INTERACTION OF BRADYKININ AND ITS COMPONENT AMINO ACIDS WITH LIPID MONOLAYERS

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The nonapeptide tissue hormone bradykinin (BK; Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) exhibits a broad spectrum of biological activity. Its most important properties are its effect on the cardiovascular and immune systems, on the extravasal musculature of the bronchi, and on smooth-muscle cells of the intestine and myometrium [3].

The various biological effects of bradykinin.in are mediated by specific receptors of at least two types [6]. However, the molecular mechanisms of the action of BK are still difficult to explain. Taking into account the diversity of action of BK, we postulated that one of the early stages of the action of BK on an effector cell is binding of the hormone with the lipid matrix of its plasma membrane, as has been shown for oxytocin [1]. In this paper we give proof of the possibility of such interaction.

EXPERIMENTAL METHOD

Experiments to study interaction of BK and amino acids present in the peptide chain of the hormone with lipid monolayers were carried out by monolayer techniques [4]. A solution containing 10 mM KCl and 5 mM Tris-HCl (pH 7.2-7.4) was used as the subphase. Azolectin and bradykinin used in the work were obtained from Sigma, the amino acids were of Soviet origin and of the chemically pure grade. Bradykinin and the amino acids were introduced benea.th an azolectin monolayer, formed on the surface of the electrolyte. Steps were taken to ensure that the test substances did not directly reach the phase separation boundary.

EXPERIMENTAL RESULTS

Dependence of the two-dimensional pressure and the boundary pressure jump (BPJ) on the concentration of bradykinin introduced into the subphase, with its surface occupied by the azolectin monolayer, is shown in Fig. 1. The hormone, introduced into the volume of the electrolyte in a concentration of 10^{-8} M caused a very small change in BPJ while the surface pressure of the lipid monolayer remained constant, evidently due to concentration of the BK molecules in the region of the phase boundary. With a peptide concentration of $5 \cdot 10^{-8}$ M its molecules modified the azolectin monolayer, leading to an increase of two-dimensional pressure and to a further increase in BPJ. With higher BK concentrations in the volume of the subphase, the value of both parameters of the monolayer, modified by peptide molecules, showed a dose-dependent increase The level of adsorption of the hormone, calculated for a concentration of $4.5 \cdot 10^{-6}$ M, was $3.89 \cdot 10^{-7}$ M, which agrees with the area per molecule of the peptide namely 4.3 nm^2. Over the whole range of concentrations of the peptide used, the steady state was established in the system in the course of 25-35 min.

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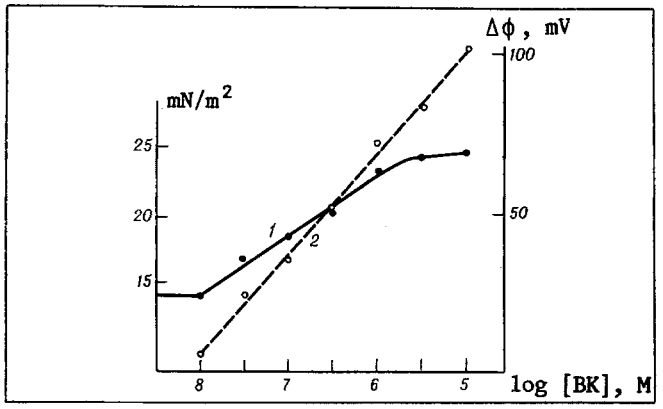

Fig. 1. Increase in two-dimensional pressure (1) and increase in BPJ (2) of azolectin monolayer after injection of bradykinin into the subphase.

TABLE 1. Changes in Surface Pressure (π) and Boundary Potential Jump (φ) of Azolectin Monolayer in Response to Injection of Amino Acids into the Subphase

Amino acids	Concentrations, M								
	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Arg, π, mN/m²	1,3	2	2,6	3	3,2	3,2	3,2	3,4	3,4
φ, mV	13	25	63	82	126	126	126	157	157
Phe, π, mN/m²	2,1	2,3	—	2,7	4,7	6,5	7,1	8,2	8,2
φ, mV	24	30	—	38	45	59	90	90	90
Pro, π, mN/m²	0,9	1,8	2,3	2,7	2,9	4,4	4,9	5,1	5,1
φ, mV	53	103	111	119	144	152	163	186	186
Ser, φ, mV	—126	—121	—121	—121	—121	—	—	—	—
Gly, φ, mV	20	20	—	—	—	—	—	56	84

Evidence of the modifying effect of bradykinin was given also by dynamic isotherms obtained as the result of successive compression widening of the monolayer structure formed. The surface collapse pressure (π) of such a monolayer, when modified by BK in a concentration of $4.5 \cdot 10^{-6}$ M, was 53 mN/m², and BPJ was 273 mV, whereas for the unmodified monolayer these parameters were 48 mN/m² and 298 mV respectively. These results also are evidence of interaction between molecules of the peptide and molecules that are components of the azolectin monolayer.

Arginine residues are located in the bradykinin molecule in positions 1 and 9 of the peptide chain. They confer a positive charge on the hormone over a wide pH range, and since the azolectin monolayer also includes in its composition a considerable quantity of negatively charged lipids [5], it can be tentatively suggested than an important role in the process of binding of BK with lipid monolayers is played by electrostatic interactions.

To discover the contribution of the various amino acid residues in the composition of BK to the mechanism of the modifying action of the hormone we studied their interaction with lipid monolayers. The experimental results are given in Table 1.

The most marked interaction with lipids was observed for phenylalanine and proline, and rather less for arginine. Depending on interaction with monolayers, the amino-acid components of the peptide chain of BK can be arranged in the following order: Phe > Pro > Arg > Ser > Gly.

The results of these investigations thus demonstrate that molecules of BK and the amino acids forming its peptide chain can interact with lipid monolayers and modify them. Interaction of BK with lipids is determined, on the one hand, by the basic properties of the peptide and, on the other hand, by the presence of negatively charged lipids in the composition of the azolectin. Such interaction evidently takes place into two stages. In the 1st stage, as a result of electrostatic interaction between arginine residues and the negatively charged lipids the peptide is "fixed" in the region of the polar heads of the lipids In the 2nd stage the hormone inserts itself into the azolectin monolayer as a result of hydrophobic interactions between residues of phenylalanine, proline, and arginine and the acyl chains of the lipids. The possibility cannot be ruled out that as a result of such insertion, BK molecules in the hydrophobic environment may acquire a biologically active conformation (quasicyclic?). The hormone also disturbs the packing of the lipids into a monolayer and bilayer, with

consequent changes in the physicochemical parameters of the membrane, and these may lead to realization of the physiological effects [2].

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EFFECT OF SODIUM HYPOCHLORITE ON OXYGEN BALANCE AND FUNCTIONAL STATE OF THE SMALL INTESTINE IN EXPERIMENTAL PERITONITIS

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The known bactericidal agent sodium hypochlorite (NaOCl) has an anticoagulant [4] action and a marked antiaggregating and disaggregating action on platelets [6]. NaOCl is effectively used in surgery to sterilize the peritoneal cavity in the case of spreading peritonitis and in the treatment of septic wounds [2, 3].

In the investigation described below the effect of NaOCl was studied on the oxygen balance and the acid—base state (ABS) of the blood and functional activity of the small intestine in spreading peritonitis, when administered intravenously and intraperitoneally.

EXPERIMENTAL METHOD

Experiments were carried out on 121 albino rats weighing 180-230 g, in three series, under combined anesthesia (5 mg diazepam + 600 mg sodium hydroxybutyrate/kg body weight intramuscularly). Parameters of the electromyogram (EMG), partial pressure of oxygen in the intestinal wall (pO_2), gas composition and ABS of the blood in intact animals, after experimental spreading peritonitis for 24 h, and also 30-40 min and 24 h after intravenous or intraperitoneal injection of NaOCl. The same parameters but when a 0.9% solution of sodium chloride (physiological saline — PS) was used served as the control. The NaOCl used in the work was obtained electrochemically [3]. A 0.1% solution of NaOCl in a dose of 10 mg/kg body weight was injected intravenously, and 3-4 ml of the 0.12% solution was injected intraperitoneally. Series I consisted of 47 intact rats (of which 19 received NaOCl intravenously and 16 intraperitoneally). Twelve animals served as the control. In series II, on 22 animals (16 experimental and six control) the effect of intraperitoneal injection of NaOCl on the course of spreading peritonitis was studied. In series III, on 52 rats with spreading peritonitis, changes in values of pO_2

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