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ORIGINAL ARTICLE

Comparative study of the interaction of synthetic methionine-enkephalin and its amidated derivate with monolayers of zwitterionic and negatively charged phospholipids

Asya Tsanova · A. Jordanova · G. As. Georgiev · T. Pajpanova · E. Golovinsky · Z. Lalchev

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Abstract Using Langmuir's monolayer technique, the surface behavior and the interaction of the synthetic neuropeptide methionine-enkephalin (Met-enk) and its amidated derivate (Met-enk-NH_2) with monolayers of the zwitterionic dimyristoylphosphatidylcholine (DMPC) and the negatively charged dimyristoylphosphatidylglycerol (DMPG) were studied. The surface tension (γ, mN/m) of DMPG and DMPC monolayers as a function of time (after injection of the peptide under the interface) was detected. The decrease in γ values showed that there was a strong penetration effect of both types of Met-enk molecules into the monolayers, being significantly stronger for the amidated derivate, Met-enk-NH_2. We suggest that the interaction between the neuropeptides and DMPC was predominantly determined by peptides amphiphilicity, while the electrostatic forces play significant role for the insertion of the cationic Met-enk-NH_2 in DMPG monolayers, especially at high packing densities. Our results demonstrate the potential of lipid monolayers formed in Langmuir's trough to be successfully used as an elegant and simple membrane models to study lipid–peptide interactions at the air/water interface.

Keywords Methionine-enkephalin · DMPC · DMPG · Langmuir's monolayer technique · Surface tension

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Introduction

The brain and the gastrointestinal tract have receptors localized in cell membranes capable of interaction with opioids like morphine. On the search for endogenous ligands for these receptors, scientists discovered two homologous pentapeptides called methionine-enkephalin (Met-enk) and leucine-enkephalin (Leu-enk) with similar amino acid sequence: Tyr-Gly-Gly-Phe-(Met or Leu) (Ganong 2001). Recently, neuropeptides like enkephalins have attracted much interest because their receptors are the sites of action of opiate drugs such as morphine and codeine (Vander et al. 2001). Enkephalins are neurotransmitters found in the human central nervous system, especially in the regions of the brain and spine associated with diffuse pain pathways (Hucho 1986; Kruk and Pycock 1991). They are involved in a wide variety of physiological processes. Circulating enkephalins are believed to play a role in the inflammatory and immune response by acting as neurotransmitters in the central nervous system (Shipp et al. 1991). They also play an important role in gastrointestinal physiology, especially in ion transport (Cheng et al. 1996). Enkephalins also modulate learning and memory (Gallagher 1982; Gallagher et al. 1983; Messing et al. 1979) as well as emotional behaviors (Nieto et al. 2005). They are involved in the central control of respiration and in inhibition of pain signals (Fuxe et al. 1988). Moreover, it is known that they have a significant role in store of

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different physiological processes like cell differentiation and regeneration, inflammation, cancer- and angiogenesis, analgesia effects, etc.

There is limited information about the exact molecular mechanisms of the interaction between enkephalins and biological membranes. To achieve their biological function, enkephalins must be transported from an aqueous phase to the lipid-rich environment of their membrane-bound receptor proteins (Liu et al. 2006). It is now known that Met-enk acts via three main subtypes of receptors referred to as μ (mu)-, δ (delta)-, and κ (kappa)-, as well as ζ (zeta)-receptors. While the first three receptor subtypes mediate the classic opioid effects of Met-enk, ζ-receptors are reported to be involved in the non-opioid actions of the peptide, i.e. the inhibitory effect on cell growth (Malendowicz et al. 2005).

In order to better understand the interaction of enkephalins with neuronal membranes, numerous experiments have been conducted using different model membranes: giant unilamellar vesicles (GUV) (Boyanov et al. 2005; Mutafchieva et al. 2005), bicelles (Marcotte et al. 2003), multilamellar vesicles (Marcotte et al. 2004), monolayers (Bourhim et al. 1993; Ege and Lee 2004), etc. It is believed that these neuropeptides interact with the nerve cell membrane in order to adopt a bioactive conformation that will then fit onto the receptors (Behnam and Deber 1984; Deber and Behnam 1985; Gysin and Schwyzer 1983; Sargent and Schwyzer 1986). According to this mechanism called "membrane catalysis," the polar head groups of the lipids at the cell membrane surface interact with these amphiphilic hormones which enter into the membrane via hydrophobic interactions and undergo a hormone conformational change. Then, the enkephalins would migrate to the receptor with the suitable structure for binding (Sargent and Schwyzer 1986).

Enkephalins act as agonists of the opioid receptors which are coupled to G-proteins. These receptors possess the same general structure of an extracellular N-terminal region, seven transmembrane domains and intracellular C-terminal tail structure (Childers 1991). The interactions of the neuropeptides with lipids are pivotal for understanding the mechanism of their biological activities. The lipid phase of the membranes not only acts as a matrix for the receptors, but also is essential for the functionality and the topological arrangement of the receptor proteins (Wakamatsu et al. 1987). With this regard, alterations in the stiffness of lipid bilayers are likely to constitute a general mechanism for modulation of membrane protein function (Lundbaek et al. 1996). This regulation is due to both specific lipid–protein interactions and general bilayer–protein interactions, which modulate the energetics and kinetics of protein conformational transitions, as well as the protein distribution between different membrane compartments (Andersen and Koeppe 2007).

The synthetic analogues of the naturally occurring opioid peptides, as well as their modified forms, could be introduced into clinical practice, since it is believed that they would be more resistant to enzyme attack, and could have stronger effect on their target cells. There are not enough literature data regarding the effect of the artificial neuropeptides to the membranes. It is found so far by Boyanov et al. (2005) that a large number of synthetic derivates of naturally occurring enkephalins is capable of penetrating the hydrophobic region of model membranes. In this respect, it is of interest to study the interaction of the synthetic opioid peptides and their derivates with lipid model membrane systems such as lipid monolayers.

Langmuir's monolayer model in combination with Wilhelmy's method for measuring the surface tension is an elegant and simple way of studying the surface behavior of different hydrophobic molecules, such as membrane lipids, as well as their interactions with a variety of membrane-active substances (e.g. peptides, proteins, etc.). Moreover, because of the simplified two-dimensional geometry and the large degree of thermodynamic and conformational control that can be exerted on the constituent molecules, monolayers of insoluble amphiphiles stand to serve as important model systems for the basic understanding of complex rheological behavior, such as non-Newtonian viscous response and multiphase flow (Yim et al. 2000).

The aim of the present work was to study the ability of the synthetic Met-enk and its amidated derivate, Met-enk-NH_2, to penetrate spread monolayers of phospholipids at the air/water interface and to compare the degree of interaction between both enkephalins and the phospholipids. In order to estimate the role of electrostatic interactions, the zwitterionic dimyristoylphosphatidylcholine (DMPC) and the negatively charged dimyristoylphosphatidylglycerol (DMPG) were chosen.

Materials and methods

Materials

To obtain methionine-enkephalins, manual stepwise solid phase techniques were applied. The peptides were synthesized on a Rink-amide resin using Fmoc strategy with DIC/HOBt activation. The crude peptides were purified by preparative TLC and their purity was checked by analytical HPLC. Electrospray ionization mass spectrometry (ESI-MS) was in agreement with the expected results. DMPC was purchased from Sigma, and DMPG was purchased from Avanti Polar Lipids. Met-enk/Met-enk-NH_2 stock solutions (10 mM) were prepared by dissolving the peptides in physiological solution (0.15 M NaCl). DMPC and DMPG (1 mg/ml) were dissolved in chloroform.

Surface tension measurements

To determine the surface activity of the enkephalins to the lipid monolayers, the decrease in surface tension (γ, mN/m) with time was monitored, and $\Delta\gamma$ ($\gamma_{monolayer} - \gamma_{monolayer+Met\text{-}enk}$, or $\gamma_{monolayer} - \gamma_{monolayer+Met-enk-NH_2}$) was calculated. All measurements were made in multi-well plate (3 × 5) MicroTrough X (Kibron Inc., Finland). The subphase volume was 500 µl. The apparatus uses the Wilhelmy method with platinum wire probe attached to the microbalance sensor head. Fixed amounts of DMPC and DMPG solutions were spread at the surface with a Hamilton microsyringe in order to reach different surface concentrations of 150, 100 and 75 $Å^2$ per lipid molecule over a subphase of 0.15 M NaCl at 25°C (the temperature was chosen to be above the main phase transition temperature of both phospholipids). The pH of the subphase was 5.77. Upon establishment of an equilibrium value of the surface tension (γ_{eq}), Met-enkephalins were injected in the subphase under the formed monolayers to final volume concentration of 0.5 and 1 mM. The surface tension was measured with accuracy of ±0.01 mN/m. The trough temperature was controlled within ±0.5°C. Each measurement was repeated at least three times with each separate sample.

Surface pressure calculations

In order to compare the penetration ability of the two forms of Met-enk studied into the formed lipid monolayers, the γ values measured were converted to surface pressure, π values and plotted as $\Delta\pi/\pi_0$, where $\Delta\pi = \gamma_{monolayer} - \gamma_{monolayer+Met\text{-}enk}$, or $\gamma_{monolayer} - \gamma_{monolayer+Met-enk-NH_2}$, and $\pi_0 = \gamma_{subphase} - \gamma_{monolayer}$. The surface pressure values at which the peptides were excluded from the monolayers ($\pi_{critical}$) were determined as π_0 values at which $\Delta\pi = 0$.

Results

In this work, we studied the interaction between the synthetic Met-enk and monolayers of the zwitterionic DMPC and the negatively charged DMPG. In addition, to observe the effect of the C-terminal amidation of the neuropeptide, the penetration capacity of the modified enkephalin (Met-enk-NH_2) into both monolayers was studied. With this regard, the change in surface tension at air/water interface with time was measured. Our data presented in Fig. 1 showed a significant decrease in γ of both lipid monolayers (at surface concentration of 150 $Å^2$ per molecule) after the addition of increasing amounts of the respective enkephalins. Moreover, the results for the interaction of the unmodified and the amidated Met-enk (at final volume concentrations of 0.5 and 1 mM) with DMPC monolayers showed that Met-enk-NH_2 lowered the surface tension to a higher degree. It was observed that γ_{eq} after the addition of Met-enk changed from 70.7 to 61.2 mN/m (for 0.5 mM enkephalin) and to 58.1 mN/m (for 1 mM enkephalin). After the addition of Met-enk-NH_2, γ_{eq} decreased to 54.6 and to 49.9 mN/m for the two enkephalin concentrations, respectively (Fig. 1a). The same effect was observed after the injection of both enkephalins under the monolayer composed of the negatively charged DMPG at the same surface concentration (γ_{eq} values changed from 69.3 to 58.2 mN/m for 0.5 mM enkephalin and to 55.4 mN/m for the 1 mM concentration of Met-enk, and to 54.0 and to 50.2 mN/m for both concentrations of Met-enk-NH_2, respectively, Fig. 1b).

The measurements of γ of phosphatidylcholine (PC), and phosphatidylglycerol monolayers at surface concentration of 100 $Å^2$ per molecule with time showed that the injection of the opioid peptides studied reduced γ to a smaller degree as compared to the larger area per phospholipid molecule. However, the tendency Met-enk-NH_2 to reduce

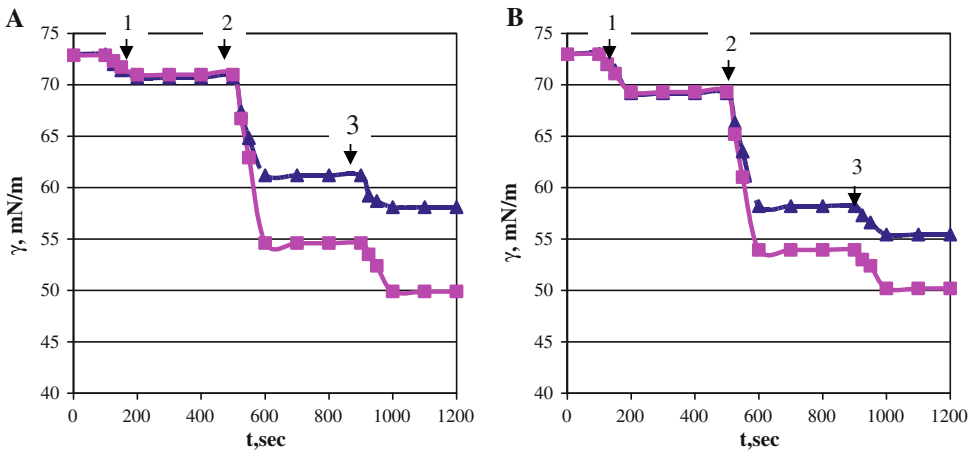

Fig. 1 Change in γ (mN/m) with time (s) after the addition of *1* DMPC (**a**) and DMPG (**b**) to surface concentrations of 150 $Å^2$ per molecule on subphases of 0.15 M NaCl at 25°C, *2* Met-enk to final volume concentration 0.5 mM, *3* Met-enk to final volume concentration 1 mM. *Triangles* Met-enk, *squares* Met-enk-NH_2

surface tension values at a higher level as compared to the unmodified enkephalin remained: Met-enk lowered γ_{eq} of DMPC from 60 to 54.3 mN/m when added to final volume concentration of 0.5 mM, and to 52.2 mN/m for 1 mM enkephalin, and Met-enk-NH_2 lowered γ_{eq} to 49.6 mN/m (Fig. 2a). For DMPG, γ_{eq} values changed from 60.5 to 53.3 mN/m for the lower Met-enk concentration, and to 52.5 mN/m for 1 mM Met-enk, and to 50.7 and 48.4 mN/m for both concentrations of Met-enk-NH_2, respectively (Fig. 2b).

The results for the interaction between enkephalins and DMPC and DMPG at surface concentration of 75 $Å^2$ per phospholipid molecule obtained are presented in Fig. 3. They imply that the penetration of Met-enk into the monolayers at this lipid concentration is impaired (γ_{eq} for both monolayers changed insignificantly). However, even at the highly packed monolayers, Met-enk-NH_2 still reduced the surface tension significantly, which was more pronounced for the DMPG monolayer (γ_{eq} changed from 47.9 to 41.8 mN/m for the amidated derivate concentration of 0.5 mM, and to 40.9 mN/m for the doubled concentration, Fig. 3b).

In order to present the degree of penetration of the methionine-enkephalin and its modified form into the monolayers of the differently charged phospholipids, the values of $\Delta\gamma$ were calculated and presented in Table 1, and the plot of $\Delta\gamma$ versus area per molecule is shown in Fig. 4. It is seen in Table 1 that $\Delta\gamma$ values increased with increasing the area per lipid molecule in all cases. Moreover, $\Delta\gamma$ for Met-enk/DMPC mixed monolayers was comparable with that of Met-enk/DMPG monolayers for both peptide concentrations indicating that Met-enk interacted with and penetrated into both monolayers equally. The same conclusion was true for the Met-enk-NH_2/DMPC and Met-enk-NH_2/DMPG monolayers at surface concentrations of 150 and 100 $Å^2$ but not for the compact monolayers of 75 $Å^2$ per phospholipid molecule. In Fig. 4, it is clearly seen the tendency $\Delta\gamma$ values of Met-enk-NH_2/DMPC monolayers to become closer to the curve of Met-enk/DMPC during DMPC monolayer compression (going from surface concentration of 150–75 $Å^2$, Fig. 4a), while in case of DMPG monolayers the curves are parallel (Fig. 4b), i.e. in more densely packed monolayer state the

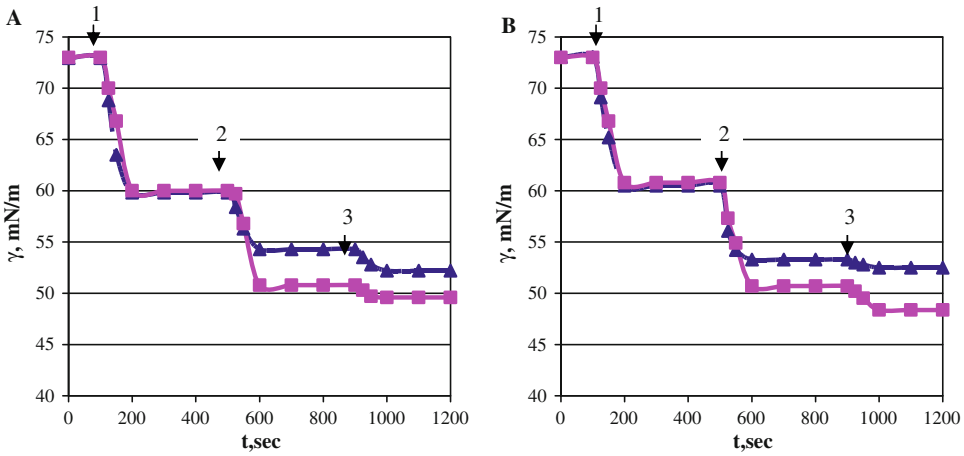

Fig. 2 Change in γ (mN/m) with time (s) after the addition of *1* DMPC (**a**) and DMPG (**b**) to surface concentrations of 100 $Å^2$ per molecule on subphases of 0.15 M NaCl at 25°C, *2* Met-enk to final volume concentration 0.5 mM, *3* Met-enk to final volume concentration 1 mM. *Triangles* Met-enk, *squares* Met-enk-NH_2

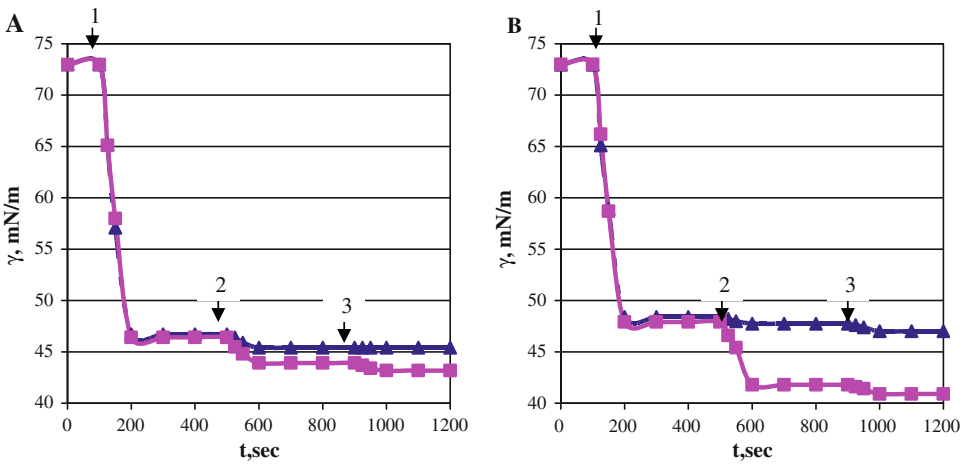

Fig. 3 Change in γ (mN/m) with time (s) after the addition of *1* DMPC (**a**) and DMPG (**b**) to surface concentrations of 75 $Å^2$ per molecule on subphases of 0.15 M NaCl at 25°C, *2* Met-enk to final volume concentration 0.5 mM, *3* Met-enk to final volume concentration 1 mM. *Triangles* Met-enk, *squares* Met-enk-NH_2

Table 1 Change in surface tension values ($\Delta\gamma$, mN/m) of monolayers composed of DMPC and DMPG with different area per lipid molecule after the addition of Met-enk, and Met-enk-NH_2

Area per lipid molecule (Å^2 per molecule)	$\Delta\gamma$ (mN/m)							
	0.5 mM Met-enk		1 mM Met-enk		0.5 mM Met-enk-NH_2		1 mM Met-enk-NH_2	
	DMPC	DMPG	DMPC	DMPG	DMPC	DMPG	DMPC	DMPG
150	9.5 ± 0.62	11.0 ± 1.42	12.6 ± 0.70	13.8 ± 1.18	16.4 ± 1.37	15.4 ± 0.93	21.1 ± 1.71	19.1 ± 0.94
100	5.5 ± 0.58	7.2 ± 0.76	7.6 ± 1.16	8.0 ± 0.94	9.2 ± 0.64	9.8 ± 1.10	10.4 ± 0.18	12.1 ± 1.68
75	1.3 ± 0.39	0.7 ± 0.18	1.3 ± 0.16	1.4 ± 0.24	2.5 ± 0.30	6.1 ± 0.73	3.2 ± 0.71	7.0 ± 0.78

Data are presented as mean ± standard error (SE) of three different experiments

amidated derivate penetrates stronger than Met-enk to negatively charged monolayers. Thus, Fig. 4 reveals data that generally the amidated Met-enk interacted stronger than Met-enk with both zwitterionic DMPC and negatively charged DMPG; however, at higher lipid surface concentrations (75 Å^2), its penetration to DMPG monolayers was facilitated, probably by electrostatic forces.

Finally, to compare the degree of interaction between both enkephalins studied and the phospholipid monolayers, the change in surface pressure ($\Delta\pi = \gamma_{monolayer} - \gamma_{monolayer+Met\text{-}enk}$, or $\gamma_{monolayer} - \gamma_{monolayer+Met-enk-NH_2}$, mN/m) of the film after the injection of Met-enk/Met-enk-NH_2 versus surface pressure of the pure lipid monolayers, π_0 ($\pi_0 = \gamma_{subphase} - \gamma_{monolayer}$, mN/m) was plotted (Fig. 5). Our data showed that the modified form of the peptide was excluded from the monolayers of both lipids at higher surface pressure values than that of Met-enk. For instance, $\pi_{critical}$ values (the approximated pressure at which no insertion of peptides was observed, $\Delta\pi = 0$) for DMPC monolayers were 29 and 33 mN/m for Met-enk and Met-enk-NH_2, respectively (Fig. 5a). For the DMPG monolayers, the difference between the penetration capability of the two enkephalins increased and the $\pi_{critical}$ values determined were 27 mN/m for the unmodified peptide, and 45 mN/m for the amidated derivate, which proved the ability of the modified Met-enk-NH_2 to penetrate significantly stronger than Met-enk to charged monolayers with high surface molecular density (Fig. 5b).

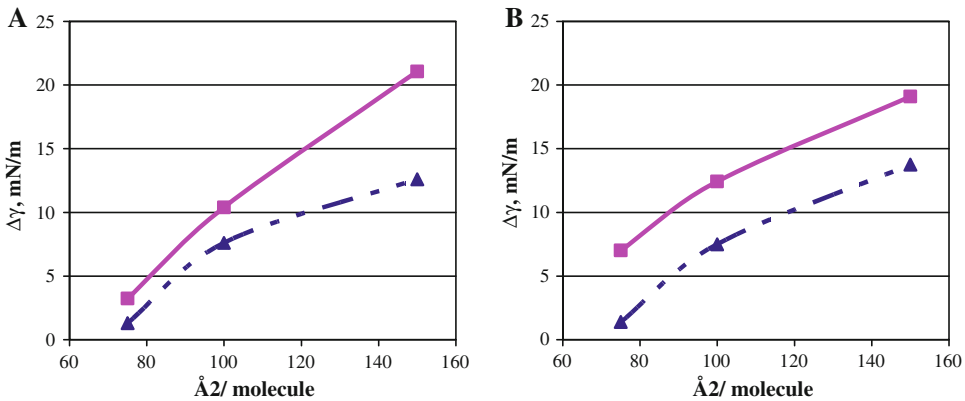

Fig. 4 Change in $\Delta\gamma$ (mN/m) with area per lipid molecule (Å^2 per molecule) after the injection of Met-enk, and Met-enk-NH_2 to final volume concentration of 1 mM under a monolayer of DMPC (**a**) and DMPG (**b**). *Triangles* Met-enk, *squares* Met-enk-NH_2

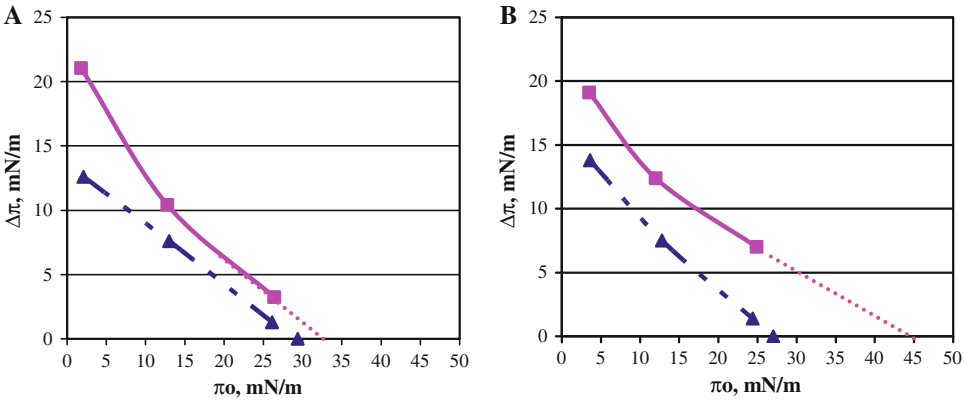

Fig. 5 Change in $\Delta\pi$ (mN/m) with π_0 (mN/m) after the injection of Met-enk, and Met-enk-NH_2 to final volume concentration of 1 mM under a monolayer of DMPC (**a**) and DMPG (**b**). *Triangles* Met-enk, *squares* Met-enk-NH_2

Discussion

Endogenous phospholipids in vivo, such as those in the microenvironment of the enkephalin receptor, could conceivably play any of several roles in mediating the transfer of a neurotransmitter or peptide hormone from the aqueous to the membrane phase. These could include: facilitating the capture, entry, and concentration of the aqueous-soluble hormone or neurotransmitter into the lipid-rich environment of the receptor; and/or orienting the peptide in the membrane vis a vis the receptor by restricting molecular motions (Deber and Behnam 1984). With this regard, the goal of the present study was to compare the interaction of the synthetic Met-enk and its C-terminal amidated derivate with monolayers composed of two differently charged phospholipids, DMPC and DMPG as a function of the lipid surface concentration and the enkephalin concentration in the subphase. Although phosphatidylglycerol is a ubiquitous lipid that can be the main component of some bacterial membranes, it is found also in membranes of plants and animals where it appears to perform specific functions: e.g., it is one of the major components of the pulmonary surfactant (Christie 2010; Hallman and Gluck 1976; Pfleger et al. 1972). Because of its net charge phosphatidylglycerol is a preferred lipid to mimic membranes with a negative surface charge. According to Träuble (1976), the pK_a of the PG phosphate group is 2.9. Therefore, at pH 5.77 of the subphase, most of the DMPG molecules are deprotonated. In addition, its head group size is similar to that of PC, and accordingly the main phase transition temperatures T_t from the lamellar gel phase L_β or ripple phase $P_{\beta'}$ to the fluid lamellar phase L_α are similar (Struppe et al. 2000). The lipid surface concentrations were chosen to be 150, 100 and 75 Å since the aim of the present study was to observe how did the penetration ability of the peptides changes with the compression of the monolayers, starting from a "diluted" monolayer to a surface concentration showing surface pressure values near to 25–30 mN/m (Fig. 3), near to that of the native membranes (Ege and Lee 2004; Evans and Skalak 1980; Ishitsuka et al. 2006; Marsh 1996; Seelig 1987).

Our results revealed that the synthetic neuropeptides, Met-enk and Met-enk-NH_2, interacted with and penetrated into both monolayers, DMPC and DMPG, depending on the given surface area per lipid molecule and the concentration of the peptides, and that these effects were more pronounced for Met-enk-NH_2. The decrease in monolayer surface tension after the injection of the peptides was much more pronounced at surface concentration of 150 Å^2 per molecule of both lipids, and was reduced with lowering the area per lipid molecule. The latter effect was due to the more "diluted" state of DMPC and DMPG monolayers at the larger area per molecule and, therefore, the amphiphilic peptides could easily penetrate the monolayers.

The reduction of the given area per lipid molecule to 100 Å^2 resulted in a decrease in γ values of both phospholipid monolayers to a smaller degree as compared with the larger area per phospholipid molecule. This effect was logical, since with lowering the given area per lipid molecule the number of neuropeptide molecules that could penetrate the monolayer decreased, as compared to the larger area per molecule, which could explain the smaller decrease in γ values.

At high surface concentration, the compact ordering of the lipid molecules at 75 Å^2 per molecule impaired the penetration of the unmodified neuropeptide into the monolayers of both lipids. However, even at this high surface concentration, Met-enk-NH_2 was able to penetrate both monolayers. A possible explanation of the fact that the amidated enkephalin penetrated the monolayers to a higher degree as compared to the unmodified peptide is that at the pH 5.77 of the subphase the synthetic Met-enk is near to its isoelectric point 5.695. Therefore, at this pH, most of the peptide molecules were in its zwitterionic form, while the addition of an amino group to the C-terminus of the pentapeptide converted the zwitterionic molecule to a cation that is capable to interact electrostatically with the negative phosphate group of the phospholipids.

In addition, the comparison between the change in γ values of PC and phosphatidylglycerol monolayers after the injection of Met-enk into the subphase did not show any difference. These results indicated that the charge of the phospholipid head groups most probably had an insignificant effect on the interaction of the synthetic methionine-enkephalin with the negatively charged DMPG and the zwitterionic DMPC, at the pH studied, and that most probably the change in γ values was a result predominantly of a nonspecific adsorption (Table 1; Fig. 4). In contrast, the derivate of Met-enk showed different behavior to DMPC and DMPG monolayers. It is seen (Table 1; Fig. 4) that the amidated derivate revealed significantly higher $\Delta\gamma$ values for DMPG monolayers at surface lipid concentration of 75 Å^2 as compared to DMPC monolayers. These observations were confirmed by the determination of the maximum surface pressure at which the peptides were not able to penetrate into the monolayers (Fig. 5). Significantly, higher $\pi_{critical}$ values and penetration capacity for the amidated peptide in comparison with Met-enk to DMPC and especially to the negatively charged DMPG monolayers were seen. Our results were in agreement with the findings of Jaikaran et al. (1995) who observed that gramicidin derivates with C-terminal amino groups incorporate readily and refold quickly when added to dioleoylphosphatidylcholine lipid vesicles from concentrated methanol solutions. They suggested that the amino groups at C-terminus of gramicidin interact with lipid phosphate groups (more strongly

than carboxylates interact with phospholipid head groups) and provide some sort of anchor for the C-terminal end of the peptides during the refolding process. According to our results, most probably at 75 $Å^2$ per molecule occurs repulsion between the negative charge of the terminal carboxyl group and the negative charge of the lipid phosphate group, which could explain why the unmodified form of Met-enk was excluded from both phospholipid monolayers studied at higher surface pressure, in contrast to the amidated derivate. With this regard, the blocking of the peptide C-terminus with the amino group resulted in stronger penetration of the modified opioid even at higher surface pressure. In addition, the positive charge of the amidated Met-enk facilitated its interaction with the negatively charged DMPG head groups at low lipid surface concentrations as well. Our findings suggested that the interaction between peptides and phospholipids was due to a combination between electrostatic attractions in case of the Met-enk-NH_2 and hydrophobic interactions between the hydrophobic amino acids of the amphiphilic peptides and the phospholipids acyl chains. It is generally accepted that electrostatic interactions with negatively charged lipids are important for the association of enkephalins with membranes (Deber and Behnam 1984; Milon et al. 1990), while site-specific hydrophobic contacts facilitate its entry, concentration, and orientation into the lipid phase (Deber and Behnam 1984; Marcotte et al. 2003). Marcotte et al. (2003) found that the insertion of Met-enk into zwitterionic and negatively charged bicelles was attributed to the balance between electrostatic and hydrophobic forces. In their recent NMR spectroscopic studies, Marcotte et al. (2004) conclude that the association of enkephalins to anionic vesicles appears to be electrostatically driven as concluded by the increase in the proportion of hydrogen-bonded lipid head group carbonyl groups. Our results agree with this as the difference in the penetration capability between Met-enk-NH_2 and Met-enk increases from DMPC to DMPG monolayers (Fig. 5). Marcotte et al. (2004) found that the peptides were able to only slightly perturb the organization of the acyl chains in the membrane gel phase. In our experimental conditions, both DMPC and DMPG monolayers are in liquid-expanded state where the distance between the molecules is increased as compared to the gel phase. This might enhance the penetration of the amphiphilic peptides into the acyl chain region of the monolayers and can increase the impact of the hydrophobic interactions in comparison with the gel phase vesicles.

The different interactions to phospholipids of the peptides studied suggest differences in the surface morphology of the monolayer films at air/water interface which is the aim of our further investigations.

Conclusions

Based on the results obtained, we suggest that the surface interactions between the neuropeptides and both phospholipids were predominantly due to hydrophobic interactions. Electrostatic forces probably play role only at compact ordered lipid monolayers for the blocked peptide C-terminus by the amino group of methionine-enkephalin-NH_2. Moreover, the amidation of Met-enk results in converting the zwitterionic Met-enk into a cation, and therefore facilitates its penetration into the membrane lipid monolayers. Our results demonstrated as well that lipid monolayers formed in Langmuir's trough could be successfully used as an elegant and simple membrane models to study lipid–peptide (especially newly synthesized peptide analogues) interactions at the air/water interface.

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