A SPECIFIC BIOLOGICAL METHOD OF DETERMINING FREE KININS IN THE VENOUS BLOOD OF MAN AND ANIMALS*

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A method of determining free kinins in whole heparinized blood, untreated by chemical reagents, obtained from peripheral veins. The method is based on the selective sensitivity of an isolated strip of cat jejunum to bradykinin. By immersing a strip of jejunum in 6-10 ml blood containing 8-hydroxyquinoline it was found that the mean content of free kinins per ml healthy human blood was 3.4 ng, compared with 5.3 ng for dogs, 2.0 ng for cats, 5.4 ng for rabbits, 3.2 ng for guinea pigs, and 4.0 ng for rats.

Previous investigations [1] showed that methods of measuring the concentration of free kinins in plasma, including the treatment of whole blood by chemical reagents, lead to inaccuracies because the residual activity of serotonin (which is liberated in large quantities during destruction of platelets) is taken for the action of bradykinin or kallidin.

For this reason considerable importance is attached to the communication of Ferreira and Vane [3] reporting the specific sensitivity of the cat jejunum to the action of bradykinin and kallidin, thereby enabling very small quantities of kinins (0.1 ng/ml) to be detected.

The object of the present investigation was to adapt this method for the determination of free kinins, not in circulating blood, but in heparinized human blood and blood of laboratory animals taken from veins (or arteries).

EXPERIMENTAL METHOD

Experiments were carried out on isolated longitudinal strips (5-7 mm wide, 3-5 cm long) of jejunum of 31 healthy cats of both sexes weighing 2-4 kg. The cats were killed by ether anesthesia or by stunning and subsequent exsanguination by division of the carotid arteries. The strip was taken from a segment of jejunum excised at a distance of up to 25 cm from its beginning, and placed in 6-10 ml Krebs's solution, at a temperature of 35-37°, of the following composition (in g/liter): NaCl 6.9, KCl 0.35, $CaCl_2 \cdot 6H_2O$ 0.5, KH_2PO_4 0.16, $MgSO_4 \cdot 7H_2O$ 0.29, glucose 1.0, $NaHCO_3$ 2.1. The response of this strip of jejunum to the action of bradykinin, histamine, serotonin, angiotensin, and heparin (substances which may be circulating in the blood stream at any one time) was tested by adding them to the Krebs's solution in a volume of 0.1-0.5 ml. The sensitivity of the jejunum was estimated from its threshold contraction in response to the smallest doses of the tested substances 2 min after their addition. After the strip had been rinsed in Krebs's solution, the bath was filled with 6-10 ml of freshly obtained, heparinized (1-3 units/ml) venous blood con-

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TABLE 1. Effect of Added Bradykinin on Contraction of an Isolated Strip of Cat Jejunum Immersed in Blood

Test object	Amplitude of contraction of strip (in mm)
Bradykinin (1.25 ng/ml)	13
Human blood (10 ml)	33
Human blood (10 ml) + bradykinin (1.25 ng/ml)	46
Bradykinin (25 ng/ml)	15
Rabbit blood (10 ml)	1.5
Rabbit blood (10 ml) + bradykinin (25 ng/ml)	16.5

taining 8-hydroxyquinoline or O-phenanthrolin ($1 \cdot 10^{-3}$ M). Blood was taken from a human or animal vein by a 10-ml syringe, or was applied to the jejunum through a polyethylene tube and catheter. All the glassware in contact with the blood was first treated with paraffin wax or silicone. Quantitative relationships were determined between the bradykinin concentration and the amplitude of contraction of the strip and calibration curves were plotted to enable the concentration of free kinins in the blood to be calculated. The part of the curve for which the bradykinin concentration was directly proportional to the amplitude of contraction of the strip was used for the calculations.

The following substances were used in the investigation: bradykinin (Sandoz, Switzerland), bradykinin triacetate, synthesized in the Laboratory of Peptide Synthesis of the Institute of Chemistry of Natural Compounds, Academy of Sciences of the USSR, histamine hydrochloride, serotonin creatine-sulfate, valine-5-angiotensin-II-amide, soybean trypsin inhibitor (STI) (Reanal, Hungary) and O-phenanthrolin (Chemapol, Prague, Czechoslovakia).

EXPERIMENTAL RESULTS AND DISCUSSION

The measurements showed that the initial segment of small intestine (the first 25 cm) responds by a threshold contraction on the average ($M \pm m$) to 0.7 ± 0.09 ng bradykinin/ml Krebs's solution (variation from 0.025 to 2 ng/ml). In bradykinin concentrations from 1-8 times above the threshold dose the amplitude of contraction of the strip was directly proportional to the quantity of the preparation added. In higher concentrations of bradykinin (10-12 threshold doses) this regular relationship was disturbed. The sensitivity of the jejunum to Sandoz bradykinin and to bradykinin triacetate was identical.

In 68% of the experiments the strip of jejunum (the first 25 cm) did not react to histamine (250-1000 ng/ml), in 80% it did not respond to serotonin (500-1400 ng/ml), and in 100% it did not respond to angiotensin (120-500 ng/ml). In 32 and 20% of experiments respectively the strip of jejunum contracted only slightly to addition of histamine and serotonin in doses of 100-200 ng/ml. Higher sensitivity of the jejunum to histamine was found, as a rule, at a distance of 30 cm from its origin. A very small contraction sometimes appeared under the influence of large doses of heparin (50 units/ml). Tipindole (a Soviet serotonin antagonist), in doses of $1 \cdot 10^{-5}$–$3 \cdot 10^{-5}$ g/ml, acted on the cat jejunum not as an antagonist, but as a synergist. The sensitivity of strips of jejunum to bradykinin remained unchanged during keeping for 2-5 days in a refrigerator (at 4°C). In occasional experiments this time was increased to 15 days. Tachyphylaxis to bradykinin developed in only one of the 31 experiments.

By using strips of cat jejunum responding selectively to bradykinin and not responding by contraction to the action of serotonin, histamine, angiotensin, and heparin, the concentration (in ng/ml) of free kinins in human blood and the blood of some animals was determined. In freshly obtained blood from the ulnar vein of 12 clinically healthy persons aged 17-60 years it was ($M \pm m$) 3.4 ± 0.31, from a vein of the forelimb of 7 dogs it was 5.3 ± 0.43, from the marginal vein of the ear of 12 rabbits 5.4 ± 0.46, from the inferior vena cava of 8 cats 2.0 ± 0.38, of 8 guinea pigs 3.2 ± 0.5, and 6 rats 4.0 ± 0.44 ng/ml.

The specificity of the method of measuring the concentration of free kinins as described above was confirmed by detection of bradykinin added to whole heparinized human and rabbit blood in 100% of cases (Table 1).

The results given in Table 1 indicate a proportionate increase in the strength of contraction of the jejunum as a result of summation of the effects of added bradykinin and that already existing in the blood.

A series of tests were carried out to determine whether the freshly collected blood could be stored and, if so, under what conditions. Human and rabbit blood was kept for 0.5-3 h at room temperature (18-20°C) in a water bath at 36°C, or in a refrigerator at 2-3°C, in the absence or presence of STI (100 μg/ml) and of carboxypeptidase N inhibitors (8-hydroxyquinoline, O-phenanthrolin, $1 \cdot 10^{-3}$ M). The investigations

showed that the optimal conditions for preservation of the concentration of free kinins for 30-50 min are obtained by keeping the blood at room temperature in the presence of 8-hydroxyquinoline and of STI (or without STI). If blood containing only 8-hydroxyquinoline was kept at 36° or 2°C (followed by warming on a water bath), in many experiments the formation of new kinins took place. In some experiments O-phenanthrolin significantly reduced the sensitivity of the jejunum to bradykinin.

Repeated determinations of bradykinin in rabbit and human venous blood during the first 30 min of its keeping showed that the relative magnitude of the standard error of a single measurement ($\pm\sigma$) was 6.1 and 6.9% respectively.

The results described above indicate that this method can be used to determine minimal quantities (0.1-1 ng/ml) of free kinins in blood taken from human blood vessels and from various parts of the circulation in animals. For instance, blood from the carotid arteries of rabbits (6 experiments) contained less free kinins (1.4 ng/ml) than blood from the marginal vein of the ear (5.4 ng/ml).

The use of a segment of cat jejunum has the advantage over the use of the rat uterus that the latter contracts under the influence of serotonin and angiotensin also. In addition, the cat jejunum retains its sensitivity to bradykinin unchanged for a long time, but it can be used after storage in a refrigerator for 2-5 days. Attention is directed to results [2] indicating an increase in the sensitivity of the intestine by the action of dimercaprol (50 units/ml). However, this is a matter for further study.

LITERATURE CITED

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