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IONOPHORETIC ACTIVITY IN PANCREATIC ISLETS[1]

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SUMMARY: Extracts of pancreatic islets stimulate the translocation of calcium from an aqueous into an organic immiscible phase. This ionophoretic activity, which is derived mainly from membrane-rich subcellular fractions, displays several features in common with that of A23187 in the same model. The phenomenon of calcium translocation caused by either the islet extract or the antibiotic ionophore represents a power function of the concentration of ionophoretic material; it is saturable at high calcium concentrations, affected by the concentration of Na^+ and pH of the aqueous phase, increased at low temperature, and inhibited by suloctidil, the latter inhibitory effect being antagonized by calcium itself. These findings underline the potential significance of native ionophores in the regulation of calcium movements across membrane systems in the islet cells.

The regulation of calcium fluxes across membranes in the pancreatic B-cell is an essential determinant of insulin release (1). It was recently postulated that nutrient secretagogues, such as glucose, may affect the movements of calcium by modulating the activity of native ionophoretic systems (2, 3). The present study aims at characterizing the ionophoretic capacity of islet extracts.

METHODS

Pancreatic islets were isolated from fed female albino rats (4). In most experiments, a group of 400 or more islets was homogenized by sonification (twice 10 sec) in a mixture of chloroform and methanol (2/1, v/v; 100 islets/ml). After centrifugation (5 min, 700 g), the organic mixture was washed with H_2O (1.0 ml of organic phase + 0.25 ml H_2O), treated with anhydrous Na_2SO_4 and dried by heating at 60°C. The dry matter was solubilized in a mixture of toluene and butanol (7/3, v/v). In a limited series of experiments, subcellular fractions isolated as described in the sub-title of Fig. 2 were treated in the same manner.

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The ionophoretic activity (5) of the toluene/butanol mixture was measured by mixing a sample (0.2 ml) of the islet extract in a small tube and at room temperature with 0.2 ml of an aqueous phase. Except if otherwise stated, the aqueous phase consisted of a triethanolamine buffer (20 mM, pH 7.0) containing NaCl (20 mM), variable amounts of $^{40}CaCl_2$ and a trace amount of $^{45}CaCl_2$ (5.0 µCi/ml). An aliquot (0.1 ml) of the supernatant immiscible organic phase was eventually examined for its radioactive content by liquid scintillation. The amount of calcium translocated into the organic phase was usually expressed relative to the number of extracted islets present in each tube. Results derived from two or more individual measurements were expressed as the mean (± SEM). Except if otherwise stated, comparison of data was restricted to results obtained within the same experiment(s), a single islet extract being used in each individual experiment. The amount of calcium recovered in the organic phase when the latter contained no islet extract is only mentioned if the experimental data found in the presence of the islet extract were not corrected for such a blank value.

RESULTS

In a series of 7 separate experiments performed under similar conditions (calcium concentration of the initial aqueous phase : 0.1 mM; 30 to 70 extracted islets per tube), the amount of calcium translocated into the immiscible organic phase averaged 2.39 ± 0.39 pmol/islet. The amount of translocated calcium (T) was not proportional to the concentration of insular material (M). When the initial aqueous phase contained calcium at a 0.1 mM concentration, the amount of translocated calcium increased from 1.76 ± 0.01 to 5.22 ± 0.08 pmol/islet, as the concentration of insular material was raised from 1.5 to 600 extracted islets per tube (Fig. 1A). The experimental data were compatible with the equation : $T = aM^b$, in which the factor b amounted, under the present experimental conditions to 1.14 ± 0.06. The coefficient of correlation between logT and logM amounted to 0.9981.

At a given concentration of insular material (150 extracted islets/tube), the amount of translocated calcium increased from 0.55 ± 0.05 to 26.0 ± 3.2 pmol/islet, as the concentration of calcium in the initial aqueous phase was increased from 5.0 µM

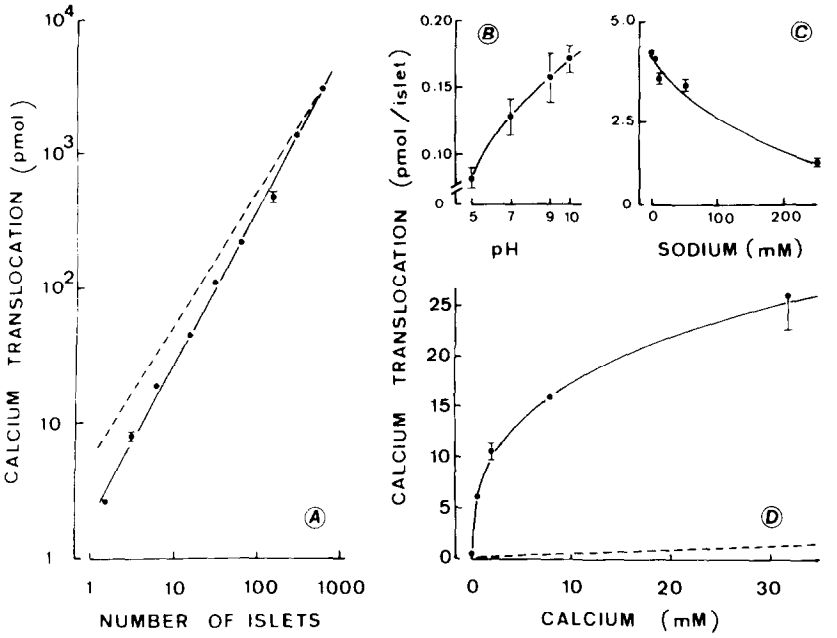

Fig. 1. Ionophoretic activity of islet extracts. (A) Effect of increasing concentrations of insular material (number of extracted islets per tube) upon the amount of calcium translocated from the aqueous phase (initial calcium concentration : 0.1 mM) into the organic immiscible phase (logarithmic scale for both parameters). The solid line corresponds to the regression line calculated from the experimental data. The dotted line corresponds to a theoretical rule of direct proportionality between the two parameters with a coefficient of proportionality identical to that seen at the highest concentration of insular material. (B) Effect of changes in pH of the initial aqueous solution upon the amount of translocated calcium. The aqueous phase consisted of a Hepes buffer (25 mM) containing NaCl (115 mM), KCl (5 mM) and $CaCl_2$ (6 µM); its pH was adjusted by addition of variable amounts of NaOH (0 to 27 mM). The organic phase contained 25 extracted islets per tube. Pooled data from 6 separate experiments. (C) Effect of increasing concentrations of NaCl in the initial aqueous phase upon the amount of translocated calcium. The aqueous phase consisted of the usual triethanolamine buffer (20 mM; pH 7.0), except for its NaCl content. The organic phase contained 30 extracted islets per tube. (D) Effect of increasing concentrations of $CaCl_2$ (5.0 µM to 32.0 mM) upon the amount of translocated calcium (solid line). The organic phase contained 150 extracted islets per tube. The dotted line corresponds to the amount of calcium translocated in the absence of islets extract, but is expressed relative to the same number of islets as that otherwise used in this experiment. In all panels, the mean value is derived from 2-3 individual observations, the SEM being only represented when it exceeded the size of the mean point.

to 32.0 mM (Fig. 1D). The experimental data suggested the existence of a saturation phenomenon with, under the present experi-

mental conditions, an apparent "Km" close to 4.0 mM in terms of calcium concentration in the initial aqueous phase.

The amount of calcium translocated into the immiscible phase depended on the pH of the initial aqueous phase, there being a two-fold increase in such a translocation as the pH of the aqueous phase was increased from 5.0 to 10.0 (Fig. 1B).

Increasing concentrations of NaCl inhibited in a dose-related fashion the translocation of calcium, as little as 10 mM NaCl decreasing the amount of translocated calcium from a control value of 4.56 ± 0.02 to 3.77 ± 0.14 pmol/islet (Fig. 1C). At a 250 mM concentration of NaCl, the amount of translocated calcium averaged only 26.8 percent of the control value found in the absence of NaCl.

The amount of calcium translocated was higher at cold than at room temperature. Adding 30 extracted islets per tube and at a 0.1 mM concentration of calcium in the initial aqueous phase, the amount of translocated calcium increased from 1.57 ± 0.08 to 2.10 ± 0.03 pmol/islet, as the temperature was reduced from 23°C to 4°C. A 32-percent increase in calcium translocation at the low temperature was also observed at a higher initial calcium concentration (0.5 mM).

The organic calcium antagonist suloctidil inhibited in a dose-related fashion the phenomenon of calcium translocation (Table 1). At a 1.5 mM concentration of suloctidil in the initial organic phase and with 70 extracted islets per tube, the translocation of calcium was virtually abolished, the amount of translocated calcium being barely higher than the blank readings obtained in the absence of the islet extract. At a lower concentration of suloctidil (0.3 mM), the relative magnitude of the drug-induced decrease in calcium translocation depended on the

Table 1. Effect of increasing concentrations of suloctidil in the initial organic phase upon the amount of translocated calcium at two concentrations of calcium in the initial aqueous phase (0.1 and 8.0 mM).

Calcium (mM)	Suloctidil (mM)	Calcium translocation (pmol/islet)	(% of control)
0.1	-	2.72 ± 0.40	100.0
0.1	0.3	0.31 ± 0.01	11.4 ± 0.1
0.1	1.5	0.05 ± 0.01	1.9 ± 0.1
8.0	-	12.93 ± 0.02	100.0
8.0	0.3	3.79 ± 0.19	29.3 ± 1.5
8.0	1.5	1.69 ± 0.38	13.1 ± 2.9

The organic phase contained 70 extracted islets per tube. The results are expressed in absolute terms or relative to the mean control value found in the absence of verapamil. The amount of calcium translocated in the absence of insular material at the highest verapamil concentration (1.5 mM) corresponded to about 0.02 and 0.71 pmol/islet at calcium 0.1 and 8.0 mM, respectively, when expressed relative to the same number of islets as that otherwise used in this experiment.

calcium concentration of the initial aqueous phase. Thus, relative to the control value found in the absence of suloctidil, the amount of calcium translocated in the presence of the drug (0.3 mM) decreased from 29.3 ± 1.5 to 11.4 ± 0.1 percent, as the calcium concentration of the aqueous phase was lowered from 8.0 to 0.1 mM (Table 1).

The ionophoretic capacity of subcellular fractions was highest in those fractions containing most of the mitochondria and secretory granules, as judged from the distribution of glutamate dehydrogenase and insulin, respectively (Fig. 2A). Using ^{125}I-labelled wheat germ agglutin as a probe for plasma membrane-containing material, elevated ionophoretic activity was found in the membrane-enriched fractions (Fig. 2B).

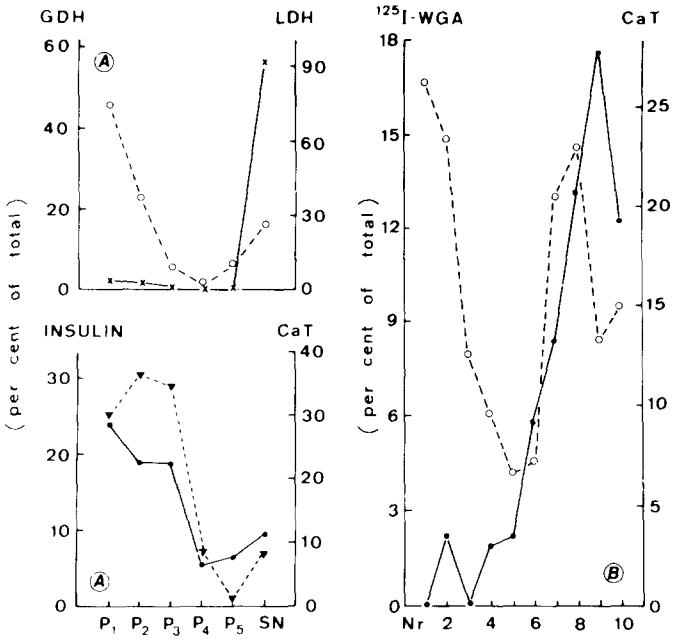

Fig. 2. Ionophoretic activity in islet subcellular fractions. (A) Islets were homogenized in 0.5 ml of a Hepes buffer (5 mM; pH 7.2) containing sucrose (300 mM), EGTA (1 mM), dithiothreitol (1 mM) and bovine albumin (2 mg/ml). Intact cells and cell debris were removed by a first 10 min centrifugation at 700 g. A series of pellets (P1 to P5) were obtained by successive centrifugations of 10 min each at 3,400 (P1), 10,300 (P2), 21,000 (P3) and 215,000 (P5)g, respectively. Each pellet was resuspended in 0.5 ml of the Hepes buffer, centrifuged at 215,000 g to remove contaminating soluble matter, again resuspended in 0.5 ml of the Hepes buffer, and homogenized by sonification. These homogenates and the final supernatant (SN) were examined for their lactic dehydrogenase (LDH; crosses and solid line) and glutamate dehydrogenase (GDH; open circles and dotted line) activities and insulin content (triangles and dotted line) by methods described elsewhere (13, 14, 15). An aliquot (0.2 ml) of each subcellular homogenate was vigorously mixed for 2 min with 0.8 ml of a chloroform-methanol mixture (2/1,v/v), the organic phase being then treated, as described in the Material and Methods section, for extraction of ionophoretic material. The enzymatical activities, insulin content and ionophoretic activity (CaT; closed circles and solid line) of each fraction are expressed in percent of the integrated value for each parameter. (B) Membrane-enriched fractions were obtained after incubation of an islet homogenate with ^{125}I-wheat-germ agglutinin and separation on a sucrose gradient (gradient A in ref. 16). Each of the ten fractions (Nr 1 to 10) removed from the sucrose gradient was examined for its radioactive content (^{125}I-WGA; open circles and dotted line) and extracted, as described above, for characterization of its ionophoretic capacity (CaT; closed circles and solid line). Results are expressed as in Fig. 2A.

DISCUSSION

The findings illustrated in Fig. 1 indicate that it is possible to extract from pancreatic islets a material able to evoke the translocation of calcium into an organic phase. The data illustrated in Fig. 2 suggest that the ionophoretic material is associated mainly with membrane-rich subcellular fractions.

The ionophoretic activity found in the islet extract displays several features also observed in the same model with the antibiotic ionophore A23187 (6). At non-saturating calcium concentrations, the amount of calcium translocated into the immiscible phase is a power function of the concentration of ionophoretic material (Fig. 1A). In the case of A23187 (or X537A), this behaviour was attributed to the fact that each atom of calcium is complexed by two molecules of ionophore. At a fixed concentration of ionophoretic material and increasing concentrations of calcium, the translocation of calcium appeared as a saturable phenomenon (Fig. 1D). With A23187, saturation is reached at calcium concentrations close to 20 mM. With X537A, saturation is only reached at much higher calcium concentrations (about 150 mM). Incidentally, the apparent Km for this dose-related response depends on the concentration of ionophoretic material used, another feature attributable to the above-mentioned stochiometry (6). The ionophoretic activity of the islet extract like that of A23187 was enhanced at high pH (Fig. 1B) and decreased at high sodium concentrations (Fig. 1C). In the case of the islet extract, the inhibitory effect of sodium could conceivably be attributed to competition between sodium and calcium, as observed with the ionophore X537A which translocates both cations. However, the translocation of calcium mediated by A23187, which is unable to translocate sodium, is also decreased in a dose-related fashion at increasing concen-

trations of sodium (Couturier & Malaisse, unpublished observations). The translocation of calcium evoked by either the islet extract or A23187 is higher at low (4°C) than room (23°C) temperature, an effect tentatively ascribed to a disproportionate change in the rate constants for calcium-ionophore association and dissociation, respectively (6). The organic calcium-antagonist suloctidil inhibits calcium translocation evoked by either the islet extract (Table 1) or A23187 (7). In both cases, calcium itself antagonizes the inhibitory effect of the calcium-antagonist so that, at intermediate concentrations of the drug, the relative magnitude of inhibition is less marked at high than at low calcium concentrations (5).

Several of the present findings are compatible with the view that native ionophores present in the islet cells may participate in the regulation of calcium transport across membrane systems. First, the effect of pH could account for the decrease in calcium fractional outflow rate which is observed in response to intracellular acidification of perifused islets (Carpinelli & Malaisse, unpublished observations). Second, the inhibitory action of suloctidil could explain the effect of the drug to inhibit calcium net uptake and insulin release in intact islets (7). In the intact islets, like in the present model, the inhibitory effect of organic calcium antagonists is minimized by raising the extracellular calcium concentrations (5, 8). Third, ionophores may translocate calcium against its chemical gradient by a mechanism of sodium-calcium counter-transport (9); the extrusion of calcium from the islet cells was recently found to be mediated, in part at least, by such a counter-transport process (10).

The presence of ionophoretic activity in biological material is not specific of the pancreatic islets and, in view of the great

number of organic molecules which display an ionophoretic capacity (11), e.g. phospholipids (12), it may be important to assess the respective contribution of different purified compounds which could be isolated from the islet extract. However, in view of cooperativity between distinct ionophores in mediating calcium translocation (Couturier & Malaisse, unpublished observation), it was equally important to assess the overall activity of the membrane-associated material in its actual heterogeneity. In this respect, the present procedure offers the immediate opportunity to scrutinize the influence of insulinotropic agents and potential second messengers upon the activity of native ionophores in pancreatic islets.

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