EXISTENCE OF A BRADYKININ-LIKE SUBSTANCE, AND INFLUENCE OF CARBACHOL AND ATROPINE ON KININ-FORMING AND DESTROYING ACTIVITIES, AND KININ AND KININOGEN CONTENTS IN RAT STOMACH

Motoki KOBAYASHI, Tadahiro SHIKIMI*, Sadao MIYATA**
and Katsuya OHATA

Department of Pharmacology, Kyoto College of Pharmacy,
Yamashina, Kyoto 607, Japan

Accepted May 27, 1980

The presence of kinin-forming enzyme (KFE) in the rat stomach was reported in our previous paper (1). Singh (2) has reported that vagal stimulation induced the release of a bradykinin-like substance and substance P from the frog stomach muscle. These peptides seem to be vagal transmitters which form the basis of spastic states of the frog stomach muscle. In the present study, an attempt was made to confirm the existence of bradykinin-like substance and the influences of carbachol and atropine on KFE and kininase activities in the rat stomach. Kinin and kininogen contents were also investigated.

Male Wistar rats weighing about 150 g were fasted for 18 hr, and exsanguinated from

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* Present address: Department of Pharmacology, Shimane Medical University, Izumo, Shimane 693, Japan
** Present address: Department of Pharmacology, Hyogo College of Medicine, Nishinomiya, Hyogo 663, Japan
the carotid artery. The stomach was extirpated and washed with Ringer's solution at 0°C. The tissue was separated into the fore- and glandular stomach. The glandular stomach was separated in the muscular layer and mucous membrane. These tissues were homogenized in 10 volume of 0.2% acetic acid at 0°C and the homogenate was heated in a boiling water bath for 30 min. The denaturated homogenate was adjusted to pH 7.8 with 1 N NaOH. After centrifugation at 700 g for 10 min, the supernatant fraction was evaporated to dryness at below 35°C. The dried residue was extracted with the same volume of methanol as 0.2% acetic acid used above. The methanol extract was evaporated to dryness, and the dried material was dissolved in 0.9% saline. The solution was used as the test sample for the biological identification.

To elucidate the possible role of the kinin system in the stomach, the following experiments were carried out. Rats were fasted for 18 hr, and carbachol (0.4 mg/kg), atropine sulfate (0.8 mg/kg) or 0.9% saline was given s.c. At 0, 1 and 2 hr after this administration, the muscular layer of the glandular stomach was extirpated. The content of kinin-like substance in the tissue was determined with the extracts prepared by the same method as the test sample described above, using isolated rat uterine tissue. The content of kininogen in the muscular layer of the glandular stomach was determined in the denaturated homogenates prepared by the same procedure as stated above, according to the method of Diniz and Carvalho (3). The KFE activity was determined by the same method as in our previous paper, using the substrate II (1). The KFE activity was expressed in terms of bradykinin equivalent formed/g tissue/min. The kininase activity was determined as follows: The muscular layer of the glandular stomach was homogenized in 10 volume of 0.32 M sucrose solution at 0°C. After centrifugation at 700 g for 10 min, the supernatant fraction was used to determine the activity of kininase. With 1.0 ml of the bradykinin solution (2.5 µg/ml in 0.9% saline), 0.1 ml of the enzyme preparation diluted 20 fold with 0.32 M sucrose solution and 0.5 ml of 0.1 M phosphate buffer (pH 7.8) were incubated at 37°C for 10 min. The reaction was terminated by boiling for 5 min. After centrifugation at 700 g for 10 min, bradykinin remaining in the supernatant fraction was assayed with the isolated rat uterine tissue. The optimum pH of kininase in the rat stomach was 7.8. The kininase activity was expressed in terms of per cent of bradykinin activity abolished. In a separate experiment, the effects of carbachol and atropine on the gastric acid secretion was determined by the method of Shay et al. (4). The rats were fasted for 18 hr and the pylorus was ligated under ether anesthesia. Immediately thereafter, the drug was given s.c. At 1 and 2 hr after administration, the whole stomach was extirpated, the gastric juice was washed out with 5 ml of distilled water and the hydrogen ion in the gastric juice was measured by titration with 0.01 N NaOH. The acidity was expressed in terms of µ equivalent (µeq) of HCl secreted/gastric juice/1 or 2 hr after administration. Bioassays using the isolated rat uterine and duodenal tissues, and on the rabbit blood pressure, were carried out by the same method as used in a previous paper (1).

We found that a kinin-like substance was present in the rat stomach, as determined according to the original idea of Schachter and Thain who used the word 'kinin' to indicate
locally active substances, peptidic in nature and affecting the smooth muscle and the blood
pressure (5). The test sample contracted the isolated rat uterine tissue in the presence of
atropine (10^{-6} g/ml) and dibenamine (10^{-7} g/ml), relaxed the isolated rat duodenal tissue
in the additional presence of propranolol (10^{-8} g/ml) and diphenhydramine (10^{-8} g/ml), and
produced a fall on rabbit blood pressure after intravenous administration of atropine (2
mg/kg, at 30 sec), propranolol (0.3 mg/kg, at 30 sec) and dibenamine (5 mg/kg, at 40 min).
The uterine contractile activity of the test sample was abolished by chymotrypsin treatment
but not by trypsin. On the basis of the observations described above, the biologically active
substance contained in the test sample was found to be the bradykinin-like substance, as
classified by Bertaccini (6).

The content of the bradykinin-like substance in the glandular stomach was almost four
times that found in the forestomach, i.e. 0.17 ug of bradykinin equivalent/g tissue in the
glandular stomach, while in the forestomach, 0.04 ug/g tissue. In the glandular stomach,
the amount of the substance was highest in the muscular layer (0.14 ug/g tissue) as compared
with the mucous membrane (0.02 ug/g tissue). These results suggest a functional role of
this bradykinin-like substance.

Carbachol and atropine appeared to be ineffective on KFE and kininase activities, and
kinin and kininogen contents in the muscular layer of the glandular stomach, as shown in
Table 1 and Fig. 1. Carbachol increased the gastric acid secretion to 571 (200±15 peq/1 hr,
p<0.001) and 430% (301±61 peq/2 hr, p<0.01) of that of the control (35±3 peq/1 hr,
70±14 peq/2 hr), whereas atropine decreased this secretion to 29 (10±5 peq/1 hr, p<0.05)
and 21% (15±5 peq/2 hr, p<0.001), at 1 and 2 hr after administration, respectively.

A relationship between the influence on the kinin system in the muscular layer of the

table 1. Influence of carbachol and atropine on kinin-forming enzyme and kininase
activities

<table>
<thead>
<tr>
<th>Treatment (s.c.)</th>
<th>Activity of kinin-forming enzyme</th>
<th>Activity of kininase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time after injection</td>
<td>0 hr</td>
</tr>
<tr>
<td>Carbachol 0.4 mg/kg</td>
<td>—</td>
<td>24.2±5.0</td>
</tr>
<tr>
<td>Atropine sulfate 0.8 mg/kg</td>
<td>—</td>
<td>24.0±6.0</td>
</tr>
<tr>
<td>0.9% saline</td>
<td>29.0±8.1</td>
<td>28.4±9.9</td>
</tr>
<tr>
<td>Carbachol 0.4 mg/kg</td>
<td>—</td>
<td>31±9</td>
</tr>
<tr>
<td>Atropine sulfate 0.8 mg/kg</td>
<td>—</td>
<td>27±6</td>
</tr>
<tr>
<td>0.9% saline</td>
<td>27±6</td>
<td>24±3</td>
</tr>
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The kinin-forming enzyme and kininase activities were determined using the muscular
layer of the glandular stomach of rats. The kinin-forming enzyme and kininase activities
were expressed as ng bradykinin equivalent formed/g tissue/min and per cent of brady-
kinin activity abolished, respectively. All values represent means of four experiments
±S.E. There was no statistical significance in differences between the values obtained
with the drugs and those obtained with 0.9% saline.
glandular stomach and that on the gastric acid secretion, by carbachol and atropine, was not observed. Thus, it does not seem likely that regulation involves cholinergic receptors in the muscular layer of the rat glandular stomach.

**Fig. 1.** Influence of carbachol and atropine on the contents of bradykinin-like substance and kininogen. The contents of bradykinin-like substance and kininogen were determined using the muscular layer of the glandular stomach of rats. The contents were evaluated in terms of μg bradykinin equivalent/g tissue. Vertical bars represent S.E., Each group included four animals. There was no statistical significance in differences between the values obtained with the drugs and those obtained with 0.9% saline.

Acknowledgement: We thank Messrs. H. Okugawa and Y. Tou for technical assistance.

**REFERENCES**


