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THE INFLUENCE OF STEROLS ON THE SENSITIVITY OF LIPID BILAYERS TO MELITTIN

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SUMMARY: The sensitivity of planar lipid bilayers to the permeabalizing effect of melittin was evaluated when sterols of varying structure were incorporated into the membrane. The addition of increasing amount of cholesterol (0-50 mole %) decreased the sensitivity of membranes formed from negatively charged phospholipids to melittin but did not (in amount of up to 66 mole %) change the sensitivity of membranes formed from zwitterionic lipids. 7-Dehydrocholesterol, stigmasterol and ergosterol had the same ability as that of cholesterol to decrease the membrane sensitivity to melittin, while lanosterol had no effect on the sensitivity of membranes to melittin. The results suggest that the effect of sterols is complex and cannot be explained only by a direct interaction of melittin with cholesterol, by a decrease of membrane fluidity, or by changes in distribution of surface charge.

Melittin, a major component of bee venom, is a peptide composed of 26 amino acids, exhibiting a pronounced amphiphilic structure, which favors the interaction of melittin with membranes (1). At high concentrations melittin induces lysis of membranes (2, 3). At sublytic concentrations, melittin forms voltage-dependent ion channels in planar lipid bilayers (4-6).

The influence of cholesterol on the channel-forming activity of melittin has not been evaluated, though it is well known that sterols can influence the sensitivity of membranes to a wide array of antibiotics and toxins, enhancing the effect of some compounds (7-9) and decreasing the effectiveness of the others (10-12). The structural features of the sterol molecule, such as the number and position of double bonds in the tetracyclic nucleus or branching of the alkyl side chain, markedly influences the ability of sterols to alter the sensitivity of the membranes to membrane-active compounds (12-15).

The present study evaluates the sensitivity of planar lipid bilayers formed from neutral and negatively charged phospholipids to the permeabalizing effect of melittin in the presence and absence of sterols of varying structure.

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MATERIALS AND METHODS Synthetic 1,2-dioleoyl-*sn*-glycero-3-[phospho-L-serine] (sodium salt) (DOPS); 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE); 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC);and 1,2-dioleoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (DOPG) were obtained from Avanti Polar Lipids, Inc. (Pelham, AL). All electrolytes were reagent grade. All water was distilled and deionized. Salt solutions for bilayer experiments were buffered by MOPS (5 mM in 1M NaCl) to pH 6.5. Melittin, HPLC-purified; cholesterol, 99%+ purity; 7-dehydrocholesterol, 98% purity; ergosterol, 90% purity; stigmasterol, 96% purity; and lanosterol, 97% purity, were purchased from Sigma Chemical Company (St.Louis, MO). The structural formulas of the sterols used in this study are presented in Figure 1.

"Solvent-free" membranes were prepared as described by Montal and Mueller (16). A detailed description of methods used for membrane preparation and single channel data analysis may be found elsewhere (17, 18).

The concentration of melittin that affected bilayer conductivity was determined by measuring the effect of increases of melittin concentration on several membranes of the same lipid composition. Melittin was added from stock solutions (1-100 μg/ml) in ethanol only to the *cis* side of each membrane by step-wise increases in concentration in the following sequence: 0.01; 0.02; 0.04; 0.08; 0.2; 0.4; 0.8 μg/ml. After finding the concentration that induced an increase in bilayer conductivity of any amplitude in the range 100 pS - 10 nS, and where this conductivity did not decrease during a 20 minute interval, several (3-5) membranes were tested with this melittin concentration to determine the reproducibility of the observation. If the effect was not reproducible, the step-wise increase in melittin concentration was continued. The entire procedure was repeated 3-5 times. The average concentration step that was between the minimal and the maximal one was assumed to represent a threshold concentration level for the effect, and is referred to in this study as the "active concentration", which may be considered as a measure of the sensitivity of the membranes to melittin.

RESULTS AND DISCUSSION

The melittin molecule has an effective charge about +2 (though a formal net charge of +6) (1), therefore, we compared the sensitivity to melittin of bilayers formed from zwitterionic phospholipids (an equimolar mixture of DOPE and DOPC) with that of bilayers formed from

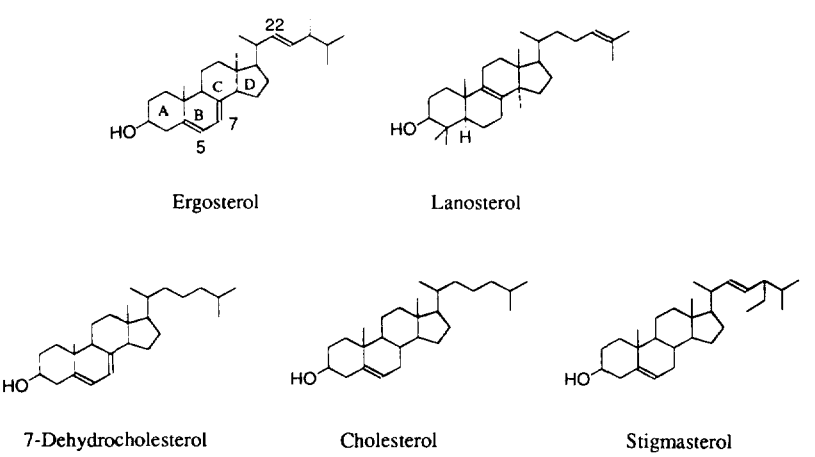

Figure 1. Structures of the sterols used in this study.

either pure negatively charged lipid (DOPS) or from the mixture of zwitterionic phospholipids with negatively charged DOPG, all with and without the addition of cholesterol. The active concentrations of melittin for the bilayers formed from zwitterionic lipids, without the addition of negatively charged lipid, were 20 times higher than those for bilayers formed either from negatively charged DOPS or from the mixture of zwitterionic phospholipids with negatively charged DOPG (Table 1). The addition of cholesterol to bilayers formed only from zwitterionic lipids did not influence membrane sensitivity to melittin, while addition of cholesterol to the negatively charged lipids decreased the sensitivity of these negative bilayers to melittin (Table 1). Also, in both types of membranes with negative electric charge, cholesterol induced the same suppressive effect on the membrane's sensitivity to melittin. The dependence of the active concentration of melittin on the content of cholesterol in bilayers formed from mixtures of DOPS and cholesterol is presented in Figure 2. It is apparent that augmenting the DOPS bilayer with cholesterol (from 0 to 50 mole%) markedly decreases the sensitivity of the bilayer to melittin. These observations suggest that the presence of negative charge is crucial for the high sensitivity of membranes to melittin as well as for the expression of the suppressive effect of cholesterol on this sensitivity, probably, through the influence of cholesterol on electrostatic interaction between positively charged molecules of melittin and negatively charged phospholipids. However, the mechanism of this influence is not clear, because it has been demonstrated that incorporation of the sterols into the bilayers containing charged phospholipids produced no

TABLE 1. ACTIVE CONCENTRATIONS OF MELITTIN FOR BILAYERS OF DIFFERENT STEROL AND PHOSPHOLIPID COMPOSITIONS

DOPC mole %	DOPE mole %	DOPG mole %	DOPS mole %	Sterol mole %	Melittin, active conc.* μg/ml
0	0	0	100	0	0.02 (0.01-0.04)
0	0	0	50	Cholesterol 50	0.4 (0.2-0.8)
37.5	37.5	25	0	0	0.02 (0.01-0.04)
12.5	12.5	25	0	Cholesterol 50	0.4 (0.2-0.8)
50	50	0	0	0	0.4 (0.2-0.8)
25	25	0	0	Cholesterol 50	0.4 (0.2-0.8)
0	0	0	50	7-Dehydrocholesterol 50	0.4 (0.2-0.8)
0	0	0	50	Ergosterol 50	0.4 (0.2-0.8)
0	0	0	50	Stigmasterol 50	0.4 (0.2-0.8)
0	0	0	50	Lanosterol 50	0.02 (0.01-0.04)
12.5	12.5	25	0	Lanosterol 50	0.02 (0.01-0.04)

*The minimal and maximal concentrations of melittin that produced a sustained increase in conductivity are shown in brackets.

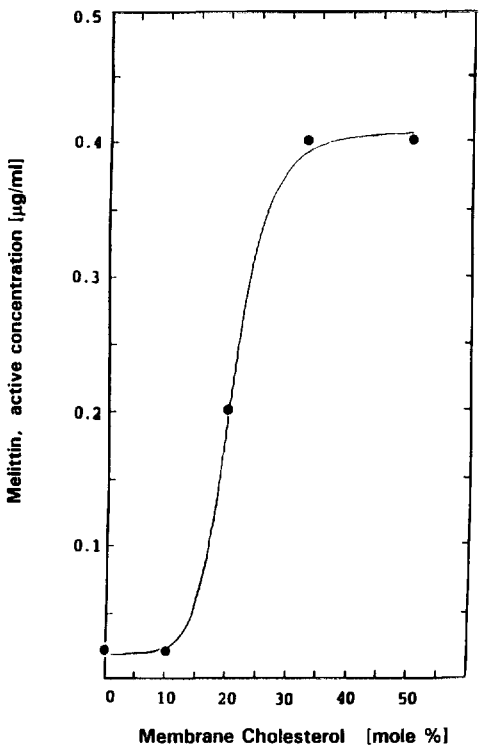

Figure 2. Active concentration of melittin as a function of the mole percent of cholesterol in DOPS bilayers. Melittin was added to the *cis* side of the bilayer in the bathing solution (1M NaCl, 5mM MOPS, pH 6.5). The transmembrane voltage was 100 mV, positive on the *cis* side.

change in surface potential (19). Another possible explanation for this phenomena may be associated with the limited solubility of cholesterol in negatively charged phospholipids compared with its solubility in neutral phospholipids (20).Having taken into account also the fact that at high concentrations cholesterol forms a separate phase in bilayers (21), Schagina and co-workers suggested (12) that the presence of cholesterol in a separate phase was necessary for the inactivation of gramicidin A channels, and a direct interaction of gramicidin A channels with a cholesterol in this separate phase was responsible for channel inactivation. The larger amount of cholesterol within a separate phase in the negatively charged membranes, compared with that in the neutral membranes, may be responsible for the decrease in sensitivity of negatively charged bilayers. However, we observed that the sensitivity of bilayers formed from a mixture of DOPC:DOPE:Cholesterol = 1:1:4 (i.e. 66 mole % of cholesterol) was the same as that of bilayers containing lesser amounts (0-50 mole %) of cholesterol (data not shown). This observation suggests that the effect of cholesterol on the sensitivity of bilayers to melittin is not

connected with the formation of a separate phase of cholesterol, and, thus, it is unlikely that a direct interaction between melittin and cholesterol is responsible for the cholesterol effect.

Our results (Table 1) show that 7-dehydrocholesterol, stigmasterol and ergosterol have the same ability to decrease membrane sensitivity to melittin as cholesterol has. These results demonstrate that neither the presence of the 5,7-dien system in ring B of the sterol nucleus (7-dehydrocholesterol and ergosterol) nor the branching of the side chain of the sterol molecule and placement of a double bond in position C_{22}-C_{23} (ergosterol and stigmasterol) influence the ability of sterols to decrease the sensitivity of DOPS bilayers to melittin. This is in contrast to the strong effect of the 5,7-dien system on the sensitivity of membranes to polyene antibiotics (13, 14) and to gramicidin A (12) and the major effect of the structure of the side chain of the sterol molecule on the sensitivity of membranes to iturin A (15). Lanosterol had no effect on the sensitivity of DOPS membranes to melittin, probably due to the presence of three additional (compared with other sterols in this study) methyl groups in the tetracyclic nucleus. Since the effects of cholesterol, 7-dehydrocholesterol, stigmasterol and ergosterol on physical properties of bilayers (such as mobility of the fatty acid residues) are similar, though not identical (14, 22), while those of lanosterol are quite different (23), it is possible that it is the ability of the sterols to decrease the mobility of fatty acid residues in membrane phospholipids that is responsible for the modulation of membrane sensitivity to melittin. Even though this explanation may be viable, there remains the question of why cholesterol did not decrease melittin sensitivity of bilayers formed from neutral phospholipids.

Analyses of our data and the data of others suggest that the decrease of bilayer sensitivity to melittin by cholesterol has a complex cause and cannot be simply dependent on either a direct interaction of melittin with cholesterol, a decrease of membrane fluidity, or changes in distribution of surface charge.

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