interaction between kinin releasing enzymes and the synthesized bradykinin derivatives

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Received for publication August 16, 1966

Methionyllysyl-, lysyl- and bradykinin are produced from serum bradykininogen by the action of kinin releasing enzymes, i.e. trypsin, certain snake venoms, or kallikreins. Bovine serum bradykininogen have been isolated in pure form and the action of bradykinin releasing enzymes on the bradykinogen have liberated kinins (1, 2). The amino acid sequence being adjacent to the C-terminus arginine of methionyl-lysylbradykinin moiety in bovine bradykininogen have been elucidated as follows : Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ser-Val-Glu(NH2), and synthesized bradykinyl-Ser have been shown to be applicable for synthetic bradykininogen (3).

In the present communication, the bradykininogen-like characters of synthesized Gly-Gly-Lys-6-0-acetyl-Ser-bradykinin, 6-0-acetyl-Ser-bradykinyl-Gly-Val-Glu(NH2), and bradykinyl-Gly** have been identified by a combination of thin-layer chromatography and biological testing.

Enzymatic digestion of the synthesized bradykinin derivatives was carried out according to the directions given by Suzuki et al. (1) The incubation mixture contained the synthesized bradykinin derivatives (0.1-0.5 μ mole), enzyme solution (0.1 ml), and 0.05 M ammonium formate (pH 8.0) (0.3 ml) in total volume of 0.6 ml. Ten μg of crystalline trypsin*** [EC 3.4.4.4], and 20 μg of hog pancreatic kallikrein [EC 3.4.4.21] (100 units per mg) were used respectively. Hog pancreatic kallikrein solution was heated for 3 minutes in a boiling water bath to inactivate some contaminated kininase just

REFERENCE


INTERACTION BETWEEN KININ RELEASING ENZYMES AND THE SYNTHESIZED BRADYKININ DERIVATIVES*

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** Synthesis of these peptides will be reported in separate paper.
*** Trypsin was purchased from Nutritional Biochemicals Co. Lot 4655.
before incubation (1). The reaction mixture were incubated at 37°C for 50 minutes, freeze-dried immediately, and lyophilized. The reaction mixtures so obtained were submitted to thin-layer chromatography with Waley's solvent system (4), BuOH-pyridine-AcOH-H2O (30:20:6:24), and the bradykinin-like activity of the reaction mixtures was assayed by Magnus method on the isolated guinea pig ileum. Biological potency ratios were calculated from the dose response curves for the action of bradykinin, bradykinin derivatives, and the incubation mixtures. These results here obtained were shown in Fig. 1 and Table 1 respectively.

The consecutive numbers in the Figure and the Table correspond to bradykinin (I), 6-0-acetyl-Ser-bradykinin (II), 6-0-acetyl-Ser-bradykinyl-Gly-Val-Glu (NH2) (III), Gly-Gly-Lys-bradykinin (IV), bradykinyl-Gly (V), Gly (VI), Gly-Val-Glu (NH2) (VII), incubation mixtures of 6-0-acetyl-Ser-bradykinyl-Gly-Val-Glu (NH2) with trypsin (VIII), incubation mixture of 6-0-acetyl-Ser-bradykinyl-Gly-Val-Glu (NH2) with hog pancreatic kallikrein (IX), incubation mixture of Gly-Gly-Lys-6-0-acetyl-Ser-bradykinin with trypsin (X), incubation mixture of Gly-Gly-Lys-6-0-acetyl-Ser-bradykinin with hog pancreatic kallikrein (XI), incubation mixture of bradykinyl-Gly with trypsin (XII), and incubation mixture of bradykinyl-Gly with hog pancreatic kallikrein (XIII) respectively.

From these results, it was recognized that trypsin liberated bradykinin in large quantities from 6-0-acetyl-Ser-bradykinyl-Gly-Val-Glu (NH2) and liberated a marked bradykinin-like active material from Gly-Gly-Lys-6-0-acetyl-Ser-bradykinin. But now it has been investigating that trypsin hydrolysed partially the peptide bond of Lys-Arg in the substrate (IV), trypsin liberated quantitatively 6-0-acetyl-Ser-bradykinin, or the liberated Gly-Gly-Lys inhibited partially the biological activity of the produced bradykinin or its 0-acetyl compound. Gly-Gly-Lys-6-0-acetyl-Ser-bradykinin can be a useful substrate to distinguish between trypsin and hog pancreatic kallikrein.

**Fig. 1.** Thin-layer chromatogram of bradykinin derivatives, the incubation mixtures of the bradykinin derivatives with kinin releasing enzymes, and the presumable peptides and amino acids released by the enzymes.

a) Ninhydrin stain of these spots changed from yellow to violet.

**Table 1.** Comparison for the contractile activity of bradykinin derivatives, the incubation mixtures of the bradykinin derivatives with kinin releasing enzymes, and the presumable peptides and amino acids released by the enzymes on the isolated guinea pig ileum.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Bradykinin-like activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1000</td>
</tr>
<tr>
<td>II</td>
<td>359</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>28</td>
</tr>
<tr>
<td>V</td>
<td>2</td>
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<td>VI</td>
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<td>VII</td>
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<td>IX</td>
<td>7</td>
</tr>
<tr>
<td>X</td>
<td>326</td>
</tr>
<tr>
<td>XI</td>
<td>19</td>
</tr>
<tr>
<td>XII</td>
<td>3</td>
</tr>
<tr>
<td>XIII</td>
<td>2</td>
</tr>
</tbody>
</table>

* : Judged on the basis of the mole concentration of the samples in tissue bath which caused half-maximal contraction.

** : These compound showed also no effect on the biological potency of bradykinin in equal mole concentration of both bradykinin and the compound.
The authors thank Prof. T. Suzuki, Institute for Protein Research, University of Osaka for kind gift of a purified hog pancreatic kallikrein.

REFERENCES

BLOCKADE WITH PROPRANOLOL OF THE POSITIVE INOTROPIC ACTION OF SEROTONIN IN THE DOG

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Received for publication August 24, 1966

The positive inotropic action of serotonin (5-hydroxytryptamine) has extensively been demonstrated in the isolated perfused heart of cats, dogs, and rabbits (1, 2), in the isolated auricle of the rabbit’s heart (3, 4), in the cat papillary muscle (5), and in the heart of dog in situ (6). In reserpinized rabbits, however, serotonin does not produce a positive inotropic action (4). Accordingly, the serotonin-induced increase in myocardial contractile force is presumably mediated by noradrenaline and adrenaline, which were liberated by the administration of serotonin. If this assumption is correct, beta-adrenergic blocking agents should be expected to block the positive inotropic action of serotonin.

The present investigation was undertaken to examine this possibility, by comparing the presence or absence of a beta-adrenergic blocking drug on the positive inotropic action of serotonin.

Normal dogs (8-11 kg) were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). Under artificial respiration, the chest was opened and a strain-gage arch (7, 8) was sutured to the right ventricular muscle for the measurement of the myocardial contractile force. Blood pressure was recorded by means of a pressure transducer from the right femoral artery. Propranolol (9) was chosen as the beta-adrenergic blocking agent in the present experiment, owing to the potent ability to block the beta-adrenergic receptors (10). A single dose of serotonin in the form of serotonin creatinine sulfate was injected at a rate of 50 µg/kg. A similar experiment was conducted in reserpinized dogs (9-12 kg). Reserpine (0.3 mg/kg) was given subcutaneously 24 hours prior to the experiment in 6 dogs, in an attempt to deplete the catecholamine stores (11). In 1 dog, a larger dose of reserpine (0.5 mg/kg) was given subcutaneously 48 hours and 24 hours before the experiment. Anesthetic doses of pentobarbital sodium for the reserpinized dogs were about 1/3 to 1/2 of those for normal dogs.

As shown in the upper panel of Fig. 1, an intravenous injection of 50 µg/kg of serotonin into a normal dog, produced a transitory increase in the myocardial contractile force, and an increase in blood pressure corresponding to the positive inotropic response. A small fluctuation in blood pressure was observed immediately before a marked pressor response to serotonin. Propranolol (0.5 mg/kg), which was injected slowly into the vein, decreased the myocardial contractile force by about 50%,