vation of the enzyme. The effective concentration of PMA at the cells involved in the adenosine/histamine interaction in our slice preparation is unknown. However, the inhibitory effect on adenosine-induced cyclic AMP accumulation consistently observed with concentrations of PMA ≥ 10 nM argues strongly that PMA does penetrate to the relevant cells. This argument holds even more strongly at higher concentrations of PMA at which the inhibition is greater and there seems no reason to doubt that protein kinase C would be activated, irrespective of whether it has any role in the inhibitory effect. In view of the marked inhibition we have not examined the effect of concentrations of PMA above 1 μM, but in summary, the lack of any potentiation of the adenosine response by lower, and probably physiologically more relevant, concentrations, alone or in combination with A23187, suggests that stimulation of protein kinase C, with or without raised intracellular calcium, is not the mechanism by which histamine and other H_1-agonists have their effect in potentiating cyclic AMP accumulation.

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Effect of gentamicin on the transition temperature and permeability to glycerol of phosphatidylinositol-containing liposomes

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Gentamicin, a cationic aminoglycoside antibiotic widely used in the treatment of gram negative infections [1], accumulates in renal proximal tubular cells [2] where it causes biochemical and structural changes which eventuate in cell necrosis [3, 4]. At physiologic pH, gentamicin carries a net charge of +3.5 and is thought to interact electrostatically with fixed anionic sites on the brush border membrane [2]. These fixed anionic sites may be acidic phospholipids, especially phosphoinositides [5–9], which play an important role in the biochemical and biophysical characteristics of membranes [9, 10]. Binding of gentamicin to these lipids may alter the properties of the membrane, and such alterations may be relevant to the pathogenesis of gentamicin nephrotoxicity. We investigated the effects of gentamicin on the differential scanning calorimetry (DSC*) properties and glycerol permeability of dipalmitoyl phosphatidylcholine (DPPC): phosphatidylinositol (PI) liposomes.

Materials and methods

DPPC and PI (bovine brain) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). Gentamicin sulfate was a gift from the Schering Corp. (Bloomfield, NJ).

DSC studies. Multilamellar liposomes of DPPC and DPPC:PI (1:1) were prepared according to published methods [11]. Liposomes, 15 μmol lipid/ml, were incubated with and without gentamicin, 0 to 10^{-4} M, at 50° for 2 hr. Liposomes (20-μl samples) were transferred to aluminium pans and scanned 5° to 60° at a rate of 5°/min in a Perkin–Elmer differential scanning calorimeter in both the heating and cooling modes which gave similar results. The instrument was calibrated with benzoic acid and indium.

Glycerol permeability. Multilamellar liposomes of DPPC:PI (1:1) were prepared as above in 0.15 M KCl, 10 mM Tris, pH 7.0, and incubated with and without gentamicin, 10^{-4} M, at 50° for 1 hr. Liposomes (20 μl) were added to 1 ml of 0.3 M glycerol, and the change in absorbance at 450 nm due to liposome swelling was monitored. The relative permeability coefficient (P) was calculated as $\bar{P} = dA/A_o^2 t^{-1}$ where dA = change of absorbance over time and A_o = initial absorbance [11, 12]. Measurements were done at temperatures ranging between 25° and 32° which is below the T_m of DPPC:PI liposomes. Although most studies of permeability to non-electrolytes have been performed at temperatures above the T_m, studies of non-electrolyte permeation of lipid bilayers can be performed below the T_m [12, 13]. We chose the latter for convenience.

* Abbreviations: DSC, differential scanning calorimetry; E_a, activation energy; DPPC, dipalmitoyl phosphatidylcholine; PI, phosphatidylinositol; and T_m, transition temperature.

Results and discussion

The thermal phase transition (T_m) of DPPC liposomes occurred at 41.5° with an enthalpy (ΔH) of 7.8 ± 0.3 kcal/mol, which is similar to published data [14]. The T_m and ΔH of DPPC liposomes were not altered by 10^{-4} M gentamicin. As seen in Fig. 1, the mixing of PI with DPPC lowered the T_m to 34.5°. The presence of a single homogeneous transition peak indicates uniform dispersion of the lipids. Gentamicin caused an increase in the T_m of DPPC:PI liposomes which was first discernible at 10^{-7} M (Fig. 1). At 10^{-4} M gentamicin, the T_m rose to 38.5°, still below the T_m of pure DPPC liposomes. Similar results were reported by Ganesan *et al.* [6] and Wang *et al.* [7], using neomycin. These observations are consistent with the concept that neutralization of the anionic charge on PI due to an electrostatic interaction with the cationic amino groups of the drug leads to increased lipid packing [6, 7, 9]. Further support for the idea that gentamicin interaction with acidic phospholipids leads to increased microviscosity is obtained from studies of the effect of gentamicin on the fluorescence polarization of diphenylhexatriene in biological membranes [15] and from studies showing stabilization of lysosomal membranes by gentamicin [16, 17].

Gentamicin decreased the glycerol permeability of DPPC:PI liposomes but not that of pure DPPC liposomes (data not shown). From the Arrhenius plot of the data (Fig. 2), we calculated the energy of activation, E_a, for glycerol permeation in DPPC:PI liposomes to be 15.2 kcal/mol, which is similar to published values for phospholipid vesicles [11, 18]. Gentamicin caused an increase in the E_a to 19.7 kcal/mol ($P < 0.025$).

While the decreased glycerol permeation can be explained as a consequence of decreased membrane fluidity, the increased E_a for permeation cannot be explained by this phenomenon because it is not dependent on the microviscosity of the membrane. Rather, the E_a for permeation is a measure of the ability of the membrane to dehydrate the permeant [19]. The hydrophilic region of the membrane, which consists of the phospholipid headgroups and the carbonyl ester groups [20], has been shown to participate in the dehydration of the permeant. It has been shown that enhancement of hydrogen bonding in the carbonyl ester region leads not only to increased microviscosity but also to an increase in E_a for non-electrolyte permeability [20]. The increased E_a for glycerol permeability in DPPC:PI liposomes caused by gentamicin may result from hydrogen bonding between one or more amino groups of gentamicin and the carbonyl ester groups in the "hydrogen belt". The increased E_a of 4.5 kcal/mol is consistent with an increase of 1–2 hydrogen bonds [21]. These data for the binding of gentamicin to acidic phospholipids of membranes suggest that such binding alters the functional properties of the membrane.

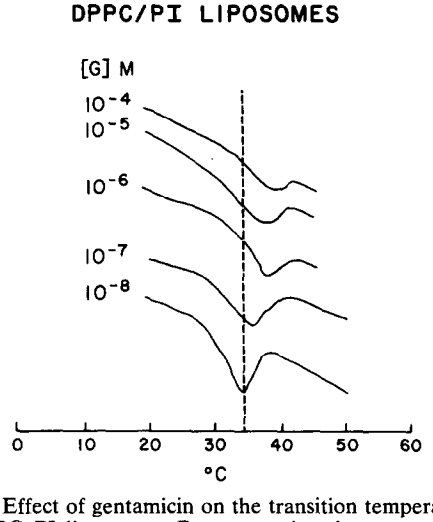

Fig. 1. Effect of gentamicin on the transition temperature of DPPC:PI liposomes. Representative thermograms in the cooling mode are shown.

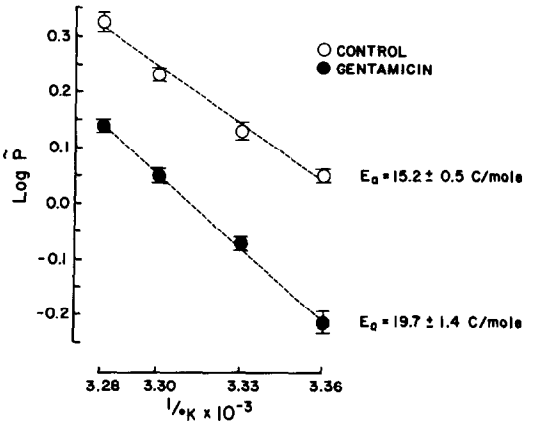

Fig. 2. Arrhenius plot of the relative glycerol permeability coefficient ($\bar{P}$) of DPPC:PI liposomes in the presence and absence of 10^{-4} M gentamicin. Data represent mean ± SE, N = 8. E_a, activation energy; C, kilocalorie.

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Effect of a single subtoxic dose of aflatoxin B_1 (AFB_1) on glucose-6-phosphate dehydrogenase in mouse liver

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A large body of data links aflatoxin B_1 (AFB_1*) to hepatocarcinogenicity and non-specific degeneration of the liver [1–4]. The toxic effects, however, have only been observed with high levels of AFB_1 that are not normally attained in food. *In vitro* studies have indicated that, in human liver, conversion of AFB_1 to several monohydroxylated aflatoxins B_1 by NADPH-dependent microsomal enzymes represents a major detoxification process [5, 6]. Alternatively, AFB_1 may be activated to form highly reactive 8,9-epoxy AFB_1, a metabolite thought to be the ultimate toxic agent, which in turn may be converted to either 8,9-dihydroxy AFB_1, another probable toxic metabolite, or to glutathione–AFB_1 conjugate, whose formation is believed to be another major detoxification process [7]. Glutathione reductase, a widely distributed enzyme which catalyzes the conversion of oxidized glutathione to reduced glutathione (GSH) at the expense of NADPH, maintains the intracellular concentrations of GSH [8]. Hence, it is conceivable that the rate of detoxification reactions depends on the availability of NADPH, most of which is derived from the hexose monophosphate shunt pathway. We have therefore explored the possibility of induction of the rate-limiting enzyme glucose-6-phosphate dehydrogenase by a subtoxic level of AFB_1.

Materials and methods

Chemicals. AFB_1, mercaptoethanol, glucose-6-phosphate, NADP, $MgCl_2$, Tris–HCl, KH_2PO_4 and EDTA were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. The AFB_1 was dissolved in dimethyl sulfoxide (DMSO) (Fisher Scientific Chemical Co., Fair Lawn, NJ, U.S.A.) (5.56 mg/ml).

Treatment of animals. Male CFW Swiss mice (6–8 weeks) were used. Experimental mice were given a single intraperitoneal injection of AFB_1 (3.0 mg AFB_1/kg body weight in 0.02 ml DMSO). Control mice were injected with either 0.02 ml DMSO or phosphate-buffered saline (PBS), pH 7.4. All animals were fed normal mouse chow with free access to water. Body weights of the animals were recorded daily. At days 0, 7, 14, and 21, mice from each group were killed by cervical dislocation, and the liver glucose-6-phosphate dehydrogenate (G6PD) was partially purified.

Partial purification of liver G6PD. The liver from each mouse was homogenized in ice-cold KH_2PO_4–1.0 mM EDTA–5 mM mercaptoethanol–5% glycerol buffer (pH 7.4) (3 ml/g liver tissue) with a Potter–Elvehjem homogenizer for 60 sec. The homogenate was centrifuged in the cold at 400 *g* for 10 min. The supernatant fraction was recentrifuged at 10,000 *g* for 20 min, and aliquots of the resulting crude supernatant fraction (S-10) were analyzed for G6PD activity as described in the legend for Table 1. Pooled fractions of the supernatant fraction (S-10) were used for the partial purification of G6PD as previously described [9]. Briefly, the pooled S-10 fraction was filtered through several layers of cheesecloth. To the filtrate $(NH_4)_2SO_4$ (50% saturation) was added dropwise while stirring at 0–4°. The mixture was centrifuged at 5000 *g* for 10 min, and the precipitate was redissolved in the homogenizing buffer. The precipitation step was repeated and dialyzed against the homogenizing buffer minus EDTA for 17–20 hr in the cold. The partially purified G6PD was kept at −20° until use. Enzyme activities were determined as previously described [10], and protein was measured by a modification of the Lowry method [11].

Results and discussion

To test the possibility that a subtoxic dose of AFB_1 induces the synthesis of glucose-6-phosphate dehydrogenase (G6PD) in the mouse liver, 3.0 mg/kg AFB_1 was injected intraperitoneally (LD_{50} i.p. in adult mouse: 60 mg/kg). The G6PD was prepared from the liver, and the enzyme activities were measured both in the crude fraction and the partially purified fraction. As shown in Table 1, in the PBS control and DMSO-treated mice, the mean G6PD value in the crude fraction on day 7 were 0.46 ± 0.07 and 0.44 ± 0.15 nmol/mg protein respectively. The corresponding value in the mice injected with AFB_1 was 1.26 ± 0.44 nmol/mg. Thus, the intraperitoneal injection of AFB_1 significantly stimulated ($P \leq 0.05$) the G6PD. A subsequent decline in the G6PD levels may have coincided with metabolic inactivation of AFB_1. The results with the partially purified G6PD also indicate the stimulatory effect of AFB_1 although the increase was about twice that of the PBS control. With exception of the samples derived from the DMSO-treated mice, there was noted a substantial loss of the enzyme activities, particularly in the 7-day sample from the AFB_1-treated animal. A possible explanation for the loss of the enzyme activity is that the G6PD isolated from the animal sources including mouse liver is labile [12]. Attempts to minimize the loss of the enzyme activity by addition of 5% glycerol/5 mM mercaptoethanol/1 mM EDTA in the assay solution, however, were unsuccessful.

* Abbreviations: AFB_1, aflatoxin B_1; Tris, Tris(hydroxymethyl)methylamine; GSH, reduced glutathione; DMSO, dimethyl sulfoxide; and NADPH, reduced nicotinamide adenine dinucleotide phosphate.