10. A. Rosowsky, J. E. Wright, C. A. Cucchi, K. Boeheim and E. Frei, III, *Biochem. Pharmac.* **35**, 356 (1986).
11. E. Frei, III, A. Rosowsky, J. E. Wright, C. A. Cucchi, J. A. Lippke, W. A. Haseltine, T. Ervin and J. Jolivet, *Proc. natn. Acad. Sci. U.S.A.* **81**, 2873 (1984).
12. M. R. Osborne, *Comput. Biol. Med.* **12**, 159 (1982).
13. N. T. Van, M. Raber, G. H. Barrows and B. Barlogie, *Science* **224**, 876 (1984).

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Decrease in lipid mobility in rat erythrocyte membrane after amiodarone chronic treatment

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Amiodarone is used as an antianginal and antiarrhythmic drug. Although its efficacy in the treatment of a wide spectrum of arrhythmias is well documented [1], its mode of action is poorly understood. *In vitro*, amiodarone displays several properties suggesting extremely low water-solubility and significant hydrophobic behaviour. These properties include micelle formation [2], spreading at the air–water interface [3] and high value of partition coefficient [4]. Studies on purified membranes [5] and on liposomes [6] indicate that amiodarone decreases lipid dynamics with a threshold concentration of 10^{-6} M and suggest that the compound is a rigid molecule deeply buried in the hydrocarbon core of the lipid bilayer. On the other hand, data from the literature [7, 8] indicate that effective treatment of arrhythmias in the human is achieved for a plasma concentration of 1.50–3.80 10^{-6} M. On the basis of these data, we decided to look for a possible effect of amiodarone on the lipid dynamics in rat erythrocyte membrane after chronic treatment.

Methods

Male Sprague–Dawley rats weighing 280–300 g were treated with amiodarone during 14 days at the doses of 50 or 150 mg/kg/day. Control group received the vehicle only (aqueous solution of gum-arabic). Blood was collected on heparin by intracardiac puncture 24 hr after the last administration. Erythrocyte ghosts were prepared according to Waelbroeck *et al.* [9]. Phospholipids and cholesterol were extracted [10] and their values determined according to standard procedures [11, 12]. Protein content was measured according to Lowry *et al.* [13]. Amiodarone was extracted and determined by high performance liquid chromatography [14]. Lipid dynamics was appreciated by fluorescence depolarization technique using 1,6 diphenylhexatriene (DPH) as fluorophore [5, 15]. Fluorescence depolarization and excited-state lifetime measurements were performed on a SLM 4800 Spectrofluorimeter. Steady-state fluorescence anisotropy (r_s) was determined by the emission intensities through two analyzers oriented respectively parallel ($I_\parallel$) and perpendicular ($I_\perp$) to the direction of polarization of the excitation light:

$$r_s = \frac{I_\parallel - I_\perp}{I_\parallel + 2I_\perp}$$

All results are expressed as means ± SEM; statistical differences were determined by unpaired Student's *t*-test.

Results and discussion

Control values of the steady-state fluorescence anisotropy (r_s) (Table 1) are in excellent agreement with published data [15–17]. Nevertheless, according to the Perrin equation [15]

$$\frac{r_o}{r_s} = 1 + C(r) \cdot T \cdot \frac{\tau}{\eta}$$

where r_o and r_s are respectively the limiting and the measured values of the fluorescence anisotropy, $C(r)$ is a structural parameter, T is the absolute temperature, τ is the excited-state lifetime of the probe and η is the viscosity of the medium, variations of r_s may result from variation of either τ or η or both. This is particularly important if one is looking for the effects of a foreign compound which may act as a quencher. In control experiments, we checked this possibility for amiodarone. Direct measurements of τ indicate that when up to 25% of amiodarone is incorporated in the lipid matrix, τ remains constant. Consequently, the observed variations in r_s values do represent variations in DHP mobility and thus in lipid dynamics.

Parameters such as the steady-state fluorescence anisotropy, r_s, and the derived order parameter S_V associated with the lipid dynamics in rat erythrocyte membrane indicate that amiodarone chronic treatment induces a decrease in lipid mobility, i.e. r_s values are increased (Table 1). To

Table 1. Effect of chronic amiodarone treatment on lipid dynamics parameters, phospholipids, cholesterol and amiodarone content in rat erythrocyte membranes

	Control (17)	Amiodarone 50 mg/kg/day (7)	Amiodarone 150 mg/kg/day (15)
r_s	0.248 ± 0.001	0.252 ± 0.001*	0.259 ± 0.001†
Amiodarone	—	0.21 ± 0.13	0.48 ± 0.15
Phospholipids	368 ± 9	357 ± 4	347 ± 8
Cholesterol	283 ± 17	283 ± 10	319 ± 22*
C/PL	0.77 ± 0.04	0.79 ± 0.03	0.92 ± 0.06*

r_s is the steady-state fluorescence anisotropy measured at 25°.
Amiodarone, phospholipids and cholesterol are expressed as 10^{-9} M/mg membrane protein.
Results are the mean ± SEM. ( ) represents number of rats * $P \leq 0.05$, † $P \leq 0.01$.

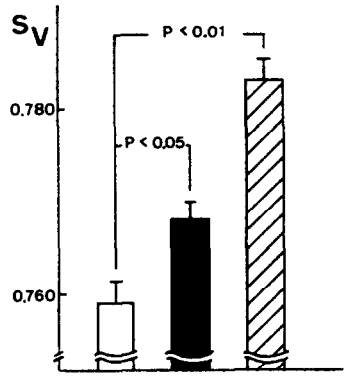

Fig. 1. Lipid order parameter S_V of the lipid matrix of erythrocyte ghosts. S_V is calculated through the equation $S_V = \frac{5}{2} r_\infty^{1/2}$ where $r_\infty = \frac{4}{3} r_s - 0.10$ [17]. The erythrocyte ghosts were prepared from blood of animals receiving the vehicle (open column), 50 mg/kg (closed column) and 150 mg/kg (hatched column) of amiodarone. Results are the mean ± SEM of 7–8 animals in each group.

this increase in r_s corresponds an increase in the degree of order S_V (Fig. 1). The effect is linked to the dose since it increases as a function of the increase of the latter. Amiodarone chronic treatment does not modify significantly the protein and phospholipid contents of the erythrocyte membranes. There is, however, an increase in the cholesterol content. As shown in Table 1, amiodarone is associated with the membrane although in a low amount. The amounts of amiodarone correspond to 0.3–0.7% of the total lipid content. In control experiments, in which radioactive amiodarone was incubated with erythrocyte prior to the membrane preparation, we found that up to 95% of the incorporated drug remained associated with the membrane during the procedure. Thus the amounts of amiodarone found are representative of the amounts of drug incorporated *in vivo*. In our experimental conditions, the measured plasma concentrations were $0.6\,10^{-6}$ and $4.1\,10^{-6}$ mol/l for rats receiving 50 and 150 mg/kg/day of amiodarone respectively for 14 days.

Determinants of lipid fluidity are the fatty composition of the lipids, the nature and ratios of the phospholipid classes and the cholesterol/phospholipid ratio. An increase in the degree of unsaturation of the phospholipid fatty acid increases lipid fluidity, whereas an increase in the sphingomyelin/phosphatidylcholine ratio and in the cholesterol/phospholipid ratio decreases membrane fluidity [15, 16]. Among these determinants, the cholesterol/phosphatidylcholine is the most critical in the control of lipid dynamics [15–17]. As several observations support the idea that compensatory changes controlling homeostasis of lipid fluidity can occur, we determined the total phospholipid and cholesterol contents in the erythrocyte membrane (Table 1). Significant change in the cholesterol content with no change in the phospholipid content occurred in our experimental conditions at the highest dose of amiodarone. Consequently the cholesterol/phospholipid ratio is increased leading to the expected increase in r_s and S_V. Whether or not amiodarone itself participates to the lipid ordering is difficult to assess. After a chronic treatment at the dose of 50 mg/kg, there is no significant variation of cholesterol and phospholipid content but r_s and S_V are already increased suggesting an effect of the drug. However, as amiodarone is present in a low amount, the lipid ordering effect of the molecule itself appears to be an insufficient explanation. Additional and/or alternative explanations could be (1) more specific interaction(s) between amiodarone and some lipids thus enhancing the lipid ordering effect of the possible complex, (2) the presence of metabolite(s) and (3) conformational changes of the proteins. During chronic treatment, a main metabolite of amiodarone namely desethylamiodarone appears at the same plasma concentration as the parent compound [18]. *In vitro*, this metabolite has qualitatively the same lipid ordering effect as amiodarone. Its presence in the erythrocyte membrane can thus contribute in addition or in synergy to the effect of amiodarone. Finally, amiodarone has been shown to partition equally in the lipid and protein of erythrocyte membranes [4]. Since the protein content of erythrocyte influences lipid microviscosity [19], a possible change in protein conformation and/or distribution within the lipid core due to amiodarone absorption could also be of relevance.

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REFERENCES

1. B. N. Singh, K. Nademanee, N. Ikeda and R. Kaman, in *New Aspects in the Treatment of Tachyarrhythmias. Role of Amiodarone* (Eds G. Breithardt and F. Loogen), pp. 46–56. Urban and Schwarzenberg, München (1983).
2. L. J. Ravin, E. G. Shami and E. S. Rattie, *J. Pharm. Sci.* **64**, 1830 (1973).
3. J. Ferreira, R. Brasseur, P. Chatelain and J. M. Ruysschaert, *J. Pharm. Pharmac.* **38**, 561 (1986).
4. P. Chatelain and R. Laruel, *J. Pharm. Sci.* **74**, 783 (1985).
5. P. Chatelain, R. Laruel and M. Gillard, *Biochem. biophys. Res. Commun.* **129**, 148 (1985).
6. P. Chatelain, J. Ferreira, R. Laruel and J. M. Ruysschaert, *Biochem. Pharmac.* **35**, 3007 (1986).
7. R. Latini, G. Tognoni and P. E. Kates, *Clin. Pharmacokinetics* **9**, 136 (1984).
8. W. J. McKenna and D. M. Krikler, *Br. Heart J.* **51**, 241 (1984).
9. M. Waelbroeck, G. Taton, M. Delhaye, P. Chatelain, J. C. Camus, R. Pochet, J. L. Leclerc, J. M. De Smet, P. Robberecht and J. Christophe, *Molec. Pharmac.* **24**, 174 (1983).
10. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
11. K. Itaya and M. Ui, *Clin. Chim. Acta.* **14**, 361 (1966).
12. P. Ott, Y. Bingelly and V. Brodbeck, *Biochim. biophys. Acta* **685**, 211 (1982).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. S. Pourbaix, Y. Berger, J. P. Desager, M. Pacco and C. Harvengt, *Clin. Pharmac. Ther.* **37**, 118 (1985).
15. M. Shinitzky and M. Inbar, *Biochim. biophys. Acta* **433**, 133 (1976).
16. R. A. Cooper, *N. Engl. J. Med.* **297**, 371 (1977).
17. W. J. Van Blitterswijk, R. P. Van Hoeven and B. W. Van Der Meer, *Biochim. biophys. Acta* **644**, 323 (1981).
18. T. A. Plomp, W. M. Wiersinga and R. A. A. Maes, *Arzneim. Forsch./Drug Res.* **35**, 122 (1985).
19. B. Aloni, M. Shinitzky and A. Lewine, *Biochim. biophys. Acta.* **348**, 438 (1974).