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The biguanide compound metformin prevents desensitization of human pancreatic islets induced by high glucose

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Abstract

Pancreatic islet desensitization by high glucose concentrations is a temporary and reversible state of beta-cell refractoriness to glucose (and possibly other secretagogues), due to repeated or prolonged pre-exposure to increased glucose concentrations. We evaluated whether the oral antidiabetic agent metformin affects this phenomenon in isolated, human pancreatic islets, and whether the possible effects of the biguanide are influenced by the presence of a sulphonylurea, glyburide. Islets prepared from five human pancreases were incubated for 24 h in M199 culture medium containing either 5.5 or 22.2 mmol/l glucose, with or without a therapeutic concentration (2.4 μg/ml) of metformin. Then, the islets were challenged with either 3.3 mmol/l glucose, 16.7 mmol/l glucose, or 3.3 mmol/l glucose + 10 mmol/l arginine, and insulin release was measured. After incubation in the absence of metformin, the human islets exposed to 22.2 mmol/l glucose showed no significant increase in insulin release when challenged with 16.7 mmol/l glucose (confirming that hyperglycemia desensitizes pancreatic beta-cells). In the presence of metformin, the islets fully maintained the ability to significantly increase their insulin release in response to glucose, even when previously exposed to 22.2 mmol/l glucose. No major effect on arginine-induced insulin release was observed, whatever the culture conditions. The protective action of metformin was observed also when glyburide was present in the incubation medium, whereas the sulphonylurea alone did not affect insulin release from the islets previously exposed to high glucose concentrations. These in vitro results suggest that metformin can prevent the desensitization of human pancreatic islets induced by prolonged exposure to increased glucose concentrations.

Keywords: Metformin; Glucose desensitization; Pancreatic islet; Glyburide

1. Introduction

The harmful consequences of high glucose concentrations on pancreatic beta-cells have been recently differentiated into glucose toxicity and glucose desensitization (Robertson et al., 1994). Glucose toxicity has been defined as a condition of irreversible damage of the beta-cells, that occurs after chronic exposure to high glucose concentrations, and is possibly due to the deleterious effects of the hexose on insulin gene transcription and/or expression (Robertson et al., 1994). The concept of glucose desensitization implies a temporary, readily induced and reversible condition of refractoriness of the beta-cell to glucose that follows repeated or prolonged exposure to hyperglycemia. Glucose desensitization has been observed in several experimental models (Weir et al., 1986; Purrello et al., 1989; Rossetti et al., 1990; Davalli et al., 1991; Eizirik et al., 1992), and seems to be due to defects at the level of insulin exocytosis mechanisms or insulin granule stores (Robertson et al., 1994). Much less information is available about the effects of high glucose concentrations on islet sensitivity to secretagogues other than glucose. In a previous paper it was suggested that insulin release in response to arginine is not changed after preculture with increased glucose levels (Davalli et al., 1991).

Glucose toxicity and glucose desensitization may adversely affect residual insulin secretion in diabetic patients as well, triggering a vicious cycle in which hyperglycemia

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is a consequence and also a cause of the altered pancreatic beta-cell function (Unger and Grundy, 1985). Indeed, a reduction of blood glucose levels by any means improves beta-cell sensitivity to glucose (Shah et al., 1989). Unfortunately, even when adequate therapy is provided, most diabetic patients experience periods of mild to severe hyperglycemia, and this could further damage the residual islet insulin secretion.

Metformin (dimethylbiguanide) is an oral antidiabetic drug that can be used, alone or in combination, to lower blood glucose in patients with non-insulin dependent diabetes (Marchetti and Navalesi, 1989; Bailey, 1996; Scheen and Lefebvre, 1998). The therapeutic action of the drug is attributed mainly to its effects at the hepatocyte level, with decreased glucose production, and the muscle cell level, with enhanced glucose uptake. Metformin is also able to affect the function of many other cell types, including pancreatic beta-cells (Gregorio et al., 1989; Marchetti et al., 1996a). Indeed, although this issue is still a matter of debate (Bailey, 1992; Hermann et al., 1994), under certain conditions the drug can influence insulin release from the perfused rat pancreas (Gregorio et al., 1989) and isolated human islets (Marchetti et al., 1996a). In view of these latter findings, we considered it of interest to evaluate whether metformin can affect the phenomenon of islet desensitization by high glucose concentrations. To address this issue, we prepared purified human islets and incubated them for 24 h with various glucose concentrations, with or without a therapeutic concentration of metformin. At the end of the incubation period, islet insulin release in response to glucose and arginine was measured. In addition, since in the treatment of non-insulin dependent diabetes metformin can be used in combination with a sulphonylurea, we also evaluated whether the possible effect of the biguanide could be affected by the presence of glyburide in the incubation medium.

2. Materials and methods

2.1. Preparation of the islets

The procedures for the preparation of the islets were initially developed for the pancreas of large mammals (Marchetti et al., 1995, 1996b), and then applied, with minor modifications, to the human pancreatic gland. In the present study we used the islets from the pancreases of five human cadaver donors (three males, two female, aged 18 to 48 years), obtained through the local organ procurement organization, AIRT, with permission of our local Ethics Committee.

The enzyme collagenase (Type XI, Sigma, St. Louis, MO) was used for the digestion of the pancreas. The pancreatic duct was cannulated and the digestion solution (2.0–3.0 mg collagenase/ml, dissolved in 300 ml of Hanks' balanced salt solution, containing 2% serum) was injected (three-fold in volume the weight of the pancreas) to distend the tissue. After distension, the gland was placed into a 500-ml glass beaker, and the solution not used for distension was added to the beaker. This was placed into a shaking water bath at 37°C (120 rev/min). After 10 to 20 min the tissue was shaken with forceps, and the digestate was filtered through 300- and 90-μm mesh stainless steel filters, in sequence. The solution that passed through the filters and the tissue entrapped on the 300-μm mesh filter were placed back in the water bath, for further digestion. The tissue that remained on the 90-μm mesh filter was washed with 250 ml of 2% serum Hanks' solution at 4°C and was allowed to settle for 20 min. The same procedures of filtration and washing were repeated 20–25 and 30–40 min after the start of the incubation.

For purification, the digested tissue was pelletted at $400 \times g$ for 2 min at 4°C. Then, 1-ml aliquots were loaded into 50-ml plastic conical tubes and resuspended in 13 ml of 80% Histopaque 1.077 (Sigma) in 2% serum Hanks' solution. This layer was topped with 10 ml of 2% serum HBSS. After centrifugation at $800 \times g$ for 5 min at 4°C, the islets were recovered at the interface between the Histopaque and the Hanks' solution layers. The recovered islets (purity > 80%, the impurities being mostly small clusters of acinar tissue, lymph node cell aggregates and duct cells) were washed at $800 \times g$ for 2 min at 4°C in 10% serum Hanks' solution, and resuspended in M199 tissue culture medium (Sigma), supplemented with 10% serum and antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/ml; gentamicin, 50 μg/ml, and amphotericin B, 0.25 μg/ml). Approximately 2500 islets in 15 ml culture medium were loaded per 25-cm^2 uncoated plastic flask (BioBraun, Milan, Italy) and cultured at 37°C in a CO_2 incubator.

2.2. Insulin secretion studies

Within 7 to 12 days of isolation, the islets, which had been kept at 37°C in M199 culture medium containing 5.5 mmol/l glucose, were aliquoted into untreated petri dishes containing the supplemented M199 medium and either 5.5 or 22.2 mmol/l glucose, with or without 2.4 μg/ml metformin and/or 5 μmol/l glyburide (gifts from Laboratori Guidotti, Pisa). After a 24 h incubation at 37°C, the insulin secretory capacity of the islets in response to glucose and arginine was assessed by batch incubation methods as previously described (Marchetti et al., 1995, 1996b). Groups of islets of comparable size (approximately 100 μm in diameter) were preincubated at 37°C for 45 min in Krebs–Ringer-bicarbonate solution, supplemented with 3.3 mmol/l glucose and 0.5% bovine serum albumin. The islets were then washed and incubated at 37°C for 45 min in the Krebs–Ringer-bicarbonate solution containing either 3.3 mmol/l glucose, 3.3 mmol/glucose + 10 mmol/l arginine, or 16.7 mmol/l glucose to evaluate their sensitivity to glucose and amino acid stimulation.

Table 1
Insulin release (pmol/islet/45 min) in response to glucose from human islets previously cultured for 24 h with 5.5 or 22.2 mmol/l glucose without or with metformin

Culture conditions	Insulin release at	
	3.3 mmol/l glucose	16.7 mmol/l glucose
5.5G, M−	22.4±2.8	67.9±30.8[b]
22.2G, M−	44.8±8.4[d]	46.2±11.2
5.5G, M+	25.9±1.4	60.2±21[a]
22.2G, M+	34.6±8.4[e]	81.9±18.2 [c,f]

G: glucose.
M−/+: preculture without/with metformin.
[a,b,c] $P < 0.05$, 0.02 or 0.01 vs. release at 3.3 mmol/l glucose.
[d] $P < 0.01$ vs. 5.5G, M−.
[e] $P < 0.05$ vs. 5.5G, M+.
[f] $P < 0.05$ vs. release at 16.7 mmol/l glucose from 22.2G, M−.

Insulin release was measured by immunoassay, using commercially available kits (Medgenix, Brussels, Belgium), and expressed as pmol/islet/45 min. Owing to the inherent variability of human islet preparations, at the end of the secretion experiment we also measured the islet insulin content of some islet batches by acid alcohol extraction, and expressed insulin secretion as a percentage of the hormone content.

2.3. Analysis of results

One to five replicates with islets from single pancreas were performed for any given experimental condition, and the mean value of these replicates was used for statistical analysis. Since we studied the islets from five different pancreases, 5 is the *n* value in our experiments.

Results are given as means ± S.D. Comparison of data was made by the two-tailed Student's *t*-test, or the analysis of variance (ANOVA), when assessing differences between more than two groups.

3. Results

Insulin secretion in response to 3.3 and 16.7 mmol/l glucose from the islets cultured for 24 h with the various glucose concentrations, with or without metformin, is detailed in Table 1 (pmol/islet/45 min) and Table 2 (percent of insulin content). The release of insulin was significantly higher with 16.7 mmol/l glucose than with 3.3 mmol/l glucose islets precultured with 5.5 mmol/l glucose, either with or without metformin, and from the islets precultured with 22.2 mmol/l glucose and metformin, whereas no change in insulin secretion in response to 16.7 vs. 3.3 mmol/l glucose was observed with the islets exposed for 24 h to 22.2 mmol/l glucose without metformin.

Tables 1 and 2 also show that the secretion of insulin in response to 3.3 mmol/l glucose was higher from the islets

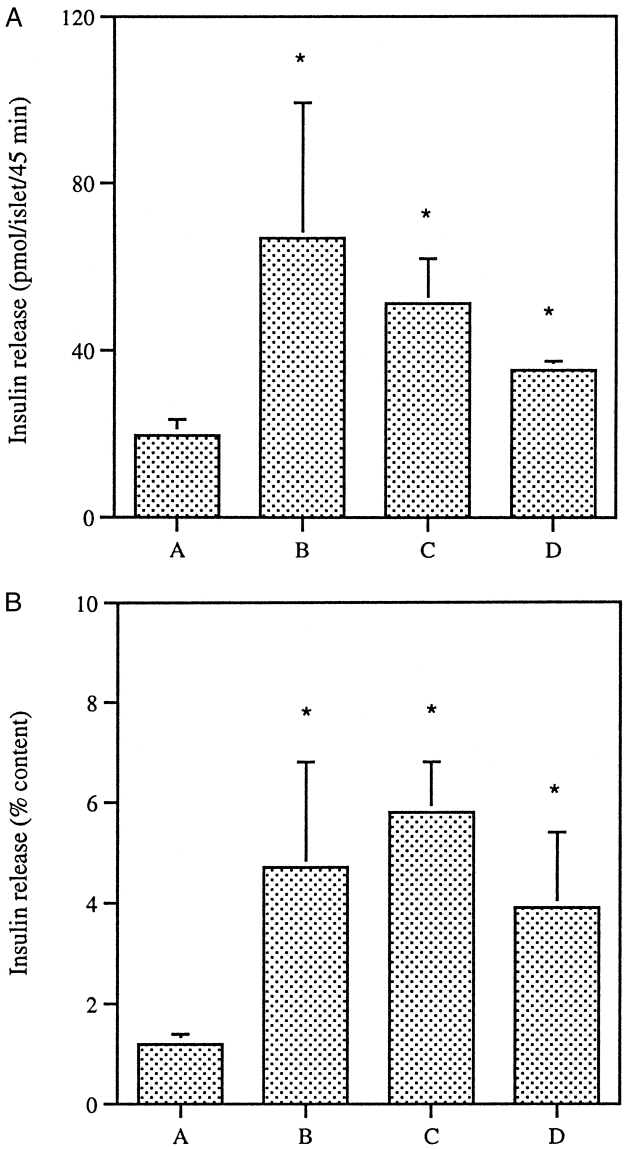

Fig. 1. Insulin release in response to 3.3 mmol/l glucose alone from human islets pre-exposed for 24 h to 5.5 mmol/l glucose (A), and in response to 3.3 mmol/l glucose + 10 mmol/l arginine from human islets pre-exposed for 24 h to 5.5 mmol/l glucose (B), 22.2 mmol/l glucose without metformin (C) or 22.2 mmol/l glucose with metformin (D). Insulin release is shown as pmol/islet/45 min in the upper panel, and as percent of islet insulin content in the lower panel. * $P < 0.05$ vs. A.

Table 2
Insulin release (percent of insulin content) in response to glucose from human islets previously cultured for 24 h with 5.5 or 22.2 mmol/l glucose with or without metformin

Culture conditions	Insulin release at	
	3.3 mmol/l glucose	16.7 mmol/l glucose
5.5G, M−	1.2±0.1	4.2±1.4[a]
22.2G, M−	5.8±1.2[b]	5.0±1.1
5.5G, M+	1.2±0.1	4.3±1.1[a]
22.2G, M+	4.7±1.1[c]	7.2±0.2[a,d]

G: glucose.
M−/+: preculture without/with metformin.
[a] $P < 0.01$ vs. 3.3 mmol/l glucose.
[b] $P < 0.02$ vs. 5.5G, M−.
[c] $P < 0.02$ vs. 5.5G, M+.
[d] $P < 0.01$ vs. release at 16.7 mmol/l glucose from 22.2G, M−.

cultured with the high glucose concentration, either with or without metformin, than it was from islets cultured with 5.5 mmol/l glucose. Insulin release in response to 16.7 mmol/l glucose was significantly higher from the islets precultured with 22.2 mmol/l glucose + metformin than it was the islets pre-exposed to 22.2 mmol/l glucose without the drug (Tables 1 and 2).

The effect of 3.3 mmol/l glucose + 10 mmol/l arginine on insulin release was evaluated with human islets precultured with 5.5 mmol/l glucose without metformin and with 22.2 mmol/l glucose with or without metformin ($n = 5$ each, Fig. 1). In this part of the experiment, insulin output in response to 3.3 mmol/l glucose alone from the cells cultured with 5.5 mmol/l glucose was 19.6 ± 3.8 pmol/islet/45 min (1.2 ± 0.2% of insulin content). With 3.3 mmol/l glucose + arginine the secretion of the hormone increased significantly and was 66.8 ± 32.2 pmol/islet/45 min (4.7 ± 2.1% of insulin content) from the islets precultured with 5.5 mmol/l glucose, 51.1 ± 10.8 pmol/islet/45 min (5.8 ± 1.0% of insulin content) from the islets previously exposed to 22.2 mmol/l glucose with metformin, and 35.3 ± 2.2 pmol/islet/45 min (3.9 ± 1.5% of insulin content) from those cultured with 22.2 mmol/l glucose without the biguanide. Although the release of insulin in response to 3.3 mmol/l glucose + arginine from the cells cultured with high glucose without metformin tended to be lower, no significant difference was found by ANOVA ($P = 0.1$) between the three groups.

The results obtained when we tested whether the effects of metformin are influenced by the presence of glyburide in the incubation medium for 24 h are detailed in Table 3. Compared to the secretion in response to 3.3 mmol/l glucose, insulin release did not increase significantly when the islets were pre-exposed for 24 h to glyburide, whereas the islets precultured in the presence of both glyburide and metformin were able to significantly enhance their insulin release in response to the glucose challenge.

The insulin content of islets after 24 h of culture decreased significantly ($P < 0.01$) upon exposure to high glucose levels, with no major difference between the batches with or without metformin. It was 10.1 ± 3.4 and 5.1 ± 2.6 ng/islet in the islets cultured with 5.5 or 22.2 mmol/l glucose without metformin, and 10.6 ± 3.8 and 5.8 ± 4.5 ng/islet in the islets cultured with the corresponding glucose concentrations and metformin.

Table 3
Insulin release (pmol/islet/45 min, pmol and percent insulin content, %) in response to glucose from human islets previously cultured for 24 h with 22.2 mmol/l glucose (22.2G) with metformin (M), glyburide (Gly), or metformin + glyburide (M + Gly)

Culture conditions	Insulin release at			
	3.3 mmol/l glucose		16.7 mmol/l glucose	
	(pmol)	(%)	(pmol)	(%)
22.2G + M	34.6 ± 8.4	5.0 ± 0.7	81.9 ± 18.2[a]	7.2 ± 0.2[a]
22.2G + Gly	32.0 ± 12.1	5.1 ± 1.4	33.6 ± 10.8	5.2 ± 1.7
22.2G + M + Gly	24.4 ± 6.5	4.7 ± 0.7	49.9 ± 11.6[a]	8.1 ± 2.3[b]

[a] $P < 0.01$.
[b] $P < 0.02$ vs. 3.3 mmol/l glucose.

4. Discussion

This study confirms that human islets exposed to high glucose loose the ability to increase their insulin release at increased glucose concentrations, i.e., they become glucose desensitized (Robertson et al., 1994). In keeping with a previous report (Davalli et al., 1991), we also found that the human islets cultured with 22.2 mmol/l glucose did not change their sensitivity to arginine stimulation significantly, even though a trend to a decrease in insulin secretion in response to 3.3 mmol/l + 10 mmol/l arginine was observed for the islets cultured for 24 h with the high glucose concentration.

The present study shows that the presence of a therapeutic concentration of metformin in the high glucose culture medium can prevent islet desensitization, at least within the limit of 24 h. Indeed, the islets cultured with 22.2 mmol/l glucose + metformin were able to significantly increase their insulin release upon challenge with the tested secretagogues. Since we observed that the islet insulin content was significantly and similarly decreased in the islets cultured with higher glucose concentrations, either with or without metformin, our data suggest that a decrease in intracellular insulin stores may indeed be one of the mechanisms underlying glucose desensitization (Marynissen et al., 1990; Sako and Grill, 1990; Zawalich et al., 1990), and that, if so, the protective effect of metformin does not seem to act at this level. Interestingly, metformin did not affect insulin release at a low (3.3 mmol/l) glucose concentration, but enabled the islets to increase insulin secretion in response to 16.7 mmol/l glucose (see Tables 1 and 2). Therefore, the drug does not seem to act on the mechanisms (still to be clearly defined) leading to increased 'basal' insulin release from islets pre-exposed to high glucose (Purrello et al., 1996). Rather, metformin maintains islet sensitivity to acute stimulation with high glucose concentrations, and this suggests possible actions of the drug at the level of intracellular glucose metabolism. It has been previously shown that the biguanide increases the release of lactate from islets, which in turn can lead to augmented insulin secretion (Best et al., 1989). Other possible mechanisms could be involved. For example, it has been recently shown that hyperglycemia causes alterations in the pattern of K^+ and Ca^{2+} flux through the plasma membrane of pancreatic islet cells (Anello et al., 1996). In particular, high glucose concentrations cause a partial inhibition of ATP-dependent K^+ channels, thus reducing K^+ efflux. This, in turn, leads to

decreased Ca^{2+} uptake by the beta cells and eventually to diminished insulin secretion. Metformin could beneficially act on these latter mechanisms. The drug, like all guanidine derivatives, has lipophilic properties (Dunn and Peters, 1995; Scheen, 1996). This allows the compound to bind to hydrophobic structures, such as the phospholipids of biological membranes, and possibly affect cell membrane properties. Indeed, guanidine derivatives have been reported to increase K^+ fluxes and to displace bound Ca^{2+} (Davidoff, 1973).

An additional result of some importance is that the action of metformin on the islets pre-exposed to high glucose was maintained when glyburide was also present in the incubation medium for 24 h. However, glyburide alone did not affect the phenomenon of glucose-induced islet desensitization. Whereas a more detailed evaluation of the effect of pre-exposure to glyburide on insulin secretion was beyond the aim of our study, the present results may have some clinical relevance, since metformin and glyburide may be combined in the treatment of non-insulin dependent diabetes.

In conclusion, the results of the present study show that metformin may have a protective role on islet cells pre-exposed to high glucose concentrations. Since there is evidence that hyperglycemia can adversely affect residual insulin secretion in diabetic patients, which in turn worsens the progression of the disease, our in vitro findings suggest that metformin could be used to protect pancreatic beta-cell function in diabetic patients. Further studies are needed to elucidate the precise mechanisms of the effects of metformin at the pancreatic level and, in this respect, to test the utility of the drug in the clinical setting.

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