Interference of Glycogenolysis with Glycolysis in Pancreatic Islets from Glucose-infused Rats

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Abstract

When pancreatic islets isolated from rats infused for 48–72 h with a hypertonic solution of D-glucose were incubated for two successive periods of 10 min each, in the presence first of 16.7 mM and then 2.8 mM D-[U-^{14}C]glucose, the total output of L-lactic acid during the second incubation was as high as that recorded during the first incubation, while the specific radioactivity of L-lactic acid dramatically decreased during the second incubation. In islets from normoglycemic rats, however, the total output of L-lactic acid decreased and its specific radioactivity modestly increased as the concentration of D-glucose was lowered from 16.7 to 2.8 mM. Such contrasting results indicate that in the glycogen-rich islets isolated from glucose-infused rats, the fall in extracellular D-glucose concentration was not accompanied by a parallel fall in glycolytic flux, the decreased utilization of exogenous D-[U-^{14}C]glucose coinciding with stimulation of glycogenolysis. This unusual metabolic situation also coincided with a transient and paradoxical stimulation of insulin release in response to the decrease in extracellular D-glucose concentration. It is proposed, therefore, that the interference of glycogenolysis with glycolysis in pancreatic islets from glucose-infused rats participates in the paradoxical changes in insulin output which represent a typical feature of B-cell glucotoxicity. (*J. Clin. Invest.* 1993. 91:432–436.) Key words: pancreatic islets • glycogenolysis • B-cell glucotoxicity • L-lactic acid output

Introduction

In both human subjects with non-insulin-dependent diabetes and experimental models for the latter disease, chronic hyperglycemia is thought to alter the secretory behaviour of insulin-producing cells in a phenomenon of so-called B-cell glucotoxicity. Typical features of this phenomenon of B-cell glucotoxicity include, in response to a rise in D-glucose concentration, an early paradoxical fall in insulin output and/or a perturbation of the normal anomeric preference of insulin release for α-D-glucose (1, 2). It was recently proposed that glycogen accumulation in the pancreatic islet B-cell in situations of long-term hyperglycemia may account, in part at least, for these secretory anomalies (1, 2). For instance, it was speculated that no change or even a paradoxical increase of glycolytic flux would occur in response to a fall in extracellular D-glucose concentration, provided that sufficient glycogen had first accumulated in the islet cells (1, 3). Large amounts of glycogen accumulate in islets of rats infused for 1–2 d with a hypertonic solution of D-glucose (4, 5). The present study reveals that in islets isolated from the glucose-infused rats, a fall in extracellular D-glucose concentration from 16.7 to 2.8 mM indeed fails to affect over 10 min incubation the total production of L-lactic acid, despite a dramatic decrease in the contribution of exogenous D-glucose to the generation of this metabolic end-product. This unusual metabolic situation coincides with a short-lived and paradoxical stimulation of insulin release in response to the same fall in hexose concentration.

Methods

The experiments were conducted in 14 female albino rats with an initial body weight of 231±5 g. They had free access to food and water throughout the experimental procedure. A catheter was introduced through the jugular vein in the right heart under anaesthesia with ketamine (125 μg/g body wt. intraperitoneally). 3 d later, a hypertonic solution of D-glucose (1.67 M) was infused from 9 a.m. onwards for 48–72 h at a rate close to 4.6 mmol of glucose per hour. In this procedure, the mortality remained < 6%. The glycemia, measured by test-strips (Medi-Test; Macherey-Nagel, Düren, Germany) in blood samples collected from the tail of the rats, increased from an initial value of 6.1±0.2 mM (n = 13) to 16.3±1.1 mM (n = 36) during the first day, 15.9±1.2 mM (n = 36) during the second day and 15.1±1.8 mM (n = 18) during the third day, each of these mean values being derived from measurements made at ~ 12 a.m., 4 p.m., and 8 a.m. on the next day. The rats were disconnected from the infusion pump at 9 a.m. and immediately killed by decapitation.

Isolated islets were prepared by the collagenase method (6) from the pancreas of 1–3 rats, using media containing 16.7 mM D-glucose (i.e., a concentration close to that found in vivo) for inflation of the pancreas, exposure to collagenase, and collection of the islets. Groups of 40 islets each were used for the measurement of glycogen content (3). Groups of 100 islets each were placed in a perifusion chamber for measurement of insulin release, as described elsewhere (7). Last, groups of 40–80 islets each were used in the experiments designed to measure the production of L-lactic acid. For this purpose, the islets were incubated for two successive periods of 10 min each at 37°C in 0.1 ml of a Hepes-NaOH buffer (20 mM, pH 7.4) containing BSA (0.5 mg/ml), 115 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM $NaHCO_3$, and, as required, either 2.8 or 16.7 mM D-glucose, mixed in some experiments with a tracer amount of D-[U-^{14}C]glucose (10–20 μCi/ml). The incubation media were separated from the islets, heated for 10 min at 85°C and stored at −20°C. Control experiments using groups of 40–150 islets each, prepared from the pancreas of normoglycemic rats, were conducted in the same manner.

The generation of ^{14}C-labeled L-lactic acid was measured as described elsewhere (8). Briefly, aliquots (20 μl each) of the incubation media were mixed with 40 μl of a glycine-NaOH buffer (100 mM, pH 9.7) containing NAD^+ (8.0 mM), L-glutamate (24 mM), glutamate-

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alanine transaminase (8 U/ml), and, when required, lactate dehydrogenase (110 U/ml). After 60 min incubation at 20°C, ^{14}C-labeled L-alanine was separated by ion exchange chromatography (9). The amount of ^{14}C-labeled L-lactic acid generated by the islets was judged from the difference in the radioactive L-alanine content of samples incubated in the presence and absence of lactate dehydrogenase. It was corrected for the recovery (91.6±1.5%; n = 5) of L-[U-^{14}C]lactate standards (160–2,560 pmol/sample) treated in the same manner. When the initial 10 min incubation was conducted in the absence of islets, no ^{14}C-labeled L-lactic acid was generated from D-[U-^{14}C]-glucose.

A novel procedure for the assay of unlabeled L-lactic acid was designed to measure the low concentrations of this metabolite in samples of incubation media obtained after a short incubation (10 min) of the islets at either low or high D-glucose concentration. Aliquots (20 or 40 μl) of the incubation media were mixed with 40 μl of a glycine-NaOH buffer (100 mM, pH 9.7) containing NAD^+ (2 mM), ADP (1 mM), ammonium acetate (50 mM), 2-ketoglutarate (0.6 mM) mixed with a tracer amount of [5-^{14}C]2-ketoglutarate (2.5 μCi/ml), glutamate dehydrogenase (3.6 U/ml), and, when required, lactate dehydrogenase (110 U/ml). After 60 min incubation at 20°C, L-[5-^{14}C]glutamate was separated by ion exchange chromatography (9). The amount of unlabeled L-lactic acid generated by the islets was judged from the difference in the radioactive L-glutamate content of samples incubated in the presence and absence of lactate dehydrogenase. It was corrected for the corresponding difference found in samples of media incubated in the absence of islets, and expressed relative to readings obtained with standards of L-lactic acid (160–2,560 pmol/40 μl) treated in the same manner. As illustrated in Fig. 1, the generation of L-[5-^{14}C]glutamate was proportional to the amount of L-lactic acid present in each sample. The coefficient of variation, as derived from triplicate or quadruplicate measurements, progressively decreased from 6.7±1.7% to 1.9±0.4%, as the amount of L-lactic acid increased from 160 to 2,560 pmol/sample (Fig. 1, *inset*). The efficiency of the procedure, expressed as the ratio between the amount of L-[5-^{14}C]glutamate generated in the assay and the corresponding amount of L-lactic acid present in the sample averaged 32.4±1.5% (Fig. 1).

All results, including those already mentioned, are presented as the ±SEM together with the number of individual determinations (n) or degree of freedom (d.f.). The statistical significance of differences between mean values was assessed by use of Student's t test.

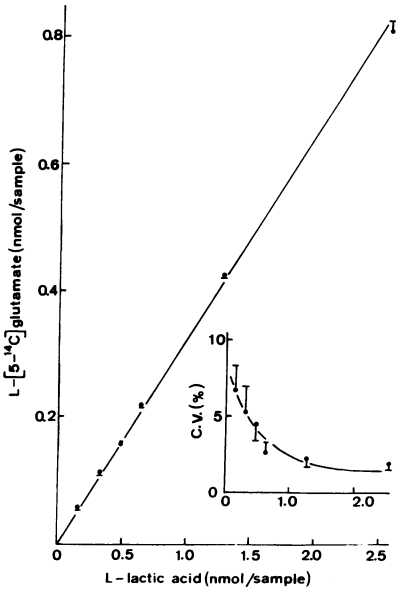

Figure 1. Assay of L-lactic acid through the generation of L-[5-^{14}C]-glutamate. In each individual experiment, the data were expressed relative to the paired reading recorded with the highest amount of L-lactic acid (2.56 nmol/sample) to establish the concentration-response relationship. The absolute value for L-[5-^{14}C]glutamate production was judged from the mean of all available readings recorded with the same high amount of L-lactic acid. (*Inset*) the coefficient of variation (*C.V.*), as derived from triplicate or quadruplicate measurements and expressed in percent, is shown as a function of the amount of L-lactic acid present in each sample (nmol/sample). ±SEM are derived from a series of 10 separate experiments.

Results

Lactic acid release by islets from normoglycemic rats. For the purpose of comparison with data collected in islets from glucose-infused rats, a first series of experiments was conducted in glycogen-free islets from control, normoglycemic, animals (5). In these experiments, all media used to inflate the pancreas, to expose it to collagenase, to isolate, and collect the islets contained 16.7 mM D-glucose.

When the islets were incubated for two successive periods of 10 min each at 37°C in the presence of 16.7 mM D-glucose, the release of lactic acid averaged 16.5±1.1 (n = 54) and 16.2±1.3 (n = 29) pmol/islet per 10 min during the first and second incubation, respectively (Fig. 2). The value recorded during the second incubation represented 101.7±4.5% of the paired value found during the first incubation (Table I). When D-[U-^{14}C]glucose was present in the incubation medium, the specific radioactivity of lactic acid only represented, during the first incubation, 56.1±2.7% of that of the exogenous hexose. The specific radioactivity of lactic acid was higher, however, during the second incubation. Comparison of data collected within each experiment indicated that the specific radioactivity of lactic acid during the first incubation averaged 79.3±5.1% (d.f. = 16; $P < 0.001$ as compared to unity) of that released by the islets during the second incubation.

When the concentration of D-glucose was lowered to 2.8 mM during the second incubation, the output of lactic acid was decreased ($P < 0.001$) to 46.4±3.2% (n = 25) of the paired value recorded during the first incubation conducted in the presence of 16.7 mM D-glucose. It averaged 7.6±0.8 pmol/islet

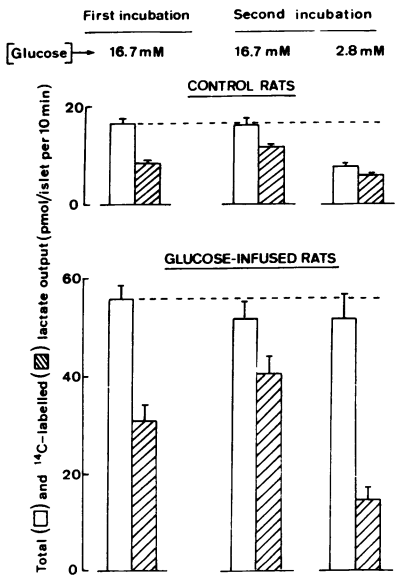

Figure 2. Total (*open columns*) and ^{14}C-labeled (*shaded columns*) L-lactic acid output from islets of control rats (*upper panel*) and glucose-infused animals (*lower panel*) incubated for two successive periods of 10 min each in the presence of 2.8 or 16.7 mM D-glucose mixed with a tracer amount of D-[U-^{14}C]-glucose. ±SEM are derived from the number of individual observations mentioned in Table I. The horizontal dashed lines refer to the mean total output of L-lactic acid during the first incubation.

Table I. Lactic Acid Output and Changes in Its Specific Radioactivity Relative to that of Exogenous D-Glucose in Islets from Control and Glucose-infused Rats over Two Successive Incubations of 10 min Each

Rats D-glucose (mM)	Control		Glucose-infused	
	16.7	2.8	16.7	2.8
First incubation				
Lactic acid output (pmol/islet per 10 min)	16.5±1.1 (54)		55.8±2.7 (66)	
Specific radioactivity (percent)	56.1±2.7 (15)		54.2±4.3 (28)	
Second incubation				
Lactic acid output (percent of paired initial value)	101.7±4.5 (29)	46.4±3.2 (25)	94.5±5.4 (33)	96.6±7.4 (33)
Specific radioactivity (percent)	68.5±4.3 (10)	70.1±5.4 (5)	74.2±5.4 (14)	19.9±1.8 (14)

per 10 min ($n = 25$). However, at the low hexose concentration, the specific radioactivity of lactic acid was undistinguishable ($P > 0.3$) from that recorded, at the same time and within the same experiment, in islets exposed to 16.7 mM D-glucose (Table I).

The time course for the changes in specific radioactivity of lactic acid released by islets incubated for two successive periods of 10 min each in the presence of 16.7 mM D-glucose is compatible with a time-related phenomenon of isotopic dilution of newly formed radioactive lactic acid by the pool of unlabeled lactic acid present inside the islet cells under steady-state conditions of exposure to a high concentration of D-glucose (10). To confirm this interpretation and to assess the possible contribution of endogenous nutrients to the de novo generation of lactic acid (11), a further control experiment was conducted over two successive incubations in the presence of 16.7 mM D-glucose, using islets which were isolated and collected in glucose-free media. Under these conditions, the specific radioactivity of lactic acid, relative to that of exogenous D-glucose, averaged 93.3±10.9 and 93.9±14.6% ($n = 5$ in both cases) during the first and second incubation. The overall mean value derived form these measurements (93.6±8.6%) was not significantly different ($P > 0.45$) from that of exogenous D-glucose, and much higher ($P < 0.001$) than that found, within the same experiment, in islets that had been exposed to D-glucose (16.7 mM) at 20°C during the 45–60 min period required to collect 750 islets and transfer them from the petri dish to the incubation vials. Indeed, in the latter case, the specific radioactivity of lactic acid did not exceed 53.8±5.0% ($n = 10$) of that of exogenous D-glucose. Incidentally, the total production of lactic acid was not significantly different under these two experimental conditions. Thus, the measurements made in islets prepared in glucose-free media averaged 87.5±10.5% (d.f. = 16; $P > 0.2$ as compared to unity) of the corresponding value found at the same time of incubation in islets exposed to D-glucose (16.7 mM) during the collection procedure.

Taken as a whole, these findings indicate that in islets prepared from normoglycemic rats and exposed to a high concentration of D-glucose throughout the experimental procedure, virtually all newly formed lactic acid is derived from exogenous D-glucose but, when generated from D-[U-^{14}C]glucose, undergoes isotopic dilution in the islet cells, so that the lactic acid released by the islets in the incubation medium does not immediately reach the same specific radioactivity as that of the exogenous hexose.

Glycogen content and lactic acid release in islets from glucose-infused rats. The glycogen content of the islets prepared from glucose-infused rats averaged, before incubation, 36.6±9.0 pmol of glucose equivalent per islet ($n = 30$).

When the islets were prepared from glucose-infused rats, two features of their metabolic behavior closely resembled that observed in islets from control rats. First, when the islets were incubated for two successive periods of 10 min each in the presence of a high concentration of D-glucose (16.7 mM), the output of lactic acid was fairly stable, the readings recorded during the second incubation averaging 94.5±5.4% ($n = 33$) of the paired value measured after the first incubation. Second, in the islets maintained throughout at high hexose concentration, the specific radioactivity of lactic acid followed a time course comparable to that found in the islets of control rats. Relative to the specific radioactivity of exogenous glucose, that of lactic acid averaged 54.2±4.3% during the first incubation and 74.2±5.4% during the second incubation. The first reading represented 71.2±5.3% (d.f. = 39; $P < 0.005$ as compared to unity) of the second one. All these values are virtually identical to those found in islets from normoglycemic rats. Such a close analogy strongly suggests that the contribution of exogenous D-glucose to the total generation of lactic acid is superimposable in islets from control and glucose-infused rats, at least when the islets are incubated at a high concentration of the hexose (16.7 mM).

In three other respects, however, the metabolic behaviour of islets from glucose-infused rats differed strikingly from that found in islets from control animals. First, during the first incubation conducted in the presence of 16.7 mM D-glucose, the absolute value for lactic acid output was much higher ($P < 0.001$) in islets from glucose-infused than control rats (Table I). Second, when the concentration of D-glucose was lowered to 2.8 mM during the second incubation, no decrease in lactic acid output was observed in islets from glucose-infused rats. Thus, relative to the paired value recorded over the first incubation, the output of lactic acid during the second incubation was virtually identical at low (96.6±7.4%) and high (94.5±5.4%) hexose concentration, in sharp contrast to the situation documented in islets from control rats. Third, when the second incubation was carried out at a low glucose concentration, the specific radioactivity of lactic acid decreased to

19.8±1.8% of that of exogenous D-glucose (Table I). As judged from group comparison within each experiment, the specific radioactivity of lactic acid released during the second incubation period only represented, in the presence of 2.8 mM D-glucose, 28.2±3.1% (d.f. = 22) of the corresponding value recorded at the same time in the presence of 16.7 mM D-glucose.

If it is assumed that, in the presence of the high concentration of D-glucose, virtually all lactic acid formed during the second incubation is derived from the exogenous hexose (see above), the data just mentioned would imply that, at the low hexose concentration, only 28.2±3.1% of the lactic acid formed during the second incubation originates from exogenous D-glucose, the complementary 71.8±3.1% being mainly attributable to glycogenolysis. Since the total production of lactic acid during the second incubation, when conducted in the presence of 2.8 mM D-glucose, averaged 51.8±4.9 pmol/islet per 10 min (n = 33), the glycogen-derived lactic acid would correspond to about 37.2±3.9 pmol/islet. In glucose-stimulated islets, the production of lactic acid corresponds to ~ 45.8±4.1% of the total hexose utilization (12). Hence, the production of lactic acid originating from glycogen would correspond to a total glycogenolysis close to 40.6±5.6 pmol of glucose equivalent per islet. The latter value is not significantly different ($P > 0.7$) from the initial glycogen content of the islets removed from glucose-infused rats.

Insulin release by perifused islets. When the islets from glucose-infused rats were first perifused for 45 min in the presence of 16.7 mM D-glucose, the output of insulin occurred at a fairly stable rate close to 8±1 μU/islet per min, well in excess of the value found at the same time in islets from control rats; i.e., about 2 μU/islet per min (13). A decrease in D-glucose concentration to 2.8 mM provoked a paradoxical increase in insulin output, which lasted for 4.8±0.7 min (n = 5), and was followed by an exponential fall in secretory rate over the ensuing 15–20 min (Fig. 3). When comparable experiments were conducted in islets from control rats, the decrease in D-glucose concentration provoked an immediate fall in insulin release, without any positive "off response" (7).

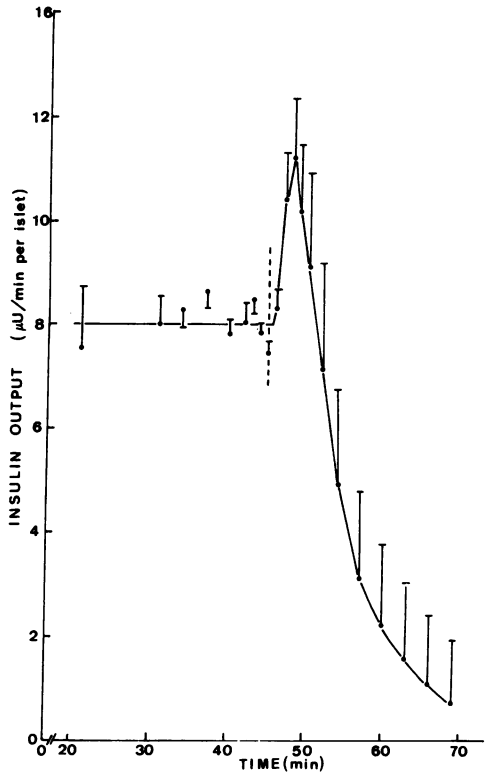

Figure 3. Insulin output in response to a fall in D-glucose concentration from 16.7 to 2.8 mM, at the time shown by the vertical dotted line, in islets from glucose-infused rats. In the left part, the horizontal line corresponds to the mean output recorded between the 21st and the 49th min of perifusion, inclusive. ±SEM refer to five individual experiments and are shown as paired differences from the mean output recorded between the 42nd and the 45th min inclusive in each individual experiment, such a reference value averaging 7.95±1.08 μU/islet per min.

Discussion

In a recent study conducted in islets first cultured for 2–5 d in the presence of a high concentration of D-glucose mixed with a tracer amount of D-[5-^{3}H]glucose, the rate of glycogenolysis was judged from the production of ^{3}HOH by the prelabeled islets (3). A comparable procedure could not be used in the present experiments. Indeed, to provoke a sizeable labeling of the glycogen accumulating in the islets, the amount of D-[5-^{3}H]glucose that should be incorporated in the hypertonic solution of D-glucose used for infusion of the rats over 2–3 d would largely exceed the budget of our laboratory. Hence, a nonradioactive approach, based on the measurement of total lactic acid output, was used here to monitor changes in glycolytic flux. By comparison of this measurement with the amount of ^{14}C-labeled lactic acid generated from exogenous D-[U-^{14}C]glucose, it became possible to estimate indirectly the rate of glycogenolysis. Incidentally, the new radioisotopic procedure here designed for the measurement of nanomolar amounts of unlabeled L-lactic acid can be recommended as an easy, sensitive, precise, and specific assay.

The present results indicate that, during incubation at a high concentration of D-glucose (16.7 mM), the utilization of the exogenous hexose is higher in islets from hyperglycemic than normoglycemic rats, in fair agreement with data obtained in islets first cultured in the presence of high concentrations of D-glucose (3). This priming effect coincided with a higher insulin output when the islets of hyperglycemic, as distinct from normoglycemic, rats were perifused at the same high concentration of D-glucose. The present results also confirm that a fall in extracellular D-glucose concentration provokes, in glycogen-rich islets, an increase in the rate of glycogenolysis (3). They reveal that this coincides with a paradoxical stimulation of insulin release, in a manner comparable to that previously documented in the isolated perfused pancreas (5, 14). The paradoxical stimulation of insulin release was of shorter duration, however, in perifused islets than in the perfused pancreas, this being conceivably attributable, in part at least, to a partial loss of glycogen during the procedure of islet isolation. Our results indeed suggest that the length of the paradoxical stimulation of insulin release is dictated by the time required to exhaust the glycogen stores. In this respect, the results here recorded in response to a fall in extracellular D-glucose concentration are reminiscent of those obtained when glycogenolysis is stimulated through an increase of the cyclic AMP concentration in the islets (15).

It could be objected that the transient stimulation of insulin release in response to a fall in D-glucose concentration did not

coincide with an increase in lactic acid production. Three factors may account for such an apparent discrepancy. First, the stimulation of insulin release lasted for no more than about 5 min, whereas the output of lactic acid was measured over 10 min incubation. It is quite conceivable, therefore, that over this period, the rate of glycolysis, at the low concentration of D-glucose (2.8 mM), was transiently higher and, thereafter, lower than that recorded during the first incubation conducted in the presence of 16.7 mM D-glucose. Under the present experimental conditions, the rate of glycogenolysis is indeed proportional to the glycogen content of the islets (3) and, hence, progressively decreases, in parallel to the progressive fall in insulin output (15), when the glycogen-rich islets are exposed to a low concentration of D-glucose. Second, since the cytosolic concentration of ATP is currently considered the essential factor coupling metabolic to cationic events in the process of nutrient-stimulated insulin release (16), it should be realized that the net production of ATP associated with the conversion of glycogen to L-lactic acid is 50% higher than that associated with the conversion of exogenous D-glucose to L-lactic acid. Third, as far as the mitochondrial generation of ATP is concerned, it is likely that it was somewhat higher during exposure of the islets for 10 min to 2.8 mM D-glucose than over the preceding 10 min of incubation in the presence of 16.7 mM D-glucose. Indeed, at the low hexose concentration, the transient stimulation of insulin release was presumably associated with a transient increase in cytosolic Ca^{2+} concentration, which in turn may be expected to cause a preferential stimulation of mitochondrial oxidative events relative to the overall glycolytic flux (17).

In conclusion, therefore, the present work provides further support to the view that an interference of glycogenolysis with glycolysis may account, in part at least, for the paradoxical changes in insulin output often observed in patients with non-insulin-dependent diabetes (18–20) and in experimental models for the latter disease (5, 14). This view does not rule out the participation of other factors; e.g., of neural, hormonal or humoral nature, as modulators of the coupling between the paradoxical metabolic and secretory responses, as proposed elsewhere (21). As a matter of fact, a paradoxical secretory response to D-glucose was recently observed in islets from normoglycemic rats exposed to dopamine (22).

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