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Pancreatic islet responsiveness to D-glucose after repeated administration of repaglinide

Aouatif Laghmich, Laurence Ladrière, Francine Malaisse-Lagae, Willy J. Malaisse *

Laboratory of Experimental Medicine, Brussels Free University, 808 Route de Lennik, B-1070 Brussels, Belgium

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Abstract

The influence of three daily oral doses of repaglinide (1.0 μg/g body wt.) on plasma insulin and glucose concentrations, pancreatic islet insulin content and both protein biosynthesis and insulin release in isolated islets incubated for 90 min in the presence of either 2.8 or 16.7 mM D-glucose was examined in both control and hereditary diabetic Goto–Kakizaki (GK) rats. In the control rats, repaglinide lowered the plasma glucose concentration, whilst failing to affect significantly the plasma insulin concentration or insulin/glucose ratio, 24 h after the last administration of the antidiabetic agent. Despite a severe decrease of islet insulin content, the ratio between insulin release and content was not altered in islets obtained from repaglinide-treated control rats and incubated in the presence of 16.7 mM D-glucose. Also the biosynthesis of islet peptides was increased at both low and high hexose concentrations. In GK rats, repaglinide administration affected neither plasma glucose nor insulin concentration, restored a normal value for the otherwise abnormally high basal insulin output, increased the 16.7 mM/2.8 mM ratio for insulin release, and again augmented protein biosynthesis at both low and high hexose concentrations. In both control and GK rats, the stress induced by bleeding and decapitation augmented plasma glucose concentration. This effect was more pronounced in GK than in control rats and, in the diabetic animals, coincided with a severe lowering of the plasma insulin/glucose ratio, suggesting a higher adrenergic sensitivity of islet cells in the GK than in control rats. The increased secretory responsiveness to glucose and increased biosynthetic activity found in islets from GK rats after repaglinide administration, are considered favourable attributes of this meglitinide analogue in the perspective of its use as an insulinotropic agent in noninsulin-dependent diabetes.

Keywords: Repaglinide; Pancreatic islet; Insulin release; GK (Goto–Kakizaki) rat

1. Introduction

The meglitinide analogue, repaglinide, is currently under investigation as an insulinotropic agent for the treatment of noninsulin-dependent diabetes mellitus. Recent studies conducted in rats have documented the immediate secretory response of the pancreatic B-cell to this agent after intravenous injection (García-Martínez et al., 1997). The profile of the changes in plasma insulin and glucose concentration after a single oral administration (Ladrière et al., 1997a) and the influence of this antidiabetic drug on the increase in glycemia and insulinemia caused by refeeding (Ladrière et al., 1997b) have also been described.

We now explored, in control and hereditary diabetic Goto–Kakizaki (GK) rats, the effect of a three-day treatment with repaglinide administered orally once daily on glycemia and insulinemia, and the biosynthetic and secretory responsiveness to D-glucose of isolated pancreatic islets. Thus, the major aim of these investigations was to assess the possible effects of repeated repaglinide administration upon the functional behaviour of islet B-cells.

2. Materials and methods

2.1. Animals

Sixteen female Wistar rats (mean age: approximately 2.5 months; Proefdierencentrum, Heverlee, Belgium) and 16 female GK rats (4 to 8 months old; bred in our laboratory) had free access to water and food. In both normal and GK groups, 8 animals were sham-treated and 8 other rats repaglinide-treated.

* Corresponding author. Tel.: +32-2-5556238; fax: +32-2-5556239.

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After an initial blood sampling from the severed end of the tail for the determination of plasma glucose (Bergmeyer and Berndt, 1974) and insulin (Leclercq-Meyer et al., 1985) concentrations, conscious animals were given intragastrically, at about 0900 h, 2 ml of a 0.5% (w/v) solution of carboxymethylcellulose (Na salt; Merck, Darmstadt, Germany) containing, as required, repaglinide (Dr. Karl Thomae, Biberach, Germany). The dose of repaglinide amounted to 1.0 μg/g body wt. The solutions were administered, according to the same schedule, after 24 and 48 h. The animals were killed 5–10 min after a last tail blood sampling, at time 72 h. Blood was also collected upon decapitation of the rats.

Islets were obtained by collagenase digestion (Malaisse-Lagae and Malaisse, 1984) from the pancreas of four animals having received the same treatment.

2.2. Insulin secretion and content

Groups of eight islets each were incubated for 90 min in 1 ml of Krebs–bicarbonate buffer (Malaisse-Lagae and Malaisse, 1984) containing 5 mg/ml bovine serum albumin, and D-glucose (2.8 or 16.7 mM). After removal of the incubation medium, the islets from each vial were resuspended in 1.0 ml of phosphate buffer (0.1 M; pH 7.4) containing bovine serum albumin (10 mg/ml) and sonicated on ice 3×10 s at 10 μm (Soniprep 150, MSE, Leicester, UK). Insulin was measured by radioimmunoassay (Malaisse-Lagae and Malaisse, 1984).

2.3. Protein biosynthesis

From each islet preparation, 10 to 12 groups of 25 islets each were incubated for 90 min at glucose 2.8 or 16.7 mM in 50 μl of the same buffer as mentioned above, but also containing 3.4 μM of L-[4-^{3}H]phenylalanine (0.1 mCi/ml; 29 Ci/mmol; Amersham, Amersham, UK). At the end of the incubation, the islets were rinsed twice with a medium similar to that used for incubation, except for the absence of radioactive phenylalanine, and presence of 1.0 mM unlabelled L-phenylalanine. They were then resuspended in 0.8 ml of 2 M acetic acid and sonicated (10 s at 10 μm).

Two aliquots (25 μl each) of the homogenate were counted for the measurement of total radioactive content. Two other aliquots (50 μl each) were added with 450 μl of a glycine–NaOH buffer (0.2 M, pH 8.8) and 500 μl of a 20% (v/v) trichloroacetic acid solution. The samples were centrifuged for 5 min at $1000 \times g$, and radioactivity was counted in 500 μl of the supernatant (trichloroacetic-acid-soluble fraction). The results were expressed as femtomoles of phenylalanine incorporated per islet, with reference to the specific radioactivity of L-[4-^{3}H]phenylalanine in the incubation medium.

2.4. Presentation of results

All results are presented as means ± S.E.M. together with the number of individual observations (n). Insulin release was expressed either as absolute values (μU/islet per 90 min) or as a fraction of the paired insulin content. The 16.7 mM/2.8 mM D-glucose ratio for secretory or biosynthetic activity was calculated in each experiment, the S.E.M. with this ratio taking into account the dispersion of individual measurements at the two hexose concentrations and being calculated as indicated elsewhere (Sener et al., 1984). The results obtained in distinct experiments were then pooled. The statistical significance of differences between mean values was assessed by means of Student's t-test.

3. Results

3.1. Metabolic and hormonal data

In the control rats examined at the start of the experiments (day 0), the plasma glucose and insulin concentrations averaged 7.85 ± 0.21 mM and 31.9 ± 3.8 μU/ml, yielding a mean paired insulin/glucose ratio of 3.67 ± 0.41 U/mol ($n = 16$ in all cases). In the older (6.4 ± 0.3 vs. 2 months) and heavier (242 ± 3 vs. 196 ± 3 g) GK rats ($n = 16$), the plasma glucose, at the start of the experiments, averaged 10.27 ± 0.37 mM, a value higher ($P < 0.001$) than that found in the control rats. The plasma insulin concentration (36.4 ± 2.8 μU/ml) and paired insulin/glucose ratio (3.55 ± 0.37 U/mol) were not significantly different, however, in GK rats and control animals (Table 1).

Over 3 days of observations, the paired change in body weight was comparable in untreated ($+1.5 \pm 1.5$ g; $n = 8$) and repaglinide-treated ($+0.1 \pm 1.7$ g; $n = 8$) control rats. In the GK rats, however, repaglinide decreased body wt. by 8.4 ± 0.8 g ($n = 8$), as distinct ($P < 0.001$) from a decrease of only 2.5 ± 1.1 g ($n = 8$) in untreated GK rats.

In the untreated control or GK rats, the plasma glucose and insulin concentrations, as well as the paired insulin/glucose ratio, were not significantly different at the start of the study and 3 days later. In the control rats, the plasma glucose concentration was decreased by 1.63 ± 0.47 mM ($n = 8$; $P < 0.02$) after three daily administrations of repaglinide, but the plasma insulin concentration and insulin/glucose ratio were not significantly affected. In the GK rats, on the contrary, repaglinide treatment resulted in an increase of plasma glucose concentration ($+2.42 \pm 1.02$ mM; $n = 8$; $P < 0.05$) and a decrease of both plasma insulin concentration (-12.5 ± 5.5 μU/ml; $n = 8$; $P < 0.06$) and paired insulin/glucose ratio (-1.63 ± 0.68 U/mol; $n = 8$; $P < 0.05$).

Handling the rats at the time of the last bleeding (72nd h) from the severed end of the tail and killing by decapitation (5–10 min later) resulted, in all cases, in an increase of plasma glucose concentration. Whether in absolute or relative terms, this effect was more pronounced in GK rats

Table 1
Metabolic and hormonal data

	Rats			
	Control		GK	
Treatment:	Nil	Repaglinide	Nil	Repaglinide
Body wt. (g)	195 ± 5 (8)	197 ± 4 (8)	243 ± 3 (8)	241 ± 5 (8)
Plasma glucose (mM)				
day 0	7.82 ± 0.20 (8)	7.88 ± 0.39 (8)	9.87 ± 0.30 (8)	10.67 ± 0.67 (8)
day 3	7.86 ± 0.16 (8)	6.26 ± 0.22 (8)	10.25 ± 0.38 (8)	13.08 ± 0.77 (8)
at killing	9.18 ± 0.40 (8)	7.21 ± 0.37 (8)	17.57 ± 0.50 (8)	23.92 ± 1.13 (8)
Plasma insulin (μU/ml)				
day 0	26.2 ± 3.9 (8)	37.6 ± 6.2 (8)	38.3 ± 2.9 (8)	34.4 ± 5.0 (8)
day 3	28.4 ± 3.5 (8)	33.3 ± 7.7 (8)	44.4 ± 4.7 (8)	21.9 ± 1.8 (8)
at killing	39.0 ± 10.3 (8)	38.8 ± 4.3 (8)	36.9 ± 11.8 (8)	24.5 ± 4.2 (8)
Plasma insulin/glucose (U/mol)				
day 0	3.11 ± 0.45 (8)	4.33 ± 0.70 (8)	3.83 ± 0.30 (8)	3.29 ± 0.66 (8)
day 3	3.41 ± 0.44 (8)	4.57 ± 0.97 (8)	4.19 ± 0.55 (8)	1.66 ± 0.22 (8)
at killing	3.46 ± 0.70 (8)	5.16 ± 0.75 (8)	1.57 ± 0.44 (8)	0.93 ± 0.14 (8)

than in control animals. Thus, in control and GK rats, respectively, the paired increment in plasma glucose concentration averaged 1.14 ± 0.19 and 9.09 ± 0.68 mM (n = 16 in both cases; $P < 0.001$), this corresponding to relative increases of 15.5 ± 2.6 and 77.8 ± 5.2% ($P < 0.001$). In both the untreated and repaglinide-treated control rats, the increase in plasma glucose concentration was not associated with any significant change in plasma insulin concentration or insulin/glucose ratio. If anything, the trend was towards an increase in the latter two variables. In GK rats, the opposite trend was observed. The paired insulin/glucose ratio was decreased in these animals by 1.54 ± 0.51 U/mol ($n = 16$; $P < 0.01$), such a fall corresponding to a 53.9 ± 8.4% paired reduction ($P < 0.001$) in such an insulinogenic index, which was now lower ($P < 0.001$) in GK rats (1.21 ± 0.55 U/mol; $n = 16$) than control animals (4.22 ± 0.55 U/mol; $n = 16$).

3.2. Secretory data

In the subpopulation of relative large islets that was collected from the collagenase-digested pancreas from control and GK rats, the insulin content, measured after a 90-min incubation, was much lower ($P < 0.001$) in repaglinide-treated than untreated animals (Table 2). The

Table 2
Secretory data

	Rats			
	Control		GK	
Treatment:	Nil	Repaglinide	Nil	Repaglinide
Islet insulin content (μU/islet)				
final content	650 ± 49 (45)	109 ± 4 (48)	1379 ± 91 (36)	353 ± 51 (34)
Insulin output (μU/islet per 90 min)				
at 2.8 mM D-glucose	26.2 ± 3.9 (22)	13.1 ± 1.7 (24)	65.7 ± 12.4 (17)	19.1 ± 4.1 (17)
at 16.7 mM D-glucose	303.4 ± 12.6 (23)	46.0 ± 7.7 (24)	185.4 ± 19.4 (18)	114.4 ± 14.3 (18)
16.7/2.8 mM ratio	11.61 ± 1.94 (43)	3.58 ± 0.84 (46)	3.39 ± 0.67 (33)	8.78 ± 1.80 (33)
Insulin output/content (% per 90 min)				
at 2.8 mM D-glucose	4.1 ± 0.6 (22)	12.6 ± 1.6 (24)	4.5 ± 0.8 (17)	6.1 ± 0.8 (17)
at 16.7 mM D-glucose	52.3 ± 2.9 (23)	43.1 ± 4.3 (24)	13.9 ± 1.4 (18)	47.7 ± 7.1 (18)
16.7/2.8 mM ratio	12.60 ± 1.83 (43)	3.47 ± 0.54 (46)	3.64 ± 0.70 (33)	8.05 ± 1.79 (33)
Insulin output + content (μU/islet)				
at 2.8 mM D-glucose	726 ± 44 (42)	119 ± 4 (46)	1494 ± 133 (33)	316 ± 38 (32)
at 16.7 mM D-glucose	908 ± 86 (44)	157 ± 10 (46)	1513 ± 139 (34)	514 ± 72 (33)

insulin content was higher ($P < 0.001$) in GK than in control rats, whether in the untreated or repaglinide-treated animals.

In the untreated control rats, a rise in D-glucose concentration from 2.8 to 16.7 mM increased the rate of insulin release from 26.2 ± 3.9 to 303.4 ± 12.6 μU/islet per 90 min. The absolute values for both basal and glucose-stimulated insulin output were much lower ($P < 0.005$ or less) in the repaglinide-treated control rats (Table 2). This was no longer the case, however, when the output of insulin was expressed relative to the final insulin content of the islets. The 16.7 mM/2.8 mM ratio for insulin release was lower ($P < 0.001$) in repaglinide-treated animals than in untreated control rats, as judged from either the absolute values for secretory rates or the ratio between hormonal output and content.

The secretory data collected with GK rats differed from those for control animals in several respects. First, in untreated animals, the absolute value for basal insulin release was higher ($P < 0.005$) and that for glucose-stimulated hormonal output lower ($P < 0.001$) in GK than control rats. The latter, but not the former, anomaly persisted ($P < 0.001$) when the output of insulin was expressed relative to the insulin content of the islets. The 16.7 mM/2.8 mM ratio was much lower ($P < 0.001$) in GK rats than in control animals (Table 2).

Second, although repaglinide administration decreased ($P < 0.01$ or less) the absolute values for both basal and glucose-stimulated insulin release in GK rats, as in control animals, it improved the secretory responsiveness to the hexose as judged from the 16.7 mM/2.8 mM ratio ($P < 0.05$ or less) in the islets of GK rats. Thus, whilst the treatment with repaglinide normalized basal insulin release in the GK rats, as judged from either the absolute values for secretory rate or output/content ratio, it significantly increased ($P < 0.001$) the latter ratio in islets exposed to 16.7 mM D-glucose.

In the untreated control or GK rats, the total amount of insulin released in the incubation medium and recovered in the islets was not significantly different at low and high D-glucose concentrations (Table 2, last two lines). In the animals treated with repaglinide, however, this total amount of insulin was higher ($P < 0.02$ or less) with 16.7 mM than 2.8 mM D-glucose. This suggests that, in the islets of treated control or GK rats exposed to the high concentration of the hexose, the sugar markedly stimulated proinsulin biosynthesis, whilst such a phenomenon could not be detected in the islets from untreated animals.

The latter findings led us to investigate the biosynthetic activity of islets over a 90-min incubation in the presence of L-[4-^{3}H]phenylalanine (3.4 μM).

3.3. Biosynthetic data

In islets from untreated control rats, a rise in D-glucose concentration from 2.8 to 16.7 mM caused a 3- to 4-fold increase in the incorporation of L-[4-^{3}H]phenylalanine into trichloroacetic acid-precipitable material (Table 3). In these control rats, the administration of repaglinide increased ($P < 0.01$ or less) both basal and glucose-stimulated biosynthetic activity. The 16.7 mM/2.8 mM ratio in the synthesis of tritiated peptides was lower ($P < 0.05$ for compared data recorded within each experiment), however, in the islets from repaglinide-treated rats that in those of untreated animals.

The absolute values for the labelling of islet peptides at both low and high D-glucose concentrations, as well as the 16.7 mM/2.8 mM ratio for biosynthetic activity, were not significantly different in untreated control and GK rats. In the GK rats, as in control rats, the administration of repaglinide increased ($P < 0.005$ or less) both basal and glucose-stimulated biosynthetic activity and decreased ($P < 0.001$) the 16.7 mM/2.8 mM ratio for biosynthetic activity.

Table 3
Biosynthetic data

	Rats			
	Control		GK	
Treatment:	Nil	Repaglinide	Nil	Repaglinide
Trichloroacetic acid-precipitable (fmol/islet per 90 min)				
at 2.8 mM D-glucose	187.1 ± 18.2 (12)	339.4 ± 37.7 (11)	237.1 ± 19.2 (12)	561.0 ± 43.6 (11)
at 16.7 mM D-glucose	642.3 ± 44.2 (11)	821.1 ± 43.2 (12)	627.4 ± 30.7 (12)	823.0 ± 47.7 (12)
16.7/2.8 mM ratio	3.45 ± 0.34 (21)	2.63 ± 0.26 (21)	2.66 ± 0.24 (22)	1.48 ± 0.12 (21)
Trichloroacetic acid-soluble (fmol/islet per 90 min)				
at 2.8 mM D-glucose	9.2 ± 1.2 (12)	12.7 ± 1.3 (11)	9.6 ± 1.7 (12)	18.9 ± 4.8 (11)
at 16.7 mM D-glucose	13.5 ± 2.8 (11)	19.8 ± 2.4 (12)	15.1 ± 2.2 (12)	17.5 ± 3.3 (12)
Trichloroacetic acid-precipitable/total (%)				
at 2.8 mM D-glucose	94.7 ± 0.5 (12)	95.7 ± 0.3 (11)	95.8 ± 0.4 (12)	96.1 ± 1.0 (11)
at 16.7 mM D-glucose	97.6 ± 0.5 (11)	97.5 ± 0.2 (12)	97.2 ± 0.4 (12)	97.7 ± 0.4 (12)

As judged from the trichloroacetic acid-soluble material, the islet content of L-[4-^{3}H]phenylalanine was not significantly different in control and GK rats and was not significantly affected by the administration of repaglinide. This was also the case for the paired ratio between trichloroacetic acid-precipitable material and the islet total radioactive content. This ratio was usually slightly higher, however, in islets incubated with 16.7 mM than with 2.8 mM D-glucose.

4. Discussion

The present results indicated that, in control rats, the daily oral administration of repaglinide (1.0 μg/g body wt.), while it lowered the plasma glucose concentration, failed to affect the plasma insulin concentration and insulinogenic index. This was consistent with the fact that the glucose-stimulated secretion of insulin in isolated islets from repaglinide-treated rats, when expressed relative to the decreased insulin content of such islets, was also not significantly different from that for untreated control rats. This is comparable with findings (Malaisse, 1991) for rat islets after two intraperitoneal injections of glipizide (2.0 μg/g body wt.). Thus, in our control rats, the apparent repaglinide-induced decrease in B-cell secretory responsiveness to D-glucose, as suggested by the low 16.7 mM/2.8 mM ratio in insulin output, is attributable to a high value for basal insulin release relative to insulin content. Since the absolute value for basal insulin release was nevertheless lower in repaglinide-treated rats than in untreated animals, the high basal output/content ratio may well reflect the fact that this basal insulin output does not correspond solely to an active regulated secretory process. Alternatively, repaglinide might indeed augment the basal secretory activity, at least when it is expressed relative to the insulin content of the B-cells.

A different situation prevailed in GK rats. The diabetic animals used in the present study were older and heavier than the control animals. Presumably, as a consequence of these differences in age and body weight, they had a higher insulin content in the subpopulation of relatively large islets that could be collected. This coincided with a close-to-normal plasma insulin concentration and insulinogenic index. Nevertheless, the untreated GK rats were characterized by several typical features documented in previous studies and including an increased plasma glucose concentration, an abnormally high absolute value for basal insulin release and a low 16.7 mM/2.8 mM ratio for glucose-stimulated insulin release (Giroix et al., 1995; Sener et al., 1996a,b; Leclercq-Meyer and Malaisse, 1997).

Moreover, in response to repaglinide administration, the GK rats, as distinct from the control animals, failed to display a lowering in plasma glucose concentration and, despite a marked fall in the islet insulin content, seemed to have an improved secretory responsiveness of the B-cells to D-glucose. Although the latter improvement coincided with a fall in the absolute value for basal insulin output, the postulated increase in the B-cell response to the hexose would be consistent with the finding that, in islets exposed to 16.7 mM D-glucose, the insulin output/content ratio was indeed higher in repaglinide-treated GK rats than in untreated diabetic animals. Such was not the case at a low concentration of D-glucose. It should be stressed that these differences between control and GK rats could, in part at least, be related to the higher age and body weight of the GK rats.

Another striking difference between control and GK rats consisted in the changes in plasma glucose and insulin concentrations resulting from the handling of the animals at the time of bleeding from the severed end of the tail and subsequent killing by decapitation. Thus, on one hand, the increase in plasma glucose concentration resulting from this stressful manipulation was much more pronounced in GK rats than in control animals, whether in absolute or relative terms. On the other hand, the stress-induced hyperglycemia coincided with an unaltered plasma insulin concentration and insulinogenic index in control rats, while there was a marked lowering of the plasma insulin/glucose ratio in GK rats. Together, these findings suggest that the diabetic animals are more sensitive than control rats to the adrenergic stress-induced inhibition of insulin release (Wright and Malaisse, 1968), which is reminiscent of findings with denervated islets (Pipeleers et al., 1978). As a result of this difference, the paired plasma insulin/glucose ratio became much lower in GK than control rats in the stressed animals.

In vitro, in islets of control rats, repaglinide did not affect adversely the nutrient-stimulated biosynthetic activity (Viñambres et al., 1996), in contrast to repeatedly documented findings with islets exposed to hypoglycemic sulfonylureas (Morris and Korner, 1970; Schatz et al., 1972; Levy and Malaisse, 1975). In the present study, the repeated administration of repaglinide resulted in an increased biosynthetic activity in islets from both control and GK rats at both a low or a high glucose concentration. A comparable finding was recently reported for islets isolated from rats repeatedly injected with glibenclamide (Tielemans and Pipeleers, 1993). These similar findings suggest strongly that the long-term administration of these antidiabetic agents may favour the de novo biosynthesis of islet peptides.

It could be argued that the repaglinide-induced increase in the synthesis of islet peptides coincided with a decreased biosynthetic responsiveness to glucose, as suggested by the lowering of the 16.7 mM/2.8 mM ratio for the tritiation of trichloroacetic acid-precipitable material in both control and GK rats. However, this lowering was attributable to a greater increase in basal than in glucose-stimulated biosynthetic activity in the repaglinide-treated rats. The experimental data again suggest, therefore, that

repaglinide administration led to an increased basal functional activity of the islet cells, in terms of both secretory and biosynthetic variables.

This interpretation is further supported by the two following observations. First, when expressed in absolute terms, the glucose-induced increase in the synthesis of tritiated peptides was not significantly different in repaglinide-treated and untreated animals, whether in control or GK rats. Second, in the repaglinide-treated animals, incubation of the islets with a high concentration of D-glucose (16.7 mM) significantly enhanced, in both control and GK rats, the total amount of insulin either released in the incubation medium or recovered in the islets after incubation. This increase failed to achieve statistical significance for islets from untreated control or GK rats, probably because of the much higher fractional contribution of the islet insulin content to the total amount of insulin both secreted by and recovered from the islets after incubation.

In conclusion, the present data indicated that repeated administrations of repaglinide, while it lowered the insulin content of the islets, did not affect adversely the biosynthetic and secretory activity of islet cells. On the contrary, in GK rats, the meglitinide analogue normalized the otherwise abnormally high basal insulin output, increased the 16.7 mM/2.8 mM ratio for insulin release, and augmented both basal and glucose-stimulated biosynthesis of islet peptides. These results, together with the information so far available on the immediate biosynthetic (Viñambres et al., 1996) and secretory (Bakkali Nadi et al., 1994; García-Martínez et al., 1997; Ladrière et al., 1997a) responses of islet cells to repaglinide, thus argue in favour of the use of this novel insulinotropic agent in the treatment of noninsulin-dependent diabetes.

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