Polarization of Chlorotetracycline Fluorescence in Pancreatic Islet Cells and its Response to Calcium Ions and D-Glucose

By INGE-BERT TÄLJEDAL

Department of Histology, University of Umeå, S-901 87 Umeå 6, Sweden

(Received 28 June 1978)

Suspensions rich in pancreatic β -cells were prepared from non-inbred *ob/ob*-mice, incubated with 10 μ M-chlorotetracycline, and analysed for fluorescence polarization in a microscope. Throughout the temperature range 16–38°C, fluorescence was enhanced by 5 mM- Ca^{2+} in the incubation medium; 20 mM-D-glucose decreased the fluorescence measured in the presence of Ca^{2+} . Fluorescence showed a curvilinear negative regression on temperature. The curves were rectified to a virtually ideal degree by Arrhenius transformations of data. Non-parametric testing of differences between linearized regression lines forms the basis for the following conclusions. The temperature-dependence of fluorescence intensity appeared to be smaller for Ca^{2+} -specific signals than for the background fluorescence of chlorotetracycline in Ca^{2+} -deficient cells. D-Glucose significantly diminished the polarization of fluorescence in cells incubated with Ca^{2+} . It is suggested that D-glucose increases the mobility of Ca^{2+} in β -cell plasma membranes; this mobility increase may help to explain previously reported effects of D-glucose on $^{45}\text{Ca}^{2+}$ fluxes and membrane electric potential.

Glucose-stimulated insulin release depends reversibly on extracellular Ca^{2+} (Grodsky, 1972; Hellman *et al.*, 1973a) and is associated with uptake of $^{45}\text{Ca}^{2+}$ (Malaisse-Lagae & Malaisse, 1971; Hellman *et al.*, 1971, 1976a,b; Henquin & Lambert, 1975; Naber *et al.*, 1977) and increased histochemical staining for Ca^{2+} (Herman *et al.*, 1973; Schäfer & Klöppel, 1974; Ravazzola *et al.*, 1976) in the β -cells. It is generally suspected, therefore, that Ca^{2+} may mediate the signal to secretion aroused by D-glucose.

Ca^{2+} can bind to an array of molecules by ligand and ionic interactions. Experiments with $^{45}\text{Ca}^{2+}$ and various histochemical stains, as well as measurements of Ca^{2+} -dependent adenosine triphosphatase activities, attest to the multitude of compartments that need be considered as potential loci for regulatory roles of Ca^{2+} in β -cells. At the supramolecular level, at least the plasma membrane, the secretory granules, the mitochondria and the endoplasmic reticulum have been thought to interact with Ca^{2+} in such a manner as to suggest importance for insulin release (Herman *et al.*, 1973; Schäfer & Klöppel, 1974; Howell *et al.*, 1975; Hellman *et al.*, 1976b; Sehlin, 1976; Formby *et al.*, 1976; Ravazzola *et al.*, 1976). Any one of these structures may contain populations of Ca^{2+} -binding sites with distinct physiological roles.

A previous paper analysed the possibility of using the fluorescent chelate probe chlorotetracycline for microscopic measurements of Ca^{2+} interaction with β -cells (Täljedal, 1978). Low concentrations (10 μ M) of chlorotetracycline appeared predominantly to

monitor Ca^{2+} associated with the β -cell plasma membranes. The Ca^{2+} -dependent fluorescence was suppressed by D-glucose in the physiological concentration range. 3-O-Methyl-D-glucose, which does not stimulate insulin release, but fits the hexose-transport system (Hellman *et al.*, 1973b), had a significantly smaller effect. L-Glucose, lacking effect on both secretion and sugar transport, did not influence the Ca^{2+} -dependent chlorotetracycline fluorescence.

Although the fluorescence results clearly showed that D-glucose influences the interaction of extracellular Ca^{2+} with β -cells, the nature of the interaction remains an unsettled problem. Suppression of chlorotetracycline fluorescence could reflect a decrease of Ca^{2+} concentration in membrane regions probed by the drug. However, emission from the chelate is solvent-dependent and may have decreased because D-glucose decreased the hydrophobicity of the membrane micro-environment. Tighter co-ordination of Ca^{2+} to fixed membrane ligands, with decreased access of the ion to the probe, is a further conceivable alternative.

The mechanism by which D-glucose diminishes Ca^{2+} -chlorotetracycline fluorescence in the β -cells might be better understood if something were also known about the rotational freedom of the probe chelate. Rotational freedom is likely to be reflected in the degree of polarization of the fluorescent light. In the present study I have therefore examined whether fluorescence polarization can be measured in the small samples represented by mammalian islet cells. Particu-

lar attention was paid to the question of whether the polarization is influenced by the ambient D-glucose concentration.

Materials and Methods

Animals and basic techniques

Adult mice homozygous for the gene, *ob*, were obtained from a local colony (Umeå-*ob/ob*-mice) and starved overnight. Methods for preparing islet-cell suspensions with more than 90% β -cells (Lernmark, 1974) and for measuring fluorescence from cells incubated with chlorotetracycline (Täljedal, 1978) have been described. In brief, collagenase-isolated islets were broken up to a milky suspension by shaking in Ca^{2+} -free tissue-culture medium 199 (1 mM-EGTA as chelator). Cells were cleansed from debris by centrifuging through dense albumin and incubated under air at 37°C in a salt-balanced medium containing CaCl_2 , chlorotetracycline and D-glucose as required. After incubation the cells were taken up and sedimented in 3 μl capillary micro-pipettes. They were finally analysed for fluorescence under incident excitation in a Leitz MPV I microscope photometer equipped with a prism monochromator for excitation (390nm) and an interference filter for emission (530nm). The salt-balanced medium had the same composition as Krebs–Ringer bicarbonate (De Luca & Cohen, 1964), except that bicarbonate was replaced by 20 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.4.

It may be argued that fluorescence in the absence of Ca^{2+} is a background phenomenon that ought to be subtracted in each experiment for proper analysis of the Ca^{2+} -dependent signals. The argument requires that fluorescence in the presence of Ca^{2+} can be regarded as deriving from two independent populations of probe molecules in the cells, and that the signals from these populations are additive. It is not known for certain whether these requirements are fulfilled, but nothing empirical conflicts with the possibility of additivity. The chlorotetracycline fluorescence in islet cells increases linearly with Ca^{2+} concentration at approx. 5 mM; with a fixed Ca^{2+} concentration the fluorescence is linearly related to the thickness of the cell pellet within a wide range (Täljedal, 1978). In view of these considerations it was decided to calculate polarization values both without and with prior correction for the fluorescence not depending on extracellular Ca^{2+} . Data were always corrected for the weak background fluorescence of islet cells not exposed to chlorotetracycline. For these purposes, each experiment comprised parallel incubations of islet cells in medium containing no chlorotetracycline and in media containing 10 μM -chlorotetracycline in combination with 0 mM- Ca^{2+} (1 mM-EGTA), or with 5 mM- Ca^{2+} , or with 5 mM- Ca^{2+} plus 20 mM-D-glucose.

Polarization equipment

The microscope previously used (Täljedal, 1978) was modified by inserting polarization filters on the excitation and emission sides of the objective lens. Because the exciting light was focused on the object by means of a ring-shaped mirror in the objective, a certain polarization of the exciting light occurred already without the polarizing filter. By placing a flat mirror on the microscopic stage and removing the analyser filter, the polarizer was adjusted to give exciting light of maximum intensity. With the polarizer fixed in this position, maximum (parallel filters) and minimum (perpendicular filters) values for emission from cells were measured by rotating the analyser. Polarization is defined as the difference between these values divided by their sum.

To check the efficiency of the filters, a mirror on the objective stage was illuminated with 530 nm through the polarizer. The signal recorded with the analyser in perpendicular position was about 1% of the light transmitted by the filters in parallel. Because the polarization filters exhibited a substantial absorbance, the intensity of the light source had to be increased in comparison with previous experiments. For signals to be of sufficient intensity when measuring on cells, the mechanical slit of the monochromator was set at 0.8 mm, corresponding to a spectral width of about 8 nm.

The cell-containing capillaries were routinely oriented on the microscopic stage such that their axes were approximately parallel to the plane of the ring-shaped mirror in the objective. Because the circular walls of capillaries could theoretically influence the polarization, control measurements were also performed with the capillaries at right angles to the position routinely employed. Neither the maximum nor the minimum fluorescence signals from chlorotetracycline in islet cells were noticeably influenced by this change of capillary orientation.

Temperature control

The temperature of the object was controlled by a microscopic stage built in the laboratory. It consisted of a brass plate under which a Peltier element was attached for cooling or heating in relation to room temperature. For cooling, heat was removed from the element by circulating water. To control the temperature of the stage thermostatically, a thermistor was fixed on its top. Because specimens to be measured were resting on object glasses, the thermistor was mounted on a strip of the same type of glass that had in turn been cemented on to the brass plate. The thermostat unit was calibrated with the aid of a second thermistor, which was used to measure the temperature of a water drop that had been placed on an object glass and oriented on the brass plate like a real preparation of cells. The thermostat had a sensitivity

of about 0.5°C in the range 16–38°C. Each preparation of chlorotetracycline-stained cells was measured at about 12 temperatures in the range 16–38°C, usually at intervals of 2°C.

Statistics

Methods outlined by Snedecor & Cochran (1967) or Diem (1962) were used.

Arrhenius plots of temperature-dependence were fitted by linear regression lines computed by the method of least squares. From a mathematical point of view, it is possible that the goodness of fit could be increased by taking into account the conceivable influence of phospholipid phase transition at some narrow temperature region within the range studied. Given the very close fit to a single straight line, however, more refined modelling of temperature-dependence would not appreciably influence the polarization estimates. Moreover, conclusions from the detection of any slight bend on the regression lines, however significant, may be hazardous in presupposing a perhaps unrealistic accuracy in the experimental temperature control.

Comparisons of regression lines were generally not based on an analysis of variance. Because symmetry in the data distributions and homogeneity of variance could not be taken for granted, it was considered safest to rely on non-parametric methods. Thus effects on slope or height were tested for significance by the two-tailed Wilcoxon's rank sum test for differences between pairs in strictly parallel incubations. Before the verdict of any test for treatment effects was accepted, data were also examined for randomness by testing the runs above or below the median. Although analyses were based on linear regression only, the mere linearization of observed

variables and the fairly large number of curves necessitated a good deal of numerical exercise. Some kind of programmable computer would therefore be desirable in repeating the work; a CompuCorp 445 Statistician was used here.

Chemicals

Chlorotetracycline was from American Cyanamid Co., Pearl River, NY, U.S.A. Tissue-culture medium 199 (Salk *et al.*, 1954) with or without Ca²⁺ was from the National Bacteriological Laboratories, Stockholm, Sweden. Collagenase was from Worthington Biochemical Corp., Freehold, NJ, U.S.A. CaCl₂ as a standardized stock solution in water was from BDH Chemicals, Poole, Dorset, U.K. Other conventional chemicals were of analytical grade.

Results

Table 1 gives mean values $\pm$ s.e.m. for measurements of chlorotetracycline fluorescence in islet cells at 18 and 37°C. Both the maximum (parallel polarization filters) and minimum (perpendicular filters) signals were enhanced by Ca²⁺ and decreased with increasing temperature. Regardless of temperature and the setting of filters, 20 mM-D-glucose decreased the fluorescence of cells incubated with Ca²⁺. This result confirms previous observations (Täljedal, 1978), which also showed that D-glucose has no effect on the background fluorescence of chlorotetracycline in Ca²⁺-deficient cells. Polarization values calculated from the actual measurements indicate that D-glucose diminished the polarization at 37°C; the effect at 18°C was not significant (Table 1).

Explicit plots of fluorescence intensity against temperature in the range 16–38°C were slightly curvilinear. A virtually perfect linearization was achieved

Table 1. Effects of Ca²⁺ and D-glucose on chlorotetracycline fluorescence in islet cells as measured with parallel and perpendicular polarization filters at two different temperatures

After preliminary incubation for 30 min in basal medium lacking Ca²⁺ (no EGTA present), islet cells were incubated for 60 min in media supplemented with 10 μ M-chlorotetracycline as well as Ca²⁺ and D-glucose as indicated. Incubations in chlorotetracycline-free medium were also performed in each experiment to allow correction for any contribution of light not representing chlorotetracycline fluorescence. Values thus corrected are given as the means $\pm$ s.e.m. for ten (0 mM-Ca²⁺, 0 mM-D-glucose; 5 mM-Ca²⁺, 0 mM-D-glucose) or nine (5 mM-Ca²⁺, 20 mM-D-glucose) different cell preparations; the difference in number is due to accidental loss of one observation. Fluorescence intensities are given as the actual meter readings (arbitrary units) for both settings of the polarization filters. Mean values $\pm$ s.e.m. are also given for the polarization estimated in each experiment. The Wilcoxon rank sum test of differences in nine pairs was used to test the effects of D-glucose in comparison with 5 mM-Ca²⁺ alone: **P* = 0.02, ***P* < 0.01.

Temperature (°C)	0 mM-Ca ²⁺ , 0 mM-D-glucose		5 mM-Ca ²⁺ , 0 mM-D-glucose		5 mM-Ca ²⁺ , 20 mM-D-glucose	
	18	37	18	37	18	37
Fluorescence measured with parallel filters	11.6 $\pm$ 1.5	6.2 $\pm$ 1.1	36.0 $\pm$ 2.5	22.9 $\pm$ 1.6	21.3 $\pm$ 1.3**	13.6 $\pm$ 1.0**
Fluorescence measured with perpendicular filters	7.2 $\pm$ 1.0	3.9 $\pm$ 0.9	23.5 $\pm$ 2.0	14.2 $\pm$ 1.1	14.1 $\pm$ 0.9**	9.1 $\pm$ 0.7**
Polarization estimated	0.25 $\pm$ 0.03	0.27 $\pm$ 0.04	0.21 $\pm$ 0.01	0.24 $\pm$ 0.02	0.20 $\pm$ 0.01	0.20 $\pm$ 0.02*

by Arrhenius transformation of data. Inspection of the lines in Fig. 1 immediately suggests the following conclusions: (1) lines obtained with parallel and perpendicular filters are approximately parallel within each group (0mM-Ca²⁺, 5mM-Ca²⁺ alone, 5mM-Ca²⁺ in combination with 20mM-D-glucose); (2) all lines obtained with 5mM-Ca²⁺ are parallel regardless of the D-glucose concentration; (3) lines obtained without Ca²⁺ differ in slope from those obtained with

Fig. 1. Effect of temperature on chlorotetracycline fluorescence in islet cells as measured with parallel (solid symbols) or perpendicular (open symbols) polarization filters

After preliminary incubation for 30 min in basal medium lacking Ca²⁺ (no EGTA present), islet cells were incubated for 60 min in media supplemented with 10 μ M-chlorotetracycline. Parallel incubations were performed in medium lacking Ca²⁺ and containing 1mM-EGTA ($\blacktriangle$, $\triangle$), in medium containing 5mM-Ca²⁺ ($\bullet$, $\circ$), and in medium containing 5mM-Ca²⁺ in combination with 20mM-D-glucose ($\blacksquare$, $\square$). To facilitate comparison of the curves, the fluorescence measured at various temperatures was corrected for non-chlorotetracycline background and expressed as a percentage of the signal obtained with parallel filters at 18°C in each experiment. Mean values for four to ten (typically ten) different preparations of islet cells were finally converted into natural logarithms and plotted against the reciprocal of absolute temperature. The correlation coefficient, r , and the equation of the regression line were, for 0mM-Ca²⁺ and parallel filters ($\blacktriangle$): $r = 0.997$, $y = 3145x - 6.187$; for 0mM-Ca²⁺ and perpendicular filters ($\triangle$): $r = 0.991$, $y = 3228x - 6.965$; for 5mM-Ca²⁺ and parallel filters ($\bullet$): $r = 0.999$, $y = 2064x - 2.485$; for 5mM-Ca²⁺ and perpendicular filters ($\circ$): $r = 0.998$, $y = 2197x - 3.384$; for 5mM-Ca²⁺ in combination with 20mM-D-glucose and parallel filters ($\blacksquare$): $r = 0.998$, $y = 2133x - 2.698$; for 5mM-Ca²⁺ in combination with 20mM-D-glucose and perpendicular filters ($\square$): $r = 0.997$, $y = 2073x - 2.915$. The open circle with a plus sign is a point common to the three curves with solid symbols (18°C with parallel filters).

5mM-Ca²⁺; (4) in the presence of 5mM-Ca²⁺, 20mM-D-glucose decreased the distance between the lines for parallel and perpendicular filters. These conclusions are reliable for the experiments performed. However, for valid generalizations beyond this set of experiments it is necessary to consider the variation between experiments. Such error estimates are not obtained from the residual variances about the lines displayed in Fig. 1; measurements at different temperatures are not independent within each experiment, and the variations between experiments tend to cancel out when one computes the mean value for any temperature. Comparison of regression lines based on all individual observations within each group, rather than on the mean values for each temperature, would also be unjustified inasmuch as analysis of variance disclosed the existence of significant slope differences among individual experiments of identical design (results not shown).

To make possible the desired testing, the individual Arrhenius plots and corresponding regression lines were therefore calculated for each experiment. Biologically relevant parameters are summarized in Table 2. For all experimental groups and both settings of the polarization filters, the correlation coefficients varied little around averages as high as 0.98–0.99. This result means that the Arrhenius plot models the experimental situation with great accuracy. The goodness of fit to mean values in Fig. 1 does not depend on the cancelling-out of upward and downward curvilinearities among the individual experiments.

In view of the high correlation coefficients, the rather wide ranges of slopes (Table 2) probably reflect the existence of true differences between replicate experiments. However, no significant difference in slope can be attributed to the setting of the polarization filters. Neither did the slope change significantly in response to 20mM-D-glucose. As regards the effect of 5mM-Ca²⁺, the changes in slope compared with 0mM-Ca²⁺ appeared highly significant. Yet in this particular case the outcome of testing should be interpreted as suggestive only, as the distribution of the results showed a significant deviation from randomness. The apparent influence of Ca²⁺ on temperature-dependence adds to earlier observations that showed that the signals measured in the presence and absence of Ca²⁺ do not differ in intensity only, but are qualitatively distinct with regard to emission spectrum and the sensitivity to sugars, methylxanthines, or Mg²⁺ deficiency (Täljedal, 1978).

Polarization, p , is directly related to the arithmetic difference, d , between the logarithms of fluorescence intensities measured with parallel, I_1 , and perpendicular, I_2 , filters:

$$d = \ln I_1 - \ln I_2 = \ln(1 + p) - \ln(1 - p) \quad (1)$$

$$p = (e^d - 1)/(e^d + 1) \quad (2)$$

Table 2. Analysis of regression lines obtained by Arrhenius transformations of the fluorescence data in each experiment. Cells were incubated as described in the legend to Fig. 1. In each of ten different experiments with 0 mM-Ca²⁺ or 5 mM-Ca²⁺, and nine different experiments with 5 mM-Ca²⁺ in combination with 20 mM-D-glucose, the results were subjected to Arrhenius transformations and the regression lines for parallel and perpendicular filters calculated. Because the shapes of data distributions are not known, median, range and mean $\pm$ S.E.M. are given to summarize information about the correlation coefficient, slope and distance between lines for parallel and perpendicular filters in each experimental group. The distance between lines is the difference between the two ordinate means for each preparation measured. In addition to data for the three experimental groups, the same statistical parameters are given for the differences in slopes and inter-line distance between groups in parallel incubations. The Wilcoxon rank sum test of differences in ten (groups 2 and 1) or nine (groups 3 and 2) pairs was used to test the effects of 5 mM-Ca²⁺ or 20 mM-D-glucose: * $P < 0.02$, ** $P < 0.01$. In spite of the low P value for effect of Ca²⁺ on slopes, this effect should be interpreted as suggestive only, as runs on the median indicated significant non-randomness in data ($P < 0.01$). The effect of D-glucose, however, passed the randomness test with $P < 0.9$.

		Correlation coefficient		10 ⁻³ × Slope		Distance between lines
		Parallel	Perpendicular	Parallel	Perpendicular	
0 mM-Ca ²⁺ (group 1)	Median	0.98	0.98	3.20	2.67	0.476
	Range	0.03	0.03	4.43	6.28	0.739
	Mean $\pm$ S.E.M.	0.98 $\pm$ 0.003	0.98 $\pm$ 0.003	3.40 $\pm$ 0.45	3.97 $\pm$ 0.76	0.553 $\pm$ 0.071
5 mM-Ca ²⁺ (group 2)	Median	0.99	0.99	2.04	2.20	0.432
	Range	0.01	0.02	0.75	1.43	0.319
	Mean $\pm$ S.E.M.	0.99 $\pm$ 0.002	0.99 $\pm$ 0.003	2.90 $\pm$ 0.08	2.25 $\pm$ 0.15	0.461 $\pm$ 0.030
5 mM-Ca ²⁺ in combination with 20 mM-D-glucose (group 3)	Median	0.99	0.99	2.06	2.04	0.410
	Range	0.03	0.19	1.66	1.33	0.341
	Mean $\pm$ S.E.M.	0.99 $\pm$ 0.003	0.97 $\pm$ 0.021	2.14 $\pm$ 0.15	1.96 $\pm$ 0.12	0.423 $\pm$ 0.034
Effect of 5 mM-Ca ²⁺ (group 2 minus group 1)	Median	—	—	-1.04	-0.74	-0.037
	Range	—	—	4.24	4.85	0.561
	Mean $\pm$ S.E.M.	—	—	-1.30 $\pm$ 0.41**	-1.72 $\pm$ 0.64**	-0.092 $\pm$ 0.055
Effect of 20 mM-D-glucose (group 3 minus group 2)	Median	—	—	0.07	0.02	-0.045
	Range	—	—	1.42	1.57	0.115
	Mean $\pm$ S.E.M.	—	—	0.04 $\pm$ 0.14	-0.29 $\pm$ 0.18	-0.040 $\pm$ 0.013*

Therefore, the similarities in slope obtained with parallel and perpendicular filters indicate that polarization was little influenced by temperature in the range 16–38°C. Moreover, the distance between lines obtained with parallel and perpendicular filters can immediately be used as a statistical test for deciding whether Ca²⁺ or D-glucose significantly influenced the polarization. From the mean distance between lines (Table 2), polarization was calculated to be 0.270 for 0 mM-Ca²⁺, 0.227 for 5 mM-Ca²⁺ alone, and 0.208 for 5 mM-Ca²⁺ in combination with 20 mM-D-glucose (eqn. 2). Non-parametric testing of the differences between interline distances (eqn. 1) in parallel experiments showed the effect of 20 mM-D-glucose to be significant, whereas that of 5 mM-Ca²⁺ was not.

In Fig. 2 and Table 3 the analysis is extended to the Ca²⁺-specific fluorescence signals computed in each experiment by subtracting the background fluorescence of chlorotetracycline independent of extracellular Ca²⁺. Again, Arrhenius plots resulted in linear regression lines with very similar slopes. However, after correction for the chlorotetracycline background, there was a significant difference in slope

between the lines obtained with perpendicular filters in the presence and absence of D-glucose. In addition, the D-glucose-induced decrease of mean interline distance (d , eqn. 1) was confirmed with increased reliability.

Polarization of the Ca²⁺-specific signals was calculated from the lines in Fig. 2 according to eqn. (2). Because D-glucose significantly affected the temperature-dependence of fluorescence with perpendicular filters, but not that with parallel filters, polarization was calculated throughout the temperature range: 0.208 (16°C) to 0.226 (38°C) for cells incubated without D-glucose, and 0.191 (16°C) to 0.169 (38°C) for cells incubated with 20 mM-D-glucose.

Discussion

High concentrations of chlorotetracycline (50–100 μ M) can stain intracellular organelles in both endocrine (Täljedal, 1978) and exocrine (Chandler & Williams, 1978) pancreas cells. The plasma membrane, or a thin zone of cytoplasm or cell coat next to the membrane, is also a site of pronounced Ca²⁺-

Fig. 2. Effect of temperature on the Ca^{2+} -specific fluorescence signals in islet cells

In each experiment and for each temperature the fluorescence signals measured in cells incubated with 5 mM- Ca^{2+} and 10 μM -chlorotetracycline (with or without 20 mM-D-glucose) were corrected by subtraction of the signals measured in cells incubated with 10 μM -chlorotetracycline in Ca^{2+} -free medium (1 mM-EGTA present). The results thus corrected were expressed as percentages of the signal obtained with parallel filters at 18°C in each experiment. Mean values of four to ten (typically nine in the presence and ten in the absence of D-glucose) different cell preparations were finally subjected to an Arrhenius transformation and plotted. The correlation coefficient, r , and the equation of the regression line were, for 0 mM-D-glucose and parallel filters (●): $r = 0.997$, $y = 1745x - 1.389$; for 0 mM-D-glucose and perpendicular filters (○): $r = 0.995$, $y = 1877x - 2.268$; for 20 mM-D-glucose and parallel filters (■): $r = 0.990$, $y = 1488x - 0.480$; for 20 mM-D-glucose and perpendicular filters (□): $r = 0.926$, $y = 1302x - 0.226$. The open circle with a plus sign is a point common to the two curves with solid symbols (18°C with parallel filters).

chlorotetracycline deposition in islet cells. As judged by reversibility experiments, the plasma membrane may in fact be the predominant site when the probe is used at a concentration as low as 10 μM (Täljedal, 1978).

As previously reported (Täljedal, 1978), D-glucose decreases the intensity of Ca^{2+} -chlorotetracycline fluorescence in islet cells. This effect is restricted to a signal that monitors Ca^{2+} taken up from the extracellular fluid and is specific for islet cells (as compared with exocrine cells or cell-free methanol). Because the cell suspensions contained more than 90% β -cells, the effect is probably representative of that cell type. Supporting the notion that the glucose-sensitive signal mainly derives from β -cell plasma membranes, the inhibitory effect of D-glucose on fluorescence intensity is proportionally greater with 10 μM -chlorotetracycline than with 100 μM -chlorotetracycline (I.-B. Täljedal, unpublished work).

As shown in the present paper, the D-glucose-induced reduction of fluorescence intensity is associated with a diminished polarization. This effect is not an artifact resulting from the decrease in fluorescence intensity; the signal from Ca^{2+} -deficient islet cells was weaker and yet was at least as polarized as the fluorescence in cells incubated with 5 mM- Ca^{2+} and chlorotetracycline in glucose-free medium. It is concluded that D-glucose somehow alters the micro-environment around the Ca^{2+} ions monitored with 10 μM -chlorotetracycline. The simplest change that would account for the diminished polarization is a decrease of micro-viscosity.

When the inhibitory effect of D-glucose on Ca^{2+} -dependent chlorotetracycline fluorescence was first discovered, it was suggested that the effect may signify a stronger co-ordination of Ca^{2+} to fixed ligands in the β -cell membranes, an increased polarity of the Ca^{2+} solvent, or less likely, a decreased concentration of Ca^{2+} in the membrane regions probed by the drug

Table 3. Slopes and inter-line distances computed for Arrhenius plots of Ca^{2+} -specific signals after correction in each experiment for the chlorotetracycline fluorescence independent of extracellular Ca^{2+}

In each of ten experiments with 0 mM-D-glucose and nine experiments with 20 mM-D-glucose, the Ca^{2+} -specific signals were corrected for chlorotetracycline fluorescence independent of extracellular Ca^{2+} as described in the legend to Fig. 2. On Arrhenius transformation of data, the slopes and distances between lines obtained with parallel and perpendicular filters were calculated, summarized, and tested for effects of D-glucose as described in Table 2. * $P < 0.02$, ** $P < 0.01$.

		$10^{-3} \times \text{Slope}$		Distance between lines
		Parallel	Perpendicular	
0 mM-D-Glucose	Median	1.77	1.85	0.408
	Range	0.96	1.42	0.283
	Mean $\pm$ S.E.M.	1.75 ± 0.08	1.91 ± 0.12	0.442 ± 0.029
20 mM-D-Glucose	Median	1.50	1.49	0.367
	Range	2.37	1.75	0.385
	Mean $\pm$ S.E.M.	1.52 ± 0.26	1.35 ± 0.18	0.365 ± 0.042
Effect of 20 mM-D-Glucose	Median	-0.09	-0.30	-0.063
	Range	2.03	1.56	0.216
	Mean $\pm$ S.E.M.	-0.23 ± 0.23	$-0.53 \pm 0.19^*$	$-0.079 \pm 0.023^{**}$

(Täljedal, 1978). The idea of enhanced Ca²⁺-ligand interaction was tentatively favoured because such a mechanism seemed to be most readily reconciled with the fact that D-glucose stimulates ⁴⁵Ca²⁺ uptake by pancreatic islets. The present data argue against the first of the explanations. Stronger co-ordination of Ca²⁺ to fixed membrane ligands would be expected to restrict the mobility of the ion and hence to increase, rather than decrease, the polarization. The estimated polarization values are of a magnitude that in other contexts has been presumed to reflect Ca²⁺ binding by ionic interaction rather than ligand interaction (Caswell, 1972).

Increased mobility of Ca²⁺ in the β -cell plasma membrane may be visualized as a diminished probability of Ca²⁺ binding to fixed negative charges relative to interaction with water. Such a relative change could involve reorganization of the anionic groups or increased hydration of the membrane, or both. Whatever the detailed molecular events induced by D-glucose, it seems likely that a greater mobility of membrane-located Ca²⁺ should facilitate diffusion of Ca²⁺ ions between the plasma membrane and one or both of its bordering phases. However, the results do not predict whether the net effect would be uptake or loss of membrane Ca²⁺. The equilibrium will depend on the affinity as well as on the number of Ca²⁺-binding groups, including water in aqueous pores. Net uptake of Ca²⁺ would tend to increase the number of fluorescent molecules, but the influence of this factor on fluorescence intensity could be counteracted by a decrease in quantum yield if uptake occurs to a region with enhanced hydrophilicity.

The physiological importance of enhanced Ca²⁺ mobility in the plasma membranes of D-glucose-stimulated β -cells requires further study. It is noteworthy, however, that a lessening of the restrictions on rotational mobility and diffusion is likely to facilitate the transmembrane Ca²⁺ transport as well. Thus the results may help to explain the following three types of D-glucose-induced phenomena in β -cells: (1) apparent enhancement of the rate constant for ⁴⁵Ca²⁺ association with a peripheral compartment in the β -cells (Hellman *et al.*, 1976b); (2) stimulation of ⁴⁵Ca²⁺ release from prelabelled islets (Malaisse *et al.*, 1973; Gylfe & Hellman, 1978; Wollheim *et al.*, 1978); (3) induction of an electric Ca²⁺ current (Dean & Matthews, 1970; Meissner & Schmelz, 1974).

This work was supported by the Swedish Medical Research Council (12x-2288), the Swedish Diabetes Association, and the J. C. Kempe Memorial Foundation.

References

- Caswell, A. H. (1972) *J. Membr. Biol.* **7**, 345–364
 Chandler, D. E. & Williams, J. A. (1978) *J. Cell Biol.* **76**, 386–399
 Dean, P. M. & Matthews, E. K. (1970) *J. Physiol. (London)* **210**, 265–275
 De Luca, H. F. & Cohen, P. P. (1964) in *Manometric Techniques* (Umbreit, W. W., Burris, R. H. & Stauffer, J. F., eds.), 4th edn., pp. 131–133, Burgess Publishing Co., Minneapolis
 Diem, K. (1962) *Documenta Geigy: Scientific Tables*, 6th edn., J. R. Geigy S. A., Basle
 Formby, B., Capito, K., Egeberg, J. & Hedeskov, C. J. (1976) *Am. J. Physiol.* **230**, 441–448
 Grodsky, G. M. (1972) *Diabetes* **21**, Suppl. 2, 584–593
 Gylfe, E. & Hellman, B. (1978) *Biochim. Biophys. Acta* **538**, 249–257
 Hellman, B., Sehlin, J. & Täljedal, I.-B. (1971) *Am. J. Physiol.* **221**, 1795–1801
 Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J. & Täljedal, I.-B. (1973a) *Biochem. J.* **132**, 775–789
 Hellman, B., Sehlin, J. & Täljedal, I.-B. (1973b) *Pflügers Arch.* **340**, 51–58
 Hellman, B., Sehlin, J. & Täljedal, I.-B. (1976a) *J. Physiol. (London)* **254**, 639–656
 Hellman, B., Sehlin, J. & Täljedal, I.-B. (1976b) *Science* **194**, 1421–1423
 Henquin, J.-C. & Lambert, A. E. (1975) *Am. J. Physiol.* **228**, 1669–1677
 Herman, L., Sato, T. & Hales, C. N. (1973) *J. Ultrastruct. Res.* **42**, 298–311
 Howell, S. L., Montague, W. & Tyhurst, M. (1975) *J. Cell Sci.* **19**, 395–409
 Lernmark, Å. (1974) *Diabetologia* **10**, 431–438
 Malaisse, W. J., Brisson, G. R. & Baird, L. E. (1973) *Am. J. Physiol.* **224**, 389–394
 Malaisse-Lagae, F. & Malaisse, W. J. (1971) *Endocrinology* **88**, 72–80
 Meissner, H. P. & Schmelz, H. (1974) *Pflügers Arch.* **351**, 195–206
 Naber, S. P., McDaniel, M. L. & Lacy, P. E. (1977) *Endocrinology* **101**, 686–693
 Ravazzola, M. F., Malaisse-Lagae, F., Amherdt, M., Perrelet, A., Malaisse, W. J. & Orci, L. (1976) *J. Cell Sci.* **21**, 107–117
 Salk, J. E., Youngner, J. S. & Ward, E. N. (1954) *Am. J. Hyg.* **60**, 214–230
 Schäfer, H.-J. & Klöppel, G. (1974) *Virchows Arch. A* **362**, 231–245
 Sehlin, J. (1976) *Biochem. J.* **156**, 63–69
 Snedecor, G. W. & Cochran, W. G. (1967) *Statistical Methods*, 6th edn., The Iowa State University Press, Ames
 Täljedal, I.-B. (1978) *J. Cell Biol.* **76**, 652–674
 Wollheim, C. B., Kikuchi, M., Renold, A. E. & Sharp, G. W. G. (1978) *J. Clin. Invest.* **62**, 451–458