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STUDIES ON KININ-LIKE SUBSTANCES IN BRAIN

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Abstract—The changes in the kininogen content, kininase activity and kinin-forming activity of rat brain on administration of drugs acting on central nervous system were examined. The kininogen content of the cerebral cortex was one-third of that in the cerebellum or brain stem. The highest kinin-forming activity was observed in the cerebral cortex with lower activities in the brain stem and cerebellum. The kininogen content of the cerebellar region decreased during convulsions caused by pentetrazol, but not by strychnine or nitrogen gas inhalation. No changes were found in the kininase activity, kinin-forming activity or kininogen content of the various regions of the brain after administration of amobarbital or inhalation of ether.

THE EXISTENCE of a bradykinin-like substance[1,2] and the enzymatic inactivation of bradykinin in the nervous tissue[3,4] have been reported. We also reported on the existence and nature of an enzyme which inactivates bradykinin in rat brain.[5–7] However, the physiological and pharmacological significances of the kinin-like substance in nervous tissue are still unknown. In 1962, Cápek[8] reported that intraventricular injection of bradykinin significantly decreased the threshold for convulsions induced by metrazol, strychnine or electric shock. Moreover, Singh[9] reported that stimulation of the vagus nerve to frog stomach muscle released a bradykinin-like substance. We reported previously that intracerebral injection of bradykinin caused excitation followed by depression in mice. We suggested that the excited state might be induced by intact bradykinin while the depressed state was induced by products split from bradykinin.[10]

The present investigation was on the dynamic turnover of kinin-like substances in rat brain under conditions induced by various drugs acting on the central nervous system.

MATERIALS AND METHODS

Preparation of regions of rat brain. Male rats of the Wistar-strain, weighing about 200 g, were used for all experiments. Rats were frozen in dry ice–acetone and then decapitated. The brain was rapidly excised and pial blood vessels were removed from appropriate areas before dissection. Particular care was taken to remove as much blood as possible. The brain was separated into the cerebral cortex, cerebellum and brain stem. The brain stem included the medulla oblongata, pons and mid-brain. Diencephalon was included in the brain stem region.

Enzyme preparation. Each part of the brain was homogenized in 9 vol. of 0·32 M sucrose solution at 0° in an all-glass Potter–Elvehjem homogenizer. The homogenates were centrifuged at 700 *g* for 10 min to remove cell debris and any red blood cells

still present. The supernatant fraction was used as the preparation of enzyme destroying kinins, that is, so-called kininase, or forming kinins.

Measurement of kininogen content. Each part of the brain was homogenized in 9 vol. of acetone at 0° for 1 min and the homogenate was filtered with suction. The resulting acetone powder was homogenized in 5 vol. of saline at 0° for 1 min and then, 45 vol. of 0·2% acetic acid were added. The mixture was heated in a boiling water bath for 60 min. The kinin-like substances were released from the denatured substrate by the method of Diniz and Carvalho[11] and were assayed on isolated rat uterus against synthetic bradykinin. The kininogen content of the tissue was evaluated in terms of bradykinin formed from it on treatment with trypsin.

Statistical analysis for laboratory data was made with Student's *t*-test.

Assay of kininase. Kininase activity was determined by adding 0·1 μg of synthetic bradykinin in 0·5 ml saline to 0·5 ml of test fluid. The mixture was incubated at 37° and samples of 0·2 ml were removed at intervals and tested on isolated rat uterus.

Assay of kinin-forming activity. The enzyme preparation was adjusted to pH 2 with 1 N HCl. One part of the resulting preparation was incubated at 37° with an equal volume of substrate solution dissolved in saline, containing 6 mg/ml of EDTA. 2 Na. The substrate kininogen was prepared by the method of Wilkens and Steger.[12] About 100 ng of kinin/mg of this substrate kininogen were released on incubation for 60 min at 37° with excess bovine trypsin. Samples were removed at intervals during incubation, titrated back to pH 7 with 1 N NaOH and tested on isolated rat uterus.

Assay of bradykinin activity. Kinin activity as bradykinin was determined on isolated uterus of virgin Wistar-strain rats, weighing 150–200 g, in a 36 ml organ bath. Oestrus was induced in the rats by intramuscular injection of 4000 U/100 g of estradiol 16–18 hr before removal of the uterus. The uterus was bathed in oxygenated de Jalon solution containing 5 μg/ml of atropine sulfate at 30°. The time of contact was 90 sec and tests were made every 4 min.

Protein determination. Protein was determined by the method of Lowry *et al.*[13]

Compounds and drugs. Synthetic bradykinin was obtained from the Institute for Protein Research, Osaka University. Crystalline trypsin and atropine sulfate were purchased from E. Merck AG (Germany).

The following drugs were used: pentetrazol (Tokyo Kasei Kogyo Co., Ltd., Tokyo), strychnine nitrate (Kansai Yakuhin, Osaka), amobarbital sodium (Nippon Shinyaku, Kyoto) and estradiol benzoate (Teikoku Zoki, Tokyo).

RESULTS

Location of kinin-forming activity. Figure 1 demonstrates the kinin-forming activity in the three regions of rat brain; the cerebral cortex, cerebellum and brain stem. The highest activity was found in the cerebral cortex, with lower activities in the brain stem and cerebellum.

Kininogen contents of the three regions of brain. It is not possible to determine the actual content of the physiological active form of kinin in brain tissue, because very little kinin is present and brain tissue contains powerful kininolytic enzymes, so-called kininase. Therefore, the kininogen content, rather than the kinin content, was measured.

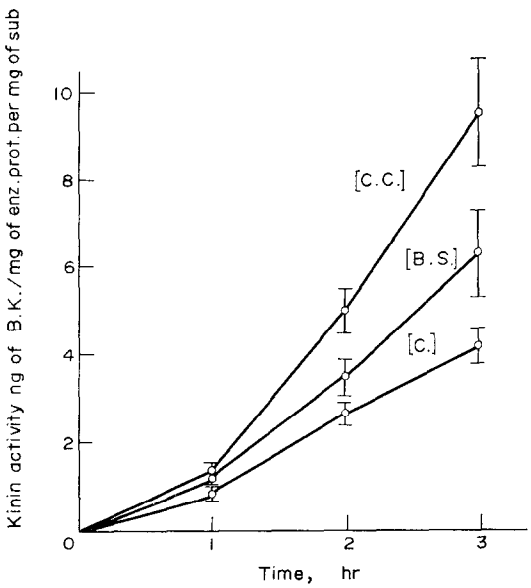

FIG. 1. Kinin-forming activities of the three regions of rat brain. Substrate kininogen (1·5 mg/ml), containing EDTA.2 Na (3 mg/ml) was incubated with acid treated enzyme preparation (supernatant fraction after centrifugation at 700 *g* for 10 min, enzyme protein 4–6 mg/ml) at 37°. Samples were taken at intervals, titrated back to pH 7·0 and then assayed on isolated rat uterus. For details see Materials and Methods. Each value represents the average and standard error of three determinations. Significant difference between (C.C.) and (C) in each time, $P < 0{\cdot}05$. (C.C.), cerebral cortex; (C), cerebellum; (B.S.), brain stem.

The kininogen content of an acetone powder of the cerebral cortex was about one-third of those of acetone powders of the cerebellum and brain stem (Fig. 2).

Effects of convulsants on the kininogen content. Pentetrazol and strychnine were injected subcutaneously at doses of LD_{100} to observe the typical effects of convulsants on the kininogen content. The effect of inhalation of nitrogen gas was also studied. The kininogen content was determined as described above immediately after the heart stopped beating.

Figure 3 shows the effect of the convulsants on the kininogen content. There were no significant differences in kininogen content between saline and pentetrazol or

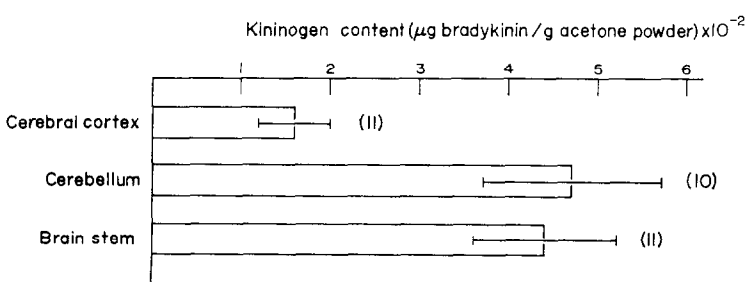

FIG. 2. Kininogen contents of the three regions of rat brain. The kininogen content was determined as described in Materials and Methods. The numbers of determinations are shown in parentheses. Each column represents the ±S.E.M. The kininogen content of cerebral cortex is significantly different from cerebellum or brain stem, $P < 0{\cdot}05$.

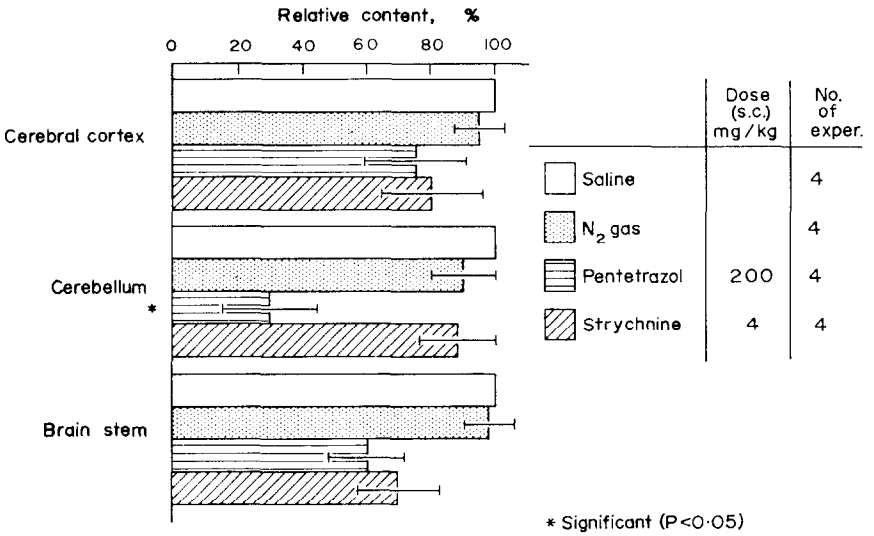

FIG. 3. Effects of convulsants on the kininogen content of rat brain. Pentetrazol (200 mg/kg) and strychnine (4 mg/kg) were given subcutaneously. After convulsive death, the kininogen content was determined as described in Materials and Methods. In every determination, each value of kininogen content of drug treated rats was expressed as a percentage of that of saline treated rats. Three rats were used in each experiment. Values are shown as ±S.E.M.

strychnine treated animals in either the cerebral cortex or the brain stem, but only remarkable change was found in the cerebellar region after injection of pentetrazol. No change was found in the kininogen contents of these three regions of the brain on anoxia induced with nitrogen gas.

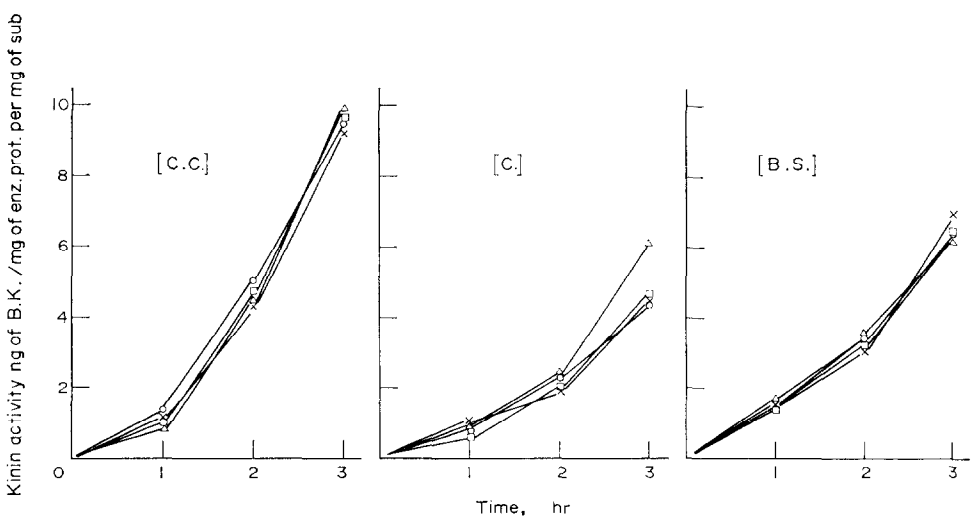

FIG. 4. Effects of convulsants on kinin-forming activities of the three regions of rat brain. Kinin-forming activity was determined as described in Fig. 1. Pentetrazol (200 mg/kg) and strychnine (4 mg/kg) were given subcutaneously. Saline was injected into control rats. Each point is the mean of three experiments. ○- - -○, Saline; □- - -□, N_2 gas inhalation; △- - -△, pentetrazol; ×- - -×, strychnine. (C.C.), cerebral cortex; (C), cerebellum; (B.S.), brain stem.

Effects of convulsants on kinin-forming activity. Figure 4 shows the effects of pentetrazol (200 mg/kg), strychnine (4 mg/kg) and nitrogen gas on the kinin-forming activity of rat brain. The respective activities in the three regions were the same in control rats and in those treated with convulsants.

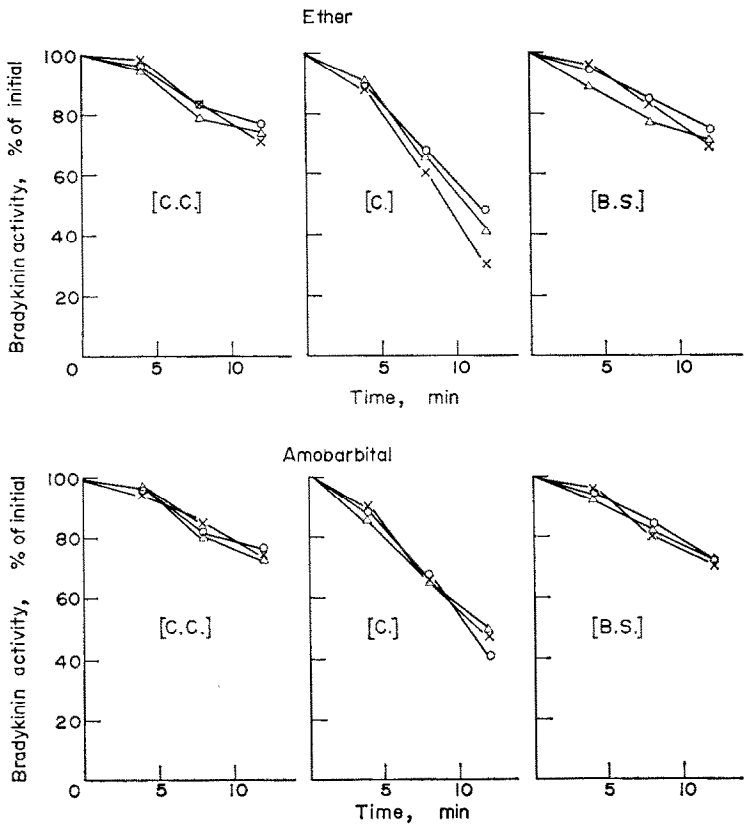

FIG. 5. Effects of ether and amobarbital on the kininase activities of the three regions of rat brain. Amobarbital sodium (80 mg/kg) was injected subcutaneously. The kininase activities of the three regions of rat brain were measured in the three behavior states described in the text. Synthetic bradykinin (0·05 μg/ml) was incubated with enzyme preparation (supernatants after centrifugation of each region of brain at 700 *g* for 10 min, enzyme protein 1·8 μg/ml) at 37°. Each point represents the mean of three observations. ○– – –○, Control; ×– – –×, anesthetized state; △– – –△, recovered state. (C.C.), cerebral cortex; (C), cerebellum; (B.S.), brain stem.

Effects of ether and amobarbital on the kinin levels. The kininogen contents, kininase activities and kinin-forming activities in the three regions of rat brain were determined in relation to the states of behavior induced by subcutaneous injection of 80 mg/kg of amobarbital or ether inhalation. Behavior was divided into the following states; control state before treatment, anesthetized state and recovered state. On amobarbital treatment, the period from 15 min after loss of the righting reflex was defined as the anesthetized state and the period from 5 min after recovery of the righting reflex was defined as the recovered state. On ether inhalation, the time within 1 min after loss

of the righting reflex was defined as the anesthetized state and the period from 5 min after recovery of the righting reflex was also defined as the recovered state.

Figure 5 shows the effects of ether and amobarbital on kininase activities in the three regions of rat brain in relation to behavior. In the three regions of rat brain, no difference in kininase activities was found in these three states. There were also no differences in kininogen contents and kinin-forming activities in these states in any of the three regions of rat brain.

DISCUSSION

Previously, we reported[5] that the kininase activity was higher in the cerebellum than in the cerebral cortex or brain stem and that the kininase activities in the latter two regions were almost the same. The present results showed that the kinin-forming activity was lowest in the cerebellum and that this region also had a higher kininogen content than the cerebral cortex. These results suggest that the physiologically active form of kinin released is more rapidly inactivated in the cerebellum than in the other two regions. It is also quite possible that in the brain stem, released kinin is not inactivated for a comparatively long time, because the kininogen content is high and enzymatic inactivation of kinin is slow. The physiological significance of these findings requires further investigation.

Hori[2] reported that the kinin-releasing activity in brain has the nature of a kallikreinogen rather than a plasminogen activator or proactivator. In our study, determination of kinin-forming activity involved comparatively prolonged acid treatment. Hardly any significant kinin formation was observed without activation of kinin-releasing enzymes and inactivation of kininase in brain tissue.

Previously, we reported[5] that the activity of the kininase in the cerebellar region increased during convulsions caused by pentetrazol or picrotoxin, but not by strychnine. The present work showed that the kininogen content decreased significantly in the cerebellar region during convulsions induced by pentetrazol, but not by strychnine. However, there was no apparent change in the kinin-forming activity in any of the regions during convulsions caused by either pentetrazol or strychnine. Previously, we also reported[10] that intracerebral injection of bradykinin caused excitation followed by depression and that kininase activity was increased in the excited state and we suggested that the excited state might be induced by intact bradykinin. On the basis of these findings, it seems from the present results that in convulsions induced by pentetrazol, a slight change in kinin-forming activity, which is so slight that it could not be detected in this study, has some effect on the kininogen content. Then the kininase activity in the cerebellar region is increased to inactivate the newly released kinin.

No changes were found in the activities of kininase or the kinin-forming enzyme or in the kininogen contents of the three regions of brain after administration of amobarbital or ether inhalation. Moreover, no relationship was found between the behavioral states induced by these drugs and the dynamic turnover of kinin-like substances.

Kinin-like substances in the cerebellar region seem to be the most susceptible to alteration in state and the significance of this may be better understood when the physiological role of the cerebellum is better known.

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