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Opposite effects of D-fructose on total versus cytosolic ATP/ADP ratio in pancreatic islet cells

Marie-Hélène Giroix [a], Eda Agascioglu [b], Berrin Oguzhan [b], Karim Louchami [b], Ying Zhang [b], Philippe Courtois [b], Willy J. Malaisse [b], Abdullah Sener [b,*]

[a] *Laboratory of Nutrition Physiopathology, CNRS UMR 7059, University of Paris 7, F-75271 Paris Cedex 05, France*
[b] *Laboratory of Experimental Hormonology, Brussels Free University, B-1070 Brussels, Belgium*

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Abstract

D-fructose (10 mM) augments, in rat pancreatic islets, insulin release evoked by 10 mM D-glucose. Even in the absence of D-glucose, D-fructose (100 mM) displays a positive insulinotropic action. It was now examined whether the insulinotropic action of D-fructose could be attributed to an increase in the ATP content of islet cells. After 30–60 min incubation in the presence of D-glucose and/or D-fructose, the ATP and ADP content was measured by bioluminescence in either rat isolated pancreatic islets (total ATP and ADP) or the supernatant of dispersed rat pancreatic islet cells exposed for 30 s to digitonine (cytosolic ATP and ADP). D-fructose (10 and 100 mM) was found to cause a concentration-related decrease in the total ATP and ADP content and ATP/ADP ratio below the basal values found in islets deprived of exogenous nutrient. Moreover, in the presence of 10 mM D-glucose, which augmented both the total ATP content and ATP/ADP ratio above basal value, D-fructose (10 mM) also lowered these two parameters. The cytosolic ATP/ADP ratio, however, was increased in the presence of D-glucose and/or D-fructose. Under the present experimental conditions, a sigmoidal relationship was found between such a cytosolic ATP/ADP ratio and either ^{86}Rb net uptake by dispersed islet cells or insulin release from isolated islets. These data provide, to our knowledge, the first example of a dramatic dissociation between changes in total ATP content or ATP/ADP ratio and insulin release in pancreatic islets exposed to a nutrient secretagogue. Nevertheless, the cationic and insulinotropic actions of D-glucose and/or D-fructose were tightly related to the cytosolic ATP/ADP ratio.

Keywords: Pancreatic islet; Dispersed islet cell; ATP and ADP; D-glucose and D-fructose

1. Introduction

At variance with D-glucose and D-mannose, D-fructose (15.0 mM) fails to augment significantly insulin output from pieces of rat pancreas incubated in the presence of 1.7 mM D-glucose [1]. Hence, the ketohexose was first considered to be devoid of any significant insulinotropic action. However, it was later observed that D-fructose augments insulin output at higher concentrations of D-glucose [2–5]. For instance, D-fructose (16.7 mM) causes a shift to the left of the sigmoidal relationship between insulin output from isolated rat pancreatic islets and extracellular D-glucose concentration (zero to 16.7 mM), the insulinotropic action of the ketohexose being considered to represent about 16% of that of an equimolar amount of D-glucose [3]. Further experiments dealing with the effects of D-fructose upon insulin release provided two essential additional pieces of information. First, even in the absence of any other exogenous hexose, D-fructose causes, in the 80–240 mM range, a concentration-related stimulation of insulin release above basal value [6]. Second, the increment in insulin output caused by D-fructose (8.0 and 33.0 mM) is higher in rat islets exposed to D-glucose (7.0 mM) than in islets exposed to D-mannose (14.0 mM), despite the fact that, under these experimental conditions, the two aldohexoses display close-to-equal insulinotropic potency [7].

In the light of these secretory data, the possible cause-to-effect link between the catabolism of D-fructose in islet cells and

* Corresponding author. Fax: +32 2 555 63 56.
E-mail address: abdsener@ulb.ac.be (A. Sener).

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its insulinotropic action was then investigated. Several findings support the view that, as it is currently believed in the case of D-glucose or D-mannose, the insulin secretory potential of D-fructose can indeed be accounted for by its ability to be catabolized in pancreatic islets. For instance, at a concentration of 16.7 mM, the oxidation of D-[U-^{14}C]fructose represents, in rat islets, about 16% of that found with D-[U-^{14}C]glucose in close agreement with the respective insulinotropic action of these two hexoses as mentioned above [3]. Likewise, D-mannose, even when tested at concentrations (≥10 mM) higher than D-glucose (6 mM), is less efficient than the latter hexose, in conferring to glucokinase positive cooperativity towards D-fructose [8], in fair agreement with the secretory findings mentioned above.

In the process of nutrient-stimulated insulin release, an increased generation of ATP is thought to couple the metabolism of nutrient secretagogues to their insulinotropic action [9]. Such is the case for both the so-called K^+-ATP channel-dependent and independent modalities of coupling between nutrient catabolism and insulin secretion [10]. Within the limits of present knowledge, further ionic and motile events in the process of nutrient-stimulated insulin release appear grossly comparable in the case of D-fructose and other nutrient secretagogues, such as D-glucose. Thus, the effects of D-fructose (240 mM) upon ^{86}Rb and ^{45}Ca efflux from prelabelled perifused rat islets are similar, if not identical, to those caused by D-glucose (11 and 80 mM) [11]. Moreover, the release of insulin evoked by D-fructose (240 mM) is partially inhibited by D-mannoheptulose (1.0 mM), which also inhibits D-fructose phosphorylation by glucokinase [12], suppressed by KCN (2 mM), enhanced by glibenclamide (5 μM), abolished in the absence of extracellular Ca^{2+}, potentiated by forskolin (0.01 mM) and theophylline (1.4 mM), and doubled by cytochalasin B (0.02 mM). These findings suggest that the stimulation of insulin release by D-fructose corresponds to an active secretory process modulated by the metabolic fate of the ketohexose, the availability of ATP, the activity of ATP-sensitive K^+ channels, the extracellular concentration of Ca^{2+}, the cell content in cyclic AMP and the motile events under control of the microfilamentous cell web [13].

We have now investigated whether D-fructose also shares with D-glucose the property of increasing the ATP/ADP ratio in pancreatic islet cells. The results of the latter study are presented in this report.

2. Materials and methods

2.1. Preparation of pancreatic islets and dispersed islet cells

All experiments were conducted in pancreatic islets or dispersed islet cells prepared from fed female Wistar rats. The protocol of the present study was approved by the Commission d'Ethique et du Bien-Etre Animal of our Faculty.

The islets were isolated from the pancreas of 2–3 animals using a collagenase (EC 3.4.24.3 from *Clostridium histolyticum*, type P; Roche Diagnostics, Mannheim, Germany) digestion technique [14] and passed through a 400 μm mesh nylon filter (Nytal, Thal, Switzerland) to discard large exocrine aggregates. The islets were then placed in iced Hanks' solution and isolated in two consecutive collections by hand-picking under control of a dissecting microscope, to minimize exocrine contamination.

Dispersed pancreatic islet cells were obtained by dispase digestion of isolated islets according to a previously described method [15], with a few modifications. Briefly, groups of 800 islets each were incubated 2–3 min at 30 °C in 25 ml of a calcium-free HEPES-buffered Earle's dissociation medium (NaCl 120 mM, KCl 5.2 mM, $MgSO_4$ 0.8 mM, NaH_2PO_4 0.9 mM, $NaHCO_3$ 13.8 mM, HEPES 10 mM; pH 7.3–7.4) containing bovine serum albumin (2.4 mg/ml; Fraction V, RIA grade, Sigma, St. Louis, MO), EGTA (3.0 mM), D-glucose (5.3 mM) and dispase (0.5 mg/ml; Dispase II, neutral protease from *Bacillus polymyxa*, grade II, Roche Diagnostics). The separation of islet cells was completed by sucking up and driving back the cell suspension about 10 times through an 18-gauge needle, the optimal separation of cells being assessed by phase contrast microscope observation. The suspension of islet cells was then passed through a 70 μm mesh nylon filter (Falcon Products, Franklin Lanes, NJ) and carefully layered at the bottom of a conic 50-ml tube (Falcon Products) containing 6.0 ml of the calcium-free HEPES-buffered Earle's dissociation medium described above, except for the absence of EGTA and dispase but presence of Percoll (Amersham) to reach a 1.045 density. After 20 min centrifugation at 35×*g* and 4 °C the supernatant was discarded, the cell pellet being then resuspended in 10 ml of dispase-free dissociation medium, again centrifuged (5 min at 4 °C and 40×*g*) and resuspended in 2.0 ml of an incubation medium (see below). After verification of the viability by neutral red staining and counting on a Neubauer slide, the dispersed islet cells were eventually resuspended in a bicarbonate- and HEPES-buffered salt-balanced medium [16] supplemented with 5 mg/ml bovine serum albumin, and equilibrated against a mixture of O_2/CO_2 (19/1, v/v), in a ratio equivalent to 0.4×10^6 cells/ml. Incidentally, in this dispersion procedure the yield of cells was $(1.54\pm0.09)\times10^3$ cells/islet (n=4).

2.2. Incubation procedure designed to determine the total adenine nucleotide content of pancreatic islets

Batches of 12 freshly isolated islets each, were incubated for 60 min at 37 °C in 0.36 ml of a bicarbonate- and HEPES-buffered salt-balanced medium [16] supplemented with bovine serum albumin (5 mg/ml) and the tested agent(s) and equilibrated against a mixture of O_2/CO_2 (19/1, v/v). The incubation was halted and extraction procedure of adenine nucleotides was achieved by addition of 0.12 ml of a solution of NaOH (160 mM) containing 0.4 mM ammonium monovanadate (ATPase inhibitor), 6.0 mM EDTA and Triton X-100 (0.0004%, w/v). After 10 min incubation at 85 °C, in order to denature the enzymes and protect adenine nucleotides from degradation, and 5 min centrifugation at 20 °C and 1000×*g*, the supernatant was sonicated (3 times for 10 s) on ice and neutralized by addition of 30 μl of HCl (650 mM). Then, 0.12 ml of an imidazole buffer (262 mM, pH 7.00) containing KCl (394 mM) and $MgCl_2$ (10 mM) was added to all tubes (islet samples, control media without islet, ATP and ADP standards) and in each of them two aliquots (0.27 ml each) were taken and frozen until ATP assay. On the day of this assay (see below) one of the two aliquots was mixed with 30 μl of an imidazole buffer (100 mM, pH 7.75) while the other was mixed with 30 μl of the same buffer also containing 3.0 mM phospho-enol-pyruvate (Roche Diagnostics) and 0.02 mg of rabbit muscle pyruvate kinase (E.C. 2.7.1.40; 200 units/mg at 25 °C; Roche Diagnostics) to convert ADP into ATP. After 30 min incubation at 30 °C, the reaction was halted by heating for 10 min at 85 °C. The samples were cooled, centrifuged for 1 min in Beckman microfuge, and ATP was assayed on aliquot portions (100 μl each).

2.3. Incubation procedure designed to determine the cytosolic adenine nucleotide content of dispersed islet cells

Aliquots of cell suspension (50 μl, corresponding to 20,000 cells, each) were placed in small polythene tube (Beckman Microfuge tubes) and incubated for 30–60 min at 37 °C in the presence of an equal volume of the cell suspension medium (see above), but containing the appropriate concentration of the agent(s) to be tested. At the end of incubation, permeabilization of the cells was achieved at 20–24 °C by adding 0.1 ml of an ice-cold cell suspension medium enriched with 6.0 mM EDTA, 0.2 mM ammonium monovanadate and digitonine (1.0 mg/ml), freshly suspended by

sonication. Immediately thereafter, 0.1 ml of dibutylphthalate (density = 1.04; Sigma) was carefully layered on top. Exactly 30 s after digitonin treatment, the tubes were centrifuged for 10 s in a Beckman Microfuge (model 11; Beckman Instruments, Inc., Palo Alto, CA). An aliquot (0.12 ml) of the supernatant, representing the cytosolic fraction [12], was incubated for 10 min at 70 °C and then stored at −20 °C until the day of the ATP assay. Prior to ATP assay (see below), two aliquots of the extract (50 μl each) were incubated for 30 min at 30 °C with 20 μl of an imidazole buffer (87.5 mM; pH 7.75) containing 87.5 mM of KCl and 7 mM of $MgCl_2$ and supplemented with either 1.75 mM of phospho-enol-pyruvate or with both phospho-enol-pyruvate (1.75 mM) and 0.02 mg/ml rabbit muscle pyruvate kinase (EC 2.7.1.40; 200 U/mg at 25 °C) to convert ADP into ATP, for ATP assay and the combined assay of ATP plus ADP, respectively. The reaction was halted by heating for 10 min at 70 °C. The samples were cooled and centrifuged for 1 min in Beckman microfuge. ATP was then assayed on 40 μl, as described below.

2.4. ATP assay

ATP was assayed by a luminometric method [17,18]. The samples were mixed with 200 μl of a commercially available lyophilized ATP monitoring reagent containing firefly luciferase and luciferin (ATP Bioluminescence Assay KIT CLS II, Roche Diagnostics) at first reconstituted in an imidazole buffer (100 mM, pH 7.75). The emitted light was measured in a luminometer (LKB 1250 luminometer). The adenine nucleotide content in samples was determined after correction of the control (no islet or cell) and calculated by reference to ATP and ADP standards treated in the same manner as the samples. ADP was calculated from the measurements of ATP and ATP plus ADP contents by difference.

In control experiments, it was first verified that, in the measurement of cytosolic ATP, internal standards (1.25 pmol per assay) yielded a mean value representing 110.8 ± 3.9% (n = 10) of that obtained with the external standards (100.0 ± 0.6; n = 6), the latter two values not being significantly different from one another. Likewise, in further control experiments conducted in dispersed islet cells, no significant difference was observed for the internal standards under the five experimental conditions tested in this study (no exogenous hexose, 10 mM D-glucose, 10 mM D-fructose, 10 mM of both D-glucose and D-fructose, and 100 mM D-fructose), and this whether in the assay of cytosolic or total ATP. The overall mean value for the measurements of such internal standards in the latter case (total ATP) averaged 99.4 ± 6.0% (n = 10) of that obtained in the former case (cytosolic ATP).

2.5. Rb^+ net uptake by dispersed islet cells

For measuring the net uptake of $^{86}Rb^+$, groups of 2.10^4 dispersed islet cells each were incubated for 90 min at 37 °C in 0.1 ml of a bicarbonate- and HEPES-buffered salt-balanced medium (see above) containing bovine serum albumin (1.0 mg/ml), $^{86}RbCl$ (56.4 μCi/ml, 0.6 μCi/mmol; Perkin Elmer, Boston, MA, USA) and the tested hexose(s). After incubation, 0.15 ml of a mixture of dibutylphthalate and di-isononylphthalate (10:3; v/v) was added to each tube, which was then centrifuged for 3 min at 5000 × g. The bottom of the tube containing the cell pellet was then cut, placed in a counting vial containing 5.0 ml of scintillation fluid (Universal, I.C.N., Costa Mesa, CA, USA) and, after mixing, examined for its radioactive content. After correction for the blank value found under the same experimental conditions in the absence of islet cells, the results were expressed as pmol of K^+ per 10^3 cells by reference to the $^{86}Rb^+/^{39}K^+$ ratio found in the incubation medium.

2.6. Data presentation and statistical analysis

All results, including those already mentioned, are given as mean values (±S.E.M.) together with the number of individual determinations (n).

Each individual value refers to a separate group of islets or cell preparation. The results related to islets were analyzed, as described elsewhere [19]. Briefly, the mean values found within each of five experiments for each variable (ATP content, ADP content, ATP + ADP content, ATP/ADP paired ratio) under the five experimental conditions under consideration were averaged as a reference value relative to which all individual measurements within that same experiment were normalized. The normalized results from distinct experiments were then pooled together, and eventually reconverted to absolute values after multiplication by the mean of the five corresponding reference values established in each experiment [19]. The results related to dispersed islet cells are presented as the mean value (±S.E.M.) of the absolute individual measurements. The statistical significance of differences between mean values was assessed by Student's t-test or univariate variance analysis (ANOVA) with Bonferroni's post test, using Prism™ version 4.00 for GraphPad software (San Diego, CA, USA).

3. Results

3.1. Isolated islets

The islet ATP content was about 70% higher (P < 0.001) in islets exposed to 10.0 mM D-glucose than in islets deprived for 60 min of exogenous nutrient (Table 1). D-fructose, however, caused a concentration-related decrease of the ATP content, which averaged at 10.0 and 100.0 mM D-fructose, respectively, 80.2 ± 6.5% (n = 30; P < 0.02) and 53.3 ± 4.2% (n = 29; P < 0.001) of the mean corresponding basal value (no exogenous nutrient) recorded within the same experiment(s). In the presence of D-glucose (10.0 mM), D-fructose (10.0 mM) also decreased (P < 0.01) the islet ATP content, which averaged 75.3 ± 5.1% (n = 30) of the mean corresponding value recorded within the same experiment(s) in the sole presence of the aldohexose (100.0 ± 7.2; n = 30) but remained nevertheless higher (P < 0.02) than basal value.

In the sole presence of D-glucose (10.0 mM) or in the presence of both D-glucose and D-fructose (10.0 mM each), the islet ADP content was not significantly different (P > 0.2 or more) from basal value. D-fructose (10.0 and 100.0 mM), however, lowered the islet ADP content to, respectively, 78.0 ± 5.9% (n = 28; P < 0.005) and 69.7 ± 5.2% (n = 29; P < 0.001) of the mean basal value recorded within the same experiment(s). The latter two percentages were not significantly different (P > 0.25) from one another.

As expected from these findings, the islet content in ATP + ADP was higher (P < 0.005) in islets exposed to D-glucose (10.0 M) than in islets deprived of exogenous nutrient.

Table 1
Total ATP and ADP content and ATP/ADP ratio in rat pancreatic islets

D-glucose (mM)	–	10.0	–	–	10.0
D-fructose (mM)	–	–	10.0	100.0	10.0
ATP (pmol/islet)	2.05 ± 0.16 (29)	3.50 ± 0 28 (30)	1.67 ± 0.12 (30)	1.10 ± 0.07 (29)	2.58 ± 0.15 (30)
ADP (pmol/islet)	1.72 ± 0.07 (27)	1.66 ± 0.14 (30)	1.33 ± 0.09 (28)	1.20 ± 0.08 (29)	1.57 ± 0.11 (30)
ATP + ADP (pmol/islet)	3.91 ± 0.18 (27)	5.18 ± 0.37 (30)	2.91 ± 0.15 (28)	2.17 ± 0.13 (29)	4.23 ± 0.22 (30)
ATP/ADP (ratio)	1.40 ± 0.11 (26)	2.47 ± 0.16 (30)	1.31 ± 0.07 (28)	1.21 ± 0.11 (29)	1.91 ± 0.12 (30)

Data are means ± S.E.M. with the number of individual determinations indicated in parentheses.

D-fructose, on the contrary, caused a concentration related decrease in the ATP+ADP islet content. Relative to the basal value recorded within the same experiment(s), such a content indeed decreased ($P<0.001$) to 76.7±4.9% ($n=28$) at 10.0 mM D-fructose, and was further lowered ($P<0.001$) to 57.1±3.5% ($n=29$) at 100.0 mM D-fructose. In the presence of both D-glucose and D-fructose (10.0 mM each), the ATP+ADP content was lower ($P<0.04$) than in the sole presence of the aldohexose and no more significantly different ($P>0.2$) from basal value.

Relative to basal value, D-glucose (10.0 mM) increased by about 75% the paired ATP/ADP islet ratio ($P<0.001$). D-fructose (10.0 and 100.0 mM), however, failed to affect significantly the ATP/ADP ratio ($P > 0.2$ or more), the trend being towards a lowering of such a ratio below basal value. Thus, the paired ATP/ADP ratio averaged, in the presence of 10.0 and 100.0 mM D-fructose, respectively, 93.8±5.2% ($n=28$) and 86.1±8.0% ($n=29$) of the mean value found in islets deprived of exogenous nutrient (100.0±7.7%; $n=26$). In the presence of D-glucose (10.0 mM), D-fructose (also 10.0 mM) significantly lowered ($P<0.01$) the ATP/ADP ratio, which remained nevertheless higher ($P<0.005$) than basal value (Table 1).

Fig. 1 illustrates the opposite effects of D-fructose upon ATP/ADP ratio and insulin output in rat pancreatic islets. D-fructose (100.0 mM), which tends to lower the ATP/ADP ratio below basal value, augments significantly ($P<0.001$) insulin output. Likewise, at a 10.0 mM concentration, D-fructose lowers the ATP/ADP ratio of islets exposed to 10.0 mM D-glucose, but increases ($P<0.001$) the secretory response to the aldohexose.

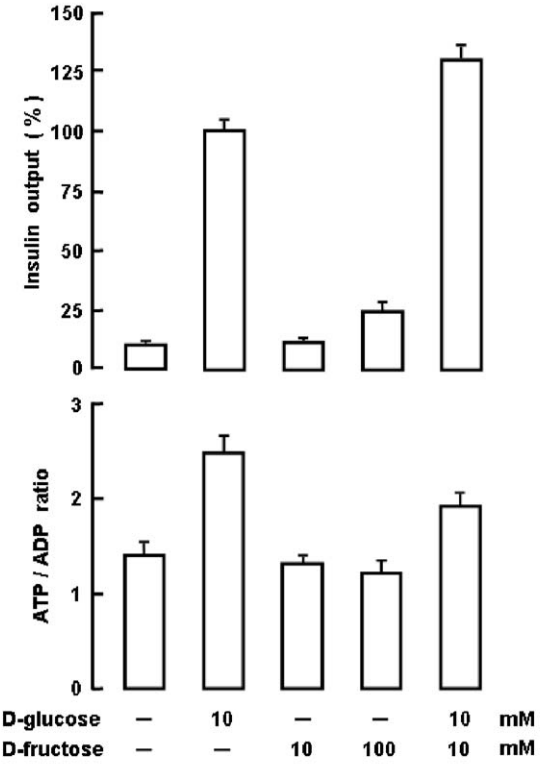

Fig. 1. Comparison between the effects of D-glucose and D-fructose upon insulin output [6,43,44] and ATP/ADP ratio in rat pancreatic islets. In the upper panel, the output of insulin is expressed relative to the value recorded, within the same experiment(s), in the presence of 10.0 mM D-glucose, i.e., 5.40±0.21 ng/islet per 120 min ($n=84$).

3.2. Dispersed islet cells

Whether in the absence or presence of D-fructose (10.0 mM), the cytosolic ATP content of the dispersed islet cells was 2.5 times higher ($P<0.001$) in the presence of D-glucose (also 10.0 mM) than in its absence. At the 10.0 mM concentration, however, the ketohexose failed to affect significantly ($P > 0.7$) the cytosolic ATP content, whether in the absence or presence of D-glucose. Nevertheless, at a 100.0 mM concentration, D-fructose increased by about 50% ($P<0.001$) the cytosolic ATP content. There was a significant correlation ($r=0.9569$; $P<0.02$) between the mean values for the cytosolic ATP content in dispersed islet cells and the corresponding values for insulin output from isolated islets.

Relative to basal value (0.72±0.06 pmol/10^3 cells; $n=12$), the cytosolic ADP content failed to be significantly affected ($P > 0.4$) by 10.0 mM D-glucose (0.81±0.12 pmol/10^3 cells; $n=12$). In the presence of D-fructose, however, the mean cytosolic ADP content was always lower than the corresponding mean basal value recorded within the same experiment(s). Such was the case whether at 10.0 or 100.0 mM D-fructose and whether in the absence or presence of D-glucose. Thus, the cytosolic ADP content averaged in the presence of 10.0 mM D-fructose 81.8±9.0% ($n=12$; $P > 0.1$), in the presence of 100.0 mM D-fructose 73.5±7.7% ($n=12$; $P<0.025$), and in the presence of both D-glucose and D-fructose (10.0 mM each) 72.7±8.9% ($n=12$; $P<0.05$) of the mean corresponding basal values (no exogenous hexose) found within the same experiment(s) (100.0±7.8%; $n=12$). As expected from these findings, the cytosolic ADP content was also significantly lower ($P<0.05$) in the islet cells exposed to either 100.0 mM D-fructose or both D-glucose and D-fructose (10.0 mM each) than in cells exposed to 10.0 mM D-glucose (Table 2).

D-glucose (10.0 mM) augmented ($P<0.001$) the cytosolic content in ATP+ADP from a basal value (no exogenous nutrient) of 1.81±0.09 pmol per 10^3 cells to 3.59±0.14 pmol per 10^3 cells ($n=12$ in both cases). Whether in the absence or presence of D-glucose, the ATP+ADP cytosolic content was slightly lower in the presence of D-fructose (10.0 mM) than in its absence (Table 2). Thus, the individual values recorded in the presence of the ketohexose averaged 91.3±2.5% ($n=24$; $P<0.005$) of the mean corresponding values found within the same experiment(s) at the same concentration of D-glucose in the absence of D-fructose (100.0±1.6%; $n=24$). Unexpectedly, however, at a higher concentration (100.0 mM) of D-fructose, the ATP+ADP cytosolic content was significantly higher ($P<0.001$) than that found in the absence of any exogenous nutrient, averaging 122.2±4.4% ($n=12$) of the mean corresponding basal values found within the same experiment(s) in the absence of any exogenous hexose (100.0±2.6%; $n=12$).

Table 2
Cytosolic ATP and ADP content and ATP/ADP ratio in dispersed rat islet cells

D-glucose (mM)	–	10.0	–	–	10.0
D-fructose (mM)	–	–	10.0	100.0	10.0
ATP (pmol/10^3 cells)	1.10±0.06 (12)	2.77±0.16 (12)	1.09±0.07 (12)	1.69±0.12 (12)	2.70±0.09 (12)
ADP (pmol/10^3 cells)	0.72±0.06 (12)	0.81±0.12 (12)	0.59±0.07 (12)	0.53±0.06 (12)	0.52±0.06 (12)
ATP+ADP (pmol/10^3 cells)	1.81±0.09 (12)	3.59±0.14 (12)	1.68±0.12 (12)	2.22±0.13 (12)	3.22±0.13 (12)
ATP/ADP (ratio)	1.64±0.17 (12)	4.38±0.67 (12)	2.14±0.27 (12)	3.79±0.58 (12)	6.16±0.84 (12)

Data are means±S.E.M. with the number of individual determinations indicated in parentheses.

As indicated in Table 2, the paired ATP/ADP cytosolic ratio failed to be significantly increased above basal value in the islet cells incubated in the sole presence of 10.0 mM D-fructose, which also fails to stimulate insulin release in rat islets. In the presence of D-fructose (100.0 mM), D-glucose (10.0 mM) or both D-glucose and D-fructose (10.0 mM each), however, such a paired ATP/ADP cytosolic ratio was significantly higher ($P<0.005$ or less) than basal value. Once again, there was a significant correlation ($r=0.9191$; $P<0.05$) between the mean values for the cytosolic ATP/ADP ratio in the dispersed islet cells and the corresponding values for insulin output from isolated islets. As illustrated in Fig. 2, the relationship between these two variables displayed an apparently sigmoidal pattern with a threshold value for the stimulation of insulin release above basal value at an ATP/ADP cytosolic ratio in excess of that found in the cells exposed to 10.0 mM D-fructose.

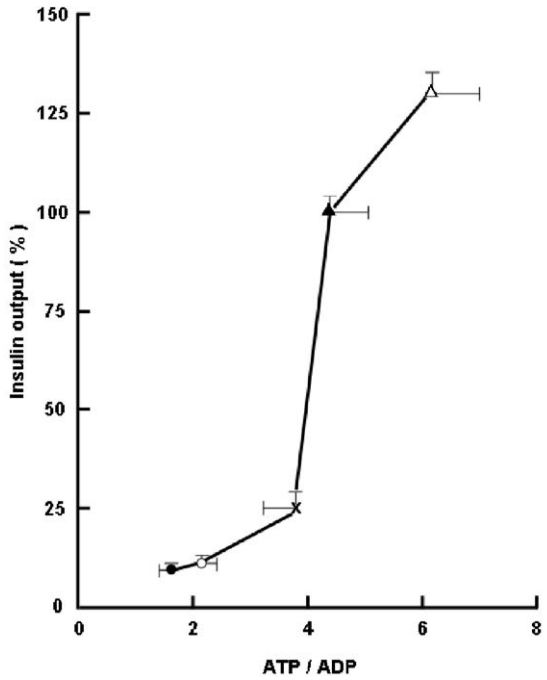

Fig. 2. Comparison between insulin output from isolated rat pancreatic islets and cytosolic ATP/ADP ratio in dispersed rat islet cells (see Table 2) incubated in the absence (circles and cross) or presence (triangles) of 10.0 mM D-glucose and absence (closed symbols) or presence of 10.0 mM D-fructose (open symbols) or 100.0 mM D-fructose (cross). Mean values (±S.E.M.) for insulin output refer to 14–85 observations and are expressed relative to the mean insulin release recorded within the same experiment(s) in the sole presence of 10.0 mM D-glucose (see Fig. 1).

In order to establish a comparison between biophysical and biochemical data both collected in dispersed islet cells, the net uptake or ^{86}Rb was measured in dispersed islet cells incubated for 90 min at 37 °C in a salt-balanced medium containing a tracer amount of $^{86}Rb^+$, the results being expressed as pmol of K^+ with the same $^{86}Rb^+/^{40}K^+$ ratio as in the incubation medium. In a first series of experiments illustrated in Fig. 3 (panel A), the net uptake of $^{86}Rb^+$ was found to increase as a function of the extracellular concentration of D-glucose, the mean results obtained at 8.3, 11.1 and 16.7 mM D-glucose being no more significantly different from one another. The concentration–response relationship illustrated in Fig. 3 (panel A) represents a mirror image of that characterizing the concentration-related effect of D-glucose upon $^{86}Rb^+$ fractional outflow rate from prelabelled and perifused islets [20], in agreement with the knowledge that D-glucose increases the K^+ content of islet cells by decreased K^+ conductance, without any obvious effect on the rate of K^+ inflow into islet cells [21].

In the second series of experiments, the net uptake of $^{86}Rb^+$ was measured under the same five environmental conditions as those used to measure the effect of D-glucose and/or D-fructose upon the cytosolic content of adenine nucleotides in dispersed islet cells. In the absence of D-glucose, D-fructose (10.0 mM) slightly decreased ($P<0.02$) $^{86}Rb^+$ net uptake from a basal value of 141.0±3.7 pmol/10^3 cells ($n=20$) to 126.9±3.8 pmol/10^3 cells ($n=25$). In the presence of D-glucose (10.0 mM), however, D-fructose (also 10.0 mM) increased the net uptake of $^{86}Rb^+$ ($P<0.05$) from 246.3±3.8 pmol/10^3 cells ($n=20$) to 286.2±19.7 pmol/10^3 cells ($n=18$). There was a highly significant correlation between the mean values for $^{86}Rb^+$ net uptake and cytosolic ATP content (Fig. 3, panel B). Moreover, the relationship between $^{86}Rb^+$ net uptake and cytosolic ATP/ADP ratio displayed a sigmoidal pattern, reminiscent of that documented in Fig. 2 (Fig. 3, panel C).

4. Discussion

As a rule, there is a close parallelism between the estimated rate of ATP generation in pancreatic islet cells exposed to exogenous nutrients and their insulinotropic capacity. This presumably cause-to-effect relationship was first supported by measurements of O_2 uptake by isolated islets [22,23]. In the most recent report on this issue, the same approach was again used, together with the measurement of cytochromes in perifused rat islets, to investigate the regulation of ATP/ADP

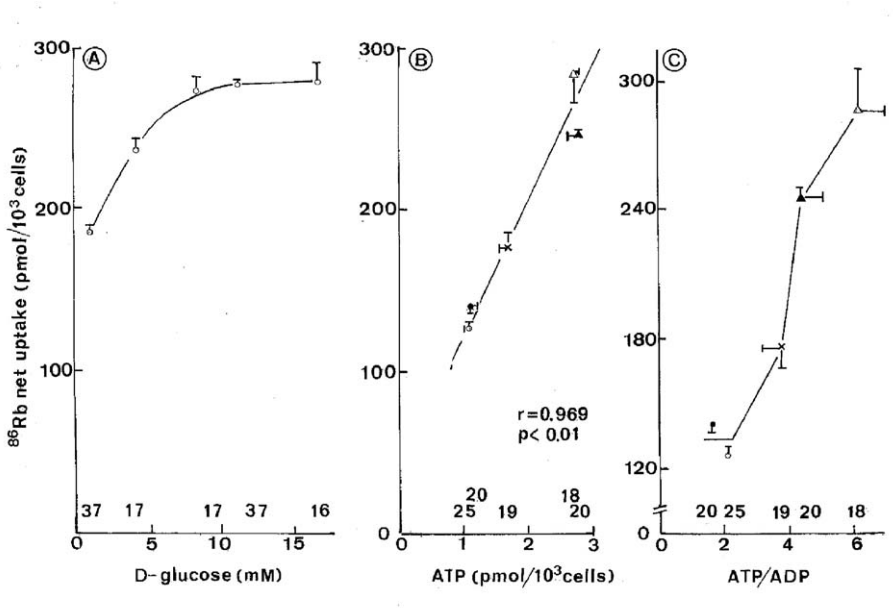

Fig. 3. ^{86}Rb net uptake (pmol/10^3 cells) by dispersed islet cells incubated for 90 min at increasing concentrations of D-glucose (panel A). Correlation between the mean values for ^{86}Rb net uptake and cytosolic ATP content (Table 2) in dispersed islet cells incubated in the absence or presence of D-glucose (10 mM) and/or D-fructose (10 or 100 mM; same symbols as in Fig. 2), the slope of the oblique line corresponds to $\sqrt{\Sigma y^2/\Sigma x^2}$ (panel B). Relationship between ^{86}Rb net uptake (same data as in panel B) and the cytosolic ATP/ADP ratio (Table 2) in dispersed islet cells. Mean values (±S.E.M.) are shown together with the number of separate determinations of ^{86}Rb uptake as indicated at the bottom of each panel.

ratio in the islet cells [24]. The oxidation of ^{14}C-labelled nutrients, the identification and quantification of selected metabolic-end-products and the possible changes in the catabolism of endogenous fatty acids and amino acids were also often considered to document parallel changes in ATP generation and insulin release in islets exposed to various nutrient secretagogues [25–28]. For the same purpose, ATP levels were continuously monitored in insulin secreting cells expressing cytosolic firefly luciferase [29]. Last, direct measurements of ATP, ADP and AMP in both the cytosol and intracellular organelles provided further support to the fuel hypothesis for nutrient-stimulated insulin secretion [30,31].

This study aimed at exploring the possible participation of changes in the adenine nucleotide content of islet cells to the insulinotropic action of D-fructose. The measurements of ATP and ADP were made after 60 min incubation in pancreatic islets, i.e. at the mid time of the 120 min period otherwise used to assess insulin release from such islets. Likewise, in the dispersed islet cells, the ATP and ADP content was measured after 30–60 min incubation, in the middle of the 90 min period used for the study of $^{86}Rb^+$ net uptake by such cells. It should be emphasized, therefore, that all these data refer to a close-to-steady state situation, relevant to the sustained stimulation of insulin release over a period of 120 min, as observed after intragastric administration of D-fructose to overnight fasted rats [32].

The present findings unambiguously reveal that, in the case of D-fructose, there is no parallelism between the changes evoked by the ketohexose in the total islet content of ATP and ADP and its insulinotropic action (Fig. 1). Thus, while D-fructose (10 mM) augments significantly, in rat pancreatic islets, insulin release evoked by 10 mM D-glucose, it lowers the total ATP content and ATP/ADP ratio of islets exposed to the aldohexose. Likewise, in the absence of any other exogenous nutrient, D-fructose (100 mM), which displays a positive insulinotropic action, fails to augment, or even lowers, the total islet ATP content and ATP/ADP ratio. In the latter situation, it could be objected that the release of insulin induced by D-fructose reflects an osmotic effect of the sugar at the high concentration used. The following two considerations argue against such a view. First, the transport of D-fructose, like that of D-glucose, into the islet cells is efficient enough to lead to rapid equilibration between intracellular and extracellular hexose concentrations [33–35]. Second, both 3-*O*-methyl-D-glucose and D-galactose (240 mM each) fail to augment insulin output above basal value [13]. Incidentally, the partial removal of extracellular Na^+, usually made to compensate for an increase in substrate concentration to maintain iso-osmolarity, could be questioned since Na^+ itself modifies both the metabolism of D-glucose in islet cells and the release of insulin [36]. Last, as already underlined in Introduction of this report, the release of insulin evoked by high concentrations of D-fructose displays the typical features of an active secretory process.

Differences between the metabolic and functional responses of rat pancreatic islets to D-glucose versus D-fructose were already previously observed. First, in an attempt to assess the validity of a proposal according to which the insulinotropic action of D-fructose may be related to its capacity to generate D-fructose 1-phosphate and, hence, to cause activation of glucokinase at the intervention of its regulatory protein [37], we observed that the oxidation of 8.3 mM D-[U-^{14}C]glucose is higher ($P<0.03$) than the sum of that of 6.0 mM D-[U-^{14}C]

glucose and 80.0 mM D-[U-^{14}C]fructose when both hexoses are present together in the incubation medium, whilst the release of insulin is about four times higher in the presence of both D-glucose (6.0 mM) and D-fructose (80.0 mM) than in the sole presence of 8.3 mM D-glucose [38]. These findings already suggested that the insulinotropic action of D-fructose could not be fully accounted for by its capacity to act as a fuel in islet cells, as if it were to involve the generation of a second messenger distinct from those coupling factors currently implied in the process of nutrient-stimulated insulin release. Second, Miwa and Taniguchi [39] reported that the release of insulin evoked by 20 mM D-fructose in the presence of 10 mM D-glucose was comparable to that evoked by 20 mM D-glucose alone, whether in rat islets incubated in a salt-balanced medium containing 5.4 mM K^+ (ATP-sensitive K_{ATP} channel-dependent secretory pathway) or in islets exposed to diazoxide (0.15 mM) in the presence of 50.0 mM K^+ (ATP-sensitive K_{ATP} channel-independent secretory pathway). Yet, it can be calculated from their data that, in the salt-balanced medium, the oxidation of the [U-^{14}C]labelled hexoses was significantly lower ($P<0.01$) in the presence of both D-fructose (20 mM) and D-glucose (10 mM), i.e. 16.3 ± 1.1 pmol/islet per 30 min, than in the sole presence of 20 mM D-glucose (21.5 ± 1.3 pmol/islet per 30 min). Under these two conditions, however, the islet content in D-fructose 1,6-bisphosphate, as well as that of tritiated inositol triphosphate (generated in islets prelabelled with *myo*-[2-^{3}H]inositol), were virtually identical, thus leading to the proposal that these two metabolites are involve in the fructose potentiation of glucose-induced insulin secretion. Last, D-fructose, when tested in the 80 to 240 mM concentration range, fails to stimulate rat islet biosynthetic activity, in sharp contrast to both the situation found in islets exposed to D-glucose (e.g., 16.7 mM) and the insulin secretory response of the islets under the same experimental conditions. This dissociation between biosynthetic and secretory data again supports the view that the insulinotropic action of the ketohexose does not entail the same metabolic determinants as those operative in glucose-stimulated islets [40].

Nevertheless, in the present study, a vastly different situation was found when the cytosolic ATP/ADP ratio was measured, instead of the total ATP/ADP ratio. The measurement of cytosolic ATP and ADP was conducted in dispersed islet cells rather than isolated islets in order to optimize the digitonine-induced release of cytosolic adenine nucleotides. The results indicated that the insulin secretory response to the two hexoses under consideration, as assessed in intact islets, was tightly related to the cytosolic ATP/ADP ratio (Fig. 2). This finding is consistent with the observation that the cationic determinants of the insulinotropic action of D-fructose are similar, if not identical, to those involved in the process of glucose-stimulated insulin secretion [11].

It could be objected that the measurements of cytosolic adenine nucleotides in dispersed islet cells were not compared to secretory data collected in the same cells. Considering the poor secretory response of dispersed islet cells to insulin secretagogues [41,42], an alternative approach, consisting in the measurement of $^{86}Rb^+$ net uptake, was used in the present study to assess, in such cells, the possible relationship between the changes in cytosolic adenine nucleotides caused by D-glucose and/or D-fructose and a cationic variable playing a key role in the process of nutrient-stimulated insulin release [20,21]. The data illustrated in Fig. 3 unambiguously document the existence of a close relationship between metabolic and ionic events in the dispersed islet cells.

The present findings favour, therefore, the view that an increase in cytosolic ATP/ADP ratio represents a key determinant of the secretory response to D-glucose and/or D-fructose. They do not rule out, however, the participation of other factor(s) in the functional response of islet cells to these hexoses, as indeed suggested by the opposite effects of D-fructose upon $^{86}Rb^+$ net uptake by dispersed islet cells incubated either in the absence or presence of D-glucose. Further investigations are required to identify the mechanisms by which D-fructose affects unfavourably, in pancreatic islet cells, the total ATP and ADP content, as well as ATP/ADP ratio, whilst nevertheless increasing the cytosolic ATP/ADP ratio. Meanwhile, the present data afford, to our knowledge, the first example of a dramatic dissociation between the effects of a given nutrient secretagogue on total and cytosolic adenine nucleotide content. In our opinion, relevance of this finding to the regulation of insulin secretion by circulating nutrients under physiological conditions should not be overlooked, especially in the light of a recent report documenting the changes in insulin secretion evoked in vivo by the oral administration of D-fructose, given either as the hexose itself, whether alone or together with D-glucose, or in the form of the sucrose disaccharide [32].

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