Biochemical Pharmacology, Vol. 45, No. 1, pp. 183–189, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

CHLOROQUINE STABILIZATION OF PHOSPHOLIPID MEMBRANES AGAINST DIACYLGLYCEROL-INDUCED PERTURBATION

RAPHAEL ZIDOVETZKI,*† IRWIN W. SHERMAN,* MICHAEL CARDENAS* and DAN B. BORCHARDT‡

*Department of Biology and ‡Department of Chemistry, University of California, Riverside, CA 92521, U.S.A.

(*Received* 27 *April* 1992; *accepted* 27 *August* 1992)

Abstract—The effects of 1-stearoyl,2-*sn*-arachidonoylglycerol (SAG) and the antimalarial drug chloroquine on lipid bilayer structure were studied by ^{2}H-NMR spectroscopy. Model lipid systems were established with compositions similar to those of normal human erythrocytes, malaria-infected erythrocytes, or malaria parasite membranes. The ^{2}H-NMR spectra of the membranes formed from the lipids extracted from normal human erythrocytes were similar to those obtained using the corresponding lipid mixtures. The order parameters of the model "infected" and model "parasite" membranes were reduced markedly relative to that of normal erythrocytes. Addition of SAG induced formation of non-bilayer lipid phases in all lipid systems. Only a small decrease in the order parameters of the acyl side chains of the phosphatidylserine, but not of the phosphatidylcholine component of the lipid membranes, was observed upon the addition of chloroquine. A dramatic effect was observed upon the addition of chloroquine to the SAG-containing membranes: this antimalarial almost totally abolished the formation of SAG-induced non-bilayer lipid phases. Since SAG, endogenously formed in erythrocyte membranes, is a potent activator of phospholipase A_2, this membrane-stabilizing action of chloroquine may partially account for the phospholipase A_2-inhibiting properties of this drug, and, consequently, for both its therapeutic and toxic modes of action.

Chloroquine (Fig. 1), the most widely used antimalarial drug [1, 2], has also been used for the treatment of rheumatoid arthritis [3] and ischemia [4]. Although the mechanism of action of chloroquine is unknown, an increasing number of investigators contend that it involves inhibition of lysosomal enzymes; for the malaria parasite this would prevent both feeding and digestion of hemoglobin [5–8; see also Ref. 9 for a different hypothesis]. Chloroquine-induced inhibition of a lysosomal enzyme, phospholipase A_2 (PL-A_2)§, has also been proposed to result in some toxic side-effects, such as the accumulation of phospholipids in various tissues (phospholipidosis) [10, 11]. Indeed, chloroquine has been shown to inhibit PL-A_2s from several different sources [12, 13]. It has also been suggested that chloroquine binds to the phospholipids and that the resulting complex is resistant to hydrolysis by PL-A_2 [14]. However, our previous NMR studies showed that chloroquine did not perturb significantly the order of the lipid acyl side chains, indicating that this molecule does not penetrate into the interior of the lipid bilayer membranes [15, 16].

The question of how chloroquine inhibits the activity of PL-A_2 remains unanswered. Many PL-A_2s, especially those of intracellular origin, are not very active against stable, unperturbed, lipid bilayers [17–19]. Numerous physicochemical studies have shown that PL-A_2s are activated by increased structural fluctuations in the lipid bilayers, induced by such agents as alcohols [20–22] or detergents [20] or by proximity to the gel to liquid crystalline phase transition temperature of the lipids [17, 18, 23, 24].

An endogenous membrane-perturbing agent, diacylglycerol (DAG), is produced during the course of a transmembrane signal transduction process, which includes increased phospholipid metabolism and activation of protein kinase C [25]. The amount of DAG in the erythrocyte membrane increases ~9-fold upon malaria infection [26]. DAG-induced membrane perturbations probably play a significant role in its mode of action [27]. DAGs also directly activate PL-A_2s from various sources [28–31]. The DAG-induced activation of PL-A_2s was correlated with the increased structural perturbations induced

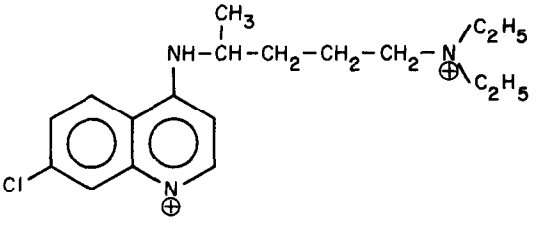

Fig. 1. Chemical structure of chloroquine.

† Corresponding author. Tel. (714) 787-5903; FAX (714) 787-4286.

§ Abbreviations: PL-A_2, phospholipase A_2; DAG, diacylglycerol; SAG, 1-stearoyl,2-*sn*-arachidonoylglycerol; DPPC-d_{62}, di(perdeuteropalmitoyl)phosphatidylcholine; DPPS-d_{62}, di(perdeuteropalmitoyl)phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; and SM, sphingomyelin.

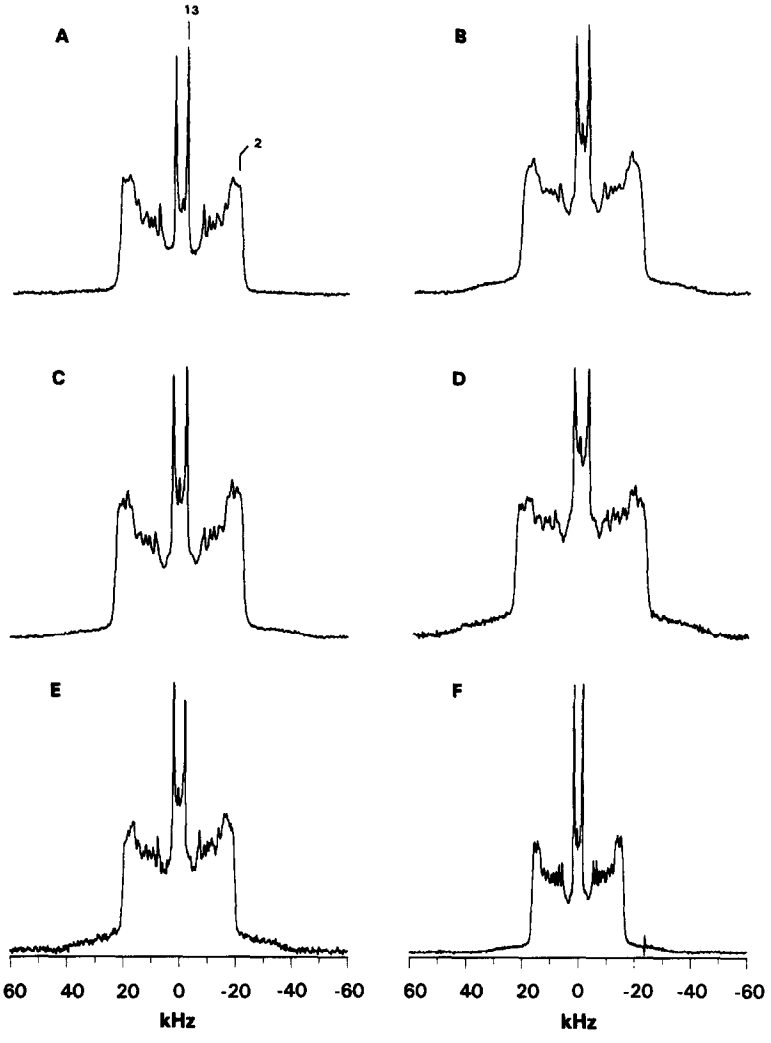

Fig. 2. ^{2}H-NMR spectra of DPPC-d_{62} or DPPS-d_{62} added to erythrocyte or model lipid mixtures at 37°. Nine peaks are resolved in these spectra; peaks 1 and 3 were further resolved from peak 2 at higher temperature. Peaks 2 (the outermost) and 13 (the innermost) are labeled on panel (A). (A) DPPC-d_{62} probe, erythrocyte lipid extracts; (B) DPPC-d_{62} probe, model "normal" membranes; (C) DPPS-d_{62} probe, erythrocyte lipid extracts; (D) DPPS-d_{62} probe, model "normal" membranes; (E) DPPC-d_{62} probe, model "infected" membranes; and (F) DPPC-d_{62} probe, model "parasite" membranes.

by DAGs into the lipid bilayer structure [28, 31–34]. In the case of DAGs with unsaturated acyl side chains, the increased structural fluctuations are associated with the DAG-induced propensity of the lipid to form non-bilayer lipid phases [32]. In the present study we show that the most common endogenously produced DAG, 1-stearoyl,2-*sn*-arachidonoylglycerol (SAG), also induces non-bilayer lipid phases in the lipid membranes formed from lipids extracted from normal human erythrocytes, as well as in model lipid membranes resembling either the membranes of normal or malaria-infected human erythrocytes. This SAG-induced formation of non-bilayer lipid phases, however, is totally abolished in the presence of chloroquine. This effect of chloroquine may play a role in the inhibition of PL-A_2 by this drug and be a part of its therapeutic and toxic modes of action.

MATERIALS AND METHODS

Preparation of the lipid extracts. Blood (type O+) was drawn from healthy donors into acid-citrate-dextrose and washed with phosphate-buffered saline (10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4) to remove the plasma and buffy coat. Lipids were extracted by the method of Bligh and Dyer [35] and an aliquot was assayed for lipid phosphorus by a modification [36] of the method of Fiske and Subbarow [37], and for cholesterol by the method of Zlatkis *et al.*, [38].

NMR experiments. Di(perdeuteropalmitoyl)-

phosphatidylcholine (DPPC-d_{62}), di(perdeuteropalmitoyl)phosphatidylserine (DPPS-d_{62}), bovine liver phosphatidylcholine (PC), bovine liver phosphatidylethanolamine (PE), bovine brain phosphatidylserine (PS) and cardiolipin were from Avanti Polar Lipids (Birmingham, AL). Sphingomyelin (SM), chloroquine diphosphate and cholesterol were from the Sigma Chemical Co. (St. Louis, MO). SAG was from Serdary Research (London, Ontario).

Model lipid membranes were made with the following composition: (i) composition modeling the normal human erythrocytes [39]: PC:PE:PS:SM:cholesterol at molar ratios of 6:6:3:4:19, (ii) the same lipids with the molar ratios of 8:5:2:2:10, modeling the malaria-infected human erythrocytes [26, 39–42], or (iii) PC:PE:SM:PS:cardiolipin:cholesterol = 10:5:1:1:1:2, modeling the malaria parasite membranes [41, 42].

Multilamellar lipid dispersions for the NMR measurements were made by first dissolving the lipids in chloroform. Either DPPC-d_{62}, or DPPS-d_{62} was added at the molar ratio of 1:8 to the phospholipids as a ^{2}H-NMR probe. The solvent was then evaporated with dry nitrogen and the sample was kept under vacuum (<1 mtorr) for at least 8 hr. The dried lipids were then hydrated with a 250 mM Tris–HCl buffer solution (pH 7.4), prepared in ^{2}H-depleted H_2O (Sigma). Chloroquine was typically added to the lipids with the hydrating buffer. In some experiments, chloroquine was added to previously hydrated lipids and the mixture was subjected to three freeze–thaw cycles to ensure proper mixing. To compensate for the acidity of chloroquine, a stock solution of Tris, pH 9.3, was used in chloroquine-containing samples to adjust the pH to 7.4. SAG was dissolved in chloroform together with the lipids prior to evaporation. The samples were typically 30% (w/w) in water.

^{2}H-NMR spectra were acquired at 11.74 T (corresponding to 500.13 MHz ^{1}H- and 76.78 MHz ^{2}H-NMR frequencies) on a General Electric GN-500 spectrometer. The spectra were acquired at 37° with a high-power probe (Doty Scientific, Columbia, SC) using the standard quadrupole echo sequence [43]. The spectral width was 0.5 to 1 MHz, refocusing time 64 μsec, and 90° pulse of 3.5 μsec. The moments of the ^{2}H-NMR spectra were calculated according to Davis [44].

RESULTS

The ^{2}H-NMR spectra of DPPC-d_{62} or DPPS-d_{62} added to the lipid extracted from normal human erythrocytes, or to the model lipid mixtures with a composition similar to that of the normal human erythrocyte, malaria-infected erythrocytes or the malaria parasite are shown in Fig. 2. The spectra are typical of the bilayer lipid conformation [45]. The spectra of the erythrocyte extracts (Fig. 2, A and C) were nearly identical to the spectra of the model lipid mixtures with a similar composition (Fig. 2, B and D), and the spectra with DPPC-d_{62} added as a probe (Fig. 2, A and B) are similar to the spectra of the DPPS-d_{62} (Fig. 2, C and D), indicating that DPPC-d_{62} and DPPS-d_{62} were well mixed with the lipid extracts or with the model lipid membranes.

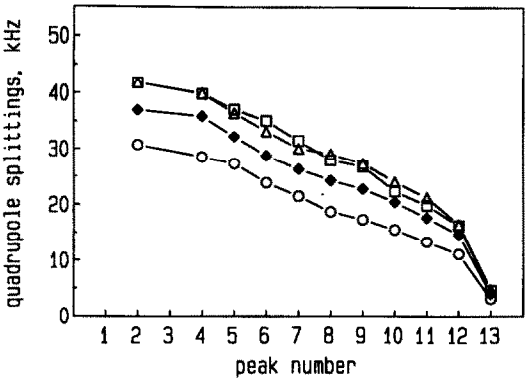

Fig. 3. Plots of the quadrupole splittings of DPPC-d_{62} incorporated into the lipid mixtures vs peak number for the lipid systems used in this study. Key: (□) normal erythrocyte extracts; (△) model "normal" membranes; (◆) model "infected" membranes; and (○), model "parasite" membranes.

Also, the values for the first through fourth moments [44] of the spectra on Fig. 2A–D were identical within the experimental error. Similarities between the spectra obtained from the lipid extracts and the model mixtures show that the latter are indeed an accurate representation of the erythrocyte membrane lipids. We also obtained nearly identical spectra between lipid extracts from erythrocytes and the model lipid membranes using POPC-d_{31} or POPS-d_{31} as ^{2}H-NMR probes (not shown). Similar spectra were also obtained in our previous study with the DPPE-d_{62} probe [16].

Also shown in Fig. 2 are the ^{2}H-NMR spectra obtained using lipid mixtures with compositions similar to the membranes of malaria-infected human erythrocytes (Fig. 2E) or to the membranes of the malaria parasite (Fig. 2F). These spectra, also indicative of the bilayer lipid conformation, were quantitatively different from the spectra obtained with normal human erythrocytes (Fig. 2A). The observed peak-to-peak quadrupole splittings, $\Delta\nu$, in the ^{2}H-NMR spectra are proportional to the order parameters of the corresponding C^2H_2 segments along the lipid acyl side chains [45]. The quadrupole splittings of the spectra obtained using the lipid extracts of normal erythrocytes were nearly identical to those of the corresponding model lipid mixtures (Fig. 3). Also, nearly identical quadrupole splittings were obtained in all lipid systems studied with either DPPC-d_{62} or DPPS-d_{62} lipid probes (not shown) again demonstrating well-mixed lipid components. The $\Delta\nu$ values obtained with model "infected" membranes were markedly lower than the values obtained with normal erythrocyte lipids (Fig. 3), reflecting the reduced ordering in the membranes of the infected cells. Even lower $\Delta\nu$ values were obtained with the model "parasite" membranes. The quadrupole splittings of the acyl side chains of DPPC-d_{62} (Fig. 3) or DPPS-d_{62} (not shown) probes in these membranes were markedly lower than those of the control or "infected" model membranes.

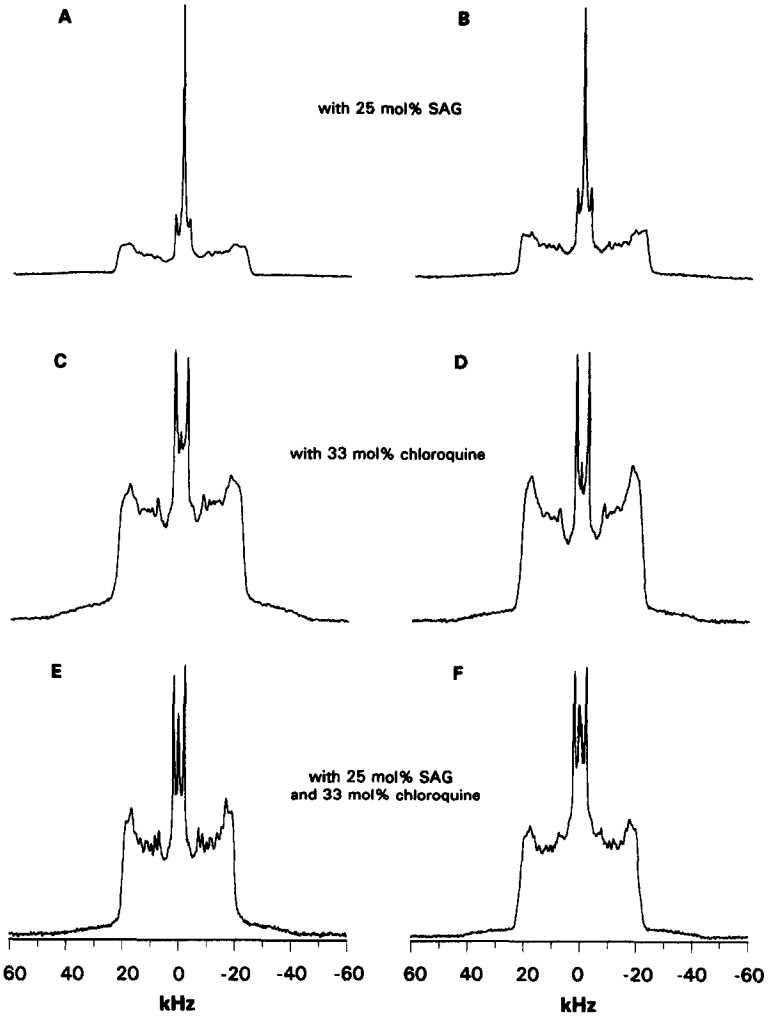

Fig. 4. ^{2}H-NMR spectra of the deuterated PC or PS probes in the model "normal" membranes in the presence of 25 mol% SAG and/or 33 mol% chloroquine. (Left) DPPC-d_{62} probe; (Right) DPPS-d_{62} probe. (A and B) with SAG; (C and D) with chloroquine; and (E and F) with SAG and chloroquine.

Effect of SAG. The addition of 25 mol% SAG to the model lipid membranes caused the appearance of a large central peak in the ^{2}H-NMR spectra of both DPPC-d_{62} and DPPS-d_{62} probes (Fig. 4, A and B). ^{31}P-NMR measurements also showed a SAG-induced peak at the "isotropic" position, corresponding to micelles, small vesicles, or cubic lipid phase. Centrifugation of the samples (100,000 *g*, 20 min) and subsequent NMR measurements showed that the supernatant contained an "isotropic" phase indicative of the microscopic nature (i.e. vesicles or micelles) of this phase. The molecular shape of SAG with its virtual absence of a headgroup suggests that the observed central peak probably represents inverted micelles. Similar results were obtained with the erythrocyte lipid extracts (not shown). The intensity of this peak comprised about 10–15% of the total intensity of ^{2}H- (Fig. 4, A and B) or ^{31}P- (not shown) NMR spectra. The intensity of this peak was similar with both types of lipids, showing that the structures of both erythrocyte and model lipids were similarly affected by SAG. Also, similar results were obtained with both DPPC-d_{62} or DPPS-d_{62} lipid probes (Fig. 4, A and B), indicating that there is no preferential separation of either the PC or PS lipid component into the non-bilayer lipid phase.

The quadrupole splittings of the spectra shown in Fig. 4B, 4D and 4F are given in Fig. 5. The order parameters of both lipid mixtures with either DPPC-d_{62} or DPPS-d_{62} lipid probes were not affected significantly by the presence of SAG. Similarly, no change in the quadrupole splittings with either DPPC-d_{62} or DPPS-d_{62} was detected upon addition of SAG to the model "infected" or "parasite" membranes (not shown).

Chloroquine. Only small changes in the ^{2}H-NMR spectra of the model lipid membranes were observed upon addition of chloroquine up to the molar ratio of 1:2 to the lipids with both DPPC-d_{62} and DPPS-d_{62} lipid probes (Fig. 4). A noticeable broadening

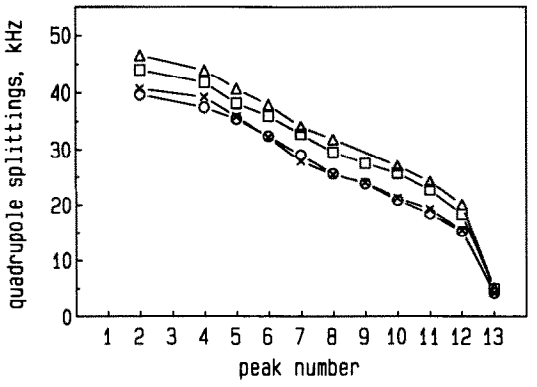

Fig. 5. Plots of the quadrupole splittings of DPPS-d_{62} probe in the model "normal" membranes in the absence or presence of 25 mol% SAG and/or 33 mol% chloroquine. Key: (□) control; (△) with SAG; (×) with chloroquine; and (○) with SAG and chloroquine.

of the individual peaks was observed without, however, significant change in the overall ^{2}H-NMR spectral shape. The broadening was also observed when DPPC with only one deuterated acyl chain was used as a probe (not shown) and was probably due to a reduced rate of motion of the phospholipid side chains in the presence of chloroquine. Small but marked decreases in the quadrupole splittings were observed in the presence of chloroquine with the DPPS-d_{62} (Fig. 5), but not with the DPPC-d_{62} (not shown) lipid probes.

Chloroquine and SAG. A dramatic effect of chloroquine was, however, observed upon addition of this drug to the SAG-containing lipids (Fig. 4, E and F). The addition of chloroquine abolished the induction of the non-bilayer lipid phases by SAG and resulted in ^{2}H-NMR spectra similar to those of the control (Fig. 2). The effect was observed with both lipid probes (Fig. 4, E and F), and also with the "infected" model membranes (not shown).

DISCUSSION

In the course of this study we developed a model membrane lipid system whereby a lipid mixture, composed of commercially available lipids, and modeling the composition of normal or malaria-infected human erythrocytes, or the membranes of the malaria parasite, could be used to study the membrane effects of drugs. We have shown here that the order parameters of either DPPC-d_{62} or DPPS-d_{62} in the "normal" model membranes are indistinguishable from those obtained with lipid extracts from normal human erythrocytes (Fig. 3). Moreover, this model system responded to the presence of SAG and chloroquine in the same way as did the lipids from the erythrocyte membranes. Among the advantages of using the model system are: markedly reduced, though not totally eliminated, need for extracting lipids from human erythrocytes, and the ability to modify lipid compositions so as to approximate various erythrocyte abnormalities, e.g. infection by the malaria parasite.

The reduced order parameters that were observed when the lipid composition modeled that of malaria-infected erythrocytes agree with studies that showed decreased membrane order parameters and increased membrane fluidity of such cells [46–48]. The sharply decreased order parameters of the model "parasite" membranes (Fig. 3) probably reflect the reduced amount of cholesterol in this system.

Increased activity of phospholipase C and DAG formation in erythrocytes were shown in earlier reports that established increased phosphatidylinositol turnover as a pathway of the transmembrane signal transduction [49]. More recently, it was shown that the DAG content of the malaria-infected erythrocytes increases ~9-fold [26]. Although the increase of DAG production is usually studied in relation to activation of protein kinase C, DAGs were shown to directly activate PL-A_2s from different sources [28–31]. This effect of DAGs was correlated with their capacity to induce defects into bilayer structure [28, 32–34]. Taking into account that typically PL-A_2s exhibit relatively low activity toward unperturbed lipid bilayers [17–19], it is likely that such a dramatic increase in the DAG content of the membranes and associated membrane structure perturbations, may be required for the "normal" activity of PL-A_2 of the malaria parasite.

Previously we showed that chloroquine does not interact strongly with the acyl side chains of PC or PE components of the membranes [15, 16]. The present study detected a small, but marked reduction in the order parameters of the acyl side chains of negatively charged PS, which agrees with the general tendency of cationic amphiphilic drugs to interact stronger with negatively charged lipids. Earlier studies proposed that the complex formed by cationic amphiphilic drugs and acidic phospholipids may be resistant to hydrolysis by PL-A_2 [50]. More recently, a similar conclusion was reached by Mingeot-Leclercq *et al.* [51]. However, Hostetler and co-workers found that degradation of neutral phospholipids is affected by cationic amphiphilic drugs to a greater degree than is degradation of acidic phospholipids [11, 52]. We also observed that chloroquine inhibition of PL-A_2 was more efficient with a PC substrate rather than with a PC/PS substrate (Zidovetzki *et al.*, unpublished observations).

The concentrations of chloroquine (30–40 mM) and SAG (15–25 mol%) used in this study are high, posing the question as to the biological relevance of these findings. The concentration of chloroquine in the food vacuoles of the malaria parasite has been calculated to be in the millimolar range [53], and in rat liver lysosomes to be > 70 mM [54]. Therefore, the chloroquine concentrations used in this study are well within this range. Additionally, an important parameter in studying the membrane effects of amphiphilic drugs is not only the total drug concentration, but also the molar ratio of drug to lipid. We observed a reversal of the SAG effect by chloroquine when chloroquine was added at slightly more than a 1:1 molar ratio to SAG which is probably the case in the cells of chloroquine-treated patients.

Moreover, from the size of the food vacuole of the malaria parasite (~2 μm, [55]) which is the site of chloroquine accumulation, and from the concentration of chloroquine at this site (taken as 1 mM) we estimate that a food vacuole contains $<4 \times 10^5$ lipid molecules and $\sim 2.6 \times 10^6$ chloroquine molecules, resulting in a chloroquine/lipid ratio more than an order of magnitude higher than the chloroquine/lipid ratio used in this study.

In summary then, although chloroquine did not interact strongly with the interior of the lipid bilayers, the addition of this drug to the SAG-containing membranes completely reversed SAG-induced formation of the non-bilayer lipid phases. This indicates that chloroquine markedly decreases SAG-induced fluctuations in the bilayer structure. The presence of such fluctuations is associated with the activation of PL-A_2. Thus, it is possible that the mechanism of PL-A_2 inhibition by chloroquine includes this "bilayer-stabilizing" effect.

Acknowledgements—This work was supported by a grant from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease (to R.Z.). The NMR work was done using a General Electric GN-500 spectrometer, funded by grants NSF DMB840491 and NIH BRSG2507.

REFERENCES

1. Rollo IM, Drugs used in the chemotherapy of malaria. In: *The Pharmacologic Basis of Therapeutics* (Eds. Gilman AG, Goodman LS and Gilman A), 6th Edn. pp. 1042–1046. Macmillan, New York, 1980.
2. White NJ, The treatment of *falciparum* malaria. *Parasitol Today* **4**: 10–14, 1988.
3. Mackenzie AH, Antimalarial drugs for rheumatoid arthritis. *Am J Med* **75** (Suppl): 48–58, 1983.
4. Kayawake S, Narbaitz R and Kako KJ, Effects of chloroquine and nifedipine on the phospholipid content and enzyme activity in the subcellular fraction of ischemic rat liver. *Basic Res Cardiol* **77**: 140–157, 1982.
5. Homewood CA, Warhurst DC, Peters W and Baggaley VC, Lysosomes, pH and the anti-malarial action of chloroquine. *Nature* **235**: 50–52, 1972.
6. Yayon A, Timberg R, Friedman S and Ginsburg H, Effects of chloroquine on the feeding mechanism of the intraerythrocytic human malarial parasite *Plasmodium falciparum*. *J Protozool* **31**: 367–372, 1984.
7. Krogstad DJ and Schlesinger PH, A perspective on antimalarial action: Effects of weak bases on *Plasmodium falciparum*. *Biochem Pharmacol* **35**: 547–552, 1986.
8. Zarchin S, Krugliak M and Ginsburg H, Digestion of the host erythrocyte by malaria parasites is the primary target for quinoline-containing antimalarials. *Biochem Pharmacol* **35**: 2435–2442, 1986.
9. Fitch C, Mode of action of antimalarial drugs. *Ciba Found Symp* **94**: 222–232, 1983.
10. Lüllmann H, Lüllmann-Rauch R and Wassermann O, Drug-induced phospholipidosis. *CRC Critical Rev Toxicol* **4**: 185–218, 1975.
11. Hostetler KY, The role of phospholipases in human diseases. In: *Phospholipids and Cellular Regulation* (Ed. Kuo JF), Vol. 1, pp. 181–206. CRC Press, Boca Raton, FL, 1985.
12. Hostetler KY, Molecular studies of the induction of cellular phospholipidosis by cationic amphiphilic drugs. *Fed Proc* **43**: 2582–2564, 1984.
13. Hostetler KY and Jellison EJ, Role of phospholipases in myocardial ischemia: Effect of cardioprotective agents on the phospholipases A of heart cytosol and sarcoplasmic reticulum *in vitro*. *Mol Cell Biochem* **88**: 77–82, 1989.
14. Ginsburg H and Geary TG, Current concepts and new ideas on the mechanism of action of quinoline-containing antimalarials. *Biochem Pharmacol* **36**: 1567–1576, 1987.
15. Zidovetzki R, Sherman IW, Atiya A and De Boeck H, A nuclear magnetic resonance study of the interactions of the antimalarials chloroquine, quinacrine, quinine and mefloquine with dipalmitoylphosphatidylcholine bilayers. *Mol Biochem Parasitol* **35**: 199–208, 1989.
16. Zidovetzki R, Sherman IW, Maguire PA and De Boeck H, A nuclear magnetic resonance study of the interactions of the antimalarials chloroquine, quinacrine, quinine and mefloquine with lipids extracted from normal human erythrocytes. *Mol Biochem Parasitol* **38**: 33–40, 1990.
17. Op den Kamp JAF, De Gier J and Van Deenen LLM, Hydrolysis of phosphatidylcholine liposomes by pancreatic phospholipase A_2 at the transition temperature. *Biochim Biophys Acta* **345**: 253–256, 1974.
18. Wilschut JC, Regts J, Westenberg H and Scherphof G, Action of phospholipases A_2 on phosphatidylcholine bilayers. Effects of phase transition, bilayer curvature and structural defects. *Biochim Biophys Acta* **508**: 185–196, 1978.
19. Lichtenberg D and Romero G, Menashe M and Biltonen RL, Hydrolysis of dipalmitoylphosphatidylcholine large unilamellar vesicles by porcine pancreatic phospholipase A_2. *J Biol Chem* **261**: 5334–5340, 1986.
20. Jain MK, Gleeson J, Upreti A and Upreti GC, Intrinsic perturbing ability of alkanols in lipid bilayers. *Biochim Biophys Acta* **509**: 1–8, 1978.
21. Upreti GC and Jain MK, Action of phospholipase A_2 on unmodified phosphatidylcholine bilayers: Organizational defects are preferred sites of action. *J Membr Biol* **55**: 113–121, 1980.
22. Kaszuba M and Hunt GRA, ^{31}P- and ^{1}H-NMR investigations of the effect of *n*-alcohols on the hydrolysis by phospholipase A_2 of phospholipid vesicular membranes. *Biochim Biophys Acta* **1030**: 88–93, 1990.
23. Noordam PC, Killian A, Oude Elferink RFM and De Gier J, Comparative study on the properties of saturated phosphatidylethanolamine and phosphatidylcholine bilayers: Barrier characteristics and susceptibility to phospholipase A_2 degradation. *Chem Phys Lipids* **31**: 191–204, 1982.
24. Menashe M, Romero G, Biltonen RL and Lichtenberg D, Hydrolysis of small unilamellar vesicles by porcine pancreatic phospholipase A_2. *J Biol Chem* **261**: 5328–5333, 1986.
25. Berridge MJ, Inositol triphosphate and diacylglycerol: Two interacting second messengers. *Annu Rev Biochem* **56**: 159–193, 1987.
26. Beaumelle BD and Vial HJ, Modification of the fatty acid composition of individual phospholipids and neutral lipids after infection of the simian erythrocyte by *Plasmodium knowlesi*. *Biochim Biophys Acta* **877**: 262–270, 1986.
27. Zidovetzki R and Lester D, The mechanism of activation of protein kinase C: A biophysical approach. *Biochim Biophys Acta* **1134**: 261–272, 1992.
28. Dawson RMC, Irvine F, Bray J and Quinn PJ, Long-chain unsaturated diacylglycerols cause a perturbation in the structure of phospholipid bilayers rendering them susceptible to phospholipase attack. *Biochem Biophys Res Commun* **125**: 836–842, 1984.
29. Kramer RM, Chegani GC and Deykin D, Stimulation

of Ca^{2+}-activated human phospholipase A_2 by diacylglycerol. *Biochem J* **248**: 779–783, 1987.
30. Kolesnik RN and Paley AE, 1,2-Diacylglycerols and phorbol esters stimulate phosphatidylcholine metabolism in GH_3 pituitary cells. Evidence for separate mechanisms of action. *J Biol Chem* **262**: 9204–9210, 1987.
31. Cunningham BA, Tsujita T and Brockman HL. Enzymatic and physical characterization of diacylglycerol–phosphatidylcholine interactions in bilayers and monolayers. *Biochemistry* **28**: 32–40, 1989.
32. De Boeck H and Zidovetzki R, Effects of diacylglycerols on the structure of phosphatidylcholine bilayers: a ^2H and ^{31}P NMR study. *Biochemistry* **28**: 7439–7446, 1989.
33. De Boeck H and Zidovetzki R, Interactions of saturated diacylglycerols with phosphatidylcholine bilayers: A ^2H NMR study. *Biochemistry*, **31**: 623–630, 1992.
34. Zidovetzki R, Laptalo L and Crawford J, Effect of diacylglycerols on the activity of cobra venom, bee venom, and pig pancreatic phospholipases A_2. *Biochemistry*, **31**: 7683–7691, 1992.
35. Bligh EG and Dyer WJ, A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**: 911–917, 1959.
36. Maguire PA and Druse MJ, The influence of cholesterol on synaptic fluidity and dopamine uptake. *Brain Res Bull* **22**: 431–437, 1989.
37. Fiske CH and Subbarow Y, The colorimetric determination of phosphorus. *J Biol Chem* **66**: 375–400, 1925.
38. Zlatkis A, Zak B and Boyle AJ, A new method for the direct determination of serum cholesterol. *J Lab Clin Med* **41**: 486–492, 1963.
39. Maguire PA and Sherman IW, Phospholipid composition, cholesterol content and cholesterol exchange in *Plasmodium falciparum*-infected red cells. *Mol Biochem Parasitol* **38**: 105–112, 1990.
40. Vial HJ and Philippot JR and Wallach DFH, A reevaluation of the status of cholesterol in erythrocytes infected by *Plasmodium knowlesi* and *P. falciparum*. *Mol Biochem Parasitol* **13**: 53–65, 1984.
41. Hsiao LL, Howard RJ, Aikawa M and Taraschi TF, Modification of host cell membrane lipid composition by the intra-erythrocytic human malaria parasite *Plasmodium falciparum*. *Biochem J* **274**: 121–132, 1991.
42. Wunderlich F, Fiebig S, Vial H and Kleinig H, Distinct lipid compositions of parasite and host cell plasma membranes from *Plasmodium chabaudi*-infected erythrocytes. *Mol Biochem Parasitol* **44**: 271–278, 1991.
43. Davis JH, Jeffrey KR, Bloom M, Valic MI and Higgs TP, Quadrupolar echo deuteron magnetic resonance spectroscopy in ordered hydrocarbon chains. *Chem Phys Lett* **42**: 390–394, 1976.
44. Davis JH, The description of membrane lipid conformation, order and dynamics by ^2H-NMR. *Biochim Biophys Acta* **737**: 117–171, 1983.
45. Seelig J, Deuterium magnetic resonance: theory and application to lipid membranes. *Q Rev Biophys* **10**: 353–418, 1977.
46. Howard RJ and Sawyer WH, Changes in the membrane microviscosity of mouse red blood cells infected with *Plasmodium berghei* detected using *n*-(9-anthroyloxy) fatty acid fluorescent probes. *Parasitology* **80**: 331–342, 1980.
47. Allred DR, Sterling CR and Morse PD II, Increased fluidity of *Plasmodium berghei*-infected mouse red blood cell membranes detected by electron spin resonance spectroscopy. *Mol Biochem Parasitol* **7**: 27–39, 1983.
48. Sherman IW and Greenan JRT, Altered red cell membrane fluidity during schizogonic development of malaria parasites (*Plasmodium falciparum and P. lophurae*). *Trans R Soc Trop Med Hyg* **78**: 641–644, 1984.
49. Allan D and Michell RH, A calcium-activated polyphosphoinositide phosphodiesterase in the plasma membrane of human and rabbit erythrocytes. *Biochim Biophys Acta* **508**: 277–286, 1978.
50. Lüllmann-Rauch R, Drug-induced lysosomal storage disorders. In: *Lysosomes in Applied Biology and Therapeutics* (Eds. Dingle JT, Jacques PJ and Shaw IH), Vol. 6, pp. 49–130. North Holland, Amsterdam, 1979.
51. Mingeot-Leclercq M-P, Schanck A, Ronveaux-Dupal M-F, Deleers M, Brasseur R, Ruysschaert J-M, Laurent G and Tulkens PM, Ultrastructural, physico-chemical and conformational study of the interactions of gentamicin and bis(beta-diethylaminoethyl-ether)hexestrol with negatively-charged phospholipid layers. *Biochem Pharmacol* **38**: 729–741, 1989.
52. Pappu A and Hostetler KY, Effect of cationic amphiphilic drugs on the hydrolysis of acidic and neutral phospholipids by liver lysosomal phospholipase A. *Biochem Pharmacol* **33**: 1639–1644, 1984.
53. Geary TG, Jensen JB and Ginsburg H, Uptake of [^3H]-chloroquine by drug-sensitive and -resistant strains of the human malaria parasite *Plasmodium falciparum*. *Biochem Pharmacol* **35**: 3805–3812, 1986.
54. Hostetler KY, Reasor M and Yazaki PJ, Chloroquine-induced phospholipid fatty liver. Measurement of drug and lipid concentrations in rat liver lysosomes. *J Biol Chem* **260**: 215–219, 1985.
55. Goldberg DE, Slater AFG, Cerami A and Henderson GB, Hemoglobin degradation in the malaria parasite *Plasmodium falciparum*: An ordered process in a unique organelle. *Proc Natl Acad Sci USA* **87**: 2931–2935, 1990.