The stimulation of insulin secretion by D-glyceraldehyde correlates with its rate of oxidation in islet cells

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D-Glyceraldehyde's capacity to mimic the effect of D-glucose on insulin secretion has not yet been sufficiently substantiated. It has been recently proposed, however, that they might act through different mechanisms in insulin-secreting tumoral cells. Therefore, we have performed a dose-related study of both the secretory and metabolic effects of D-glyceraldehyde on islets, which have been compared with those produced by D-glucose. D-Glyceraldehyde's capacity to stimulate secretion was paralleled in a dose-dependent manner by its rate of oxidation to $^{14}\text{CO}_2$. Partial inhibition of D-glyceraldehyde oxidation by β -iodoacetamide resulted in a proportional decrease in the secretory response. L-Glyceraldehyde, which was apparently very poorly oxidized by islets, did not stimulate secretion. The ratio of the maximum insulin responses to D-glyceraldehyde and D-glucose

(57 %) correlated with the ratio of their respective maximum rates of oxidation (68 %). At sub-maximal concentrations there was a potentiation of the secretagogue effects of the hexose by the triose, which was not apparent at a maximum effective dose of glucose. It is concluded that D-glyceraldehyde mimics the secretory effect of glucose because, similarly to the hexose, it is metabolized through islet aerobic glycolysis. The lower potency of D-glyceraldehyde as an insulin secretagogue than D-glucose is determined by the lower capacity of islets to oxidize the triose compared with the hexose. D-Glyceraldehyde, unlike D-glucose, is metabolized in islets to D-lactate. Alternative routes for the metabolism of D-glyceraldehyde might explain some of the secretagogue differences between the triose and the hexose.

INTRODUCTION

D-Glyceraldehyde was recognized as a nutrient secretagogue for insulin more than 20 years ago because it could be metabolized by islet cells and maintain the total content of cellular ATP within a certain concentration range [1]. The capacity of islet cells to metabolize D-glyceraldehyde and to respond with an increased insulin release was later corroborated in several reports [2–6]. Since then, it has been generally assumed that it mimics the effect of glucose on β -cells, being metabolized in the glycolytic pathway, where it would enter at the triose phosphate level after its phosphorylation by triokinase [7].

We believe, however, that this assumption has not been completely substantiated, since a correlated and dose-dependent study of both metabolic and secretory aspects of D-glyceraldehyde on islet function was not undertaken in the previously mentioned reports. Moreover, the glucose-mimicking ability of D-glyceraldehyde has recently been questioned by the following experimental evidence. First, D-glyceraldehyde can be directly oxidized by glyceraldehyde phosphate dehydrogenase, without previous phosphorylation by triokinase, giving rise to glyceralate 1-phosphate, which is probably not metabolizable in islets [7]. Second, D-glyceraldehyde is a very efficient secretagogue in insulin-secreting tumoral cells which are either sensitive or unresponsive to D-glucose in the physiological concentration range [8,9]. However, D-glyceraldehyde, unlike D-glucose, seems to be very poorly metabolized in some of these cells [10]. In contrast with D-glucose, which induces cytoplasm alkalization in β -cells [11,12] or insulin-secreting tumoral cells [13], D-glyceraldehyde produces an acidification [10,13,14]. It would then be possible for D-glyceraldehyde-mediated depolarization

and the resulting increase in cytoplasmic calcium that accompanies secretion [14,15] to be mediated through a stimulus-secretion coupling mechanism different from that used by D-glucose, at least in insulin-secreting tumoral cells [10]. We therefore aimed in the present study to investigate D-glyceraldehyde metabolism in rat islets in relation to the stimulation of insulin secretion by the triose alone or in combination with D-glucose.

EXPERIMENTAL

Chemicals

D-[U- ^{14}C]Glucose, $\text{NaH}^{14}\text{CO}_3$, Na^{125}I and the liquid-scintillation cocktail (BCS) were obtained from Amersham Iberica S.A. (Spain), and D-[U- ^{14}C]glyceraldehyde was from American Radio-labeled Chemicals (U.S.A.). Collagenase, D-lactate, fatty-acid-poor BSA, L-LDH, D-LDH and NAD^+ were obtained from Boehringer Mannheim S.A. (Spain). D- and L-glyceraldehyde were from Fluka Chemie A.G. (Switzerland): they were supplied as stock solutions containing 85 % (w/w) glyceraldehyde and 15 % water. Solvable, a CO_2 -trapping agent, was from Du Pont de Nemours (Belgium). Hepes, L-lactate, rotenone and β -iodoacetamide were from Sigma Chemical Co. (U.S.A.). All organic solvents and inorganic salts were of analytical grade from E. Merck (Germany).

Methods

Islets were isolated by collagenase digestion [16] of the pancreas

Abbreviation used: LDH, lactate dehydrogenase.

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of adult Wistar Albino rats (males of 250 g body wt.). Insulin secretion was studied in isolated islets (groups of 40) perfused at 0.5 ml/min. with Krebs–Ringer bicarbonate containing 5 mM NaHCO_3 , 20 mM Hepes and 0.5% (w/v) fatty-acid-poor BSA, which was adjusted to pH 7.4 and equilibrated with air. Islets were pre-perfused for 30 min either in the absence of nutrients or at 3 mM D-glucose, and then the medium was changed to one containing D-glyceraldehyde (1–20 mM) and/or D-glucose (3, 7 or 20 mM) for another 30 min. The perfusion medium was finally switched to restore and maintain the initial conditions for the last 25 min. The perfusate was collected every 1 min during the last 60 min and was then stored frozen at -40°C until its insulin content was measured by radioimmunoassay [17]. Pig insulin was radiolabelled with Na^{125}I [18] and used as a tracer, whereas rat insulin was used as a standard in the assay.

Substrate oxidation was measured as the production of $^{14}\text{CO}_2$ from D-[U- ^{14}C]glucose (16.6 or 2.5 mCi/mmol at 3 or 20 mM D-glucose, respectively) and D-[U- ^{14}C]glyceraldehyde (50, 10 or 5 mCi/mmol at 1, 5 or 10 mM respectively). Islets (20) were incubated with the radioisotope for 120 min at 37°C in sealed vials containing 20 μl of the same medium as that used in perfusions. Metabolism was stopped by addition of 20 μl of phosphate buffer (4 mM; pH 6) containing 20 μM rotenone and 2 mM β -iodoacetamide. The tubes containing islets plus medium were then introduced, open, into scintillation vials that contained 0.5 ml of Solvable and further incubated for 120 min at 37°C . The tubes were then removed. After addition of scintillation cocktail (5 ml of BCS) the radioactivity was measured. The radioactivity of samples of medium (5 μl in triplicate) was similarly measured after addition of Solvable and BCS, and was used to calculate the specific radioactivity of labelled D-glucose or D-glyceraldehyde. The recovery of $\text{NaH}^{14}\text{CO}_3$ was systematically checked in each experiment ($70.0 \pm 5.7\%$, $n = 4$, in a particular experiment), and was used to correct the rates of oxidation accordingly.

D- and L-Lactate production by the same group of islets was measured by an enzymic method that uses D- and L-specific LDH, respectively, to oxidize each of the stereoisomers to pyruvate at the expense of NAD^+ reduction to NADH. At an alkaline pH (9) and in the presence of hydrazine, there was an almost stoichiometric conversion of lactate into pyruvate, which was quantified by measuring NADH fluorescence [19]. There was no appreciable interference of L-lactate with D-lactate measurements, or vice versa. Briefly, islets (200 or more) were incubated in 60 μl of the same medium used in perfusions for 160 min at 37°C . Metabolism was stopped by cooling the tubes containing islets plus medium. Samples of incubation medium ($2 \times 25 \mu\text{l}$) were stored frozen (-40°C) until they were used for the assay of lactate: they were deproteinized with HClO_4 (1.35 M, 20 μl), followed by precipitation by addition of KHCO_3 and Tris (2.8 and 0.1 M, respectively; 15 μl). Supernatant samples (40 μl) were then added to 325 μl of buffer (0.5 mM glycine and 0.4 M hydrazine hydrate, pH 9) containing NAD^+ (2 mM), EDTA (0.4 mg/ml) and either D- or L-specific LDH (30 units/ml). Fluorescence was measured after 120 min of incubation at 25°C . D- and L-Lactate (1–0.125 mM) solutions in incubation medium were treated as samples and used as standards for calculations.

Total lactate was measured with a h.p.l.c. method of high sensitivity (picomolar, versus nanomolar range in the enzymic method) [20] that allowed the use of a smaller number of islets (30 islets/30 μl). As described for the enzymic method, the supernatant was deproteinized, and then samples (40 μl) were used for formation of the lactate derivative and its quantification by h.p.l.c. as has been described in detail [21].

The content of DNA was measured in all batches of islets that

were used in either oxidation or lactate-production studies. After aspiration of the incubation medium, the islets were washed with $3 \times 100 \mu\text{l}$ of 5 mM NaOH and finally disrupted by sonication. Samples (5–40 μl) were then used for DNA measurement [22].

The results are expressed as their mean values with the corresponding S.E.M., and the number of different islet preparations (n) used in calculating them is given in parentheses. Statistical comparisons between pairs of means were performed by non-paired Student's t tests. A difference between two means was considered significant when the error probability was smaller than 0.05.

RESULTS

Insulin secretion

D-Glyceraldehyde increases insulin secretion above basal values in a concentration-dependent and sustained manner (Figure 1). The initial rise in secretion induced by D-glyceraldehyde was apparently slower than that induced by D-glucose. Maximum rates of secretion were considerably lower (within 50%) in the presence of 5 mM D-glyceraldehyde than with 20 mM D-glucose. Withdrawal of the stimulus returned insulin secretion toward basal values in all cases.

Table 1 shows the total amount of insulin released by perfused islets. The maximum rate of secretion was reached at 5 mM D-glyceraldehyde; higher concentrations (10 and 20 mM) induced a significantly lower response. L-Glyceraldehyde (5 mM) did not significantly increase insulin secretion above basal values. The secretory response induced by 20 mM D-glucose was almost 2-fold higher than that obtained with 5 mM D-glyceraldehyde.

β -Iodoacetamide (1 mM), a known inhibitor of glyceraldehyde phosphate dehydrogenase, decreased to basal values the secretory response of perfused islets to 5 mM D-glyceraldehyde after a lag period of 10–15 min (Figure 2). The total amount of insulin secreted in the presence of β -iodoacetamide was significantly lower (28.5 ± 0.8 , $n = 5$, versus 43.07 ± 2.74 ng/40 islets per 30 min, $n = 5$; $P < 0.001$).

As summarized in Table 2, the perfusion of islets with 5 mM D-glyceraldehyde together with either 3 or 7 mM D-glucose

Figure 1 Insulin secretion of rat perfused islets stimulated with either D-glucose or D-glyceraldehyde

Groups of 40 islets were pre-perfused for 30 min at 3 mM D-glucose or in the absence of substrates. Then the perfusion medium was changed (5 min on the graph) to one containing 20 mM D-glucose (Δ , $n = 6$) or D-glyceraldehyde ($\circ$, 1 mM, $n = 5$; $\square$, 5 mM, $n = 4$) respectively, and maintained for 30 min. Pre-perfusion conditions were re-established again for the last 25 min. The effluent perfusate (0.5 ml/min) was collected after 25 min of pre-perfusion (0 min on the graph) and its insulin content measured at 1 min intervals. The symbols used represent the mean values obtained in a variable number of islet preparations (n), and their corresponding S.E.M. values are represented by vertical lines at periods of 5 min. Abbreviation: IRI, immunoreactive insulin.

Table 1 Total amount of insulin released by perfused islets stimulated for 30 min with either D-glucose or D- and L-glyceraldehyde

Groups of 40 islets were pre-perfused for 30 min at 3 mM D-glucose or in the absence of substrates. Then the medium was changed to one containing D-glucose and/or D-glyceraldehyde for another 30 min. Pre-perfusion conditions were then re-established and maintained for 25 min. The effluent perfusate (0.5 ml/min) was collected after 25 min of pre-perfusion and its insulin content was measured at 1 min intervals. The results represent the mean values $\pm$ their corresponding S.E.M. of the total amount of insulin secreted during 31–60 min of perfusion. The number of different islet preparations used to test each experimental condition is given in parentheses.

Secretagogue (mM)	Insulin release (ng/30 min per 40 islets)	P
D-Glucose (3)	15.5 $\pm$ 2.5 (5)	Control
D-Glucose (20)	111.0 $\pm$ 2.6 (6)***	< 0.001
D-Glyceraldehyde (1)	37.2 $\pm$ 3.0 (5)*	< 0.01
D-Glyceraldehyde (5)	63.4 $\pm$ 4.9 (4)	< 0.001
D-Glyceraldehyde (10)	47.8 $\pm$ 2.3 (3)**	< 0.001
D-Glyceraldehyde (20)	34.6 $\pm$ 1.0 (3)*	< 0.01
D-Glucose (3)	20.9 $\pm$ 2.2 (5)	Control
D-Glucose (7)	65.2 $\pm$ 2.3 (4)	< 0.001
D-Glucose (20)	158.4 $\pm$ 19.2 (5)	< 0.001
D-Glucose (3) + D-glyceraldehyde (5)	188.7 $\pm$ 15.3 (5)	< 0.001
D-Glucose (7) + D-glyceraldehyde (5)	188.7 $\pm$ 10.4 (5)	< 0.001
D-Glucose (20) + D-glyceraldehyde (5)	173.2 $\pm$ 19.2 (5)	< 0.001

* $P < 0.01$, ** $P < 0.05$ and *** $P < 0.001$, compared with D-Glyceraldehyde (5).

Figure 2 Effect of 1 mM β -iodoacetamide on D-glyceraldehyde-induced secretory response of rat perfused islets

Two groups of 40 islets were pre-perfused in parallel for 30 min in the absence of substrates. The perfusion medium was then changed (5 min on the graph) to one containing 5 mM D-glyceraldehyde alone ($\square$, $n = 5$) or in combination with 1 mM β -iodoacetamide ($\triangle$, $n = 5$) that was maintained for 30 min. Pre-perfusion conditions were then re-established for the last 25 min. See the legend of Figure 1 for other experimental details.

triggered a secretory response of the same magnitude as that obtained with 20 mM D-glucose. However, the latter response was not significantly modified by 5 mM D-glyceraldehyde.

Islet metabolism

D-[U- 14 C]Glyceraldehyde conversion into $^{14}\text{CO}_2$, measured after 120 min of incubation, increased several-fold when its concentration was changed from 1 to 5 mM, but a higher triose

Table 2 Islet oxidation rates to $^{14}\text{CO}_2$ of labelled D-glucose and D-glyceraldehyde at different concentrations

Three to four batches, each of 20 islets, were incubated at 37 $^{\circ}\text{C}$ for 120 min under the experimental conditions described below. The results represent the mean values $\pm$ their corresponding S.E.M. The number of islet preparations used to test each experimental condition is given in parentheses. Abbreviation: N.S., not significant.

D-[U- 14 C]Substrate (mM) + other modifiers	Rate of oxidation (pmol/h per islet†)	P
D-Glyceraldehyde (5)	5.0 $\pm$ 0.4 (9)	Control
D-Glyceraldehyde (10)	5.6 $\pm$ 0.2 (4)	N.S.
D-Glyceraldehyde (1)	1.9 $\pm$ 0.6 (4)	< 0.02
D-Glyceraldehyde (5) + β -iodoacetamide (1 mM)	2.3 $\pm$ 0.1 (5)	< 0.001
D-Glyceraldehyde (5) + L-glyceraldehyde (5 mM)	3.8 $\pm$ 0.3 (5)	N.S.
D-Glucose (3)	2.7 $\pm$ 0.2 (5)	< 0.05
D-Glucose (20)	10.9 $\pm$ 0.9 (7)	< 0.001

*Expressed as pmol of 'hexose' equivalents in order to allow for a direct comparison of D-glucose and D-glyceraldehyde oxidation rates.

†Average DNA content, measured in 9–12 batches each of 20 islets from nine different islet preparations, was 17.7 $\pm$ 0.5 ng/islet.

concentration (10 mM) did not result in an additional increment in the $^{14}\text{CO}_2$ -production rate (Table 2). β -Iodoacetamide (1 mM) decreased the amount of $^{14}\text{CO}_2$ produced from 5 mM D-[U- 14 C]glyceraldehyde by approx. 50%. Table 2 also shows that the amount of hexose equivalents converted into CO_2 was rather similar whether 3 mM D-glucose or 1 mM D-glyceraldehyde was used as a substrate. However, 20 mM labelled D-glucose was converted into $^{14}\text{CO}_2$ at a significantly higher rate than 5 mM D-glyceraldehyde.

There is no isotopic tracer commercially available for a direct study of L-glyceraldehyde metabolism. However, at equimolar concentrations, L-glyceraldehyde did not significantly decrease the amount of $^{14}\text{CO}_2$ produced from D-[U- 14 C]glyceraldehyde, indicating a poor intracellular dilution of D-stereoisomer-generated metabolites by the L-enantiomer.

D-Glyceraldehyde showed an apparently non-saturable rate of conversion into lactate when it was measured with a highly sensitive (picomolar range) h.p.l.c. method (Figure 3). Knowing that D-glyceraldehyde could be converted into D-lactate by tumoral insulin-secreting cells [23], we investigated the relative contribution of both stereoisomers to the total amount of lactate produced by islets with an enzymic (stereospecific) method. This is a less sensitive assay (nanomolar range) and it requires a larger number of islets (over 200) for a single measurement than the h.p.l.c. method. The results, shown in Table 3, indicate that the production of L-lactate did not increase, but slightly decreased, when both D- and L-glyceraldehyde concentrations were changed from 5 to 40 mM. By contrast, in the same concentration range, the production of D-lactate from both D- and L-glyceraldehyde increased with the triose concentration in an almost linear relationship. The amount of L-lactate produced from D-glucose was not changed by increasing the hexose concentration from 3 to 20 mM, and it was 3-fold higher than that obtained from 5 mM D-glyceraldehyde. There was no measurable production of D-lactate from D-glucose.

The total amount of lactate produced from D- and L-glyceraldehyde measured with the h.p.l.c. method was accounted for by the sum of D- and L-lactate measured enzymically. By contrast, the h.p.l.c. method gave 3-fold lower values of total lactate

Table 3 Islet production of total lactate (measured by a h.p.l.c. method) and of L- and D-lactate enantiomers (measured by an enzymic method)

A large group of islets (200 or more) obtained from each islet preparation was incubated at 37 °C for 120 min under the experimental conditions described below. Lactate was measured in the incubation medium, and it was referred to the DNA content measured in each batch of islets. The results represent the mean values $\pm$ their corresponding S.E.M. obtained in a variable number of islet preparations that is given in parentheses.

Substrate (mM)	Lactate production (pmol of 'hexose' equivalents/h per μ g of DNA)				H.p.l.c. method Total
	Enzymic (LDH) method				
	L-Lactate	<i>P</i>	D-Lactate	<i>P</i>	
D-Glyceraldehyde (5)	1130.8 $\pm$ 196.4 (5)	Control	465.1 $\pm$ 97.0 (5)	Control	1707.6 $\pm$ 250.6 (5)*
D-Glyceraldehyde (40)	712.5 $\pm$ 88.1 (9)	< 0.05	2006.1 $\pm$ 128.9 (6)	< 0.001	—
L-Glyceraldehyde (5)	1491.8 $\pm$ 188.0 (6)	Control	352.2 $\pm$ 58.6 (6)	Control	1600.7 $\pm$ 159.9 (6)**
L-Glyceraldehyde (40)	940.4 $\pm$ 92.5 (9)	< 0.02	2803.4 $\pm$ 234.9 (7)	< 0.001	—
D-Glucose (3)	2915.5 $\pm$ 823.6 (4)	Control	Not detected	—	1234.7 $\pm$ 50.2 (47)
D-Glucose (20)	3029.2 $\pm$ 361.6 (7)	N.S.	Not detected	—	1302.8 $\pm$ 48.0 (48)

* $P < 0.01$ and $P < 0.05$, compared with 3 and 20 mM D-glucose respectively.

** $P < 0.02$ and $P < 0.05$, compared with 3 and 20 mM D-glucose respectively.

Figure 3 Concentration-response relationship between D-glyceraldehyde and the total amount of lactate produced by rat islets

Two to three batches, each of 30 islets, were incubated at 37 °C for 120 min with variable concentrations of D-glyceraldehyde. Lactate was measured in the incubation medium by an h.p.l.c. method, as described in detail under 'Methods'. The number of islet preparations used at each D-glyceraldehyde concentration was usually four, but it was eight at 10 mM.

production from glucose than did the enzymic one. Therefore, the latter method seems to overestimate the amount of glucose conversion into lactate, perhaps due to the relative lack of substrate specificity of LDH.

In order to check whether D-lactate could arise from the metabolism of glyceraldehyde through the glyoxalase system [24], islet ability to transform methylglyoxal into total lactate (measured with the h.p.l.c. method) was tested. Table 4 shows that islets were able to convert methylglyoxal into lactate in an apparently linear dose-response relationship.

Table 4 Islet production of total lactate (h.p.l.c. method) from methylglyoxal

Two to three batches, each of 30 islets, were incubated at 37 °C for 120 min with the indicated concentrations of methylglyoxal. Lactate was measured in the incubation medium, and it was referred to the DNA content measured in each batch of islets. The results represent the mean values $\pm$ their corresponding S.E.M. obtained in a variable number of islet preparations that is given in parentheses.

Methylglyoxal (mM)	Lactate production (pmol/h per μ g of DNA)	P
0	943.3 $\pm$ 249.9 (6)	Control
1	4367.2 $\pm$ 947.0 (8)	< 0.01
5	19760.9 $\pm$ 2556.3 (8)	< 0.001

*Expressed as pmol of 'hexose' equivalents.

DISCUSSION

Our results confirm that D-glyceraldehyde behaves as an initiator of insulin secretion [1] in isolated rat islets: a concentration-dependent study of the secretory response of perfused islets with respect to D-glyceraldehyde has been carried out in the complete absence of glucose. It is shown that a sustained response is already generated at 1 mM D-glyceraldehyde. The magnitude of the secretory response seems to reach a maximum around 5 mM, as it decreases at higher triose concentrations (10 and 20 mM). Similar studies performed with static incubations of islets show dose-response relationships for D-glyceraldehyde stimulation of insulin secretion which are comparatively shifted to the right: 2.5 mM D-glyceraldehyde was the lowest concentration that activated secretion, which reached a maximum between 10 and 20 mM, and then it decreased at higher concentrations [2,4]. This difference might be attributed to the different methods used for studying insulin release: a similar dose-response relationship to that found in the present study was obtained previously in perfused islets [6]. The magnitude of the maximum secretory response induced by D-glyceraldehyde was approximately half of that triggered by D-glucose (20 mM), as has been previously reported in incubated islets [2,4]. Our results also corroborated in perfused islets the strong potentiation by D-glyceraldehyde of

insulin secretion at sub-maximal glucose concentrations and its inability to increase the maximum secretory response to the hexose [1,4]. A full response is obtained at a sub-stimulatory glucose concentration (3 mM) by addition of 5 mM D-glyceraldehyde. This apparently means that the secretory potencies of D-glucose and D-glyceraldehyde are not simply additive.

A dose-related study of the oxidation of D-glyceraldehyde by isolated islets has been performed in the present work, in contrast with previous reports, in which only single metabolic measurements were made [1–5]. A similarity of the dose-response relationships characterizing D-glyceraldehyde stimulation of insulin secretion and its rate of oxidation is evident: a positive concentration-dependence of both parameters was observed in the range 1–5 mM, and a maximum response of both metabolism and secretion was apparently reached at 5 mM D-glyceraldehyde, for higher triose concentrations exerted either no further effect (oxidation) or a significant decrease (secretion). β -Iodoacetamide inhibition of both the oxidation of D-glyceraldehyde and its secretory response strengthens further the idea that the triose has to be metabolized through islet glycolysis in order to stimulate secretion. Given a common metabolic mechanism for the stimulation of insulin secretion by D-glucose and D-glyceraldehyde (ATP production by aerobic glycolysis), it seems reasonable that their secretagogue effects are additive or potentiated at low concentrations, but that the maximum secretory effect of D-glucose cannot be further increased by addition of the triose because the mitochondrial oxidation of pyruvate is saturated.

Our results also provide a metabolic explanation for the different magnitude of the maximum secretory response due to D-glucose compared to D-glyceraldehyde. The maximum capacity to metabolize D-glyceraldehyde through islet glycolysis, probably set by the activity of triokinase, is approximately half of that to metabolize D-glucose, and this probably determines that the maximum secretory response to the triose can only be approximately half of that triggered by the hexose. However, at sub-maximal concentrations (3 mM D-glucose and 1 mM D-glyceraldehyde), the triose behaves as a more potent secretagogue than the hexose, even though they are oxidized at similar rates. Different rates of anaerobic glycolysis seem not to be responsible for the difference as secretagogues between the triose and the hexose at low concentrations: L-lactate production from D-glyceraldehyde is already maximum at 5 mM, and it is very close to the rate of L-lactate production from either 3 or 20 mM D-glucose. Islet L-lactate production is saturated at low substrate concentrations [25], either because LDH has a very low K_m for pyruvate, or the enzyme protein is poorly expressed in B-cells [21], or both. L-Glyceraldehyde shares with its D-enantiomer the capacity to generate L-lactate at a similar rate. Its failure to stimulate secretion may be attributed to its low rate of oxidation in islets, as judged from its weak effect on the rate of $^{14}\text{CO}_2$ production from D-[U- ^{14}C]glyceraldehyde. Previous studies have demonstrated the higher capacity of D-glyceraldehyde, as compared with its L-enantiomer, to decrease the production of $^{14}\text{CO}_2$ from D-[U- ^{14}C]glucose, due to isotopic dilution of common pools of glycolytic intermediates [2,3].

D- and L-glyceraldehyde, unlike D-glucose, share the ability of producing D-lactate in a concentration-dependent manner which does not show saturation at high concentrations. It might be speculated that, as happens in other tissues, phosphorylated D-glyceraldehyde could spontaneously oxidize to methylglyoxal, which would then be converted into D-lactate by the glyoxalase system that catalyses oxidation-reduction cycles of NADPH [24]. The demonstrated islet capacity for the concentration-dependent production of total lactate from exogenous methylglyoxal supports the presence of the glyoxalase system in islets.

Its presence has been recently evidenced in HIT-T15 cells, and D-lactate production from D- and L-glyceraldehyde has been attributed to contamination of the commercially available preparations of the trioses with methylglyoxal [23]. This seems to be a more plausible explanation, since D-glucose does not give rise to any significant production of D-lactate, as shown in [23] and the present work.

D-Glyceraldehyde can also be directly oxidized by glyceraldehyde phosphate dehydrogenase activity of islet homogenates, presumably to glyceralate 1-phosphate, which is not further metabolized [7]. The K_m of this dehydrogenase for D-glyceraldehyde (4.8 mM) is within the range of secretagogue concentrations of the triose. However, a predominant metabolism of D-glyceraldehyde by islets through this pathway, which does not provide any complementary substrate for the reoxidation of NADH, would be difficult to reconcile with a sustained rate of stimulated secretion.

In summary, the experimental data obtained in this work allow us to conclude that the oxidative metabolism of D-glyceraldehyde is mostly responsible for the stimulation of insulin secretion in isolated islets. In other words, D-glyceraldehyde uses the same mechanism of action as D-glucose for the activation of B-cells. However, D-glyceraldehyde can be metabolized through alternative routes, not shared by D-glucose, which might be responsible for some of the differences found in the secretagogue action of the triose compared with the hexose. These differences seem to be more pronounced in some insulin-secreting tumoral cell lines, in which different mechanisms of action for D-glyceraldehyde and D-glucose have been proposed [10,23,26]. As an example, D-glyceraldehyde-induced cytoplasmic acidification, which is at variance with D-glucose-induced alkalization, might be due to the production of acidic metabolites, D-lactate and glyceralate 1-phosphate, at high triose concentrations like those normally used for the stimulation of tumoral cells (10 mM or higher). A too high accumulation of non-metabolizable and acidic metabolites could also explain the observed deleterious effects of high D-glyceraldehyde concentrations on islet ATP [1] and on its own secretory response, observed above 5 mM in this work or at higher concentrations in other reports [2,4].

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