Relationship between Hypertensive Response and Brain Kinin Level in the Rat Injected Intraventricularly with Glandular Kallikrein

Kimio KARIYA and Aiko YAMAUCHI
Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Ikawadani-cho, Nishi-ku, Kobe 673, Japan
Accepted October 24, 1986

Abstract—Hypertensive action was observed in conscious rats injected intraventricularly with glandular kallikrein (EC 3.4.21.35) in a dose-dependent manner (4–16 KU), which was associated with the enhancement of brain kinin level. Concurrently administered aprotinin, a kallikrein inhibitor, led to an inhibition of these effects of kallikrein. These results suggest that the central hypertensive action of kallikrein is mediated via the kinin liberated from a kininogen in the brain.

Recently, a number of workers have obtained information about the endogeneous kallikrein-kinin system in the CNS. The existence of glandular kallikrein in the rat brain, equivalent to the urinary kallikrein, was demonstrated by Chao et al. using monoclonal antibody-affinity chromatography (1). With respect to the kinin in the CNS, recent works have shown the presence of bradykinin (BK) in the rat and guinea pig brain (2), the content in the rat brain (3) and the distribution in the rat CNS (4). Those observations roughly support the report on the immunohistochemical localization of BK in rat brain (5).

The injection of synthetic BK into the cerebral ventricles produced a hypertension and a biphasic behavioral change consisting of short-lasting excitation followed by sedation with EEG alterations (6, 7). Lately, it has been observed that intraventricular kallikrein could alter the CNS functions to evoke the behavioral and EEG changes or blood pressure increase in the conscious rat, similar to the action of intraventricular BK; these results will be published elsewhere. Thus, the purpose of this study was to determine whether the pressor response caused by the intraventricular kallikrein was exactly paralleled by changes in the brain kinin level in the rat.

Materials and Methods

Highly purified porcine pancreatic kallikrein (EC 3.4.21.35, 0.2 KU/μg protein) and aprotinin (10,000 KIU/ml) were kindly supplied by Bayer Yakuhin, Ltd. (Japan) and Hoechst Japan, Ltd., respectively. Bovine serum albumin (BSA) was purchased from Nakarai Chemicals, Ltd. (Kyoto, Japan) and BK potentiator B obtained from Peptide Institute, Inc. (Osaka, Japan). Those compounds were dissolved in artificial cerebrospinal fluid (A-CSF), pH 7.4, at the time of the treatments.

Male Sprague-Dawley rats (Clea Jcl, Japan), weighing 250–300 g, were used in all experiments. The rats were chronically implanted with the stainless steel guide cannula for the intraventricular injection, as described in the previous report (7). A volume of 5 μl was injected through the injection cannula connected to a hand-driven 10 μl syringe (Hamilton 701-N), over a period of 10 sec. Aprotinin or BK potentiator B was given simultaneously with kallikrein. Intraventricular injection and arterial blood pressure measurement during a conscious state were carried out by previously described methods (6, 7). To assess the changes in blood pressure, the area under the mean blood pressure curve...
within 20 min after the injection was evaluated as the blood pressure response.

After intraventricular injection of a compound, the rat was subjected to microwave irradiation (4.5 kW, 2 sec) by a Metabostat System (NEZ 2601, New Japan Radio Co., Ltd.) (8). The kinin in the brain homogenate was extracted with n-butanol (4) and determined using a radioimmunoassay system (3).

All the results were presented as the mean ± S.E.M. The significant differences were analysed by Student's t-test.

Results
As shown in Fig. 1, kallikrein produced a dose-dependent increase in the blood pressure response of the conscious rat (r = 0.717, n = 15, P < 0.01). In the control experiment, neither A-CSF nor 80 µg of BSA which was equivalent to the protein involved in 16 KU kallikrein had any effects on the animal behavior or the blood pressure response (A-CSF, 7.0 ± 6.4 mmHg·min, n = 6; BSA, 0.1 ± 10.1 mmHg·min, n = 6).

Figure 2 shows the changes of mean arterial blood pressure of the rat after intraventricular treatment with 8 KU of kallikrein. The blood pressure response (mmHg·min) was reflected by the area under the mean blood pressure curve within 20 min after the injection of kallikrein (●). Artificial cerebrospinal fluid (▲) or bovine serum albumin (80 µg) (▼) was intraventricularly administered as a control experiment. Each point shows the mean (± S.E.M.) of five to six rats.

Fig. 1. Hypertensive action of glandular kallikrein injected intraventricularly. The blood pressure response (mmHg·min) was reflected by the area under the mean blood pressure curve within 20 min after the injection of kallikrein (●). Artificial cerebrospinal fluid (▲) or bovine serum albumin (80 µg) (▼) was intraventricularly administered as a control experiment. Each point shows the mean (±S.E.M.) of five to six rats.

Fig. 2. Effect of the treatment with aprotinin or BK potentiator B. Aprotinin (APR) or BK potentiator B (BPB) was simultaneously injected into the cerebral ventricles with kallikrein (KAL). Each point shows the mean (±S.E.M.) of five to six rats.
with or without simultaneous administration of aprotinin or BK potentiator B. The simultaneous injection of aprotinin (16 KIU) into the cerebral ventricles depressed (P<0.05) the significant pressor response induced by 8 KU of kallikrein (8 KU kallikrein, 135.0±22.0 mmHg-min, n=6; 8 KU kallikrein and 16 KIU aprotinin, 61.2±11.8 mmHg-min, n=5). On the other hand, BK potentiator B (20 nmol) enhanced the hypertensive action of 8 KU of kallikrein as much as 3-fold (8 KU kallikrein and BK potentiator B, 433.1±88.1 mmHg-min, n=6; P<0.05 compared with 8 KU kallikrein). Neither aprotinin nor BK potentiator B had any significant effects on either the animal behavior or the blood pressure response at doses of 16 KIU (9.1±14.5 mmHg-min, n=5) and 20 nmol (−10.6±11.3 mmHg-min, n=6), respectively.

As shown in Fig. 3, the brain kinin level was progressively increased as a function of time after the intraventricular administration of kallikrein (16 KU). Five minutes after the injection, the brain kinin level was 4 times higher than the initial level (P<0.001), accompanying an increase of the blood pressure. As illustrated in Fig. 4, the intraventricular kallikrein, at doses enough to induce the pressor response, produced the dose-dependent increase in brain kinin level 5 min after the injection.

On the action of intraventricular kallikrein, the influence of a kallikrein inhibitor, aprotinin, was determined on the basis of the brain kinin level (Fig. 4). Sixteen KIU aprotinin failed to prevent the elevation of the kinin level caused by the enzyme (8 KU). However, a higher dose of aprotinin, 50 KIU, completely blocked it without affecting the basal level of the brain kinin.

**Discussion**

Although the presence and characteristics of brain kinin have already been demonstrated in the rat (2–4), no evidence has so far been observed concerning possible changes of the brain kinin level in some conditions. This paper provides evidence that the brain kinin level can rise by the application of kinin-generating enzyme into the cerebral ventricles in association with physiological changes. Namely, the intraventricular injection of glandular kallikrein produced a hypertensive response with a...
concomitant increase in the kinin content in the rat brain. Aprotinin, a kallikrein inhibitor, blocked not only the pressor response but also the increase in brain kinin level caused by the enzyme. The hypertensive action of kallikrein was also enhanced as much as 3-fold by the simultaneous treatment with BK-potentiator B, an inhibitor of kininase II. Previously, it was demonstrated that 20 nmol of the potentiator could delay by 3.5 times the degradation of BK (5 nmol) injected into the brain in a conscious rat (8). Therefore, a direct action of kallikrein could be excluded in these experiments.

Corrêa et al. (9, 10) suggested that the lateral septal area might be involved in the hypertensive action of exogenous BK which was mediated by alpha-adrenergic mechanisms. Participation of the paraventricular nucleus of the hypothalamus in the cardiovascular response accompanying emotional behavior has been established by Smith et al. using unanesthetized baboons (11). The central actions of kallikrein might be due to local generations of the kinin at these areas. Further, it is possible that the effective substrate kininogen for tissue kallikrein exists in the rat CNS, and it is involved in the CNS functions via its proteolytic products, kinins. However, neither the characteristics nor the origin of CNS kininogen is yet clear.

The above evidences suggest that a kininogen-kininogenase-kinin system exists in the CNS and that alteration of a moiety of the system results in modifications of cardiovascular regulating functions in the CNS.

References
1 Chao, J., Woodley, C., Chao, L. and Margolius, H.S.: Identification of tissue kallikrein in brain and in the cell free translation product encoded by brain mRNA. J. Biol. Chem. 258, 15173–15178 (1983)
2 Perry, D.C. and Snyder, S.H.: Identification of bradykinin in mammalian brain. J. Neurochem. 43, 1072–1080 (1984)
3 Yamauchi, A., Nakayama, A. and Kariya, K.: Determination of kinin in the rat brain by a sensitive radioimmunoassay. J. Pharmacobiodyn. 8, 607–613 (1985)
4 Kariya, K., Yamauchi, A. and Sasaki, T.: Regional distribution and characterization of kinin in the CNS of the rat. J. Neurochem. 44, 1892–1897 (1985)
5 Corrêa, F.M.A., Innis, R.B., Uhl, G.R. and Snyder, S.H.: Bradykinin-like immunoreactive neuronal systems localized histochemically in rat brain. Proc. Natl. Acad. Sci. U.S.A. 76, 1489–1493 (1979)
6 Kariya, K. and Yamauchi, A.: Effects of intraventricular injection of bradykinin on the EEG and the blood pressure in conscious rats. Neuropharmacology 20, 1221–1224 (1981)
7 Kariya, K., Yamauchi, A. and Chatani, Y.: Relationship between central actions of bradykinin and prostaglandins in the conscious rat. Neuropharmacology 21, 267–272 (1982)
8 Kariya, K., Yamauchi, A., Hattori, S., Tsuda, Y. and Okada, Y.: The disappearance rate of intraventricular bradykinin in the brain of the conscious rat. Biochem. Biophys. Res. Commun. 107, 1461–1466 (1982)
9 Corrêa, F.M.A. and Graeff, F.G.: On the mechanism of the hypertensive action of intraseptal bradykinin in the rat. Neuropharmacology 15, 713–717 (1976)
10 Corrêa, F.M.A., Ueta, J. and Pelá, I.R.: Central adrenergic mediation of the cardiovascular effect of intraventricular bradykinin. Naunyn Schmiedebergs Arch. Pharmacol. 333, 139–142 (1986)
11 Smith, O.A., Astley, C.A., DeVito, J.L., Stein, J.M. and Walsh, K.E.: Functional analysis of hypothalamic control of the cardiovascular response accompanying emotional behavior. Fed. Proc. 114, 1841–1844 (1984)