Membranotropic Activity of Optical Isomers of the Neuropeptide Kyotorphin and a Cardiotonic Agent, Suphan

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On modeled monolayer phospholipid (formed from azolectin) membranes, we studied the surface activity of optical isomers of a dipeptide, kyotorphin (Tyr-Arg), and a cardiotonic agent, suphan (N-succinyltryptophan potassium salt). It was found that the membranotropic activity of four studied isomers of kyotorphin is distributed in the order: LL > DL ≈ LD > DD, and two isomers (by tryptophan) of suphan as LL > DL. The data obtained suggest that the primary mechanism underlying binding of kyotorphin and suphan to the plasma membrane can be considered based on interaction of their molecules with the molecules of membrane phospholipids. Binding of molecules of kyotorphin and suphan by the lipid matrix to the plasma membrane and/or their incorporation into the matrix is a result of the above interaction.

INTRODUCTION

The level of biological activity of regulatory peptides and medicinals is to a considerable extent determined by the properties of their interaction with the components of the cellular plasma membrane and, in particular, with the membrane lipid matrix. Further pathways for transmission of the signals to a cell depends on this type of interaction. Recently, we studied the dependence of the membranotropic activity on the following factors: length of a peptide chain, number of charged amino acid residues, and hydrophobic level of the molecules of peptide bioregulators [1]. These results allowed us to suggest that optical isomerism also plays a certain role in such phenomena.

In our experiments described below, we aimed at studying the dependence of membranotropic properties of a dipeptide, kyotorphin (Tyr-Arg), on the amino acid chirality in its isomers. This agent displays a significant analgesic effect, which is more intensive than that of enkephalins [2]. We studied also a novel cardiotonic agent, suphan, which possess anti-arrhythmic and antihypoxic properties [3], and increases the power of heart contractions and their rate [4]. We examined four optical isomers of kyotorphin: D-Tyr-D-Arg (D-D), D-Tyr-L-Arg (D-L), L-Tyr-D-Arg (L-D), L-Tyr-L-Arg (L-L), and also L-suphan and D,L-suphan (the latter contains D- and L-tryptophan in equal rations).

METHODS

We studied the surface activity of the above dipeptides and variants of suphan and their interaction with the lipid monolayers. To measure the surface pressure on the border of separation of electrolyte solution–air phases by a Wilhelmi technique, we used a half-embedded platinum plate. The boundary step of the potential (BSP) was measured by means of a dynamic capacitor technique [5]. The sensitivity of the tests equalled 0.1 mN/m and 2 mV, respectively. The experiments were carried out at 23-24°C. As a subphase, we used the following buffers: 0.01 M Tris-HCl with pH 7.4 or 0.01 M KCl with pH 6.8; the solutions were prepared on distilled water specially purified from surface-active substances (using an Akvilegiya device). To form the monolayer membrane, azolectin (a mixture of protein phospholipids of soybean; Sigma, USA) was used.

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The level of boundary adsorption of dipeptides and suphan was calculated by Gibbs' equation:

$$B = 1/RT \cdot dP/d\ln C,$$

where R is the universal gas constant; T is absolute temperature; P is the pressure in a two-dimensional system, i.e., in the monolayer membrane; and C is the concentration of an agent. A specific area, which is occupied on the membrane by a molecule of the adsorbed substance under conditions of maximum adsorption, was calculated by the formula: $S = 1/BN$, where N is Avogadro's number.

RESULTS AND DISCUSSION

Interaction of biologically active substances with the cellular membrane, which could be considered interphase borders, is to a great extent determined by the surface-active properties of such agents. Therefore, we first of all studied the surface activity of kyotorphin isomers. It was found that these dipeptides in physiological concentrations (10^{-11} to 10^{-5} M) practically did not form their own adsorption monolayer, small amounts of the above substances only concentrated near the surface of electrolyte solution–air phase border. In this case, the BSP increased to +400 ... +600 mV, but the surface pressure in the system did not change. We observed somewhat greater activity of the L-L-type dipeptide, which formed extended monolayers with a low density (P = 1.1 mN/m; U = +574 mV, when the concentration of dipeptide in the subphase equalled 10^{-5} M). The boundary adsorption (B) of the enantiomer on the free surface of electrolyte was 6.87 × 10^{-8} M/m^2, and the specific area calculated for a molecule (S) was equal to 24.2 nm^2.

Both types of suphan possessed an insignificant surface activity, and they formed extended monolayers on the border of separation of the phases. The molecules were concentrated near the phase surface borders. This was observed from nanomolar concentrations, as was demonstrated by a BSP shift toward negative values. As for D,L-suphan, such a process was clearly pronounced only at the concentrations over 10^{-8} M; however its total effect was more profound than that of L-suphan. D,L-suphan promptly began to form a monolayer, changing the surface pressure on the boundary of separation of the phases, whereas the concentrations about 10^{-7} M promoted the L-suphan activity. At micromolar concentrations, D,L-suphan adsorption increased, and at the 10^{-5} M concentration the pressure in the monolayer (P) equalled 1.5 mN/m. For L-suphan, P reached only 1 mN/m even at the concentration of 10^{-4} M. All the above-mentioned data suggest that the surface activity of D,L-suphan exceeds the activity of L-suphan.

The presence of azolectin monomolecular membranes on the surface of electrolyte raised the rate of adsorption of suphan and kyotorphin molecules from the subphase to the monolayer, but in this case the membranotropic activity of each agent under study was different and depended on the initial density of the membrane. When the dipeptide–membrane interactions occurred at the initial membrane density of 4-5 mN/m, the membranotropic activity was clearly expressed. The lowest activity was observed for the isomer D-D-kyotorphin, which even at the concentration of 10^{-5} M evoked rather insignificant changes in the state of azolectin monolayer. The D-L and L-D isomers, which have approximately the same time for transition to a steady state (25-30 min), interacted with the azolectin monolayers somewhat intensively. Under the influence of the above two isomers, an increase in the BSP within a modified azolectin monolayer within a concentration range of these dipeptides from 10^{-7} to 10^{-6} M and the changes in the surface pressure of the membrane were nearly identical, but D-L isomer to a greater extent increased the density of the azolectin monolayers and was more active within the range of low concentrations (10^{-11} to 10^{-9} M). However, the calculated limit of the substance amount, which could be incorporated into the lipid monolayer, was nearly the same for both dipeptides if the initial parameters of monolayers were close, i.e., the level of boundary adsorption (B) for D-L isomer and for L-D isomer equalled $6.85 \cdot 10^{-8}$ and $6.35 \cdot 10^{-8}$ M/m^2, respectively.

The levorotatory amino acid (L-L)-formed isomer provided the maximum modification of azolectin monolayers. The isomer could interact with close-packed monolayers and increased the pressure in the latter up to 20-25 mN/m. At nanomolar concentrations, an increase in the surface pressure was recorded immediately after insertion of the substance into the subphase, but a stationary state was reached in 70-80 min. With increased concentration of the dipeptide, incorporation of its molecules into a lipid monolayer was initiated 10-15 min after dipeptide had been added; the time until the process termination was considerably, by about 20 min, shorter. The concentration dependence of the changes in the surface pressure of a azolectin monolayer induced by L-L dipeptide was more clearly pronounced compared with that of D-L and L-D dipeptides; the maximum adsorption occurred within the concentration range from 10^{-9} to 10^{-8} M (an increase in P by 6-8 mN/m), then its intensity dropped. In this case, the BSP (also in contrast to the above dipeptides) within the micromolar concentration range increased nearly uniformly, to +250 ... +300 mV.

Such dynamics of the changes in characteristics of the lipid monolayer under the influence of peptide is indicative of active incorporation of the latter into the lipid molecules (in the region of hydrophobic fatty acid

"tails"), while isomers of the L-D- and D-L-types are to a great extent concentrated in the region of polar lipid "heads" because of electrostatic interactions.

For extended monolayers, the calculated minimum concentration (C_{min}) of L-suphan sufficient for beginning of incorporation of its molecules into the membrane was 0.4 nM (P = 2.3 mN/m), and for monolayers with the highest density it was 1.7 nM (P = 8.5 mN/m). However, the level of adsorption in the case of interaction with more dense membranes increased twofold. The specific area per molecule of suphan at its maximum adsorption decreased and equalled 11 and 5.2 nm^2 for the extended (P = 2-3 mN/m) and more dense (P = 8-9 mN/m) monolayers, respectively. In other words, an increase in the density of packing of molecules within a lipid membrane promoted incorporation of L-suphan molecules into the lipid matrix, which reached a stationary state in 35-40 min.

Mebranotropic properties of D,L-suphan were somewhat lower. The agent practically did not incorporate into the extended monolayer membranes, but this process, as in the case of L-type agent, was intensified with membrane packing. The minimum D,L-suphan concentration, which was necessary to initiate incorporation, was 1.4 nM, but the intensity for further incorporation was two times lower than that of L-type; transition to a steady state lasted for 20-30 min.

Therefore, both L-suphan and D,L-suphan possess a proper surface activity and a capability of forming the extended adsorption monolayers on the phase border surface. The surface activity of D,L-suphan exceeded the corresponding activity of L-suphan. The existence of a phospholipid monolayer membrane catalytically intensified adsorption from the subphase volume of two suphan types under study. Adsorption is more active in the case of interaction with the dense membranes. The minimum concentrations, which provide recording of penetration of suphans into the hydrophobic phospholipid membrane zone, were 1-2 nM. It is interesting but still incomprehensible that the intensity for penetration of D,L-suphan molecules into the membrane is 2-fold lower than that for L-suphan (the B values were $3.3 \cdot 10^{-7}$ M/m^2 for L-suphan and $1.6 \cdot 10^{-7}$ M/m^2 for D,L-suphan), although the value of proper surface activity of the former compound exceeds that of the latter. At the same time, incorporation of the studied agents into the membrane at similar concentrations was finished in 20-30 min for D,L-suphan and in 30-40 min for L-suphan, which corresponds to their own surface activity. If the concentration of L-suphan in the subphase reached 10^{-6} M and more, penetration of the agent into the lipid matrix stopped. As for D,L-suphan, this process was continued at higher concentrations (up to 10^{-4} M).

Considering our data, it is difficult to agree with the hypothesis [6] that suphan is capable of penetrating into the cell via Ca channels of the plasma membrane. This fact seems highly improbable if we consider the diameter of such channels. More probably, penetration of suphan into the cell can be provided due to bounding its molecules with the molecules of phospholipids. It cannot be ruled out that suphan can be incorporated into the plasma mebrane and artificial lipid membranes in a manner similar to neurotensin [8], vasopressin [9], or substance P [10].

Thus, optical isomers of the dipeptide Tyr-Arg we studied are rather different in their membranotropic activity, and in this respect the above agents can be arranged in the following declining order: L-L > D-L ≈ L-D > D-D. As for suphan, a consequence of its types is as follows: L-suphan > D,L-suphan. Taken altogether, it demonstrates a prominent role of chirality (the optical activity) of amino acids, which are the components of bioregulator molecules, in determining the membranotropic properties of the substances. In particular, this seems quite important for a number of medicinals.

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