KALLIKREIN AS MODULATOR OF INTESTINAL SEROTONINERGIC AND BRADYKININERGIC MYOTROPIC REACTIONS

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Kallikrein is a specific enzyme which activates the kallikrein-kinin system of the blood. It is formed in large quantities in the pancreas and salivary glands. Many investigations have shown that administration of exogenous kallikrein or its inhibitor, contrykal, causes considerable modifications of the functions of the gastrointestinal tract [1, 6, 7]. It is suggested that this effect of kallikrein is due to activation or reciprocal inactivation of the kallikrein-kinin system of the blood, modifying the effector response of the digestive organs [2, 3, 6]. Meanwhile the writers previously postulated that this enzyme may also have a direct action on function of the digestive tract [1].

The aim of this investigation was to study the possible direct action of kallikrein on motor activity of the small intestine and its modulating effect in relation to physiologically active substances.

EXPERIMENTAL METHOD

Experiments were carried out on 280 isolated segments of small intestine of noninbred albino rats. Theproximal and distal portions of the duodenum, and proximal, middle, and distal portions of the jejunum and ileum were investigated. Motor activity of the segments was recorded under isometric conditions by a strain-gauge method in constant-temperature cells perfused with Krebs' solution of the following composition (in mM): NaCl – 133, $NaHCO_3$ – 16.3, KCl – 5, NaH_2PO_4 – 1.38, $CaCl_2$ – 2.8, $MgCl_2$ – 0.1, glucose – 7.8. The initial tone of the intestinal segments was assigned by a weight of 1 g.

Motor responses were transformed by means of Yu-8 strain-gauge transducers into an electric signal, which was amplified by the UTS-12-35 strain-gauge amplifier and recorded on an N-338-6P instrument. Reactivity of the segments to serotonin and bradykinin was estimated by a pharmacokinetic method, used previously to assess reactivity of cholinergic, adrenergic, and bradykininergic structures [4, 5, 8]. The dose–effect curves were plotted after addition of bradykinin or serotonin to the perfusion fluid in an increasing concentration, every 10 min. The duration of action of one dose of the substances was 2.5 min. Dose–effect curves were analyzed by the "Elektronikal-50" computer, using a program specially written in the Siberian Branch, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Reactivity of serotoninergic and bradykininergic structures of the small intestine was estimated before and during the action of kallikrein. Kallikrein was injected into the perfusion fluid up to a final concentration of 0.01 U/ml. Kallikrein was used in the form of andekalin, bradykinin as the triacetate (Hungary), and serotonin as the creatinine-sulfate (Hungary).

EXPERIMENTAL REUSLTS

The study of spontaneous motor activity of isolated segments of the rat small intenstine showed that injection of serotonin or bradykinin into the perfusion solution stopped spontaneous contractile activity and induced the development of responses characteristic of these substances. Serotonin induced a marked tonic contractile response, whereas bradykinin induced a phasic response, accompanied by contraction, immediately after relaxation. Responses to serotonin and bradykinin were dose-dependent in character and were described by the equation:

$$R = \frac{R_{max} \cdot A}{K + A},$$

where R is the magnitude of the response to addition of the agonist to the solution in a

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TABLE 1. Parameters of Bradykininergic Myotropic Reactions of Small Intenstine during Perfusion with Kallikrein in a Dose of 0.01 U/ml

Part of intestine	K, mM		E, N/mM	
	Control	Kallikrein	Control	Kallikrein
Duodenum				
Proximal portion	22,3±2,7	11,0±2,5*	81,21±8,9	137,1±19,1*
Distal	14,5±2,9	10,4±2,1	129,5±17,8	213,2±31,9*
Jejunum				
Proximal portion	11,8±1,9	18,3±3,8*	196,5±35,5	92,6±15,2*
Middle	40,9±9,3	32,7±5,3	64,7±12,7	55,8±15,2
Distal	31,6±8,2	34,6±8,9	64,7±1,3	59,6±15,2
Ileum				
Proximal portion	33,5±7,9	62,5±10,6*	44,4±10,2	25,4±5,1*
Middle	83,1±16,9	100,7±17,9	14,0±2,5	14,0±2,5
Distal	72,8±28,6	110,5±25,5	62,2±12,6	12,6±2,6*

Legend. *P < 0.05.

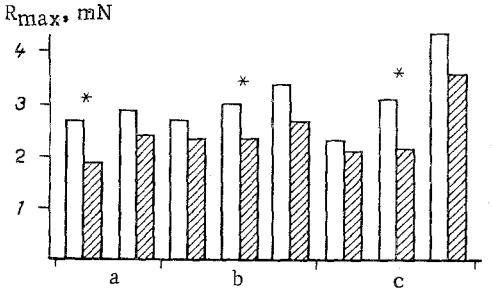

Fig. 1

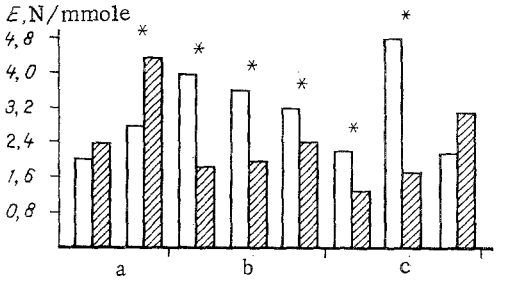

Fig. 2

Fig. 1. Changes in maximal contractile response of segments of small intenstine to serotonin during perfusion with kallikrein in a concentration of 0.01 U/ml. Here and in Figs. 2 and 3: a) duodenum, b) jejunum, c) ileum. Significant differences indicated by an asterisk.

Fig. 2. Changes effectiveness of serotoninergic reaction of segments of small intestine during perfusion with kallikrein in a concentration of 0.01 U/ml.

concentration of A; R_{max} denotes the maximal contractile response to the agonist; K is the dissociation constant of the agonist—receptor complex. Analysis of the dose—effect curves for segments of the small intestine showed that the parameters of the serotoninergic and bradykininergic reactions differed for different segments. For instance, the highest value of effectiveness of bradykininergic reactions ($E = \frac{R}{2K}$), and a low value of K, inversely proportional to the sensitivity of the bradykinin receptors, occurred in the duodenum, evidence of its high affinity for bradykinin. The effectiveness of the bradykininergic reactions was lowest in the ileum (Table 1). Differences in affinity of the different parts of the small intestine for serotonin were less marked.

Injection of kallikrein into the perfusion fluid in a dose of 0.01 U/ml, i.e., not above the limit of physiological, caused no visible changes in motor activity of isolated segments of the small intestine. Nevertheless, parameters of the serotoninergic and bradykininergic reactions were considerably modified against this background. Kallikrein reduced the value of K for duodenal segments and, at the same time, increased E. Opposite changes took place in other parts of the small intestine.

Against the background of kallikrein the parameters of the serotoninergic reactions also were changed. The value of R_{max} was reduced in all parts (Fig. 1). E, like K, changed in different directions in the distal and proximal portions. An increase in effectiveness of the serotoninergic response and a decrease in the value of K were characteristic of the duodenum (Figs. 2 and 3). Kallikrein had the opposite effect on serotonin-sensitive structures of the jejunum and ileum.

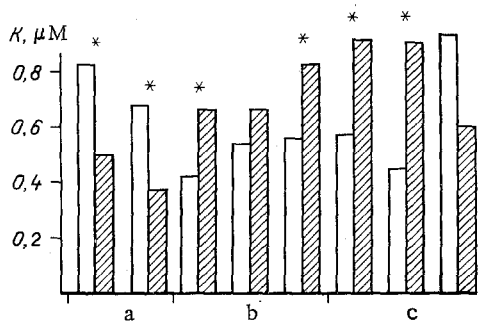

Fig. 3. Changes in dissociation constant for serotonin in segments of small intestine during perfusion with kallikrein in a concentration of 0.01 U/ml.

Thus kallikrein, in a concentration similar to that detectable under physiological conditions, has no direct effect on motor activity, but modifies the parameters of serotoninergic and bradykininergic myotropic reactions of the small intestine. These changes are expressed as an increase in the relative sensitivity and effectiveness of the response of the duodenum, and a decrease in these parameters for the jejunum and ileum. Kallikrein evidently influences the motor function of the small intestine not only indirectly, through activation of kinin production, but also directly, by modulating the reaction to serotonin and bradykinin.

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