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EFFECT OF ALAMETHICIN, GRAMICIDIN S AND MELITTIN UPON THE PARTICULATE GUANYLATE CYCLASE FROM RAT LUNG

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Summary

The channel-forming antibiotic alamethicin activated rat lung particulate guanylate cyclase (GTP pyrophosphate-lyase (cyclizing) EC 4.6.1.2), and the activated enzyme was further stimulated by sodium nitroprusside when a thiol such as 2-mercaptoethanol was present. Similar effects were seen with the antibiotic gramicidin S and with melittin, a polypeptide purified from bee venom. All of these agents are amphiphilic polypeptides. Nitroprusside was not able to stimulate both particulate and soluble enzyme treated with the nonionic amphiphile, Lubrol PX, suggesting that the membrane-active polypeptides had a different mechanism of action. These polypeptides are known to alter the membrane matrix by binding to phospholipid, and we suggest that this alteration allowed greater access of substrate and of nitroprusside to the enzyme. Lubrol PX, however, may interact preferentially with the enzyme, and thus block nitroprusside activation. The most potent of these agents was melittin, which stimulated nitroprusside activation at a concentration which had little effect by itself (7 μM), and at which others have demonstrated lytic effects on cells.

Introduction

Guanylate cyclase (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2) has been found in both soluble and insoluble forms in most mammalian tissue homogenates [1—4]. The soluble enzyme has been reported to be activated by nitric oxide [5] and compounds capable of generating nitric oxide such as nitroprusside [6,7] and carcinogenic nitrosamines [6,8]. However, the

particulate enzyme was stimulated to a much lower extent than the soluble enzyme [5,7]. Only nonionic detergents [3,9,10] and naturally occurring amphiphiles like lysolecithin [11,12] have been found to stimulate consistently the activity of particulate guanylate cyclases. Most of the particulate activity is latent, but it can be assayed in the presence of nonionic detergents [9—12].

Recently, a channel-forming ionophore, alamethicin, was reported to activate the adenylate cyclase and ($Na^+ + K^+$)-ATPase activities in cardiac sarcolemmal vesicles [13]. The authors suggested that the channels formed through the vesicle membrane by alamethicin are of sufficient size to allow entry of ATP and thus effectively eliminate the sidedness of these membrane-bound enzymes. We therefore investigated the possibility that alamethicin and similar polypeptides would enable the assay of the latent activity of membrane-bound guanylate cyclase. We found that not only did alamethicin stimulate the activity of particulate rat lung guanylate cyclase, but that this stimulated activity could be further increased by sodium nitroprusside. Similar effects were found with gramicidin S and melittin.

Materials and Methods

GTP, polymyxin E, showdomycin and gramicidin S were purchased from P-L Biochemicals; [α-^{32}P]GTP and cyclic [^{3}H]GMP were obtained from New England Nuclear. Cyclic GMP, creatine phosphate, creatine phosphokinase, gramicidin D, polymyxin B, nonactin, valinomycin and other unspecified biochemicals were from Sigma. Alamethicin was the generous gift of Dr. George B. Whitfield of the Upjohn Company. Bio-Gel A-5m was obtained from Bio-Rad. The sources of other reagents have been reported [14].

Enzyme preparation

The male Sprague-Dawley rats used were from our own out-bred colony, the strain originating at Charles-River Laboratories. The animals (100—250 g) were decapitated and the lungs perfused in situ with 10 ml cold buffer (50 mM Tris-HCl, pH 7.6, 30 mM 2-mercaptoethanol) by injecting into the right ventricle. The lungs were removed, washed twice in buffer, blotted dry, weighed and placed in 3 vols. buffer (w/v). The tissue was homogenized for 10 s at 10 000 rev./min with a Willems Polytron equipped with a PT 20 ST generator. The homogenate was filtered through a 40-mesh stainless steel wire screen and then centrifuged for 10 min at 8000 $\times g$ in the SS-34 rotor of a Sorvall refrigerated centrifuge. The supernatant solution so obtained was recentrifuged at 160 900 $\times g$ for 40 min in the 65 rotor of a Beckman L5-65 Ultracentrifuge. The pellet from this centrifugation was resuspended in fresh buffer (microsomal fraction), while the supernatant solution was used as the soluble enzyme. In order to remove contaminating soluble enzyme from the microsomes, this suspension was applied to a 1.6 $\times$ 18 cm Bio-Gel A-5m column, eluted with the homogenizing buffer, and the fractions containing the void volume pooled.

Guanylate cyclase assay

The enzyme preparations were kept in ice until assayed. 25-μl aliquots (10—30 μg enzyme protein) were added to prewarmed reaction tubes. The tubes

already contained any additions of antibiotic or Lubrol PX, usually in 25 μl and the mixture was incubated for 4—5 min, after which the constituents of the guanylate cyclase reaction mixture were added. When sodium nitroprusside was used to stimulate activity, it was added at this time. The reaction mixture comprised (in final a volume of 75 μl) the pretreated enzyme, 1.2 mM [α-^{32}P]-GTP (approx. 500 000 cpm), 6 mM $MnCl_2$, 10 mM 2-mercaptoethanol, 50 mM Tris-HCl (pH 7.6), 5 mM cyclic GMP, 15 mM creatine phosphate and 10 units of creatine phosphokinase. After 5—7 min reaction time the reaction was stopped with 0.15 ml of 1.0 M $HClO_4$, the tubes were placed in ice and 0.3 ml of H_2O containing approx. 30 000 cpm cyclic [^{3}H]GMP was added. The cyclic [^{32}P]GMP was purified from the reaction mixture using the two-step procedure of White and Karr [14], and counted as previously described [15]. Proteins were determined by the method of Lowry et al. [16], after precipitation with silicotungstic acid [17]. All assays were performed in triplicate, and activities are given as the mean ± S.E.

Results

Effect of alamethicin on guanylate cyclases

Fig. 1 shows the effect of alamethicin on the activity of the particulate and soluble guanylate cyclases, 2 mg/ml alamethicin increased particulate activity about 200% whereas with the soluble enzyme the maximal increase was only

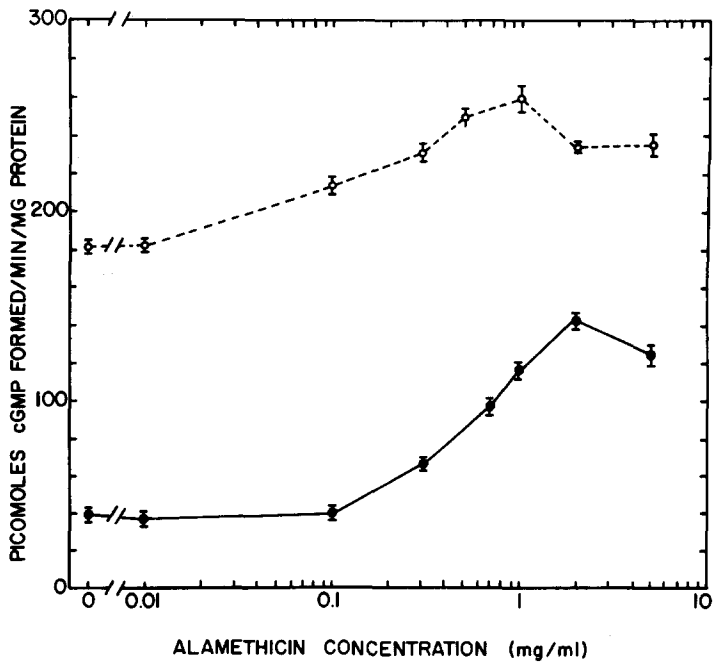

Fig. 1. The effect of alamethicin on particulate (•) and soluble (○) guanylate cyclases. Microsomal and soluble fractions were prepared from a rat lung homogenate. Reaction tubes containing aliquots of the lung fractions and the indicated concentrations of alamethicin were kept in ice for 10 min, after which they were transferred to a 30° C water bath for a 3 min warm-up and assayed for 6 min.

35%. As previously mentioned, nitric oxide and nitroso compounds including nitroprusside can effect very large increases in soluble guanylate cyclase but only small stimulations (1- to 3-fold) have been reported for the particulate enzyme from all tissues except brain, where a 10- to 15-fold stimulation was found with nitric oxide [15], and a 5-fold stimulation with nitroprusside [7]. The stimulatory effect of 1 mM nitroprusside on particulate rat lung enzyme is shown in Table I. To avoid interference with contaminating soluble enzyme, this microsomal preparation was gel filtered on Bio-Gel A-5m. Nitroprusside activated the enzyme 2- to 2.5-fold. Alamethicin produced a greater activation at 37°C (3.4-fold) than at 30°C (2.1-fold). However, when both nitroprusside and alamethicin were added, the activity resulting was higher than that found in the presence of Lubrol PX. It therefore appeared that alamethicin treatment had allowed nitroprusside access the latent enzyme. However, Lubrol PX, which solubilized particulate enzyme [10], blocked nitroprusside activation (data not shown).

A remaining possibility was that alamethicin, like Lubrol PX, could solubilize particulate activity, but that in this case the solubilized activity would be nitroprusside activated. In experiments not shown, we found that alamethicin did solubilize about 20% of the particulate activity, with little solubilization of protein; however, this activity was not activated by nitroprusside. Therefore, alamethicin potentiation of nitroprusside activation had primarily to involve particulate activity.

Effects of other antibiotics

From the previous results it appeared that alamethicin was unmasking latent guanylate cyclase activity, as had been previously demonstrated with adenylate cyclase [13]. One possible explanation for this effect was that alamethicin had increased the permeability of the microsomal vesicles, and thus increased the substrate availability to the enzyme. Chapman and associates have shown that interaction of alamethicin with phospholipid bilayers induced reaggregation of the phospholipid molecules, with a concomitant reduction in motions of the lipid alkyl chains [18—20]. They suggested that this reorganization might be

TABLE I

SODIUM NITROPRUSSIDE ACTIVATION OF ALAMETHICIN TREATED PARTICULATE GUANYLATE CYCLASE

Aliquots of gel filtered microsomal preparations were preincubated where indicated with no addition, with 1 mg/ml alamethicin or with 0.9% Lubrol PX. Activity was then assayed with (+SNP) and without (—SNP) 1 mM sodium nitroprusside for 6 min at 30°C and for 5 min at 37°C. Values shown are the mean ± S.E.

Enzyme treatment	Cyclic GMP formation (pmol/min per mg protein)			
	Assayed at 30°C		Assayed at 37°C	
	—SNP	+SNP	—SNP	+SNP
None	211.6 ± 4.2	549.8 ± 18	419.8 ± 2.14	1055.8 ± 39
Alamethicin	452.2 ± 10	1449.8 ± 143	1441.2 ± 67.8	2596.0 ± 43
Lubrol PX	1154.7 ± 19.6		2211.2 ± 28.3	

relevant to the ion transport properties of alamethicin. Since other antibiotics such as valinomycin and gramicidin have similar activities [24], we compared their effects upon guanylate cyclase with that of alamethicin. Table II shows that the neutral carriers valinomycin (a depsipeptide) and nonactin (a macroterolide) did not increase activity, with or without nitroprusside. Neither did showdomycin, a membrane active nucleoside. The amphipathic antibiotic polymyxin B (contains a polypeptide ring attached to a polypeptide chain terminating with a branched 8—9-carbon fatty acid residue) activated the basal activity, but this was not greatly potentiated by nitroprusside. Polymyxin E had no effect, nor did the channel-forming linear polypeptide containing hydrophilic amino acids, gramicidin D. However, the cyclic polypeptide gramicidin S strongly stimulated activity, biphasically (Fig. 2). Maximal nitroprusside stimulation occurred with 0.07 mg gramicidin S/ml, however, without nitroprusside maximal activation occurred at a higher gramicidin S concentration. Markedly different results were obtained with the soluble enzyme (Fig. 3). Gramicidin S had only an inhibitory effect upon activity, the effect being most pronounced in the presence of nitroprusside. Gramicidin S differs from alamethicin in that it interacts with phospholipid only electrostatically [22], while alamethicin interaction has a hydrophobic component [20]. Furthermore gramicidin S has no ion transport activity [19]. We conclude therefore, that the ability of antibiotics to activate guanylate cyclase was not related to pore-forming ability, but rather to their interaction with and rearrangement of the cell membrane lipid matrix.

TABLE II

EFFECTS OF SEVERAL ANTIBIOTICS ON PARTICULATE GUANYLATE CYCLASE

Aliquots of a gel filtered microsomal preparation were preincubated with each antibiotic for 5 min at 37°C and then assayed for 6 min, with (+SNP) and without (—SNP) 1 mM sodium nitroprusside. Values shown are the mean ± S.E.

Enzyme treatment Antibiotic	mg/ml	Cyclic GMP formation (pmol/min per mg protein)	
		—SNP	+SNP
None	—	180.7 ± 5.2	518.6 ± 9.3
Lubrol PX	1.8%	1185.3 ± 3.7	1067.6 ± 53.6
Valinomycin	0.07	164.5 ± 7.3	485.3 ± 33
	0.5	119.6 ± 3.9	415.9 ± 6
Gramicidin D	0.07	149.2 ± 6.7	479.0 ± 4.3
	0.5	84.4 ± 5.4	330.8 ± 3.7
Gramicidin S	0.07	1027.9 ± 23	1868.2 ± 32
	0.5	386.0 ± 19.2	343.0 ± 18
Polymyxin B	0.07	411.6 ± 4	594.8 ± 14.3
	0.5	386.0 ± 7.5	479.8 ± 16
Polymyxin E	0.07	160.6 ± 8.2	513.9 ± 14.7
	0.5	152.2 ± 5.4	495.7 ± 18
Showdomycin	0.07	142.1 ± 4.8	468 ± 4.7
	0.5	86.8 ± 6.5	359.9 ± 12.7
Nonactin	0.07	138.8 ± 2.7	451.7 ± 6.5
	0.5	76.7 ± 7.3	275.1 ± 2.4

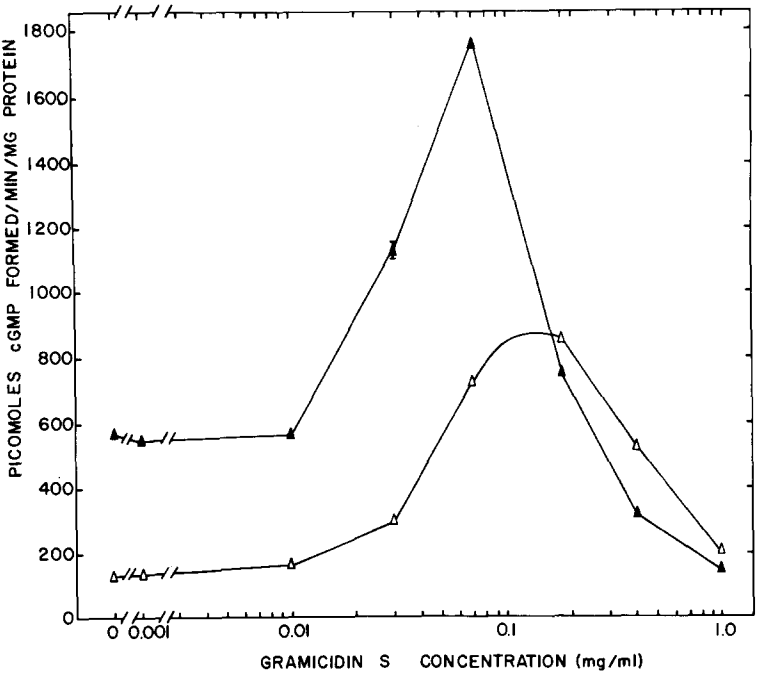

Fig. 2. The effect of gramicidin S on particulate guanylate cyclase. Aliquots of a gel-filtered microsomal preparation were preincubated with the indicated concentrations of gramicidin S for 5 min and assayed for 6 min at 37° C. Reactions were performed with (▲) or without (△) 1 mM sodium nitroprusside.

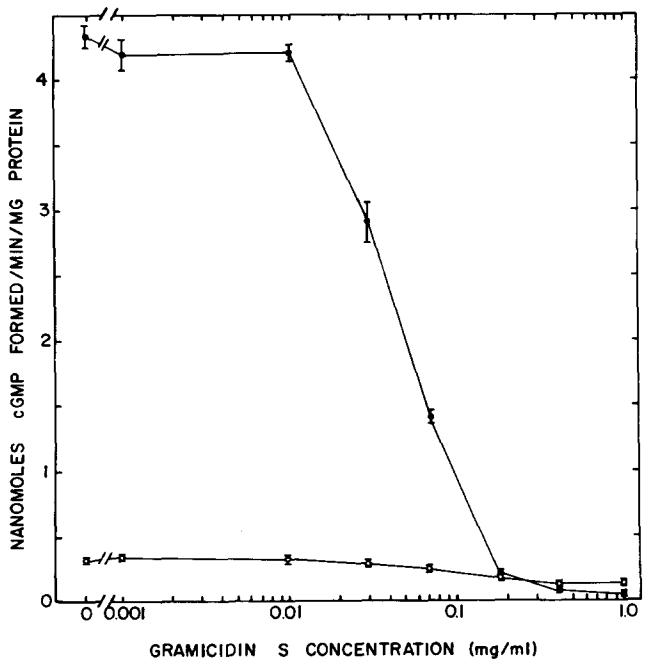

Fig. 3. Effect of gramicidin S on soluble guanylate cylase. Aliquots of soluble enzyme were pretreated with gramicidin S and assayed at 30° C, as in Fig. 2.

Effects of melittin

The previous conclusion was further supported by studies with the linear polypeptide from bee venom, melittin (26 amino acids). Melittin is not known to have pore-forming ability but has been shown to bind to phospholipids by hydrophobic interactions, in turn disturbing the organization of the apolar acyl chains [23—25]. As seen in Fig. 4. melittin by itself activated the enzyme in a concentration dependent manner. However, only small amounts of melittin were required for maximal nitroprusside stimulation. Thus at the lowest melittin concentration used, 20 μg/ml, which tripled activity, the inclusion of nitroprusside produced an additional doubling of activity. This appeared to be close to the maximal attainable activity since it was not greatly increased by higher concentrations of melittin, either with or without nitroprusside. The enzyme became progressively unstable above 150 μg melittin/ml, and nitroprusside activation disappeared at 225 μg/ml. Both melittin and alamethicin treatment induced permanent changes in the microsomes, since recentrifuged and resuspended particles were activated by nitroprusside 4.2- and 4.1-fold, respectively, as compared to 2.8-fold activation of comparable particles from untreated microsomes (data not shown). When soluble enzyme was treated

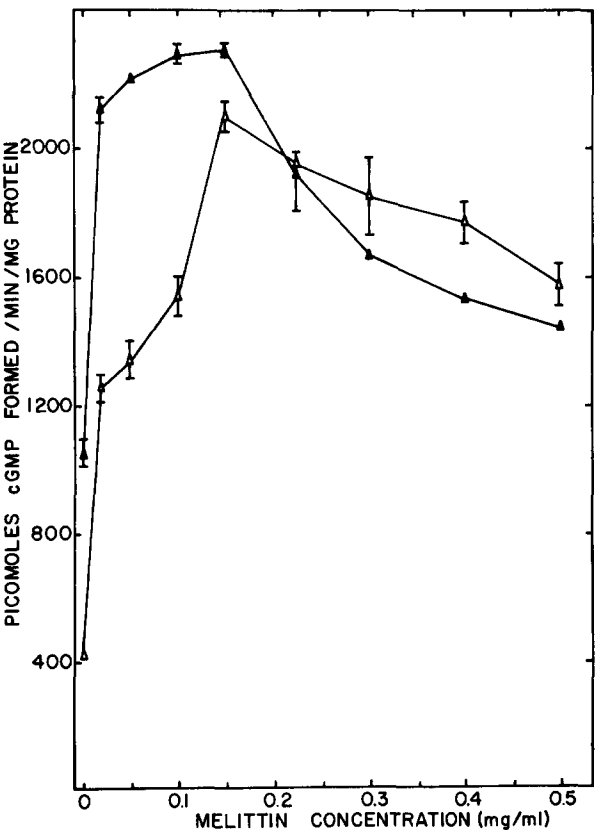

Fig. 4. Effect of melittin on particulate guanylate cyclase. The experimental procedure is described in Fig. 2.

TABLE III

EFFECT OF 2-MERCAPTOETHANOL ON SODIUM NITROPRUSSIDE ACTIVATION OF PARTICULATE GUANYLATE CYCLASE

A gel filtered microsomal preparation was prepared without 2-mercaptoethanol in the column buffer. The enzyme was pretreated with the indicated agents for 5 min and assayed for 6 min, at 37°C, with (+SNP) or without (—SNP) 1 mM sodium nitroprusside, and with or without 10 mM 2-mercaptoethanol. Values shown are the mean ± S.E.

Enzyme treatment	Cyclic GMP formed (pmol/min per mg protein)			
	Assayed without 2-mercaptoethanol		Assayed with 2-mercaptoethanol	
	—SNP	+SNP	—SNP	+SNP
Experiment 1				
None	109.3 ± 2.2	166.5 ± 5.2	121.1 ± 2.2	322.3 ± 9
Lubrol PX (0.9%)	809.0 ± 11.2		707.9 ± 26.3	
Alamethicin (1 mg/ml)	423.1 ± 28.9	428.5 ± 14.5	416.8 ± 11.4	1110.2 ± 25.1
Experiment 2				
None	122.6 ± 2	142.3 ± 4.6	127.3 ± 3.8	267.9 ± 8.6
Lubrol PX (1.2%)	765.5 ± 6.4	735.7 ± 5.4	847.3 ± 7.3	842.9 ± 10.4
Gramicidin S (75 μg/ml)	344.4 ± 16.3	386.5 ± 6.9	334.2 ± 6.0	813.3 ± 36.9

with melittin (data not shown), the results resembled those obtained with gramicidin S (Fig. 2), with melittin being 2.9-fold more toxic to nitroprusside activated enzyme than gramicidin S.

Potentiation of nitroprusside activation by 2-mercaptoethanol

We have previously shown that thiols such as 2-mercaptoethanol potentiate the activation of the soluble rat lung guanylate cyclase by nitroprusside [26], which was the reason that we included this thiol during the preparation of the microsomal enzyme. In order to examine this requirement, we omitted 2-mercaptoethanol from the column buffer during preparation of gel filtered microsomes. The results in Table III show that while nitroprusside alone could effect a small and variable increase in activity (1.2- to 1.5-fold) this stimulation was essentially abolished after alamethicin or gramicidin S treatment. However, when the antibiotic treated particles were stimulated with nitroprusside in the presence of 2-mercaptoethanol, activity increased 2.6-fold with alamethicin and 2.4-fold with gramicidin S. It appeared that after antibiotic treatment, thiol was absolutely required for nitroprusside activation.

Discussion

Results from this investigation suggest that alamethicin, gramicidin S and melittin induce changes in the microenvironment of the particulate guanylate cyclase that result in increased activity, and that this activated enzyme may be further activated by nitroprusside. It is this latter effect that distinguishes these polypeptide amphiphiles from the nonionic detergents, which also activate guanylate cyclase. Nonionic detergents such as Lubrol PX have been shown by Helenius and Simons [27] to bind to proteins with a hydrophobic character.

They suggest that when such proteins are membrane-bound, the hydrophobic regions on the proteins are occupied by lipid. Solubilization of these proteins by nonionic detergents therefore, consists of an exchange of bound detergent for bound lipid resulting in a complex with the hydrophilic part of the detergent molecule exposed to the aqueous medium, thus rendering the protein soluble. Presumably it is the ability of Lubrol PX to bind to solubilized guanylate cyclase that prevents nitroprusside activation. We have also found that nitroprusside activation of the soluble guanylate cyclase is blocked by Lubrol PX, as have Struck and Glossman [28]. In contrast with Lubrol PX, the membrane active polypeptides appear to interact preferentially with the membrane phospholipid rather than the protein [18—25]. This interaction may be entirely electrostatic, as in the case of gramicidin S [22], or it may involve both electrostatic and hydrophobic components as in the case of polymyxin B [22], alamethicin [21] and melittin [23]. Finer et al. [19] showed that both gramicidin S and alamethicin were capable of breaking down the bilayer structure of phospholipid liposomes. However, they had very different effects upon nuclear magnetic resonance spectra. Alamethicin in very small amounts produced a broadening of the signal from the hydrocarbon chain of sonicated phospholipid, which was interpreted as due to a reduced internal motion of the phospholipid molecules. The signal was completely removed when the alamethicin to phosphatidylserine ratio was 1 : 600. The presence or absence of ions did not influence these changes. The authors suggested that alamethicin induced a rearrangement of the phospholipid-polypeptide aggregate, and in this new structure a denser packing of the phospholipid molecules might occur which, by allowing less motion of the individual molecules, would cause broadening of the phospholipid signal. Since one molecule of alamethicin induces 600 molecules of phosphatidylserine to leave the bilayer state, Hauser et al. [20] suggested that the phenomenon must be regarded as an alamethicin-induced phase transition rather than the formation of a complex. In contrast, the mixing of gramicidin S with sonicated dispersions of egg yolk lecithin did not produce line broadening or reduction in signal area of the NMR spectrum in the absence of ions, although a reduction of signal was observed in 0.2 M phosphate buffer [19]. Furthermore, gramicidin S, unlike alamethicin, when added to unsonicated egg yolk lecithin dispersion caused the broad NMR band obtained with this preparation to be resolved into a high resolution spectrum. From additional work on this system Pache et al. [22] concluded that gramicidin S solubilized lecithin, the resulting particles being of molecular weight below 100 000, and it was the marked reduction in particle size that produced high resolution NMR signals. However, because gramicidin S did not penetrate the lipid chain region of the bilayer, these chains remained tightly packed and in a bilayer form. The sensitivity of the gramicidin S-lecithin mixture to the addition of ions is an indication that the interaction was only electrostatic in nature.

In the light of the aforementioned work we concluded that the ability of alamethicin and gramicidin S to activate particulate guanylate cyclase resulted from a reorganization of the phospholipid matrix in which the enzyme was anchored, with a consequent disclosure of previously hidden enzyme. The peak activation that we obtained without nitroprusside, was 3.7-fold for alamethicin

and 6.5-fold for gramicidin S. Besch et al. [13] found a maximal 2.8-fold increase in basal adenylate cyclase activity in unfractionated vesicles derived from heart ventricle, which they interpreted as resulting from the loss of a permeability barrier to substrate. Our results with alamethicin could be interpreted in the same way. The higher activation induced by gramicidin S lead us to conclude that this antibiotic produced a more drastic membrane reorganization than does alamethicin. It should be pointed out that for peak activation we used alamethicin at 2 mg/ml (1 mM), which is 14- to 18-fold higher than the concentrations used by Besch et al. [13], while gramicidin S induced maximum activity at $6.1 \cdot 10^{-5}$ M. The rapid loss in activity when higher than optimal concentrations of gramicidin S were present could be due to enzyme destruction or to interference with the assay. We have previously shown that both lysolecithin and deoxycholate also stimulated particulate guanylate cyclase biphasically, however, when excess deoxycholate was removed from the inhibited enzyme by gel filtration, the maximum activity was restored [10]. Soluble enzyme did not respond biphasically, but was only inhibited by deoxycholate, however, in this case its removal did not restore activity. The conclusion drawn was that there was a differential sensitivity of the two enzyme fractions to negatively charged amphiphiles, since neutral amphiphiles, e.g., Lubrol PX, did not demonstrate toxic effects. A similar difference in sensitivity is apparently effected by positively charged amphiphiles, since the results in Fig. 3 show that nitroprusside-activated soluble enzyme was nearly completely inhibited by 0.2 mg gramicidin S per ml. Since this is the concentration that gave maximal activity of the particulate enzyme (Fig. 2), it cannot be interfering with the assay and must be inactivating the soluble enzyme.

The ability of melittin, in the same concentration range used here, to activate particulate guanylate cyclase from rat heart microsomes was previously reported by Lad and Shier [29]. They also observed a stimulation of adenylate cyclase at concentrations below 30 μg/ml, while higher concentrations inhibited activity. We observed maximal nitroprusside stimulation of guanylate cyclase with 20 μg of melittin/ml. This melittin concentration (7 μM) is in the same range that Sessa et al. [30] found able to effect the relase of hemoglobin from human erythrocytes and CrO_4^{2-} or glucose from liposomes. Verma et al. [25] showed by infrared dichroism measurements that 1 μM melittin effected a reorganization of the polar head groups of phosphatides, while Williams and Bell [24] could detect no change in electron spin resonance spectrum at that concentration although changes could be seen at $1 \cdot 10^{-4}$ M, and suggested that changes in ESR spectra would be seen only when a large number of melittin molecules interacting with a liposome caused disturbances of a large percentage of the spin-labelled membrane matrix. They proposed that melittin initially interacts electrostatically with phospholipid head groups, after which the hydrophobic residues of the polypeptide chain enter the hydrocarbon region of the lipid matrix and interact with the acyl chains of the phospholipid. This interaction is sufficient to stabilize the melittin position, but not great enough to induce cooperative immobilization of the matrix, as is seen with alamethicin [20]. Thus at low concentrations of melittin, little or no immobilization effect would be observed, but molecular leakages through matrix disruptions could be measured. It appears possible that such disruptions in the membrane are

responsible for the nitroprusside activation of guanylate cyclase activity that we observe with micromolar melittin. As shown in Fig. 4, melittin at 20 μg/ml stimulated nitroprusside activation 2-fold, which suggests an effect upon membrane permeability uncomplicated by more drastic reorganization. Williams and Bell [24] suggested that at higher concentrations, melittin intercalation disordered the lipid matrix sufficient to totally disrupt liposomes, and Sessa et al. [30] were able to demonstrate such liposomal disruption ($1 \cdot 10^{-4}$ M melittin) by electron microscopy. It appears that the progressive increase in basal guanylate cyclase activity that we observed with increasing concentrations of melittin (Fig. 4) may be a reflection of the progressive disruption of the membrane vesicles. The inability of nitroprusside to further activate guanylate cyclase treated with higher concentrations of melittin, may be another consequence of the amphipathic character of melittin. At saturating concentrations it can presumably bind to the hydrophobic site on guanylate cyclase, occupation of which by amphiphiles such a Lubrol PX also blocked nitroprusside activation.

Although Besch et al. [13] recommended the use of alamethicin and other channel forming ionophores in the assessment of vectorial properties of membrane bound enzyme, from our work we would recommend melittin. A comparison only of the effective concentration of alamethicin used by Besch et al. (54—72 μM) with the 7 μM melittin used here suggests a considerably higher potency and/or specificity of melittin. It is also more readily available. However, because higher concentrations appear to disrupt totally the membrane structure, and also block guanylate cyclase activation, its concentration must be carefully controlled. We did not, because of a limited supply of alamethicin, carry out experiments which would determine the effect of varying concentrations of alamethicin upon nitroprusside activation. It is possible that alamethicin might, like melittin, at concentrations too low to have an appreciable effect upon basal activity, increase vesicle permeability so as to increase nitroprusside activation. Besch et al. [13] showed that increasing amounts of alamethicin would progressively increase fluoride stimulation of adenylate cyclase. It is clear that alamethicin differs from melittin in that nitroprusside will further activate guanylate cyclase that has been maximally activated by alamethicin alone (Tables I and III). This may be due to a difference in the ability of these polypeptides to bind to the hydrophobic site of guanylate cyclase and thus block nitroprusside activation.

Although previous work has emphasized the differences between soluble and particulate guanylate cyclase [1—4,6,7], they respond to nitroprusside in a similar fashion. Thus alamethicin or gramicidin S treated microsomes required thiol for nitroprusside activation (Table III) as does soluble enzyme [26]. Also the ability of particulate enzyme to use Mg^{2+} as sole cation rather than Mn^{2+} was enhanced by nitroprusside (not shown), as it does the soluble enzyme [6,7]. Gramicidin S treatment stimulated both Mn^{2+}-dependent and Mg^{2+}-dependent activities, however the ratio of these activities was altered by nitroprusside to the same extent, whether or not gramicidin S was present.

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