BIOASSAY OF FREE KININS IN THE PERIPHERAL BLOOD

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To characterize the functional state of the kinin system of the blood under normal and various pathological conditions, experimental and clinical workers as a rule determine the separate components of this system: kallikrein and its inhibitors, kininogen, and kininases. Among the most informative indices of the intensity of kinin metabolism are data on the free kinin concentration in the blood. To determine the concentration of these biologically active enzymes in the blood, radioimmunologic [11-13] or biological [5, 9, 14] methods are used at the present time. Because of technical difficulties in the reproduction of radioimmunologic methods at the present time (instability of the immune complex, possible determination of breakdown products and biologically inactive analogs of bradykinin), biological methods are the most specific and reliable. Compared with radioimmunologic methods they do not give such a wide scatter of data for an organism in the same state. For instance, values for the free kinin content in healthy human peripheral venous blood when measured by radioimmunologic methods vary within wide limits: from 25 pg/ml to 0.07 ng/ml [11], from 0.07 to 5 ng/ml [13], and from 50 to 100 ng/ml [12]. A narrower range of values is found by bioassay: 0.96-1.21 ng/ml [14], 2.46-3.4 ng/ml [2, 4-6], and 4.24-6.1 ng/ml [1, 3].

In 1971 the present writer published a method of bioassay of total free kinins in peripheral blood [5]. This method has been widely used by the writer and other workers in clinical and experimental practice [1-6]. The basic idea of the method is fixation of kinin production by inhibition of kinin formation and destruction by the appropriate inhibitors at the time of investigation. This idea itself was based on facts obtained by several workers. First, the possibility of determining a group of biologically active substances (histamine, serotonin, adrenalin, noradrenalin, and acetylcholine) in blood taken from blood vessels by methods of superfusion of isolated organs exhibiting selective sensitivity to the mediators assayed, has been published [15]. Second, there is evidence of the existence of a physiological organ (a cat jejunum) with specific sensitivity to the constrictor action of kinin [8] and that the latter can be determined in the presence of kininase inhibitors in the fluid surrounding the isolated strip of jejunum [9]. Third, the results of investigations of the response of the guinea pig ileum to bradykinin when given by infusion of solutions containing different concentrations of two chelate kininase inhibitors [10] have been published and it has been shown, that, in principle, kinins can be assayed quantitatively by the use of concentrations which only inhibit kininases but do not affect the contractile properties of the muscle fiber.

The attractiveness of this idea was that kinins were determined in whole blood, untreated with chemical reagents, containing three additional substances, two of which (heparin and soy trypsin inhibitor, STI) were inert as regards their effect on the intestine and on kinin production, and the necessary doses of kininase inhibitor could be carefully selected. Concentrations of 8-hydroxyquinoline equal to 0.05-1.10 ml of the saturated solution to 1 ml blood, found by the writer in 1971 for strips of cat jejunum, in most experiments enabled the free kinin concentration in the blood to be determined with great accuracy and reliability. However, it was subsequently noted that in some cases the sensitivity of the strip of jenumun to bradykinin could decrease after two or more blood assays.

Since methods of bioassay of free kinins based on the principle described above are frequently used in research [14], it was decided to determine the precise possibilities of working with the most frequently used chelate and thiol kininase inhibitors and also to obtain

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further evidence that the biologically active substances assayed on an isolated strip of cat jejunum are in fact kinins.

EXPERIMENTAL METHOD

Experiments were carried out by the method described previously [5] with certain additions. In particular, the jejunum was removed from cats anesthetized with pentobarbital (30-50 mg/kg body weight); a gas mixture consisting of 95% O_2 and 5% CO_2 was passed continuously through the nutrient Krebs' solution (or blood) at the rate of 20-40 bubbles/min. The bradykinin triacetate used was from Reanal, Hungary, the STI from the same firm, chymotrypsin from Spofa, Czechoslovakia, heparin from Polfa, Poland, and the 8-hydroxyquinoline, EDTA (Trilon B) and unithiol (BAL or 2,3-dimercaptopropanol), as 5% solution in ampuls, were of USSR origin.

EXPERIMENTAL RESULTS

Most of the experimental data (chymograms of strips of jejunum from 249 cats) collected by the writer and other workers [4, 11] showed that the concentrations of 8-hydroxyquinoline used in the method lowered the sensitivity of the jejunum to bradykinin in 47% of cases on average by 31%, and in 15% of this number of cats by 50-60%. In 53% of animals the above-mentioned concentrations of 8-hyxroxyquinoline did not change the sensitivity of the strip of jejunum to bradykinin. The use of 8-hydroxyquinoline in concentrations of between 0.03 and 0.05 ml of saturated solution to 1 ml blood widens the field of application of the method by reducing the number of tests accompanied by a change in the sensitivity of the strip of jejunum to bradykinin. Another widely used chelate inhibitor of blood kininases, namely EDTA, tested in concentrations of 0.5 to 1 mg/ml, reduced the sensitivity of the jejunum even more — by 50-60%. The inhibitory action of orthophenanthroline (by 60-80%) was demonstrated by the writers as long ago as in 1971 [5]. It can thus be concluded that of the three chelate kininase inhibitors tested, the most acceptable is 8-hydroxyquinoline. However, when working with this substance, additional control determinations of the sensitivity of the strip of jejunum to bradykinin against the background of 8-hydroxyquinoline must be carried out in each experiment, followed by the introduction of an appropriate correction for the reduced sensitivity (if it is reduced), and also with an increase in the time intervals between determinations to 10-15 min, limitation of the number of blood samples tested (to 3 or 4), and replacement of the jujunum.

Tests of the thiol kininase inhibitor BAL or unithiol, recommended in the literature [9], showed that inhibition of human and rabbit blood kininase takes place in the presence of this inhibitor in doses corresponding to concentrations of 0.5-2 mg/ml blood. These concentrations were able to reveal 90-100% of synthetic bradykinin added beforehand to venous blood in doses of 2 to 40 ng/ml.

In the presence of higher activity of blood kininases, such as may arise under pathological conditions and which are found in rats (as shown by special investigations), concentrations of unithiol corresponding to 2 mg/ml blood must be used.

Further tests on strips of jejunum, from 36 cats showed that in doses of 0.1 to 10 mg/ml Krebs' solution, unithiol did not change the sensitivity of the jejunum to bradykinin. However, in 50% of cats concentrations of unithiol inhibiting the action of the blood kininases themselves induce threshold contractions of the strip of jejunum. For this reason, just as during work with 8-hydroxyquinoline, in each experiment additional control assays has to be carried out to detect the effect of the contractile properties of the unithiol itself on the strip of jejunum, with introduction of the appropriate correction for its enhanced contractile activity (if such activity was present). In 50% of cats doses of unithiol corresponding to 2 mg/ml Krebs' solution did not change the contractile properties of this biological test object. The reliability of the results when unithiol was used as blood kininase inhibitor was determined in parallel investigations on blood samples containing 8-hydroxyquinoline from 10 healthy rabbits (4.2 ± 0.44 and 4.3 ± 0.38 ng/ml respectively), in 6 patients with stage II of chronic bronchiectatic pneumonia (8.1 ± 0.90 and 7.8 ± 0.82 ng/ml) and in five patients with infectious-allergic myocarditis (2.5 ± 0.10 and 2.8 ± 0.30 ng/ml).

It was next found that unithiol, in a concentration of 2 mg/ml, preserves the free kinins at their initial level when blood is stored at room temperature for 3 h in the case of rabbit and human blood, and for 90 min in the case of rat blood. In the present investigation free

TABLE 1. Concentration of Free Kinins in Healthy Human Blood and Protein-Free TCA Filtrate from It (M ± m; n = 6)

Test Object	Free kinins, ng/ml
Whole blood containing heparin (3-5 units/ml) STI (100-200 µg/ml), and unithiol (2 mg/ml)	3.10 ± 0.21
Protein-free TCA filtrate (final TCA concentration 5%)	2.7 ± 0.19
The same TCA filtrate after boiling in a water bath for 5 min	3.0 ± 0.23
The same TCA filtrate after boiling, incubated with chymotrypsin at 37°C for 20 min, and boiled again to inactivate chymotrypsin	Not found

Legend: TCA) trichloracetic acid.

kinins were usually determined in freshly taken blood, and not more than 8 to 10 measurements were made in the course of the day at intervals of 10-15 min.

The specificity of bioassay of free blood kinins was confirmed by the selective sensitivity of the cat jejunum to kinins alone [5]. This feature is due to the presence of special receptors, known as B_2 receptors, in the jejunum [7]. In the present investigation fresh evidence was obtained that the substances assayed were kinins. Special experiments showed that weighed samples of chymotrypsin (500 µg/ml), while not changing the contractile properties and sensitivity of the jejunum to bradykinin, caused total inactivation of the biological activity of the test blood samples (Table 1).

By using this biological method of infusion of the isolated strip of cat jejunum with heparinized blood and by using STI as inhibitors of kinin formation in a dose of 100-200 µg to 1 ml blood, and using 8-hydroxyquinoline in concentrations of 0.03 to 0.05 ml of the saturated solution to 1 ml blood or unithiol in a concentration of 2.0 mg/ml blood as inhibitor of kinin breakdown, total free kinins can be determined with great reliability. An advantage of the method described above is its high specificity; its disadvantage is that in 47% of cases when working with 8-hydroxyquinoline and in 50% of cases when working with unithiol the number of determinations must be limited, the time intervals between determinations must be increased, and a correction must be introduced for the change in sensitivity of the jejunum to bradykinin or for the magnitude of contraction of the jejunum in response to unithiol. Despite these disadvantages, the method is highly informative. It is very valuable when studying the pathogenetic role and diagnostic importance of kinins in various inflammatory-allergic diseases of the respiratory and circulatory organs and, in particular, in cases of flare-up of indolent and latent conditions.

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