STUDIES ON KININ-FORMING ENZYME IN RAT STOMACH

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The activity of kinin-forming enzyme (KFE) is usually estimated by assaying the kinins formed during incubation with a suitable source of kininogen at pH 7.8-8.5 (1, 2, 3). This communication describes the optimum pH and the distribution of KFE in the rat stomach.

Male Wistar rats weighing about 150 g were fasted for 18 hr and exsanguinated from the carotid artery. The stomach was extirpated and washed with cold Ringer's solution. After removal of the serosal membrane, the tissue was homogenized in 10 volumes of cold 0.32 M sucrose solution. The homogenate was adjusted to pH 2.0 with 1 N HCl, and incubation was carried out for 20 min at 37 °C to abolish the kininase activity, then pH was adjusted to 7.0 with 1 N NaOH. After centrifugation at 700 g for 10 min, the supernatant fraction was used as KFE preparation. One of the substrates for KFE was prepared from rat plasma by the method of Marin-Grez et al. (4) and used as substrate I. Another substrate for KFE was prepared from rat stomach as follows: the stomach stripped of serosal membrane was homogenized in 10 volumes of cold 0.32 M sucrose solution. The homogenate was centrifuged at 700 g for 10 min. The supernatant fraction was heated in a boiling water bath for 10 min, then adjusted to pH 4.8 with 1 N HCl and used as substrate II. The substrate I or II was incubated for 60 min at 37 °C with excess bovine trypsin. The reaction was terminated by boiling for 5 min and the reaction mixture was centrifuged at 10,000 g for 10 min. The supernatant fractions were assayed with the isolated rat uterine tissue to determine the amount of kinin released. The substrate I or II was capable of yielding 400 ng or 300 ng of bradykinin equivalent per mg or ml, respectively.

The optimum pH of KFE was determined as follows: one ml of the KFE preparation and 1.0 ml of 0.1 M glycine buffer of various pH were incubated with 5.0 ml of the substrate I (0.4 mg/ml) or 5.0 ml of distilled water at 37 °C for 30 min. The reaction was terminated by boiling for 5 min and the reaction mixture was centrifuged at 10,000 g for 10 min. The supernatant fraction was adjusted to pH 8.0 with 1 N HCl or NaOH and assayed with the isolated rat uterine tissue. The activity of KFE was expressed in terms of bradykinin

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equivalent formed in the reaction mixture. The distribution of KFE in the rat stomach was examined as follows: one ml of the KFE preparation from each region of the rat stomach and 1.0 ml of 0.1 M phosphate buffer (pH 4.8) were incubated with 3.0 ml of the substrate II at 37°C for 2 hr. The reaction was terminated by boiling for 5 min, and pH was adjusted to 8.0 with 1 N NaOH, then the reaction mixture was centrifuged at 700 g for 10 min. The supernatant fraction was assayed with the isolated rat uterine tissue. The activity of KFE was expressed in terms of bradykinin equivalent formed per gram weight of wet tissue in 1 min. The regional activity (RA) of KFE was calculated by multiplying the activity by the weight percent of wet tissue (WP).

In the experiment using the substrate I or II, a linear relationship between the amount of bradykinin-like substance liberated and incubation time, or enzyme concentration, was observed until the substrates were consumed up to 20% of the original amount. Bioassays were carried out as follows: the test sample was assayed with the isolated rat uterine, guinea pig ileum, or rat duodenal tissue against standard bradykinin. The uterine horn was excised from virgin Wistar rats in induced estrus (estradiol 40,000 IU/kg i.m., 16–20 hr prior to sacrifice), weighing 150–200 g. The uterine or duodenal tissue was suspended in a 10 ml bath filled with aerated De Jalon’s solution containing atropine (10⁻⁶ g/ml) and dibenamine (10⁻⁷ g/ml), or additional propranolol (10⁻⁸ g/ml) and diphenhydramine (10⁻⁸ g/ml), respectively at 30°C. The guinea pig ileal tissue was suspended in a 10 ml bath filled with aerated Tyrode’s solution containing atropine (10⁻⁶ g/ml) and dibenamine (10⁻⁷ g/ml). The contact time was 90 sec, and the interval between each application was 5 min. Responses were recorded on a kymograph with a lever.

![Fig. 1. Effect of pH on kinin-forming activity. One ml of enzyme preparation from rat stomach and 1.0 ml of glycine buffer of various pH were incubated with 5.0 ml of substrate prepared from rat plasma (A) or with 5.0 ml of distilled water (B) for 30 min at 37°C. Line C shows the actual formation of kinin from the substrate prepared from rat plasma (A minus B). The activity of kinin-forming enzyme was expressed as ng bradykinin equivalent formed in the reaction mixture.](image-url)
TABLE I. Distribution of kinin-forming enzyme in rat stomach

<table>
<thead>
<tr>
<th></th>
<th>Activity</th>
<th>WP</th>
<th>RA (%)</th>
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<tbody>
<tr>
<td>Forestomach</td>
<td>4.7±4.2</td>
<td>38.4±1.6</td>
<td>180(6.1)</td>
</tr>
<tr>
<td>Glandular stomach</td>
<td>muscular layer</td>
<td>45.7±11.2</td>
<td>53.2±1.5</td>
</tr>
<tr>
<td></td>
<td>mucous membrane</td>
<td>37.5±11.2</td>
<td>8.4±0.4</td>
</tr>
</tbody>
</table>

The activities of kinin-forming enzyme in rat stomach were expressed as ng bradykinin equivalent formed per gram weight of wet tissue in 1 min. WP: weight percent of wet tissue, RA: regional activity. Values show means of four experiments ± S.E.

The blood pressure was recorded from the carotid artery of the urethanized (1.5-2.0 g/kg s.c.) rabbits weighing about 3 kg. The test samples were administered into the cannula tied to the vena marginalis.

Figure 1 shows the effect of pH on the formation of kinin. Using the substrate I, the apparent optimum pH of KFE was observed at pH 4.5-5.5 (Fig. 1-A). The KFE preparation itself was found to form a considerable amount of kinin in the reaction mixture to which distilled water had been added in place of the substrate I at pH 4.5-5.5 (Fig. 1-B). The actual formation of kinin from the substrate I is shown in line C, subtracting B from A. The optimum pH in line B and C was observed at pH 4.5-5.5 which was distinct from that of KFE (pH 11.0 or 8.5) in the rat stomach or the rat ileum, as reported by Uchida et al. (5) or Zeitlin (6) respectively. In a separate experiment using the substrate II or the substrate prepared from human plasma in the same procedure as the substrate I, the optimum pH was also found to be 4.5-5.5. These results suggest the possibility that the tissue KFE whose optimum pH is between 4.5-5.5 and the tissue substrate for the enzyme exist in the rat stomach. The substance formed at pH 4.8, using the substrate I or II, was extracted by the method of Abe et al. (7). The classification of this substance was a bradykinin-like substance, based on the observations that, (a) it contracted rat uterine and guinea pig ileac tissue in the presence of atropine and dibenamine, (b) it relaxed rat duodenal tissue in the presence of atropine, dibenamine, propranolol and diphenhydramine, (c) its contractile activity on rat uterine tissue was abolished by the chymotrypsin treatment but not by the trypsin, and (d) it produced a fall in rabbit blood pressure after intravenous administration of atropine (2 mg/kg), propranolol (0.3 mg/kg) and dibenamine (5 mg/kg).

Table I demonstrates the distribution of the KFE in the rat stomach, using the substrate II at pH 4.8. The activity in the glandular stomach was higher than that in the forestomach. In the glandular stomach, no significant difference in the concentration of the enzyme was observed between the muscular layer and the mucous membrane. These distributions of KFE are probably relevant to specific functions of various parts of the stomach.

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REFERENCES

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BUTANOL EXTRACTS FROM MYELIN FRAGMENTS-V.
5-HYDROXYTRYPTAMINE BINDING TO THE MITOCHONDRIAL BUTANOL EXTRACTS AND THE INTER-RELATIONSHIP WITH MYELIN BUTANOL EXTRACTS

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In previous studies (1–4), we demonstrated that the butanol extracts from myelin fragments possessed high selectivity and specificity for 5-HT binding, and that these extracts contained basic proteins, DM-20 and proteolipid protein. We also found that the reconstituted system with crude basic proteins and lipids from myelin showed a saturable binding capacity for 5-HT (3). Basic proteins are one of the main structural proteins of myelin and induce an autoimmune disease, i.e., experimental allergic encephalomyelitis. For the subcellular distribution of basic proteins, it has been reported that these proteins are mainly located in myelin rather than other subcellular organelles, e.g., mitochondria, microsomes, synaptosomes, etc. (5). To obtain more detailed information on the interaction between basic proteins and 5-HT binding affinity, we planned experiments in which 5-HT bound to the butanol extracts from mitochondria.

Male Wistar rats (150–200 g) were decapitated and the brain stems removed. The myelin- and mitochondria-rich fractions were isolated from the homogenate (10% in 0.32 M sucrose) by the method of Whittaker et al. (6) and purity of both fractions was examined by electron microscopy (7). The resultant pellet of mitochondria fraction was suspended in 50% sucrose (2 ml/g stem) and extracted with 10 vol. of water-saturated butanol for 2 hr at room temperature. The butanol phase thus obtained was concentrated under N₂ at 38°C