK and NT were dissolved in an artificial cerebrospinal fluid and 5  $\mu\text{l}$  of each

peptide solution were administered into the lateral cerebral ventricle of the rat. Injection doses of the peptides were as follows: ANG II, 10 ng; BK, 1  $\mu$ g; Leu-ENK, 100  $\mu$ g; NT, 10  $\mu$ g. Five  $\mu$ l of artificial cerebrospinal fluid were injected intracerebroventricularly as a control. Blood samples for plasma renin assay were collected 15 and 5 min before ICV injection and 5, 15 and 30 min after the injection. PRA was measured by radioimmuno-logical microassay.<sup>22)</sup> The mean value of PRA at 15 and 5 min before ICV injection was used as the pretreatment value.

Results were expressed as means $\pm$ SEM. Statistical analyses were performed by means of analysis of variance with subsequent Tukey's test for comparison between groups and paired t-test for comparison within groups.

## RESULTS

### *Pretreatment values of PRA, blood pressure and heart rate*

PRA, mean blood pressure (MBP) and heart rate of the 3 groups before ICV injection of the peptides are shown in Table I. 2K-1C hypertensive rats in acute and chronic phases showed significantly elevated PRA compared with the normal rats. PRA in chronic 2K-1C hypertensive rats was slightly lower than that in acute phase, but the difference was not significant. MBP in 2K-1C hypertensive rats was significantly higher than that in normal rats. There was a significant difference in MBP between acute 2K-1C hypertensive rats and chronic ones. Heart rate in acute 2K-1C hypertensive rats was significantly faster than those in normal rats and chronic 2K-1C hypertensive rats.

### *Plasma renin activity after ICV injection*

ICV injection of artificial cerebrospinal fluid showed no significant change in PRA in any of the 3 groups. ICV injection of ANG II reduced

Table I. Plasma Renin Activity, Mean Blood Pressure and Heart Rate before Intracerebroventricular Injection

Groups	PRA (ng ANG I/ml/h)	MBP (mmHg)	Heart rate (beats/min)
normal rats	41.5 $\pm$ 1.8	103.5 $\pm$ 1.4	357 $\pm$ 7
acute 2K-1C rats	117.7 $\pm$ 10.7*	163.5 $\pm$ 2.3*†	378 $\pm$ 6*†
chronic 2K-1C rats	100.4 $\pm$ 6.9*	174.6 $\pm$ 2.3*	361 $\pm$ 5

\*  $p < 0.05$  compared with normal rats.

†  $p < 0.05$  compared with chronic 2K-1C hypertensive rats.

n=30 for each group.

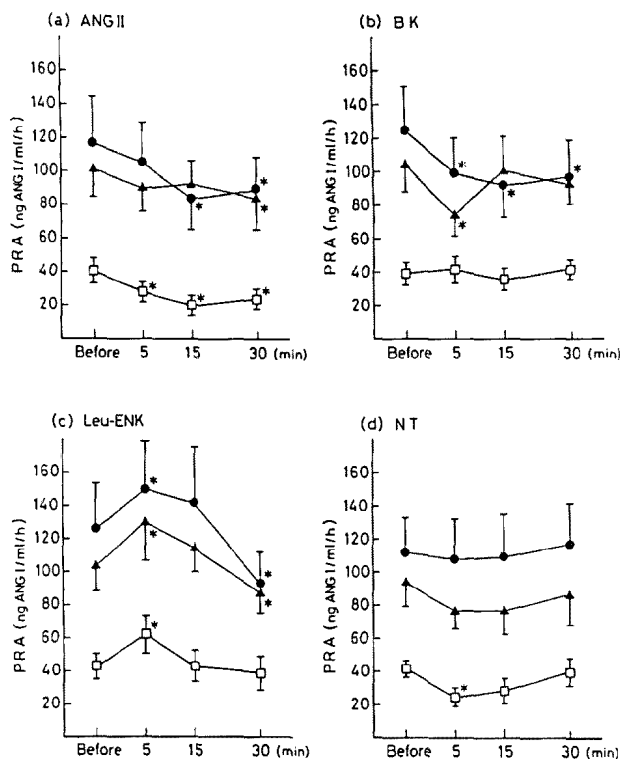


Fig. 1. Changes in PRA after ICV injections of angiotensin II (a), bradykinin (b), leucine-enkephalin (c) and neurotensin (d) in normal rats (□), acute (●) and chronic (▲) 2K-1C hypertensive rats. Values are means  $\pm$  SEM,  $n=6$  for all points. Asterisks indicate significant differences from pretreatment values ( $p < 0.05$ ).

PRA in all 3 groups. Significant reductions of PRA were observed at 5, 15 and 30 min after ICV injection in normal rats, at 15 and 30 min in acute 2K-1C hypertensive rats and at 30 min in chronic hypertensive rats. At 30 min after ICV injection of ANG II, PRA values of normal rats, acute and chronic 2K-1C hypertensive rats were  $62.2 \pm 7.8\%$ ,  $77.2 \pm 6.3\%$  and  $82.4 \pm 7.5\%$  of the pretreatment value, respectively. The reduction of PRA in 2K-1C hypertensive rats was attenuated compared with that in normal rats. In 2K-1C hypertensive rats, PRA was less reduced in the chronic phase than in the acute phase.

After ICV injection of BK, PRA showed no significant change in normal rats. In 2K-1C hypertensive rats, however, ICV injection of BK produced a significant decrease in PRA. In the acute phase, significant reductions in PRA were observed at 5, 15 and 30 min after the injection and PRA values were  $83.9 \pm 3.3\%$ ,  $76.8 \pm 4.0\%$  and  $78.5 \pm 5.2\%$  of the pretreatment value, respectively. In the chronic phase, significant reduction in PRA was ob-

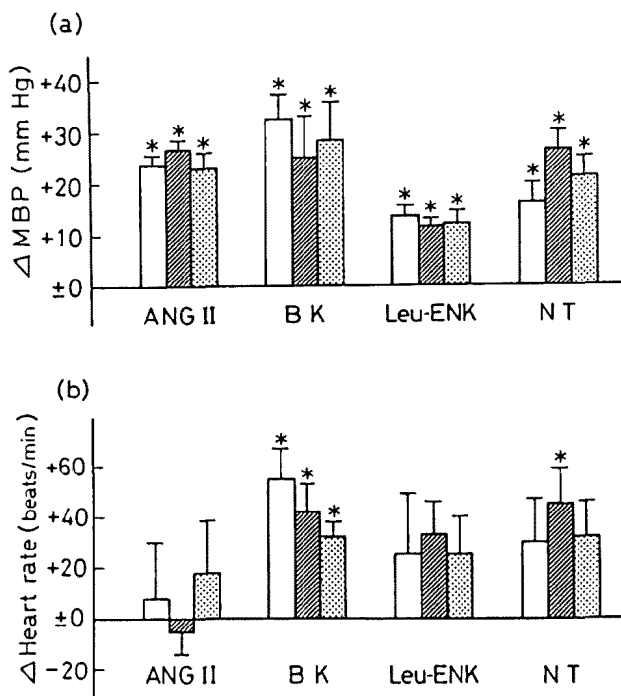


Fig. 2. (a) The maximal increase in MBP and (b) the change in heart rate after ICV injections of angiotensin II, bradykinin, leucine-enkephalin and neurotensin in normal rats (□), acute (▨) and chronic (▤) 2K-1C hypertensive rats. Asterisks indicate significant differences from pretreatment values ( $p < 0.05$ ).

served only at 5 min and the PRA value was  $71.3 \pm 5.9\%$  of the pretreatment value.

ICV injection of Leu-ENK produced significant increases in PRA at 5 min in normal rats and in acute and chronic 2K-1C hypertensive rats; PRA values were  $141.0 \pm 11.6\%$ ,  $123.0 \pm 6.0\%$  and  $123.3 \pm 5.7\%$  of the pretreatment values, respectively. In acute and chronic 2K-1C hypertensive rats PRA values at 30 min were significantly decreased to  $71.9 \pm 2.7\%$  and  $85.3 \pm 3.0\%$  of pretreatment values, respectively.

After ICV injection of NT, PRA was significantly decreased at 5 min in normal rats. In both acute and chronic 2K-1C hypertensive rats, ICV injection of NT did not produce a significant change in PRA. The changes in PRA after ICV injection of these peptides are shown in Fig. 1.

#### *Blood pressure and heart rate after ICV injection*

ICV injection of artificial cerebrospinal fluid had no influence on either blood pressure or heart rate. ICV injection of ANG II, BK, Leu-ENK

and NT all produced pressor responses in normal rats and in 2K-1C hypertensive rats. The magnitude of the increase in blood pressure caused by these peptides did not differ significantly among normal rats, acute and chronic 2K-1C hypertensive rats. The maximal increases in MBP after ICV injection of the peptides are shown in Fig. 2(a).

Heart rate was measured at the time when the maximal pressor response was observed. Heart rate did not significantly change in any of the 3 groups after ICV injection of either ANG II or Leu-ENK. ICV injection of BK significantly increased heart rate in all 3 groups. Heart rate significantly increased after ICV injection of NT in acute 2K-1C hypertensive rats, but did not significantly change in normal rats or in chronic 2K-1C hypertensive rats. The changes in heart rate after ICV injection of the peptides are shown in Fig. 2(b).

#### DISCUSSION

ICV injections of ANG II, BK, Leu-ENK and NT showed pressor responses in 2K-1C hypertensive as reported previously.<sup>16)</sup> Spontaneously hypertensive rats exhibit a marked supersensitivity to the central pressor responses of angiotensin, bradykinin and enkephalins.<sup>6),23)</sup> Chronic two-kidney, two clip hypertensive rats had a significantly increased pressor response after central administration of ANG II compared with normotensive rats.<sup>24)</sup> Our experiments showed no significant difference between normal rats and 2K-1C hypertensive rats in pressor responses after ICV injection of ANG II, BK, Leu-ENK or NT.

There are at least two efferent pathways by which the central nervous system influences renin secretion. One is the sympathetic nervous system and the other involves vasopressin which exerts an inhibitory control over renin secretion. There are several reports indicating that central administration of ANG II, BK, Leu-ENK or NT causes sympathetic nerve activation.<sup>25)–28)</sup> On the other hand, it is suggested that central administration of these peptides produces vasopressin release.<sup>25),29)–31)</sup> The effect of central peptidergic stimulation on renin secretion may depend on the extent of sympathetic nerve activation and vasopressin release. But the possibility that the other mechanisms may exist is not excluded.

There is no report on the change in PRA following ICV injections of ANG II, BK, Leu-ENK or NT in 2K-1C hypertensive rats. In this experiment, ANG II and BK significantly suppressed PRA, NT did not affect PRA and Leu-ENK produced a significant increase in PRA followed by a significant decrease in PRA. Suzuki et al<sup>10)</sup> reported that ICV injection of sara-

lasiin or captopril resulted in a significant decrease in blood pressure and PRA in 2K-1C hypertensive rats. These results suggested that the decrease in PRA due to the blockade of the brain renin-angiotensin system attenuated the sympathetic nerve activation. Our results suggest that the release of vasopressin caused by centrally administered ANG II probably has an advantage over the stimulation of the sympathetic nervous system with regard to renin secretion.

We have reported that ICV injections of ANG II, BK, Leu-ENK or NT in sodium-restricted rats whose PRA is significantly elevated cause changes in PRA similar to those observed in normal rats.<sup>32)</sup> In conscious 2K-1C hypertensive rats with elevated PRA, central administration of these peptides caused changes in PRA which showed some differences compared with normal rats. Since pressor responses after ICV injection of these peptides were similar in normal and 2K-1C hypertensive rats, these differences in PRA response might be due to peripheral mechanisms such as sensitivity of the kidney to sympathetic nerve activation and plasma vasopressin concentration. In 2K-1C hypertensive rats, compared to normal rats, suppression in PRA by ICV injections of ANG II or NT were attenuated or abolished. But BK and Leu-ENK produced significant reductions in PRA only in 2K-1C hypertensive rats. The mechanism by which the difference was caused is not clear in this experiment.

In conclusion, the results indicate that the effects of these four centrally administered peptides on blood pressure, heart rate and PRA in acute and chronic 2K-1C hypertensive rats were not essentially different from those in normal rats, though the effect of BK on PRA in 2K-1C hypertensive rats was different from that in normal rats.

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