Biochemical Pharmacology, Vol. 34, No. 21, pp. 3831–3836, 1985.
Printed in Great Britain.

0006–2952/85 $3.00 + 0.00

STRUCTURAL CONSIDERATIONS FOR CALCIUM IONOPHORESIS BY PROSTAGLANDINS

MICHEL DELEERS,* PIERRE GROGNET† and ROBERT BRASSEUR‡

Laboratories of Experimental Medicine and ‡Macromolecules at Interfaces, School of Medicine and Faculty of Sciences, Université Libre de Bruxelles, B-1000 and B-1050, Brussels, Belgium

(*Received* 29 *March* 1985; *accepted* 28 *May* 1985)

Abstract—The prostaglandins PGB_2, PGE_2 and $PGF_{2\alpha}$ were found to translocate calcium in a modified Pressman cell. At pH 7.40, PGB_2 was more potent than PGE_2 and than $PGF_{2\alpha}$. When incorporated at a 1% molar ratio in liposomes made of cholesterol and different diacyl phosphatidyl choline, prostaglandins are able to mediate a slow calcium exchange diffusion. A significant prostaglandin-mediated calcium release that depends on the lipid matrix rigidity is observable at 37° but not at 22°. Conformational analysis of the complex formed by two molecules of prostaglandins and one calcium atom, either at a simulated membrane–water interface or in a simulated bulk lipid phase reveals rigid complexes with great distances between hydrophilic and hydrophobic gravity centres that predict low ionophoretic properties.

In recent years much attention has been paid to the metabolism of arachidonic acid which after stimulation of secretory cells generates leukotrienes and prostaglandins via the lipoxygenase and cycloxygenase pathways [1–4]. This membrane phospholipid turnover is generally followed by an enhancement in Ca fluxes. It has been proposed that some phospholipids, prostaglandins and leukotrienes could act as endogenous ionophores [5–12]. More precisely, prostaglandins E_2 and $F_{2\alpha}$ were suggested to be ionophores since their action on sarcoplasmic reticulum vesicles resembled that of A23187 and X537A ionophores [6]. Prostaglandins B_2 and E_2 were effectively found to translocate Ca^{2+} in a Pressman cell [7]. However, several authors have shown that only a polymeric derivative of PGB_1 was able to mediate Ca^{2+} transport in liposomes whilst none of the prostaglandins they tested translocate Ca^{2+} in this system [8]. As for phosphatidic acid [9, 13, 14], there are controversial debate about the ionophoretic properties of prostaglandins.

The aim of this study is to show that prostaglandins may mediate to a different but small extent, a translocation of Ca^{2+} through an organic phase and a release of Ca^{2+} from liposomes. The interaction of different prostaglandins with Ca^{2+} is also studied by theoretical conformational analysis providing results in fair agreement with the results of Ca^{2+} translocation and transport.

MATERIALS AND METHODS

The prostaglandins B_2, E_2 and $F_{2\alpha}$ (PGB_2, PGE_2 and $PGF_{2\alpha}$), the lipids distearoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine (DSPC, DPPC and DMPC) and cholesterol (chol) were obtained from Sigma Chemical Co. (St Louis, MO). $^{45}Ca^{2+}$ in the $CaCl_2$ form was obtained from New England Nuclear (Boston, MA).

The methods of measuring Ca^{2+} translocation from one aqueous phase into another across an organic phase [15] and of measuring the exchange diffusion of Ca^{2+} in liposomes [16–18] have been described in full detail in prior publications. Briefly, an organic mixture containing the prostaglandins is transferred between two tubes after vigorous mixing for 1 min to ensure equilibrium partition between the two phases. Paired samples are removed from the aqueous media and examined for their radioactive content [15, 18]. For the exchange diffusion of Ca^{2+} in liposomes incubated in a medium deprived of $^{45}Ca^{2+}$, the radioactive content in liposomes is examined as a function of time [16–18]. The final lipid concentration (PC + chol) is 5 mg/ml (approximately 6.5 mM).

The method used for the theoretical conformational analysis of the different Ca–prostaglandins complexes is based on a semi-empirical method described elsewhere [19–21]. Briefly, the total conformational energy, which represents the sum of the contributions resulting from the Van der Waals interactions, the torsional potential and the electrostatic interactions, is calculated for a large number of conformations in a systematic analysis that takes all torsional angles and all atoms into account. The conformations yielding the lowest internal energy were then submitted to an energy function minimization procedure in a bulk lipid phase or at a simulated lipid–water interface [20, 21], taking into account the values of hydrophilic and hydrophobic gravity centres [21–23]. Calculations were made on a CDC cyber 170 coupled to a drawing table Calcomp 1051 utilizing the Pluto drawing program [24].

* Present address: UCB Pharma, Bldg R2, Department of Biochemistry, chemin du Foriest, B-1420 Braine l'Alleud, Belgium. To whom correspondence should be addressed.

† Present address: Ixelles Hospital, Department of Biochemical Analysis, B-1050 Brussels, Belgium.

RESULTS

Ca^{2+} *translocation across an organic phase*

Prostaglandins at a 4 mM concentration transported calcium across an organic phase (toluene/butanol, 7/3, v/v) from a triethanolamine–HCl buffer (20 mM, pH 7.4) containing 20 μM $CaCl_2$ on both sides with a tracer amount of $^{45}Ca^{2+}$ on one side (Fig. 1). Under these conditions, PGB_2 was more potent than $PGF_{2\alpha}$ and than PGE_2.

Ca^{2+} *exchange diffusion in liposomes*

Prostaglandins incorporated in membranes of lipid vesicles at a final concentration of 1 mole per 100 moles of total lipids, significantly stimulated $^{45}Ca^{2+}$ outflow from liposomes formed of DSPC:chol (2/1 molar ratio) (Fig. 2), DPPC:chol (2/1 molar ratio) (not shown) and DMPC:chol (2/1 molar ratio) (Fig. 3) when incubated at 37°. When DPPC:chol liposomes containing the prostaglandins were incubated at 22°, no Ca^{2+} outflow could be significantly detected (data not shown).

Conformation of Ca–*prostaglandins complexes*

The molecular structures [25], the numbering of the torsional angles, together with the all *trans* conformations of half of the three prostaglandins complexes taken as our initial configurations are illustrated in Fig. 4. Each complex has 23 or 25 rotational angles when one considers the PG–Ca–PG complexes. If all angles of Pg_2–Ca complexes were affected by systematic 60° changes, up to 7.89 10^{17} or 2.84 10^{19} conformations could be designated. A

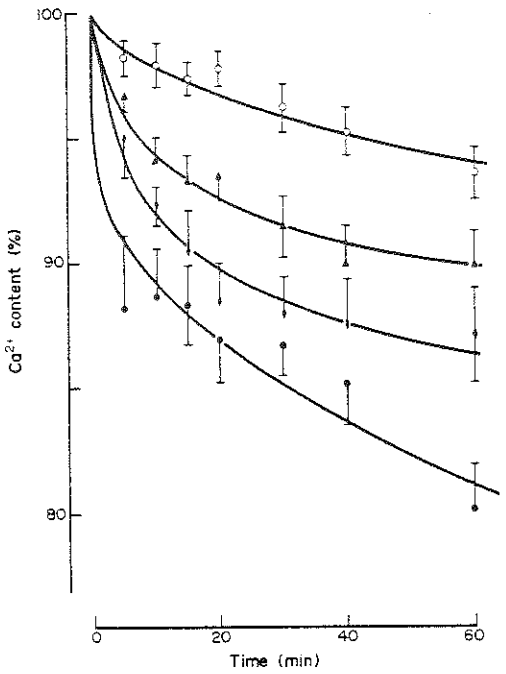

Fig. 2. Outflow of $^{45}Ca^{2+}$ from DSPC:chol (2:1 molar ratio) liposomes containing either PGB_2 (closed circles), $PGF_{2\alpha}$ (closed diamonds) or PGE_2 (closed triangles) at a 1% molar concentration vs control liposomes (open circles). The $^{45}Ca^{2+}$ content of the liposomes is expressed in % of the initial content. Mean values (±S.E.M.) refer to 4 or 12 (control) individual observation.

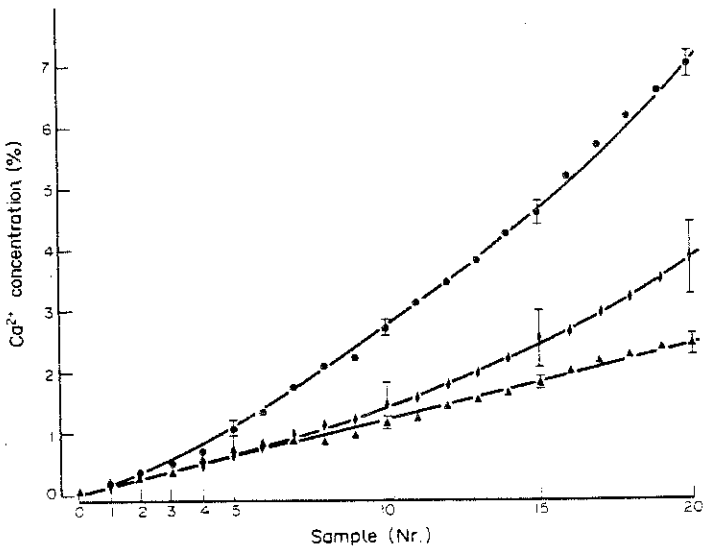

Fig. 1. The translocation of $^{45}Ca^{2+}$ was mediated by an organic phase containing as required PGB_2 (closed circles), $PGF_{2\alpha}$ (closed diamonds) and PGE_2 (closed triangles) at 4 mM concentration, and occurred between aqueous media initially containing 20 μM $CaCl_2$. The $^{45}Ca^{2+}$ content of the aqueous media was measured in the phase initially deprived of $^{45}Ca^{2+}$ after each back and forth transfer of the organic phase, and is expressed as percentage of the total radioactive content of the first compartment. Mean values (±S.E.M.) refer to 3 individual observations.

Table 1. Probabilities (in %) of the most probable configurations obtained after successive analysis on the 3 complexes and bearing on the indicated torsional angles (see Fig. 4)

Torsional angles	PGB_2	PGE_2	$PGF_{2\alpha}$
O–Ca–O, 1, 2, 3, 1′, 2′ and 3′	50	84	57
4, 5, 7, 4′, 5′ and 7′	100	100	52
8, 9, 10, 8′, 9′ and 10′	91	36	96
11, 12, (13), 11′, 12′ and (13′)	100	100	100
Probabilities products	45.5	30.2	28.5
Hydrophilic–hydrophobic distance in bulk hydrophobic	2.55	3.12	2.04
Hydrophilic–hydrophobic distance at membrane interface	3.90	2.91	2.64

The torsional angles 1′ to 13′ are the symmetrical angles of angles 1 to 13 defined in Fig. 4 (point symmetry on Ca^{2+}).

more economic procedure was used therefore [26], systematic analysis being carried out on different parts of the complexes. In this procedure, the presence of all atoms is considered while only small parts of the whole complex are twisted. All the conformations obtained are eventually submitted to the internal energy calculation. Table 1 summarizes the most probable configurations obtained after four successive systematic analyses in a medium of low dielectric constant representative of the hydrophobic part of a membrane and in a medium of dielectric constant that simulates the lipid–water interface [20, 21]. Figure 5 illustrates the most probable conformations of PGB_2, PGE_2 and $PGF_{2\alpha}$ complexes after application of the minimization procedure at the membrane–water interface. No obvious differences could be seen between the bulk lipid phase complexes (Fig. 6) and the interfacial complexes since the distance between hydrophilic and hydrophobic gravity centres are weakly affected.

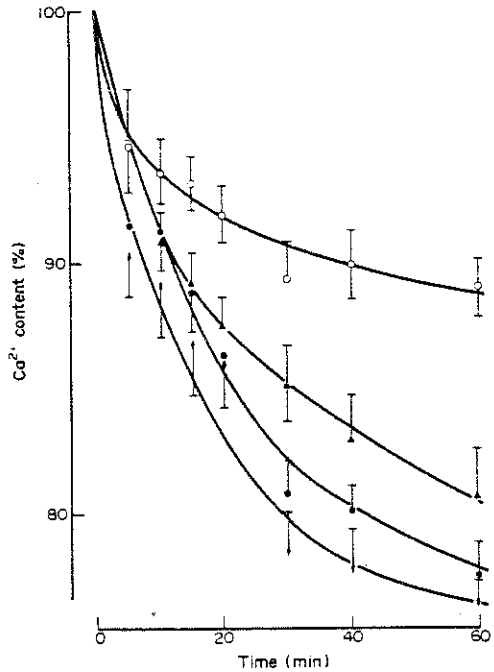

Fig. 3. Outflow of $^{45}Ca^{2+}$ from DMPC:chol (2:1 molar ratio) liposomes containing either PGB_2 (closed circles), $PGF_{2\alpha}$ (closed diamonds) or PGE_2 (closed triangles) at a 1% molar concentration vs control liposomes (open circles). The $^{45}Ca^{2+}$ content of the liposomes is expressed in % of the initial content. Mean values (±S.E.M.) refer to 8 or 18 (control) individual observation.

DISCUSSION

It has been suggested that prostaglandins may act as Ca^{2+} ionophores [6] and demonstrated that they effectively transport Ca^{2+} through organic phases [6, 7]. We have shown here that prostaglandins may act as weak Ca^{2+} ionophores depending on their chemical structures. Indeed, PGB_2 appears more active than $PGF_{2\alpha}$ which is itself more potent than

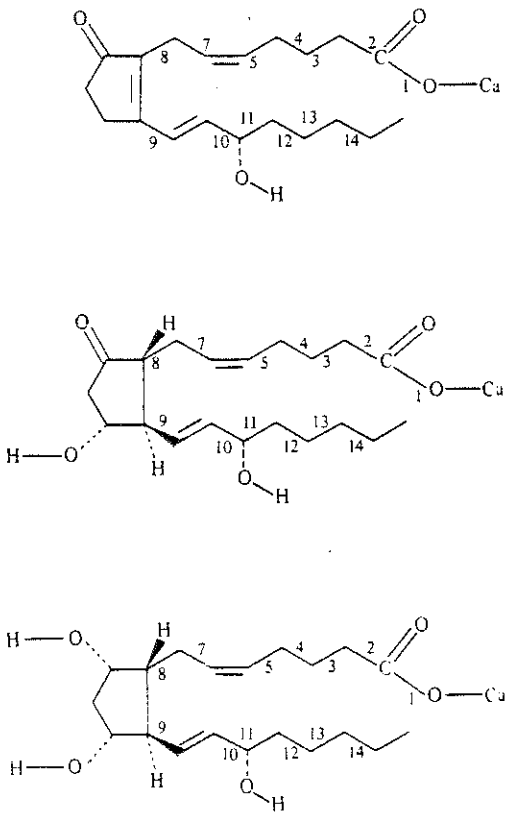

Fig. 4. Initial all-*trans* conformations of half of the complexes formed by PGB_2 (top), PGE_2 (middle) and $PGF_{2\alpha}$ (bottom) with calcium together with the numbering of the torsional angles. Conformations are taken from ref. 25.

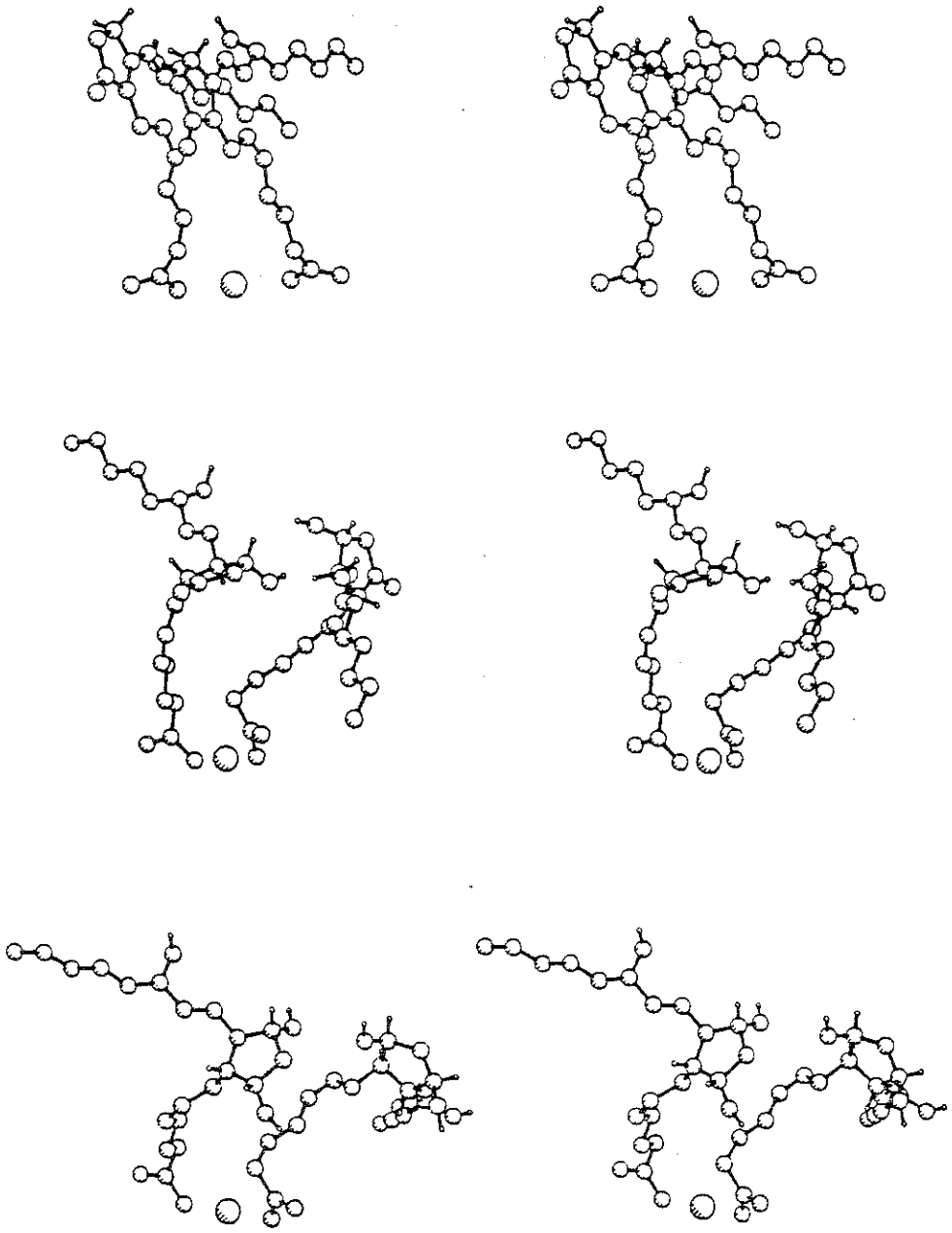

Fig. 5. Stereoscopic view of the conformation of PGB_2 (top), PGE_2 (middle) and $PGF_{2\alpha}$ (bottom) complexes with Ca at the simulated lipid–water interface (imaginary plane through Ca separating two spaces of dielectric constant of 3 above and 30 under the plane) when transfer energies between the two media [25] are taken into account. The distances between hydrophilic and hydrophobic centres are 3.90, 2.91 and 2.65 Å respectively.

PGE_2 (Figs 1 and 2). Prostaglandin-mediated Ca^{2+} release is observable at 37° but not at 22°. DPPC–chol liposomes containing 0.3, 1 or 3% molar prostaglandins were effectively stable for Ca^{2+} efflux at 22°, showing neither that these membranes are lysed by the presence of PGs nor that micelles formed by high concentrations of PGs could destabilize the membrane (data not shown).

As stated by others [8], the activity of prostaglandins is very weak in regard to that one can obtain with calcium ionophores like A23187 [8, 16], ionomycin [9, 17] or FOD [18]. It is, however, conceivable that the ionophoretic activity of prostaglandins may be better by acting with the "endogenous ionophores" by forming cooperative ionophoretic complexes [20, 27, 28]. It could be argued here that 1 mole PG per 100 moles of lipids is a high concentration but the final concentration of prostaglandins is 65 μM so that they respond to all the criteria proposed by others [9] for testing calcium ionophoresis except that the permselectivity has not been tested.

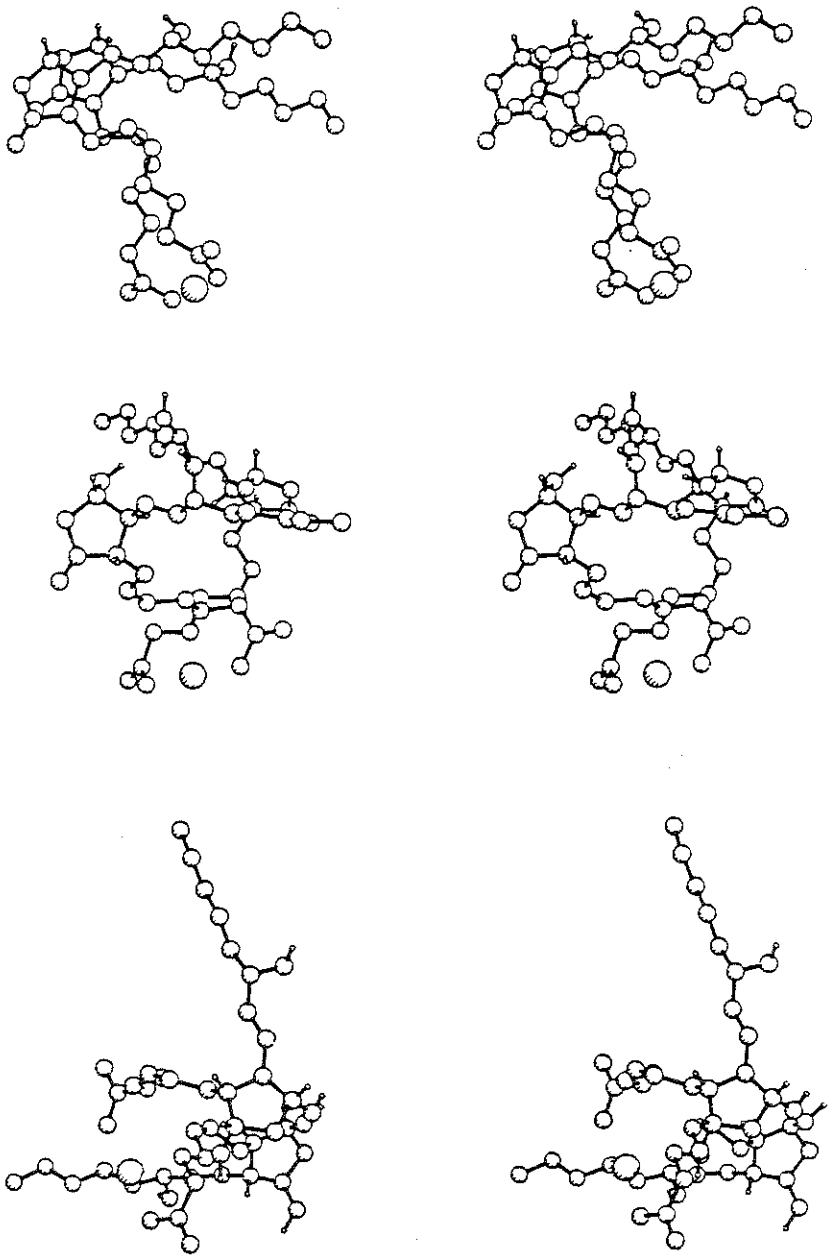

Fig. 6. Steroscopic view of the conformations of the prostaglandins–Ca complexes (same presentation as in Fig. 5) but calculated in a continuous medium of dielectric constant of 3. The distances between the hydrophilic and hydrophobic gravity centres are 2.55, 3.12 and 2.04 Å respectively.

It could also be noted that the calciphoretic properties of prostaglandins have unsuccessfully been tested in lipid vesicles [8, 9], but these works were done at room temperature. We were effectively unable to significantly detect a $^{45}Ca^{2+}$ exchange-diffusion while this is possible at 37° and our method allows to determine transport rate as low than 0.1 mmole of Ca^{2+} per mole of lipid per min [12, 18, 29] also recently reported by others [14]. It is clear from Fig. 1 that the differences in calciphoretic activities between the PGs are significant, so that it is unlikely that these activities are solely attributable to a Ca^{2+}-ferrying by their carboxylic moieties. It is true, however, that in liposomes, when judged between the 10th and the 60th min, only the differences between PGB_2 and PGE_2 ($P < 0.025$) and between PGE_2 and $PGF_{2\alpha}$ ($P < 0.050$) in DSPC–chol are significant. In DMPC–chol liposomes, these differences are less significant ($P < 0.100$ for both) while the differences between PGB_2 and $PGF_{2\alpha}$ are never significant in both types of liposomes.

The conformational analysis reveals that the $(PG)_2$–Ca complexes are quite rigid and that the distances between their hydrophilic and hydrophobic gravity centres are weakly affected by the passage from the simulated interface into the simulated bulk

lipid phase. These distances remain too large to obtain good permeant species [26, 30]. Indeed, the conformation of calcium ionophores demonstrates an important diminution of this distance when the complex is pushed into the membrane core. Conformation of *cis*-leukotriene B_4–Ca complexes reveals, for instance, an important lowering of this distance [26] and this molecule has been demonstrated to be a potent Ca^{2+} ionophore [10]. The results of conformational analysis are effectively in good agreement with the low calciphoretic properties of the PGs. Moreover, if one consider the diminution of the hydrophilic–hydrophobic gravity centres distance, the conformation results parallel the potency of the prostaglandins seen in the experiments. PGB_2 shows a diminution of 1.35 Å while $PGF_{2\alpha}$ and PGE_2 give diminution of 0.60 and −0.21 Å respectively.

In conclusion, we have attempted to show in this study that some prostaglandins may demonstrate weak but different calciphoretic properties that are well correlated by conformational analysis of the different PG–Ca complexes at the simulated membrane–water interface and in the bulk lipid phase. This does not mean that PGs have to be considered as calcium ionophores, but that they may for instance, be transported from their sites of synthesis as Ca^{2+}-complexes. It could also be that their Ca binding properties may favour their interaction with membrane receptors and by doing so may affect the calcium mobilization in cells.

Acknowledgements—We thank Professor W. J. Malaisse for supervising this work and Mrs M. Mahy for expert technical assistance. We thank the computing centre of the Université Libre de Bruxelles. This work was supported in part by a grant from the Action Recherches Concertées.

REFERENCES

1. E. G. Lapetina, *Trends pharmac. Sci.* **3**, 115 (1982).
2. J. W. Putney, *Life Sci.* **29**, 1183 (1981).
3. J. W. Putney, L. M. De Witt, P. C. Hoyle and J. S. McKinney, *Cell Calcium* **2**, 561 (1981).
4. M. J. Berridge, *Molec. Cell. Endocr.* **24**, 141 (1981).
5. C. A. Tyson, M. Van de Zande and D. E. Green, *J. biol. Chem.* **251**, 1326 (1976).
6. M. E. Carsten and J. D. Miller, *J. biol. Chem.* **252**, 1576 (1977).
7. M. E. Carsten and J. D. Miller, *Archs Biochem. Biophys.* **185**, 282 (1978).
8. G. Weissmann, P. Anderson, C. Serhan, E. Samuelsson and E. Goodman, *Proc. natn. Acad. Sci. U.S.A.* **77**, 1506 (1980).
9. C. Serhan, P. Anderson, E. Goodman, P. Dunham and G. Weissman, *J. biol. Chem.* **256**, 2736 (1981).
10. C. N. Serhan, J. Fridovitch, E. J. Goetzl, P. B. Dunham and G. Weissman, *J. biol. Chem.* **257**, 4746 (1982).
11. I. Valverde and W. J. Malaisse, *Biochem. biophys. Res. Commun.* **89**, 386 (1979).
12. M. Deleers, M. Mahy and W. J. Malaisse, *Biochemistry Int.* **4**, 47 (1982).
13. R. P. Holmes and N. L. Yoss, *Nature, Lond.* **305**, 637 (1983).
14. R. Nayar, L. D. Mayer, M. J. Hope and P. R. Cullis, *Biochim. biophys. Acta* **777**, 343 (1984).
15. R. Anjaneyulu, K. Anjaneyulu, E. Couturier and W. J. Malaisse, *Biochem. Pharmac.* **29**, 1879 (1980).
16. M. Deleers and W. J. Malaisse, *Biochem. biophys. Res. Commun.* **95**, 650 (1980).
17. M. Deleers, E. Couturier and W. J. Malaisse, *Cell Calcium* **2**, 159 (1981).
18. M. Deleers, R. Brasseur and W. J. Malaisse, *Chem. Phys. Lipids* **33**, 11 (1983).
19. R. Brasseur, M. Deleers, W. J. Malaisse and J. M. Ruysschaert, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2895 (1982).
20. M. Deleers, R. Brasseur, J. M. Ruysschaert and W. J. Malaisse, *Biophys. Chem.* **17**, 313 (1983).
21. R. Brasseur, M. Deleers and W. J. Malaisse, *Biochem. Pharmac.* **32**, 437 (1983).
22. C. Tanford, *Hydrophobic Effects: Formation of Micelles and Biological Membranes*. John Wiley, New York (1972).
23. R. Brasseur, C. Vandenbosh, H. Van den Bossche and J. M. Ruysschaert, *Biochem. Pharmac.* **32**, 2715 (1983).
24. B. C. Motherwell and C. Clegg, *Pluto*. Cambridge University Press, London (1978).
25. *The Merck Index*, 9th Edn (Eds. M. Windholz, S. Budavari, L. Y. Stroumtsos and M. N. Fertig). Merck, Rahway (1976).
26. R. Brasseur and M. Deleers, *Proc. natn. Acad. Sci. U.S.A.* **81**, 3370 (1984).
27. M. Deleers, M. Gelbcke and W. J. Malaisse, *Proc. natn. Acad. Sci. U.S.A.* **78**, 279 (1981).
28. M. Deleers, P. Grognet, M. Mahy, R. Brasseur and W. J. Malaisse, *Res. Commun. Chem. Pathol. Pharmac.* **41**, 407 (1983).
29. M. Deleers, *Arch Int. Physiol. Biochim.* **92**, BP5 (1984).
30. R. Brasseur, M. Deleers and J. M. Ruysschaert, *Biosci. Repts* **4**, 651 (1984).