Transport of Pr^{3+} by hypoglycemic sulfonylureas across liposomal membranes

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1. INTRODUCTION

Hypoglycemic sulfonylureas provoke insulin release apparently by stimulating the inflow of Ca^{2+} into pancreatic islet cells [1,2]. The mechanism by which hypoglycemic sulfonylureas facilitate Ca^{2+} inflow into the B cell is not elucidated. It is conceivable but remains to be proved that such a facilitation is somehow related to the ionophoretic capacity of hypoglycemic sulfonylureas [3]. These agents are able to translocate Ca^{2+} into or across a hydrophobic domain [4]. Hypoglycemic sulfonylureas act synergistically with the ionophore A23187 [5], and possibly native ionophores [6], a situation attributable to the formation of a hybrid complex in which each Ca^{2+} ion is bound to both one molecule of A23187 and one molecule of the hypoglycemic agent [7].

Several studies have shown that ^1H-NMR can be used to measure the transport of paramagnetic ions, as a model for Ca^{2+} transport, across phospholipid membranes [8–13]. Thus, in the absence of ionophore, the paramagnetic ion Pr^{3+} shifts the trimethylammonium proton signal on the sole external surface of phosphatidylcholine liposomes, without penetrating into the liposomal lumen [8,11,13]. This study indicates that hypoglycemic sulfonylureas act as Pr^{3+}-ionophore in liposomes.

2. MATERIALS AND METHODS

DL-α-dipalmitoyl-phosphatidylcholine (DPPC)

was purchased from Sigma (St Louis, MO). Praseodymium chloride ($PrCl_3 \cdot 6H_2O$) was obtained from Aldrich (Beerse). Glibenclamide and gliclazide were kindly provided by Hoechst Belgium and Servier Benelux (Brussels), respectively.

Monolamellar and oligolamellar lipid vesicles were prepared by sonicating 100 mg of DPPC (4-times for 3 min) in 4 ml of D_2O (99.8%; Merck, Darmstadt). The solution contained NaOH (10 μM) to minimize the lowering of pH upon addition of $PrCl_3$. Under these conditions, the internal and external pH averaged 9.0 ± 0.1 and 7.4 ± 0.1, respectively, after addition of $PrCl_3$. We used a Branson sonifier (type B12) fitted with a normal tip at 65 W. Glibenclamide or gliclazide were added to the liposomal suspension from either a stock solution in dimethylsulfoxide (final concentration of the solvent $\leqslant 1\%$, v/v) or a dry film prepared in the NMR tubes by evaporation of the solvent (chloroform) under a stream of N_2 [13]. The two procedures yielded essentially the same results. The nominal concentration of sulfonylurea ranged from 0.6 to 2.0 mM (when expressed relative to the volume of the liposomal suspension) or 1.8 to 6.0 mol/100 mol of lipid (when expressed relative to the amount of DPPC present in each sample). When examining the influence of hypoglycemic sulfonylureas upon Pr^{3+} transport, the drugs were added to the liposomal suspension a few min prior to the addition of $PrCl_3$. The hypoglycemic agents failed to affect the NMR spectra in the absence of $PrCl_3$. In all experiments, $PrCl_3$ (10 μl, 300 mM) was added to yield an initial extravesicular con-

centration close to 6 mM.

The NMR spectra were recorded at 60°C in a Jeol X 100 spectrometer at 100 MHz in 5-mm NMR tubes containing 0.5 ml of the liposomal suspension. The rate of Pr^{3+} transport was judged from either the shift in frequency (expressed in Hz) of the intravesicular toward extravesicular head group signals or the increase in the extravesicular/intravesicular ratio for the height of the head group signals (expressed as % increase relative to the initial value).

3. RESULTS

Fig.1 illustrates the NMR spectra of DPPC liposomes in the absence and presence of $PrCl_3$. The spectrum recorded after addition of $PrCl_3$ remained unchanged for at least 2 h in the absence of sulfonylurea, indicating stability of the liposomal preparation. The extravesicular/intravesicular ratio for the height of head group signals was close to 1.5, which would correspond to a mean liposomal diameter close to 30 nm in a homogeneous population of unilamellar liposomes [11,13].

Fig.2 illustrates the changes in the NMR spectra of liposomes incubated with hypoglycemic sulfonylureas in the presence of $PrCl_3$. The rate of Pr^{3+} transport into the vesicles was not vastly different whether judged from the shift in frequency

or increase in the height ratio of the head group signals. Thus, in a series of 19 individual determinations, the regression line relating the shift in frequency (S, expressed as Hz) to the increase in height ratio (H, expressed as %) corresponded to the following equation:

$$S = 0.212 \, H - 0.004$$

with a coefficient of correlation between the two measurements amounting to 0.958. The shift in frequency was used in the further analysis of experimental results.

Fig.3 (left panel) illustrates the time course for the accumulation of Pr^{3+} in the vesicle lumen as judged from the shift in frequency. The mean rate of Pr^{3+} transport, as judged from the 3 first readings in each experiment averaged 0.260 ± 0.021 and 1.040 ± 0.159 Hz/min in the presence of gliclazide 3 and 6 mol/100 mol of lipid, respectively, and 0.453 ± 0.024 and 1.492 ± 0.151 Hz/min in the presence of glibenclamide 1.8 and 6 mol/100 mol of lipid, respectively. On a molar basis, glibenclamide thus appeared more efficient than gliclazide in mediating Pr^{3+} transport. The rate of Pr^{3+} transport was proportional to the drug concentration in the presence of glibenclamide (fig.3, right panel). In the presence of gliclazide, however, the rate of Pr^{3+} transport relative to the concentration of sulfonylurea (mol/100 mol of lipid) increased from 87 ± 7 to 173 ± 27 mHz/min
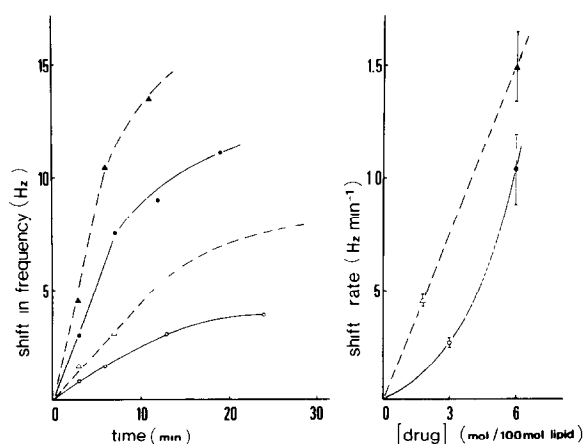

Fig.1. ¹H-NMR spectrum of DPPC vesicles prior to (upper trace) and after (lower trace) addition of $PrCl_3$. Chemical shifts are shown in Hz with reference to H_2O.
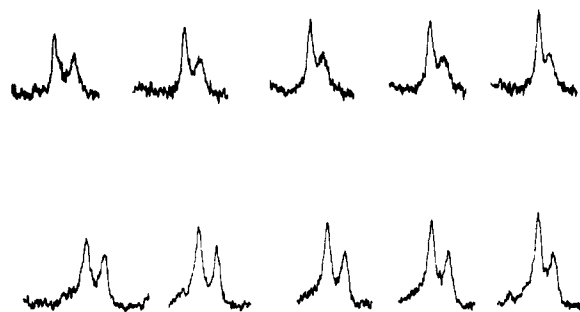

Fig.2. Changes in ¹H-NMR spectra of head group signals from DPPC vesicles incubated in the presence of either gliclazide (6 mol/100 mol of lipid; upper traces) or glibenclamide (1.8 mol/100 mol of lipid; lower traces), together with $PrCl_3$. Readings were performed 3, 7, 12 and 19 min (upper traces), or 3, 7, 14 and 27 min (lower traces) after the first reading (left traces), recorded shortly (≤ 2 min) after addition of $PrCl_3$ to the liposomal suspension.
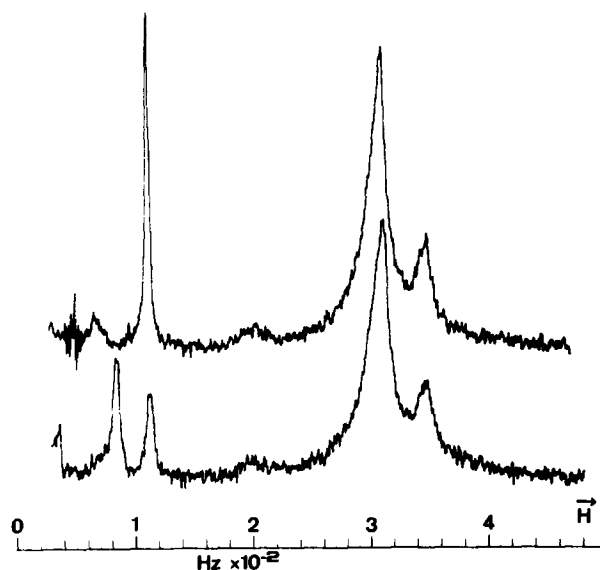
Fig.3. Time course (left) and dose–action relationship (right) for the effect of glibenclamide (triangles) and gliclazide (circles) upon the shift in head group signals in DPPC liposomes exposed to $PrCl_3$. The shift in frequency (left panel) was measured at 2 concentrations of each drug (see right panel), the regression line being derived from the first 2 (closed symbols) or 3 (open symbols) measurements performed after the initial reading (time zero). In the right panel, mean values (± SEM) for the shift rate are derived from the first 3 measurements performed after the initial reading.

($P < 0.05$) as the concentration of the drug was raised from 3 to 6 mol/100 mol of lipid.

4. DISCUSSION

The present results demonstrate that two distinct hypoglycemic sulfonylureas are able to transport Pr^{3+} across the phospholipid boundary of liposomes. This is consistent with a preliminary report indicating that gliclazide facilitates the release of ^{45}Ca from liposomes [14].

The rate of Pr^{3+} transport relative to the molar concentration of the drug was one to two orders of magnitude lower with glibenclamide than with 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-octane-4,6-dione or A23187 [13]. This difference in efficiency coincides with a low affinity for Ca^{2+} of hypoglycemic sulfonylureas (Keq about 10^{-14}) [7,15] as compared to such ionophores as X537A

(Keq = 10^{-10}) and A23187 (Keq = 4.10^{-7}) [16,17].

In this perspective, it could be objected that the concentration of sulfonylurea used in the present experiments appears much higher than that used in studies performed with isolated pancreatic islets. Such is not the case, however, if reference is made to the concentration of drug/unit membrane area. In the present system, the amount of glibenclamide added to each sample (0.3–1.0 μmol) relative to the area of the vesicles (about 3 m^2, assuming a value of 4 mg phospholipid/m^2 of bilayer) yielded a ratio of 0.10–0.33 μmol/m^2. This is comparable to the situation found in islets incubated in the presence of 5–16 μM glibenclamide (i.e., 2.4–8.0 μg/ml). In this range of concentrations, the binding of glibenclamide to isolated islets increases from about 0.18–0.56 μmol/g dry weight [18]. Since the total area of plasma membrane in each islet (about 1.1 μg dry weight; see [19] is about 1.86 mm^2 [20], the binding of glibenclamide indeed corresponds to 0.10–0.33 μmol/m^2.

In conclusion, the present findings indicate that hypoglycemic sulfonylureas such as gliclazide and glibenclamide are able to transport Pr^{3+} across a lipid membrane. Since there is usually an analogy between the transport of Pr^{3+} and other cations, it is tempting to suggest, but remains to be proved, that hypoglycemic sulfonylureas may act in a comparable manner in facilitating cation transport across the B-cell plasma membrane.

REFERENCES

[1] Malaisse, W.J., Mahy, M., Brisson, G.R. and Malaisse-Lagae, F. (1972) Eur. J. Clin. Invest. 2, 85–90.

[2] Lebrun, P., Malaisse, W.J. and Herchuelz, A. (1983) Diabetes, in press.

[3] Couturier, E. and Malaisse, W.J. (1980) Diabetologia 19, 335–340.

[4] Couturier, E. and Malaisse, W.J. (1980) Arch. Int. Pharmacodyn. 245, 323–334.

[5] Couturier, E. and Malaisse, W.J. (1980) Br. J. Pharmacol. 71, 315–320.

[6] Anjaneyulu, R., Anjaneyulu, K., Couturier, E. and Malaisse, W.J. (1980) Biochem. Pharmacol. 29, 1879–1882.

[7] Deleers, M., Brasseur, R., Gelbcke, M. and Malaisse, W.J. (1982) J. Inorg. Biochem. 16, 215–225.

[8] Fernandez, M.S., Celis, H. and Montal, M. (1973) Biochim. Biophys. Acta 323, 600–605.

[9] Hunt, G.R.A. (1975) FEBS Lett. 58, 194–196.

[10] Hunt, G.R.A., Tipping, L.R.H. and Belmont, M.R. (1978) Biophys. Chem. 8, 341–352.

[11] Hunt, G.R.A. and Tipping, L.R.H. (1978) Biochim. Biophys. Acta 507, 242–261.

[12] Bergelson, L.D. (1978) Meth. Membrane Biol. 9, Plenum, London.

[13] Hunt, G.R.A. (1980) Chem. Phys. Lipids 27, 353–364.

[14] Deleers, M., Couturier, E., Mahy, M. and Malaisse, W.J. (1980) Arch. Int. Pharmacodyn. 246, 170–172.

[15] Deleers, M., Couturier, E., Mahy, M., Gelbcke, M. and Malaisse, W.J. (1981) Ann. Endocrinol. 42, 297–299.

[16] Reed, P.W. and Lardy, H. (1972) J. Biol. Chem. 247, 6970–6977.

[17] Johnson, S.J., Herrin, J., Liu, S.J. and Paul, I.C. (1970) J. Am. Chem. Soc. 92, 4428–4435.

[18] Hellman, B., Sehlin, J. and Täljedal, I.-B. (1973) Diabetologia 9, 210–216.

[19] Sener, A. and Malaisse, W.J. (1978) Diab. Métab. 4, 127–133.

[20] Malaisse, W.J., Lebrun, P. and Herchuelz, A. (1983) Pflügers Arch., in press.