CHANGES IN ACETYCHOLINE RECEPTORS AND ADRENORECEPTORS UNDER THE INFLUENCE OF BLOOD PROTEOLYTIC ENZYMES

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KEY WORDS: kallikrein; thrombin; plasmin; acetylcholine receptors; adrenoreceptors.

The writers showed previously that proteolytic enzymes of the kallikrein-kinin, clotting, and fibrinolytic systems modify the reactivity of blood vessels to neurohumoral influences [4, 5]. Since kallikrein and thrombin do not affect contractility of smooth muscle cells [3] it can be postulated that their action is due to modification of a specific component of vascular reactivity, of which reception processes are the principal determinant.

The aim of this investigation was to study the effect of kallikrein, thrombin, and plasmin on function of vascular acetylcholine receptors and adrenoreceptors.

EXPERIMENTAL METHOD

Experiments were carried out on segments of guinea pig portal vein 10-12 mm long. Segments of the vessels were placed in a constant temperature bath containing oxygenated Krebs' solution. One end of the vessel was fixed rigidly, the other was attached to the differential capacitive transducer of an INN-ZU apparatus (OKBA, branch of the "Khimavtomatika" Research-Production Combine, Barnaul), which is capable of recording isometric contractions of smooth muscles. The original tension of the vascular fragment was 200-250 mg. The difference between the force developed by the vessel in response to injection of increasing doses of acetylcholine or noradrenalin and the original tension was taken as the magnitude of the effect. Function of acetylcholine receptors and adrenoreceptors was assessed by a pharmacokinetic method [1, 6]. Concentration–effect curves were plotted for each segment before and after incubation of the vessel for 10 min with one of the proteolytic enzymes. In this way not only could the apparent dissociation constant of the agonist–receptor complex (K), the reciprocal of which characterizes receptor sensitivity, be determined quantitatively, but changes in the maximal effect P_M, which indirectly indicates the number of actively functioning receptors, could be recorded [1, 2, 7, 8]. Proteolytic enzymes were used in the following doses: kallikrein (from Winthrop, England) 0.1 U/ml, thrombin (Kaunas) 1 U/ml, fibrinolysin 10 U/ml. In the doses used the proteolytic enzymes did not induce contractile reactions.

EXPERIMENTAL RESULTS

In all experiments smooth muscles of the portal vein contracted proportionally to the dose of acetylcholine and noradrenalin added. The force of isometric contraction was clearly dependent on the concentration of the agonists after treatment of the vessels with kallikrein and thrombin also. Both before and after treatment with these proteolytic enzymes the experimental points reflecing dependence of the effect on the concentration of agonists coincided with the theoretical curves plotted by the equation $P=\frac{P_M K}{P+A}$, which reflects the kinetics of cholinergic and adrenergic reactions [1, 6]. In this way the parameters of interaction of acetylcholine and noradrenalin with the angioreceptors could be calculated.

After treatment with plasmin the kinetics of interaction between agonists and receptors was disturbed. No significant relationship could be found between the concentrations of acetylcholine or noradrenalin and the magnitude of the response in this case.

Meanwhile in all experiments plasmin sharply reduced the amplitude of the contractile responses to different concentrations of acetylcholine or noradrenalin.

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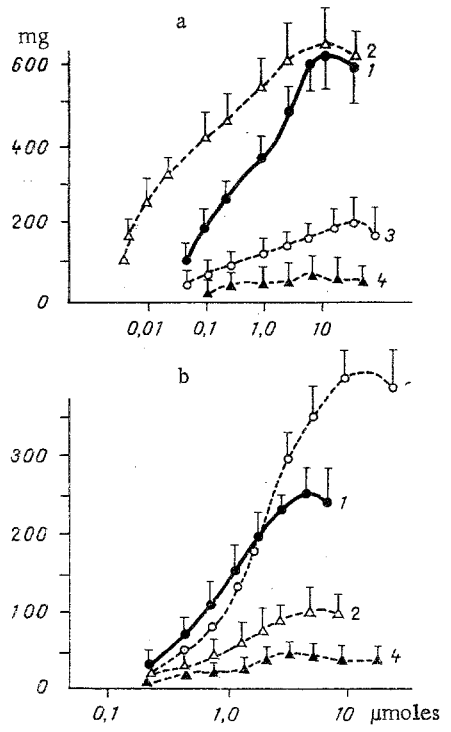

Fig. 1. Dependence of isometric contraction of isolated portal vein on concentration of acetylcholine (A) and noradrenalin (B). 1) Control; 2, 3, 4) treatment with thrombin, kallikrein, and plasmin respectively.

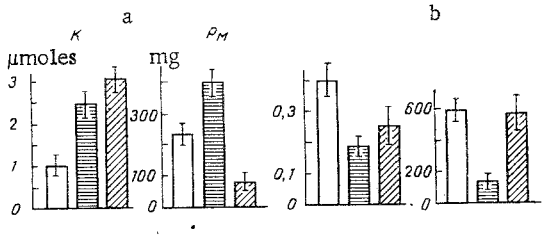

Fig. 2. Parameters of interaction of noradrenalin (a) and acetylcholine (b) with receptors of isolated vessels. Unshaded columns — control; horizontally shaded — after treatment with kallikrein; obliquely shaded — after treatment with thrombin.

Kallikrein had a different action. Under the influence of this proteolytic enzyme the effectiveness of small and average doses of noradrenalin was reduced. Meanwhile the maximal isometric contraction developed in response to high concentrations of the catecholamine was significantly increased. After treatment of the vessel with kallikrein, however, the dose of noradrenalin inducing the maximal effect was higher. In control experiments these concentrations of the catecholamine had a toxic action [1]. Kallikrein had the opposite effect on the contractile responses to acetylcholine.

Thrombin had qualitatively different modulating effects. This proteolytic enzyme reduced the amplitude of contractile respones of noradrenalin over the whole dose range. The contractile effects of acetylcholine, on the other hand, were increased by the action of thrombin. However, the magnitude of the maximal force developed in response to the action of this agonist was indistinguishable from the control (Fig. 1).

The opposite character of the effects of kallikrein and thrombin on the amplitude of contractile responses to acetylcholine and noradrenalin, and their unequal modulating effect on different doses of these agoinists were due, as these experiments showed, to the simultaneous opposite changes in sensitivity and number of active receptors.

Kallikrein reduced the sensitivity of the adrenoreceptors by 2.3 times, as shown by an increase in the apparent dissociation constant of the noradrenalin — receptor complex from

1.09 ± 0.14 to 2.46 ± 0.4 μmoles (Fig. 2; $P < 0.01$). Sensitivity of acetylcholine receptors, on the other hand, was doubled. Kallikrein also caused opposite changes in the number of active receptors. Analysis of the parameter P_M showed that under the influence of kallikrein the number of actively functioning adrenoreceptors was increased by 61% whereas the number of active acetylcholine receptors was reduced by 80%.

After incubation of the isolated vessel with thrombin the number of adrenoreceptors was reduced by two-thirds, as shown by a decrease in the value of P_M from 251.6 ± 35.4 to 79.5 ± 22.7 mg ($P < 0.001$). The sensitivity of the receptors to noradrenalin also was reduced by two-thirds. This was reflected in an increase in the parameter K from 1.09 ± 0.14 to 3.22 ± 0.51 μmoles ($P < 0.001$). The number of active acetylcholine receptors was unchanged by the action of thrombin. Their sensitivity increased by 67%, as shown by a decrease in K from 0.76 ± 0.15 to 0.25 ± 0.07 μmole ($P < 0.002$).

Proteolytic enzymes of the blood thus behave as an important component of neurohumoral regulation and help to maintain vascular tone at a level which corresponds to the characteristics of the blood flowing through them.

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EFFECT OF DIBUNOL LINIMENT ON POST-TRAUMATIC REGENERATION OF THE MOUSE SKIN

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KEY WORDS: skin wounds; cell profileration; regeneration; 4-methyl-2,6-di-tert-butylphenol (dibunol).

Dibunol (4-methyl-2,6,di-tert-butylphenol) has been suggested for use in the treatment of several diseases [5, 6]. Depending on the dose used dibunol either stimulates proliferative activity of cells or has a cytotoxic action [1]. When dibunol emulsion was used to stimulate healing of skin lesions, epithelization of indolent defects was observed with the formation of a delicate and unobtrusive scar [2, 3]. The mechanism of this wound healing effect is not quite clear. When dibunol was used to treat radiation ulcers of the skin in rats, dystrophic changes in the tissue were less marked than in the control and growth of epithelium of hair follicles was stimulated [3]. Healing of full-thickness skin wounds on the dorsal region of mice, rats, and hamsters terminated nearly always with the formation of a scar both in the control and under the influence of various regeneration stimulators [4].

The investigation described below was conducted to study the action of dibunol liniment on proliferative activity of cells of the epidermis and dermis and also the rate of contraction of the wound surface during post-traumatic regeneration of the skin on the dorsal region

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