Interactions of Selected Insect Neuropeptides with Synthetic Lipids

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Abstract. Interactions of the insect neuropeptide proctolin (Arg-Tyr-Leu-Pro-Thr), its [β-cyclohexyl-(4-O-methyl)^2]-L-alanine analog, and the leucopyrokinin [2–8]-fragment (Thr-Ser-Phe-Thr-Pro-Arg-Leu-NH_2), with synthetic phospholipids (DPPC, DMPE, DMPG) were studied using the microcalorimetric method. Most pronounced changes of the lipid thermotropic behavior were observed with DMPG /leucopyrokinin [2–8]-fragment mixtures. Proctolin itself was less active with all the lipids studied. The results obtained suggest that the studied peptides interact with the head group region of lipid bilayer.

Key words: Insect neuropeptides — Proctolin — Peptide-lipid interactions — Microcalorimetry

Introduction

Proctolin (Arg-Tyr-Leu-Pro-Thr) has first been isolated from the American cockroach (*Periplaneta americana*) hindgut by Brown and Starratt (1975), and then also found in six further insect orders (Brown 1977; Holman and Cook 1979). Its occurrence in other invertebrates has been discovered by Brown (1977), Adams and O'Shea (1983). Due to its biological activity with respect to insects, proctolin has been proposed as an insect neurotransmitter (Miller 1979). Further studies of the proctolin myotropic activity (reviewed by Orchard et al. 1989) have manifested its neurohormonal (or neuromodulatory) rather than neurotransmitter properties. Although the mode of proctolin action seems to be known (at least partly) now, its mechanism remains unknown up to date. Some recent data (Lange et al. 1987) suggest that proctolin might influence the phosphoinositol pathway and thus activate protein kinase C. This enzyme, however, can be expected to be activated (or modulated) also by changes in its lipid environment (Merino 1987; Epand et al. 1988). Such changes may be induced by direct interaction between the neuromodulator and the membrane lipids.

The aim of the present work was to determine whether proctolin can interact with different classes of phospholipids, and to answer the question if this presumable interaction plays any role in proctolin myotropic activity. We approached the problem by comparing results obtained for proctolin with those obtained for [Cha(4-OMe)^2]-proctolin, a neuropeptide analog which is myotropically inactive (Konopińska et al. 1986a). Additionally, we studied the interaction with the same lipids of the [2–8]-fragment of leucopyrokinin, a myotropically active substance isolated from the head of the Madeira cockroach, *Leucopheae maderae* (Nachman et al. 1986).

Materials and Methods

Synthetic phospholipids: dipalmitoyl-Lα-phosphatidylcholine (DPPC), dimyristoyl-Lα-phosphatidylethanolamine (DMPE) (both purchased from Calbiochem); and dimyristoyl-Lα-phosphatidylglycerol (DMPG - obtained from Avanti Polar Lipids Inc.) were used as delivered, without further purification. Neuropeptides were synthesized according to the procedures described elsewhere: proctolin (Konopińska et al. 1986b), leucopyrokinin [2–8]-fragment (Thr-Ser-Phe-Thr-Pro-Arg-Leu-NH_2) (Nachman et al. 1986), and [Cha(4-OMe)^2]-proctolin (Konopińska et al. 1986a). The [β-cyclohexyl-(4-O-methyl)^2]-L-alanine-proctolin analog was obtained by replacing the phenyl ring at Tyr residue in position 2 of the peptide chain by a more hydrophobic cyclohexane system. This modification of the peptide structure was prompted by the assumption that "para" substituted aromatic ring in position 2 plays essential role in the proctolin myotropic activity (Starratt and Brown 1979; Sullivan and Newcomb 1982; Konopińska et al. 1986a).

Samples for calorimetry were prepared by dispersing 2 mg of the respective lipid in 20 μl of the neuropeptide solution (Tris/HCl buffer, pH 7.5). Concentrations of the peptide solutions were chosen to obtain the desired final neuropeptide/lipid molar ratio in the sample. A standard procedure was used: the mixtures were preheated to temperatures about 10 °C above the phase transition of the respective lipid and shaken vigorously. Often, similar samples are prepared by codispersing peptides and lipids in nonpolar solvent followed by drying and hydration of the mixture. We have chosen the former method for at least two reasons. First, this method is used to insert hydrophobic molecules into bilayers (the peptides studied by us are hydrophilic); and second, in physiological tests peptides are always dissolved in aqueous media. Measurements were performed using a UNIPAN type 600 scanning microcalorimeter working in the DSC mode. The phase transition temperatures were detected with 0.1 °C accuracy, the transition enthalpy was determined with an error less than 10%. Each sample was scanned three times, and two preparations of each of the peptide/lipid combinations were run in parallel.

Results and Discussion

Amino acid sequence analysis of the peptides studied suggests their hydrophilic nature; this was partly confirmed by their good solubility in water. Molecules of such a nature are unable to penetrate the hydrophobic core of the lipid bilayer, however they are able to interact with the bilayer surface. To check whether this

Table 1. Phase transition parameters obtained for selected synthetic phospholipids mixed with insect neuropeptides. Peptide/lipid molar ratio was 0.1. For abbreviations see the text.

Lipid	Peptide	T_p [°C]	T_m [°C]	$T_{1/2}$ [°C]	ΔH [kJ/mol]
DPPC	proctolin	34.2	41.9	0.6	36.5
	[Cha(4-OMe)^2] proctolin	37.3	41.8	0.6	36.7
	leucopyrokinin [2–8] fragment	–	41.8	0.6	36.4
	none	35.5	41.8	0.6	36.5
DMPE	proctolin	–	50.4	1.0	19.1
	[Cha(4-OMe)^2]- proctolin	–	49.0	1.0	19.5
	leucopyrokinin [2–8] fragment	–	49.2	1.1	17.4
	none	–	50.1	0.9	24.7
DMPG	proctolin	15.5	25.3	4.3	28.7
	[Cha(4-OMe)^2]- proctolin	14.3	23.2	5.7	25.8
	leucopyrokinin [2–8] fragment	-	23.5	2.1	27.7
	none	18.5	25.1	1.3	28.5

also applied for the peptides studied, first mixtures were examined at the peptide/lipid molar ratio of 0.1. The experimental results are shown in Table 1; T_p and T_m represent pre- and main transition temperatures, respectively, $T_{1/2}$ is the width of the transition peak at its half-height, and ΔH is enthalpy of the transition. The data obtained by us for pure lipids are in agreement with those measured by other authors (see Cevc and Marsh 1987, and references therein). Examples of thermograms obtained for pure DMPE, DPPC, DMPG and for their mixtures with the neuropeptides studied are shown in Fig. 1. The results presented above seem to confirm the existence of interactions between the neuropeptides and some of the lipids under study. As follows from Tab.1 (also cf. thermograms in part A of Fig 1.), changes of the DPPC thermal properties induced in the presence of the peptides studied were very small or almost none. Only in the case of leucopyrokinin [2–8]-fragment disappearance of the lipid pretransition was observed. Also, the pretransition temperature shifts, recorded for proctolin and [Cha(4-OMe)^2]-proctolin

mixtures with DPPC, were accompanied by broadening and lowering of the transition peaks. Similarly as for DPPC, no pronounced changes in the phase transition character were recorded for DMPE mixtures with the neuropeptides studied (see Fig. 1*B*). A slight decrease (by approx. 1 °C) in transition temperature was observed for leucopyrokinin [2–8]-fragment- and the [Cha(4-OMe)^2]-proctolin-DMPE mixtures only.

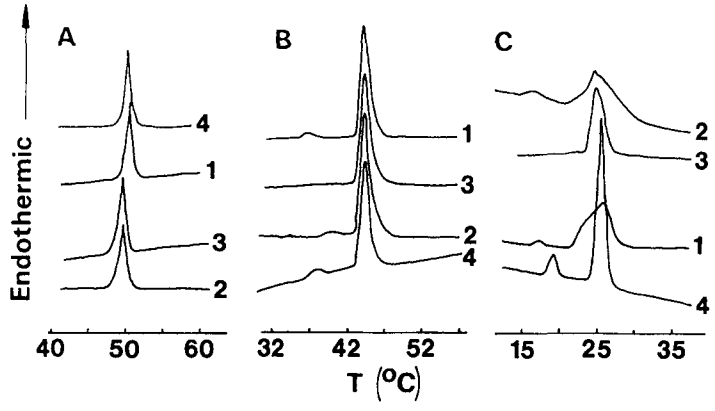

Figure 1. Examples of thermograms obtained for 0.1 mol peptide/mol lipid mixtures. *A*, mixtures with DMPE; *B*, mixtures with DPPC; *C*, mixtures with DMPG. 1, admixed proctolin; 2, [Cha(4-OMe)^2]-proctolin; 3, leucopyrokinin [2–8] fragment; 4, pure lipid. Scanning rate 1 °C/min.

Most pronounced changes of the phase transition parameters were observed for DMPG-neuropeptide mixtures (cf. thermograms in Fig 1*C*). Pretransition was abolished by the presence of leucopyrokinin [2–8]-fragment, the other two peptides caused significant decrease of the temperature of this transition (by approx. 3.0 °C and 4.2 °C for proctolin and [Cha(4-OMe)^2]-proctolin, respectively). In the presence of the leucopyrokinin [2–8]-fragment and [Cha(4-OMe)^2]-proctolin the main transition temperature was decreased by less than 2 °C. For all peptide-DMPG mixtures the transition peaks were much broader than those recorded for the pure lipid. Also, enthalpy of the main transition was slightly decreased when leucopyrokinin [2–8]-fragment or [Cha(4-OMe)^2]-proctolin were present in the mixture.

Since we were mainly interested in proctolin and its modified form ([Cha(4-OMe)^2]-proctolin), and as the preliminary results suggested interactions of both forms with DMPE and DMPG only, the second part of our work was focused on mixtures of these lipids with both forms of the peptide at various molar ratios. Differences of transition temperatures for mixtures from the value for pure lipid

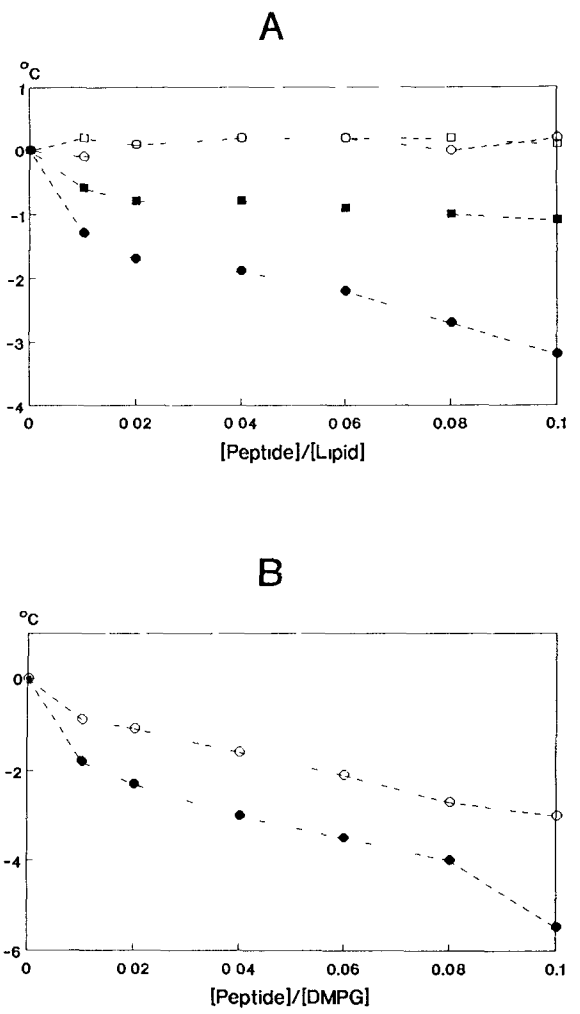

Figure 2. The dependence on peptide/lipid molar ratio of shifts from the respective temperatures for pure lipids. (*A*) main transition temperatures, (*B*) pretransition temperatures. Proctolin DMPG (empty circles); [Cha(4-OMe)^2]-proctolin DMPG (filled circles); proctolin/DMPE (empty squares); [Cha(4--OMe)^2]- proctolin/DMPE (filled squares).

(i.e., $T_{\text{mixture}} - T_{\text{pure}}$) were plotted vs. peptide/lipid molar ratio (Fig. 2*A* and 2*B*, main- and pretransition, respectively). Since there is no pretransition with phosphatidylethanolamines, Fig. 2*B* contains no data for peptide/DMPE mixtures. It appears from Fig. 2*A* that the main transition temperature for both lipids was not affected by the presence of proctolin, whereas [Cha(4-OMe)^2]-proctolin caused a decrease of T_m. This decrease was dose-dependent for peptide DMPG/mixtures, while this dependence seems to be very weak for peptide/DMPE. Two conclusions could be drawn from the T_m-peptide/lipid molar ratio relationship. Comparing the data obtained for peptide/DMPE and for peptide/DMPG mixtures it is obvi-

ous that negative charge of the lipid headgroup enhances the effectiveness of the peptide-lipid interactions. This conclusion is supported by the results presented in Fig. 3. When proctolin and its analog were codispersed with DMPG, they affected the transition half-width, whereas $T_{1/2}$ for peptide/DMPE mixtures was not changed significantly over the entire range of concentrations studied. It seems worth emphasizing that $T_{1/2}$ changed in a dose-dependent manner, even with proctolin/DMPG mixtures for which no change of T_m was recorded.

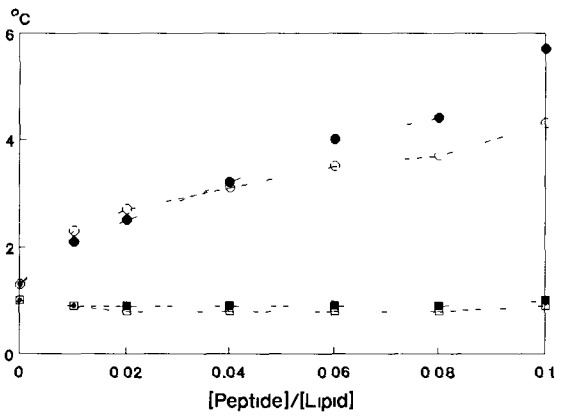

Figure 3. The dependence on peptide/lipid molar ratio of phase transition half-width. Proctolin/DMPG (empty circles); [Cha(4-OMe)^2]- proctolin/DMPG (filled circles); proctolin/DMPE (empty squares); [Cha(4 - OMe)^2] - proctolin /DMPE (filled squares)

A comparison of transition temperature shifts (Fig. 2*A*) obtained for lipid /proctolin and (the same lipid)/[Cha(4-OMe)^2]-proctolin mixtures shows that the more hydrophobic proctolin analog influences the temperature of main phase transition, while the less hydrophobic proctolin does not Also, the pretransition temperature of DMPG, which was changed in the presence of both peptides, was decreased in the presence of [Cha(4-OMe)^2]-proctolin to a greater extent than by proctolin itself (see Fig. 2*B*).

The stronger influence of the more hydrophobic compound on the lipid thermal behavior was not clearly confirmed by the observed transition enthalpies of the systems studied (see Fig. 4). Except for proctolin/DMPG mixtures, a slight decrease of ΔH was observed for all other systems examined. Since the experimental error of enthalpy determination was relatively large, this drop should be described as being dose-dependent for DMPE mixtures only. In spite of that error, we can also say that enthalpies of DMPG/[Cha(4-OMe)^2]-proctolin system were lower than that of DMPG/proctolin mixture.

The data presented above can be analyzed in two ways: either by comparing the action of the same peptide on different lipids or by comparing the influence of different peptides on the same lipid. Because all lipids used in the present experiments differ mainly in their polar parts, the first kind of analysis point to the type

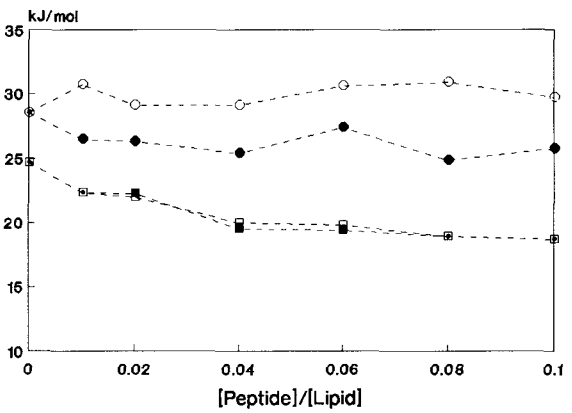

Figure 4. The dependence on peptide/lipid molar ratio of transition enthalpy. Proctolin/DMPG (empty circles); [Cha(4 - OMe)^2] - proctolin /DMPG (filled circles); proctolin/DMPE (empty squares); [Cha(4 - OMe)^2] - proctolin /DMPE (filled squares).

of the lipid head-group most suitable for neuropeptide-lipid interactions. Since the most pronounced changes of the phase transition character were detected for peptide/DMPG mixtures, we may conclude that this lipid interacts most easily with the neuropeptides under study. This is not surprising, because all peptides used in the present experiments contain arginine, which, as a positively charged amino acid, is expected to be attracted by the negatively charged molecules of DMPG. Such an interaction should, however, lead to an increase of T_m of mixtures (Van Dijck et al. 1978; Markin and Kozlov 1984), rather than to a decrease of this parameter as observed by us. This reversed effect might be explained as follows. After electrostatic incorporation into (or adsorption to) the membrane, a further process of penetration of the polar head-groups region by peptides occurs. Some results obtained by us seem to support this explanation. It is well known that pretransition (if any with the given lipid) is highly sensitive to the bilayer surface hydration (Janiak et al. 1976). The dose-dependent decrease of T_p observed by us may be due to a lowered accessibility of the bilayer to water, caused by the increasing amounts of the peptide bound to the lipid surface. Penetration of the neuropeptide into the polar region of the lipid layer is presumably followed by loosening of head-group packing, resulting in a decrease of T_m. Since the peptides studied by us possess also some hydrogen donating and/or hydrogen accepting groups, in the first step of interaction electrostatic attraction can be replaced by hydrogen bonding. This mechanism can be expected to be operative in the case of peptide DMPE/mixtures. The fact that DMPE transition enthalpies were altered by the presence of the studied peptides not so strongly as transition temperatures were, suggests (Blume 1980; Epand and Sturtevant 1984) that the headgroup region rather than the hydrocarbon one was affected by those neuropeptides. The relatively high, in comparison to other phospholipids of the same chain length and degree of unsaturation, gel-to liquid crystalline phase transition

temperatures of phosphatidylethanolamine are attributed to their strong hydrogen bond forming potency (Hauser et al 1981, Boggs 1987) It seems possible that leucopyrokinin [2–8]-fragment and [Cha(4-OMe)^2]-proctolin bind easier to the DMPE bilayer surface and modify the head-group ordering Since the head-groups of phosphatidylethanolamines are tightly packed, neuropeptides cannot penetrate DMPE bilayer easily, and this is why the observed effects of interaction are weaker than those observed for peptide/DMPG mixtures

The lack of any pronounced changes of the peptide/DPPC mixtures phase transition parameters presumably is based on the zwitterionic character of phosphatidylcholine Electrostatic interactions of peptides with the DPPC head-group dipole is probably too weak to anchor them to the bilayer surface, thus preventing any other steps of interaction

Analysis of the influences of different peptides on a given lipid shows that a more hydrophobic peptide is more efficient in disturbing the lipid structure It is known (Epand and Raymer 1987) that the more hydrophobic small peptides are, the deeper they intercalate into the lipid bilayer Since in our case the peptide molecules are only partly hydrophobic, we can expect that they are able to penetrate up to the polar-hydrocarbon interface of the lipid layer only However, the lack of any pronounced changes in the character of phase transition of zwitterionic DPPC suggests that hydrophobicity of the peptide molecules presumably plays a secondary role in their interactions with lipids Nevertheless, as it is easy to see from Fig 2, the more hydrophobic [Cha(4-OMe)^2]-proctolin interacts with DMPG stronger than proctolin itself

Some of our results seem to show that also the hydrocarbon core of the lipid bilayer is influenced by the presence of peptides in certain cases Namely, for peptide/DMPG mixtures we have observed dose-dependent increase of transition half-width, which is related to the cooperativity of transition, i e the parameter describing the interchain interactions in the hydrocarbon core of lipid bilayer (Lee 1974)

Considering the results presented above we can conclude that i) the influences of all of the peptides studied are the strongest on the charged lipid (DMPG), ii) with respect to single lipids, the most effective in all cases was leucopyrokinin [2–8] fragment, iii) the effects obtained with proctolin and [Cha(4-OMe)^2]-proctolin suggest that the presence of aromatic ring impedes neuropeptides to interact with lipids The latter ought to be related to the fact that [Cha(4-OMe)^2]-proctolin in much less bioactive than proctolin (Konopińska et al 1989) This suggests that membrane lipids are not directly involved in the proctolin action, this cannot be excluded in the case of leucopyrokinin [2–8]-fragment

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