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# PRINCIPLES OF INTERACTION BETWEEN BRADYKININ AND RECEPTORS OV VARIOUS BLOOD VESSELS

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Data now published indicate that interaction between bradykinin and smooth-muscle structures of the aorta and femoral artery of rabbits [10, 11], and of the guinea pig and cat ileum [10, 12] is described by the equation for the model of interaction between a substance and receptors suggested by Clark [9]:

$$P=\frac{P_M \cdot A}{K+A}, \quad (1)$$

where P is the effect produced by a given concentration of the agonist, $P_M$ is the maximal effect, A the concentration of the agonist, and K the apparent dissociation constant of the agonist—receptor complex (1/K is the index of sensitivity of receptors).

However, for some vessels the character of the curves reflecting the relationship between effect and concentration of bradykinin differs considerably from those predicted theoretically by equation (1) [2]. This prevents the investigator from characterizing the principles governing the kininergic reaction quantitatively and from calculating the value of the apparent dissociation constant of the bradykinin—receptor complex, which is used to compare characteristics of receptor sensitivity [6], correctly in accordance with equation (1).

The object of this investigation was to study the possibility of using certain mathematical models to describe the kinetics of interaction between bradykinin and receptors of various blood vessels. The action of bradykinin was tested on arteries and veins of the hind limb, the peritoneal cavity, and lungs of dogs and also on the portal vein of guinea pigs.

## EXPERIMENTAL METHOD

Experiments were carried out on isolated strips and segments of blood vessels. Spiral strips of arteries were prepared by Avakyan's method [1]. Preparations of the veins consisted of segments 10-15 mm long. Fragments of the vessels were placed in a constant temperature bath at 37°C with Krebs' solution of the following composition (in mM): NaCl 118.5; KCl 4.69; $NaH_2PO_4$ 1.18; $NaHCO_3$ 25.88; $MgSO_4$ 1.16; $CaCl_2$ 2.52; glucose 5.5 (pH 7.4). The solution was made up immediately before the experiment. The contractile response was recorded by an apparatus for measuring tension in a thread (INN-3U), the transducer for which is a differential capacitive transducer. The magnitude of the contractile response to bradykinin was recorded 1-1.5 h after the vessel had been placed in the constant temperature bath. Different doses of bradykinin were added in a volume of 0.5 ml. Each subsequent addition was made 20-40 min after the vessel had been rinsed 5 or 6 times to remove the previous dose. The total number of tests of the different concentrations of bradykinin on one preparation was 6-7; the duration of work with one preparation was 3-5.5 h. The solution was oxygenated. To describe the kinetics of interaction between bradykinin and the receptors, concentration—effect curves were plotted. The magnitude of the response was calculated as a percentage of maximal. The bradykinin used in the experiments was from Reanal (Hungary).

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TABLE 1. Action of Bradykinin on Isolated Vessels (M ± m)

| Name of vessel | Number of experiments | Character of response | K, μM |
|---|---|---|---|
| Portal vein of guinea pig | 10 (7) | Contraction | 0,38±0,09 |
| Portal vein of dog | 11 (7) | " | 9,36±1,95 |
| Lobar veins of dog's lungs | 18 (7) | " | 4,4±0,45 |
| Dog's femoral and saphenous veins | 25 (0) | No response | — |
| Dog's femoral artery | 12 (7) | Contraction | 1,27±0,34 |
| Subcutaneous artery of dog's hind limb | 15 (9) | " | 6,79±1,03 |
| Lobar arteries of dog's lungs | 18 (0) | Weak, constant contraction | — |
| Celiac trunk, common hepatic, anterior mesenteric arteries of dog | 30 (0) | No response | — |

Legend. Number of experiments in which dose-dependent responses were observed in parentheses.

## EXPERIMENTAL RESULTS

It will be clear from Table 1 that the ability of bradykinin to evoke myotropic effects is determined not only by whether the vessels are arteries or veins, but also by their regional specific character. Bradykinin showed marked venomotor activity on the veins of the body cavities whereas the arteries of the mesenteric region did not respond to bradykinin and arteries of the lungs responded to it only by a weak contraction that was not repeated in response to a further dose of bradykinin. Arteries of the hind limbs were highly sensitive to bradykinin, whereas the corresponding veins did not respond to it at all. The guinea pig portal vein also was highly sensitive to bradykinin and responded by a marked contraction to the peptide starting with a concentration of 0.008 μM.

The presence of a dose-dependent reaction in the experiments enabled the value of the apparent dissociation constant of the bradykinin–receptor complex to be calculated with the aid of concentration–effect graphs. Values of K found for different vessels are given in Table 1.

An experimental curve of the concentration–effect curve for the action of bradykinin on the guinea pig portal vein, plotted from the results of one experiment, is illustrated in Fig. 1. The position of the points calculated theoretically by equation (1) corresponds to the experimental curve, evidence of the receptor character of the action of bradykinin. In three of seven cases concentration–effect curves obtained in experiments on the femoral artery also were described by equation (1).

Curves reflecting the action of bradykinin on the subcutaneous artery of the hind limb (nine experiments), the femoral artery (four of seven experiments), and the portal vein (four of seven experiments) of dogs were hyperbolic in character. However, in these cases the points calculated theoretically by equation (1) did not coincide with the experimental data. Better agreement between theoretical and experimental curves was observed when the following equation was used:

$$P = \frac{P_M \cdot A^n}{K^n + A^n}, \qquad (2)$$

where n is a power index. For these experiments its value was 2.

The effect as a function of the square of concentration in a system of double reciprocal coordinates ($1/P$ and $1/A^2$), plotted from the results of one experiment on the subcutaneous artery of a dog in accordance with the equation

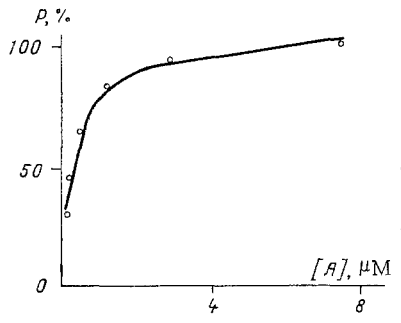


Fig. 1

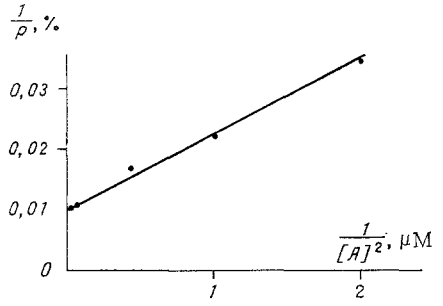


Fig. 2

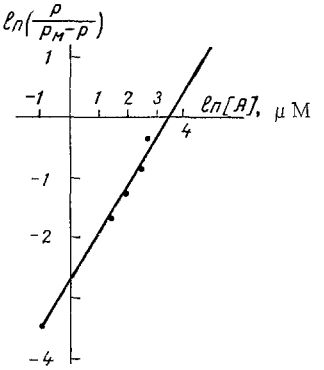


Fig. 3

Fig. 1. Experimental curve of action of bradykinin on guinea pig portal vein. Theoretical points applied to experimental curves. Abscissa, bradykinin concentration; ordinate, magnitude of response (experiment No. 7).

Fig. 2. Contractile response of subcutaneous artery of dog hind limb as a function of bradykinin concentration. Abscissa, reciprocal of square of bradykinin concentration; ordinate, reciprocal of effect (experiment No. 4).

Fig. 3. Contractile response of dog portal vein as a function of bradykinin concentration. Abscissa, logarithm of bradykinin concentration; ordinate, logarithm of quotient obtained by dividing effect due to the given bradykinin concentration by the difference between the maximal effect and the effect of the given concentration (experiment No. 5).

$$\frac{1}{P} = \frac{K^2}{P_M} \cdot \frac{1}{A^2} + \frac{1}{P_M}, \tag{3}$$

which is a transformed equation (2), for both parts of which reciprocals were used, is shown in Fig. 2. The graph in Fig. 2 shows that the experimental points fit well on a straight line. This is evidence of the receptor character of the action of this agonist [8]. Dependence of the hypotensive reaction on bradykinin concentration, which the writers described previously [4, 5], also agreed with the same equation. Curves reflecting the action of bradykinin on the veins of the lungs (seven experiments) and the portal vein (three of seven experiments) of dogs, plotted on a graph of P as a function of A, were S-shaped and were not described by any of the equations given. The use of a logistic function [7] for the graphic analysis of these results showed that the experimental points are linearized (Fig. 3), i.e., in that case the process of interaction between bradykinin and the receptors is described by the equation:

$$\ln \frac{P}{P_M - P} = n \ln |A| - \ln K, \tag{4}$$

which is one variant of the model described by equations (1) and (2). Linearization of the experimental points on the graph of the logistic function is evidence of the receptor character of action of this agonist [7]. The value of the index n is a measure of the degree of deviation of the relationship discovered from that predicted theoretically by equation (1).

To describe the kinetics of interaction of bradykinin with receptors of different blood vessels, an appropriate mathematical model must be used for each case, so that the value of the apparent dissociation constant of the bradykinin–receptor complex can be correctly determined, despite the different character of the dose-dependent curves.

The sensitivity of bradykinin receptors is determined by the region of the body and the species of animal to which the vessels belong; this is reflected in differences in the value of K and it is in harmony with existing data in the literature showing the unequal reactivity of vessels of different organs to bradykinin [3].

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# EVIDENCE FOR AN IMMUNE MECHANISM OF HEMOSTATIC ENZYME CONTROL

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The writers previously suggested the existence of an autoimmune mechanism of control of activated enzymes in the blood stream [1, 2]. It was postulated that activation of an enzyme (proteolysis or conformational changes) is accompanied by the appearance of previously latent antigenic determinants, which act as the afferent signal for triggering immune mechanisms of enzyme inhibition and elimination.

This paper describes an attempt to isolate antibodies against modified enzymes of the hemostasis system from the blood of unimmunized animals.

## EXPERIMENTAL METHOD

Bovine blood stabilized with 2% potassium oxalate solution was used. Plasma was coagulated with 1% $CaCl_2$ solution, the resulting fibrin was removed, and the serum thus obtained served as the source of active Stuart-Prower (Xa) factor (fXa). The factor was isolated by ion-exchange chromatography on DEAE-Sephadex A-50 (from Pharmacia, Sweden) using stepwise elution gradients of phosphate buffer (0.05M, pH 7.0; 0.2M, pH 7.0; 0.45M, pH 8.0). The fraction eluted with the last gradient of buffer was rechromatographed 3 times, desalted on Acrilex P-60, or dialyzed against water and lyophilized. The resulting preparation of fXa degraded BAME (from Reanal, Hungary) converted prothrombin (factor II, abbreviated to FII) into thrombin (factor IIa — fIIa) in a system of fII + fXa + $Ca^{++}$, and was homogeneous in polyacrylamide gel. Prothrombin was isolated from bovine plasma by the method in [5]. Thrombin (from the Kaunas Research Institute of Epidemiology, Microbiology, and Hygiene) was additionally purified by gel filtration on Sephadex G-100 (from Pharmacia). A 0.45% solution of NaCl, pH 7.2, was used as eluting field. Protein peaks with maximal enzyme activity in a system of fIIa + fibrinogen = fibrin, were pooled and lyophilized.

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