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Subcellular distribution and properties of the bradykinin inactivation system in rabbit brain homogenates*

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THE ADMINISTRATION of bradykinin into the cerebral ventricles of cats causes important behavioral and EEG alterations as well as a decrease of brain noradrenaline concentration.[1] These central effects of bradykinin are enhanced after the intraventricular administration of the bradykinin potentiating factor (BFP),[1] a substance extracted from the venom of *B. jararaca*[2] that inhibits the bradykininolytic activity *in vitro* of guinea pig plasma.

The present note reports the results obtained on the subcellular distribution and some properties of the enzymatic system responsible for bradykinin inactivation by rabbit brain homogenates.

Rabbits (1·5–2·0 kg) were anesthetized with pentobarbital (40 mg/kg) and their brains were removed and dissected in 0·25 M sucrose kept on ice. The minced brain with the exclusion of the stem was washed with 0·25 M sucrose and homogenized in 9 volumes of 0·25 M sucrose solution with a Potter–Elvehjem homogenizer. Subcellular fractions were obtained after successive centrifugations of the homogenate in a Sorvall refrigerated centrifuge for 30 min at 600 *g*, for 60 min at 7600 *g* and for 60 min at 25,000 *g* at 2°, to give nuclear, mitochondrial, microsomal and supernatant fraction respectively.[3] The supernatant obtained by centrifugation at 25,000 *g* for 1 hr was reduced to 1/15 of its volume, applied to a Sephadex G-100 column and eluted with Tris-HCl buffer, 0·05 M, pH 7·5.

Bradykinin inactivation was studied at apparent zero-order kinetics: 4 μg of the polypeptide plus enzyme were incubated at 37° in 2 ml acetate (pH 3·5–5·5), phosphate (pH 6·5–8·0) or borate (pH > 8) buffer at a concentration isotonic with blood. The rate of inactivation of bradykinin in the incubation media was determined by assaying, on the guinea pig ileum preparation, the remaining activity present in samples withdrawn at 6-min intervals.

After subcellular fractionation of the brain homogenate, kininase activity was mainly found in the supernatant fraction; low activity was present in the nuclear, mitochondrial and microsomal fractions (Table 1).

TABLE 1. INACTIVATION OF BRADYKININ BY FRACTIONS OBTAINED FROM HOMOGENATES OF RABBIT BRAIN TISSUE

Subcellular fractions obtained from brain tissue*	Protein/fraction (mg)	Sp. act.†
Nuclear	312	0·45
Mitochondrial	750	0·44
Microsomal	63	0·67
Supernatant	350	2·1
Total homogenate	1470	0·67
Recovery (%)	100	114

* Pool of 6 brains.

† Micrograms of bradykinin inactivated/min/mg protein.

The supernatant had optimum enzymatic activity at pH values between 7·3 and 7·6, and was inactive below pH 5·5 and above pH 8·0. This activity did not decrease upon storage for 30 days at 0° or for 15 days at 4°.

After removal of 94·9 per cent of total protein, a 10-fold purified fraction was obtained from the supernatant after filtration in Sephadex G-100.

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The alterations induced by different ions and enzyme inhibitors on the kininase activity of this purified fraction are shown in Table 2. It may be seen that 1 mM concentrations of the ions Cu^{2+} or Zn^{2+} as well as *p*-hydroxymercury benzoate completely inhibited the kininolytic activity; the same effect of these inhibitors was observed with brain neutral proteases.[4]

TABLE 2. EFFECT OF VARIOUS SUBSTANCES ON THE INACTIVATION OF BRADYKININ BY PARTIALLY PURIFIED ENZYME FROM RABBIT BRAIN TISSUE

Compound	Concentration	Inhibition (%)
Iodoacetate	1 mM	0
Ethylenediaminetetraacetate	1 mM	0
8-Hydroxyquinoline	1 mM	0
Diisopropylfluorophosphate	0·3 mM	−100
p-Hydroxymercurybenzoate	1 mM	−100
Bradykinin potentiating factor	0·1 mg/ml	−100
Trasylol	50 μ/ml	−50
Oxytocin	0·2 μ/ml	−50
Cu^{2+}	1 mM	−100
Zn^{2+}	1 mM	−100
Co^{2+}	1 mM	−37
Ca^{2+}	1 mM	−35
Mn^{2+}	1 mM	0
Mg^{2+}	1 mM	0

Complete kininase inhibition was also caused by 0·3 mM diisopropylfluorophosphate, whereas partial inhibition was verified in the presence of Trasylol (50 μml/ml, Bayer) oxytocin (0·2 unit/ml, Syntocinon-Sandoz), 1 mM Co^{2+} and 1 mM Ca^{2+}. In contrast to most of the brain peptidases,[5] EDTA, Mg^{2+} and Mn^{2+} at 1 mM concentrations were ineffective.

BPF (100 μg/ml) completely blocked the bradykininolytic activity of the purified fraction of the rabbit brain homogenate. This result suggests that the BPF potentiating effects of bradykinin on the central nervous system of cats at doses of 20 μg bradykinin and 150 μg BPF may be due to the inhibition of the destruction *in vivo* of the polypeptide by brain kininase.

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Further study on purification of hog pancreatic kallikrein

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RECENTLY many biochemists have focused their attention on the kinin-releasing system and its pharmacological, physiological and pathological effects or roles, and have isolated kallikreins from many sources. It is somewhat difficult to obtain large quantities of pure kallikreins and, since hog pancreatic