Interactions between Angiotensin and Lipid Monolayers

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Key Words: *angiotensin; lipid monolayer; plasma membrane*

Bearing in mind the surface activity of angiotensin-2 [2] and previous data on oxytocin and substance P [3-7], we proposed that primary interactions between angiotensin and cell plasma membranes may involve the lipid matrix and that the activation of intracellular processes may depend on functional specificities of the forming lipopeptide complexes. The present research was aimed at investigation of the interactions between angiotensin-2 and structural analogs of the plasma membrane lipid matrix, that is, monolayers created from various lipids in water-air and electrolyte solution-air systems.

MATERIALS AND METHODS

Angiotensin-2 (Riga) interactions with monolayers formed of lipids (Serva) were studied by methods for monolayer investigations [1-4].

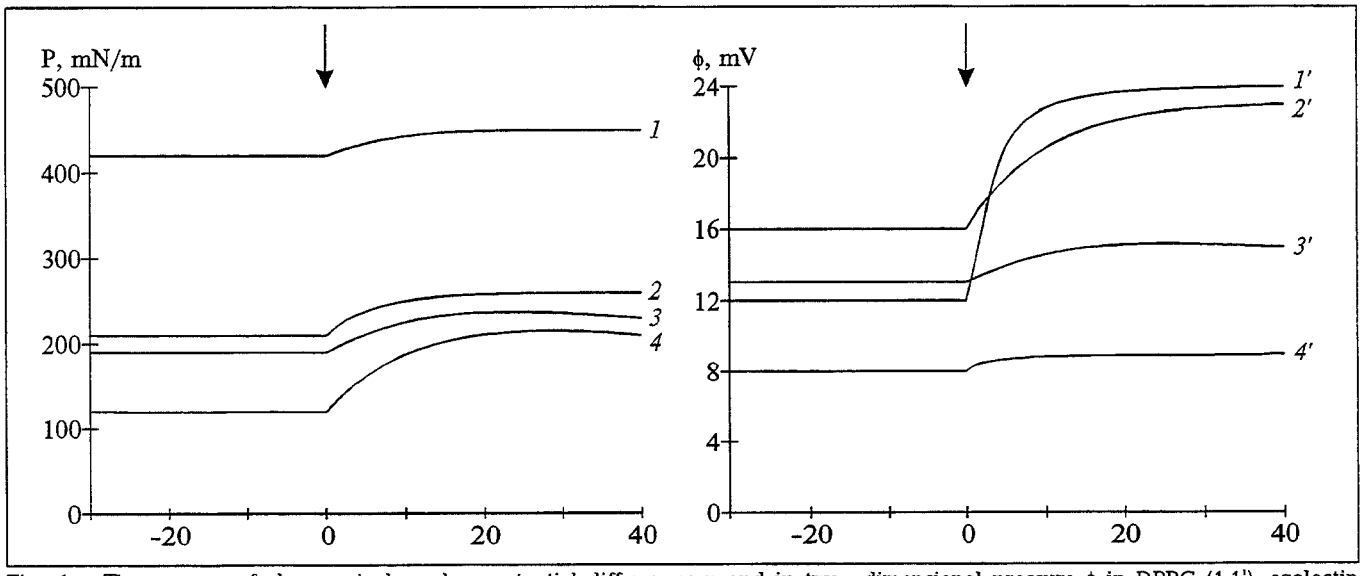

Fig. 1. Time course of changes in boundary potential differences p and in two-dimensional pressure ϕ in DPPC (1,1'), azolectin (2,2'), PS (3,3'), and PI (4,4') monolayers on salt-free subphases (arrows on all diagrams show introduction of 1.43×10^{-6} M angiotensin-2).

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RESULTS

Injection of angiotensin-2 under preformed lipid monolayers results in their interaction with pep-

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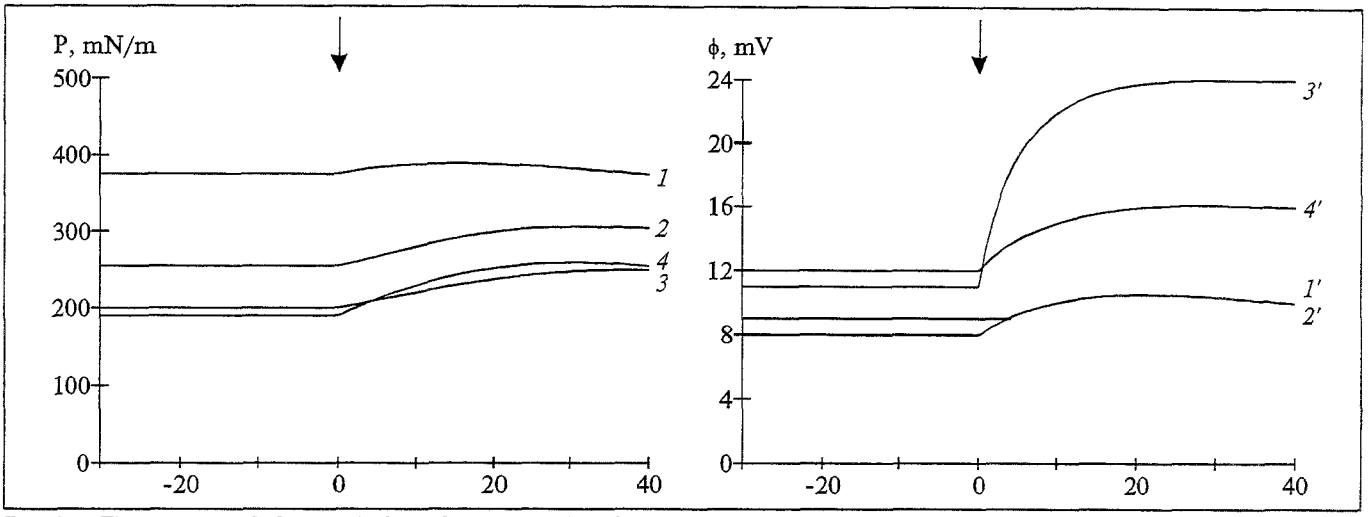

Fig. 2. Time course of changes in boundary potential differences p and in two-dimensional pressure ϕ in DPPC (1,1'), azolectin (2,2'), PS (3,3'), and PI (4,4') monolayers in the presence of 0.1 M KCl in the subphase.

tide, this being attended by a rise of two-dimensional pressure and boundary potential differences in the subphase-lipid monolayer-air systems. Other conditions being equal, the efficacy of lipopeptide interactions depends on the chemical composition of the lipids. Figure 1 presents typical chronopotentiograms and time courses of changes in two-dimensional pressure in dipalmitoylphosphatidylcholine (DPPC), phosphatidylserine (PS), phosphatidylinosite (PI), and azolectin monolayers obtained by introducing 1.43×10^{-6} M angiotensin-2 into a salt-free subphase, pH 6.2. The curves on the diagram show that peptide binding with the DPPC monolayer is poor, but with azolectin, PI, and PS monolayers it is strong. In the case with the PS monolayer the two-dimensional pressure in response to peptide introduction into the subphase increased by 4-15 mN/m in comparison with its initial value (10-16 mN/m). These data indicate that angiotensin-2 adsorption on azolectin, PS, and PI monolayers involves the penetration of peptide molecules inside the boundary membranes because polypeptide adsorption on the clean surface of the phase interface under the said concentration does not induce a reduction of the subphase tension [2].

Since angiotensin-2 interaction with lipid monolayers may be controlled by subphase ions, we undertook a series of experiments to introduce peptide under DPPG, PI, and PS monolayers whose subphases contained 0.1 M KCl and 4×10^{-2} M $CaCl_2$ (ionic strength 1=0.1 q-eq/liter). The first of these experiments (Fig. 2) showed no marked changes in the overall course of the chronopotentiogram or in the time course of two-dimensional pressure in lipid monolayer produced in response to peptide introduction into the subphase. The presence of $CaCl_2$ in the subphase, however, resulted in a noticeable reduction of the increment of the two-dimensional pressure and of the boundary potential differences (Fig. 3). This

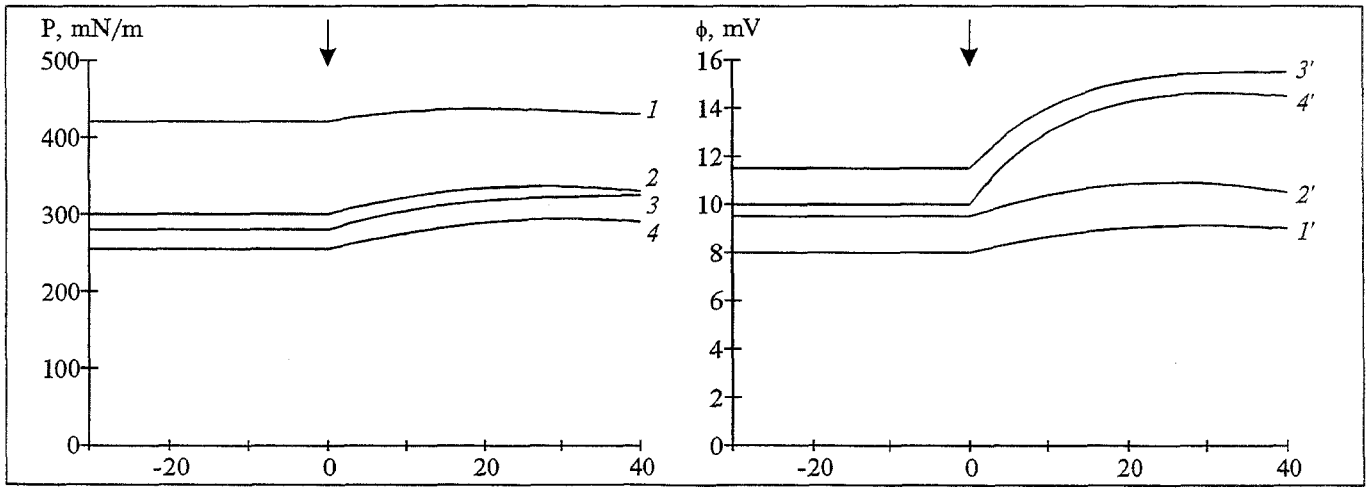

Fig. 3. Time course of changes in boundary potential differences p and in two-dimensional pressure ϕ in DPPC (1,1'), azolectin (2,2'), PS (3,3'), and PI (4,4') monolayers in the presence of 4×10^{-2} M $CaCl_2$ in the subphase.

TABLE 1. Si_p/Si_l Values in Lipoprotein Monolayers Formed as a Result of Angiotensin Adsorption under Various Experimental Conditions

Subphase composition	Monolayer composition			
	azolectin	PS	PI	DPPC
H_2O	5.4	4.3	3.6	13
KCl	4.3	4.0	3.3	42.9
$CaCl_2$	16.3	9.3	6.5	26

effect may be explained by a strong shielding of charges of lipid molecule polar heads and of ionized angiotensin sites with calcium ions. Since the boundary differences of lipid monolayer potential are created mainly at the expense of dipoles in their molecules, and angiotensin penetration into the lipid intermolecular space occurs under isochoric conditions, that is with a fixed area, stoichiometry of peptide-lipid binding may be assessed. The boundary difference of the lipid monolayer potential may be presented in this connection as the sum of components:

$$\phi=\phi_l+\phi_p \quad (1),$$

where ϕ_p and ϕ_l are components of the boundary potential differences created by the lipid and peptide molecules proper.

The value of ϕ_l in this equation should be constant due to the isochoric conditions. As for the value of ϕ_p, it will be governed by the function:

$$\phi_p=\mu_p/Si_p \quad (2),$$

where μ_p is the effective dipole moment of the angiotensin molecule and Si_p is the specific area of this peptide molecule. The value of μ_p is easily estimated from equation (2) on the basis of measurements of the boundary potential differences in an angiotensin monolayer with a known Si_p value, for example, for threshold adsorption [2]. Substituting into equation (2) the values of the increments of the boundary potential differences in lipid monolayers during interactions with angiotensin, and solving this equation with respect to Si_p, we find the specific areas per peptide molecule in the forming peptide-lipid systems. Dividing the resultant values by area size per molecule in the initial lipid monolayers (0.75 nm^2 for a two-dimensional pressure of 10 mN/m), we obtain the binding stoichiometry value.

Thus, Si_p/Si_l ratio values were found for all the systems examined (Table). The highest Si_p/Si_l values are characteristic of situations where angiotensin interacts with a neutral (DPPC) monolayer and of situations where it interacts with an azolectin monolayer for strong shielding of its negative charges with calcium ions. There are from 16 to 43 lipid molecules for every angiotensin molecule penetrating into a monolayer in such cases. The situation is different when angiotensin interacts with PS and PI monolayers for every weak (subphase-water) and moderate (subphase-0.1 M KCl) shielding of the surface charges of these monolayers. The number of lipid molecules per angiotensin molecule is reduced to 3-5 in such cases.

The data demonstrate a two-stage pattern of angiotensin-2 interaction with lipid monolayers. At stage 1 angiotensin molecules are adsorbed on lipid molecule polar heads. The rate of this stage depends on the type of lipids and on the degree of charge shielding by subphase ions. Peptide molecule adsorption on lipid monolayers seem to occur due to electrostatic interaction of positively ionized charges of the His-6 amide group and Arg-2 guanidine group with negatively charged fragments of lipid polar heads. Thus, conditions are created favoring subsequent incorporation of angiotensin molecules into the lipid matrix. The incorporation itself, occurring on account of hydrophobic interactions, is rapidly realized and results in a marked increase of the two-dimensional pressure and of the boundary potential difference in the forming lipoprotein systems.

Hence, investigation of the primary physicochemical interactions between angiotensin-2 and organized supramolecular structures - monolayers - suggests that the cell plasma membrane lipid matrix may function as a "catalyzer" of peptide-receptor interaction by changing the spatial arrangement of angiotensin-2 molecules when a hydrophilic medium is replaced by a hydrophobic one. It is also possible that the lipid matrix can perform a kind of receptor function of its own.

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