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Effect of Repaglinide on Insulin Secretion in Islets from Rats Infused for Two Days with a Hypertonic Solution of D-Glucose

Karim Louchami, Laurence Ladrière, Hassan Jijakli, and Willy J. Malaisse

Laboratory of Experimental Medicine, Brussels Free University, Brussels, Belgium

This study investigates the insulin secretory responsiveness of pancreatic islets to repaglinide in an experimental model of B-cell glucose incompetence. Rats were infused for 2 d with a 1.67 *M* solution of D-glucose administered at a rate close to 2.8 mL/h. This resulted in a modest rise in glycemia, a severe increase in plasma insulin concentration, an increased sensitivity of B-cells to adrenergic stress, an abnormally high insulin output from isolated islets perifused in the presence of 16.7 m*M* D-glucose, and a paradoxical transient increase in insulin release from the islets in response to a fall in hexose concentration. The early increment in insulin output evoked by repaglinide, in the presence of 16.7 m*M* D-glucose, was not lower in the islets from glucose-infused rats than in those from control rats. Moreover, when the meglitinide analog was administered concomitantly with the removal of D-glucose from the perifusion medium, the early response to repaglinide was further increased. Even after 24 min of glucose deprivation, the output of insulin by the islets from glucose-infused rats was higher in the presence of repaglinide than in its absence. These findings indicate that, in this model of B-cell dysfunction, the secretory responsiveness to repaglinide, as distinct from that to glucose, is fully preserved. Therefore, when taken into consideration together with prior observations, these findings argue in support of the use of this insulinotropic agent in the treatment of noninsulin-dependent diabetes.

Key Words: Repaglinide; pancreatic islets; insulin release; glucose-infused rats.

Introduction

The meglitinide analog repaglinide is currently under investigation as a potential insulinotropic tool for the treatment of noninsulin-dependent diabetes mellitus. Prior studies have already documented the stimulation of insulin release by repaglinide in isolated pancreatic islets, with emphasis on the dose–action relationship, nutrient dependency, and dynamics of the B-cell secretory response *(1,2)*. The profile of changes in plasma glucose and insulin concentration was explored in rats after either iv *(3)* or oral *(4)* administration of repaglinide. Its influence on the increase in glycemia and insulinemia caused by refeeding *(5)* and islet biosynthetic activity *(6)*, as well as the long-term effects of repaglinide on pancreatic islet function *(7)*, were also investigated.

The present study concerns another issue related to the possible use of repaglinide as an antidiabetic agent. It explores the responsiveness of isolated pancreatic islets to the meglitinide analog in an experimental model of B-cell glucotoxicity or glucose incompetence. Thus, the effect of repaglinide on insulin secretion was explored in perifused islets prepared from rats that had been infused for 2 d with a hypertonic solution of D-glucose. This experimental design is currently used to simulate the unfavorable consequences of sustained hyperglycemia and/or excessive B-cell stimulation on the metabolic and secretory behavior of the endocrine pancreas *(8,9)*.

Results

Before surgery, the body weight of the rats averaged 187 ± 2 g (Table 1). Over the ensuing 2–3 d, no significant change in body weight was observed (+2.8 ± 1.6 g; $n = 12$). Likewise, over the 2-d perfusion period, the paired change in body weight (–2.2 ± 1.9 g; $n = 12$) remained negligible.

At day zero, the plasma glucose concentration averaged 7.69 ± 0.35 m*M*. After 48 h of glucose infusion, it was increased by 1.28 ± 0.54 m*M* ($n = 12$; $p < 0.05$). The plasma insulin concentration increased to a much larger extent, the paired ratio after/before glucose infusion amounting to 2.69 ± 0.98 ($n = 11$; $p < 0.02$). This coincided with a three- to fourfold increase in the plasma insulin/glucose ratio from 3.70 ± 0.27 to 13.08 ± 4.70 U/mol (Table 1).

The handling of the rats at the time of bleeding from the severed end of the tail and killing by decapitation, although failing to affect significantly the plasma glucose concentra-

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Author to whom all correspondence and reprint requests should be addressed: Dr. W. J. Malaisse, Laboratory of Experimental Medicine, Brussels Free University, 808 Route de Lennik, B-1070 Brussels, Belgium. E-mail: malaisse@med.ulb.ac.be

Table 1
Metabolic and Hormonal Data in Glucose-Infused Rats

Body weight (g)	Before surgery	187 ± 2 (12)
	At d 0	190 ± 2 (12)
	At d 2	188 ± 3 (12)
Plasma glucose (m*M*)	At d 0	7.69 ± 0.35 (12)
	At d 2	8.97 ± 0.48 (12)
	At sacrifice	9.34 ± 0.90 (12)
Plasma insulin (μU/mL)	At d 0	28.7 ± 2.7 (12)
	At d 2	128.1 ± 51.7 (11)
	At sacrifice	66.0 ± 18.9 (12)
Insulin/glucose ratio (U/mol)	At d 0	3.70 ± 0.27 (12)
	At d 2	13.08 ± 4.70 (11)
	At sacrifice	8.09 ± 2.90 (12)
Islet insulin content (mU/islet)	After sacrifice	1.55 ± 0.18 (12)

tion, decreased the plasma insulin concentration and paired insulin/glucose ratio, respectively, to 56.9 ± 11.1% ($n = 11$; $p < 0.02$) and 59.2 ± 12.1% ($n = 11$; $p < 0.05$). The insulin content of the islets averaged 1.55 ± 0.18 mU/islet ($n = 12$).

Between the 31st and 45th min of exposure to 16.7 m*M* D-glucose, the rate of insulin release from perifused islets averaged 6.55 ± 0.34 μU/islet/min ($n = 16$), as distinct ($p < 0.001$) from a control value of 1.57 ± 0.16 μU/islet/min ($n = 8$) in islets prepared from rats that had not been infused with a solution of D-glucose.

In the islets of glucose-infused rats exposed to 16.7 m*M* D-glucose, the insulin secretory rate does not display any systematic changes as long as the concentration of the hexose remains unchanged *(10)*. The addition of repaglinide (10 μ*M*), however, provoked a rapid, but poorly sustained enhancement of glucose-induced insulin release (Fig. 1 A). The initial response to the meglitinide analog was grossly similar in the islets from glucose-infused rats and in those from normoglycemic rats. As judged from the paired difference in insulin output at min 45 and over the ensuing 5 min, the repaglinide-induced increment in secretory rate averaged 1.02 ± 0.15 μU/islet/min ($n = 8$; $p < 0.001$) and 2.12 ± 0.67 μU/islet/min ($n = 4$; $p < 0.05$) in the control and glucose-infused rats, respectively. Thereafter, however, the release of insulin often declined more rapidly in the islets from glucose-infused animals than in those from control rats (Fig. 1A). Thus, analysis of the secretory rates recorded between min 50 and 69 indicated that the slope of the regression line, when expressed relative to the repaglinide-induced increment in insulin output, as calculated by extrapolation at min 45, averaged 7.4 ± 1.5 10^{-2} / min ($n = 4$) in the glucose-infused rats, as distinct ($p < 0.02$) from only 1.6 ± 0.6 10^{-2} /min ($n = 8$) in the normoglycemic animals. As a result, the integrated value for the repaglinide-induced increment in insulin secretion over 25 min of exposure to the meglitinide analog failed to achieve statistical significance in the hyperglycemic rats (0.68 ± 0.50 μU/islet/min; $n = 4$), as distinct from control animals (0.89 ± 0.15 μU/islet/min; $n = 8$).

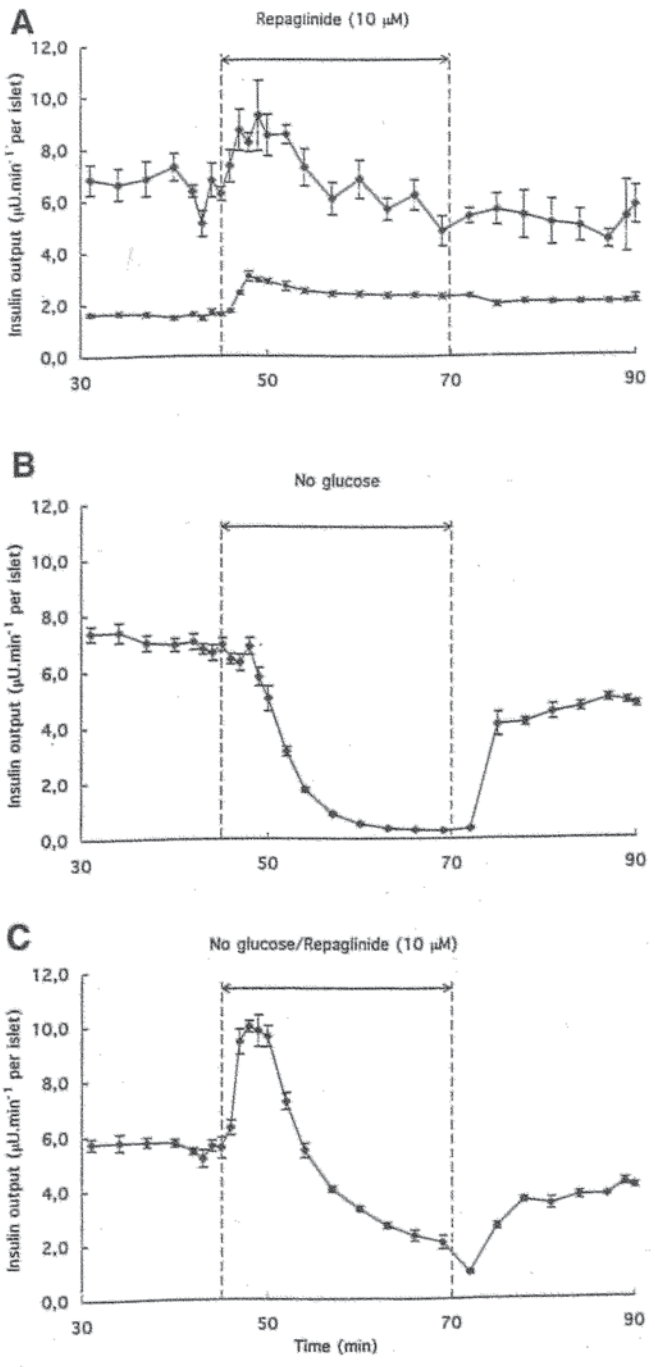

Fig. 1. Insulin release by islets from either control rats (lower tracing in **A**) or glucose-infused animals (other tracings) perifused in the presence of 16.7 m*M* D-glucose either throughout the experiment (A) or only from min 0 to 45 and 71 to 90 (**B**,**C**) and, when required, exposed to repaglinide (10 μ*M*) from min 46 to 70 (A,C). Mean values (±SEM) refer to four (upper tracing in top panel and lower panel) or eight (lower tracing in A and B) individual experiments. The vertical dotted lines indicate the time at which the composition of the perifusion medium was altered.

In order to explore further the responsiveness to repaglinide of the islets from glucose-infused rats, the

meglitinide analog was added from the 46th to 70th min, whereas D-glucose was concomitantly removed from the perifusate. For purpose of comparison, experiments were performed in which the concentration of D-glucose was also decreased form 16.7 to 0 m*M* between min 46 and 70, but in the absence of repaglinide (Fig. 1B). In the latter experiments, the fall in hexose concentration first provoked a modest and transient increase in insulin release. Thus, the peak secretory rate reached 75 ± 30 s after switching the perifusate was 0.97 ± 0.25 μU/islet/min higher ($n = 8$; $p < 0.01$) than the paired nadir value recorded at 40 ± 20 s before such a switch. The precise timing of such a short-lived increase in insulin output differed slightly from one experiment to another and, therefore, failed to be apparent when considering the overall mean values for hormonal release at each time point before and after the fall in D-glucose concentration (Fig. 1B). Thereafter, the secretory rate rapidly decreased and, at the 69th min, reached a mean value of 0.28 ± 0.04 μU/islet/min.

When repaglinide (10 μ*M*) was introduced at the same time as D-glucose was removed from the perifusate, an immediate and marked stimulation of insulin release was observed (Fig. 1C). As a matter of fact, the repaglinide-induced mean increment in insulin output, when computed over the first 5 min of exposure to the drug *(see above)*, was somewhat higher, although not significantly so, in the experiments also including a change in hexose concentration (3.44 ± 0.50 μU/islet/min; $n = 4$) than in the experiments conducted in islets from glucose-infused rats at 16.7 m*M* D-glucose throughout the perfusion period (2.12 ± 0.67 μU/islet/min; $n = 4$). In the absence of glucose, the early response to repaglinide was soon followed by a progressive decline in secretory rate. Nevertheless, even after 24 min of glucose deprivation, the mean value for insulin output remained much higher ($p < 0.001$) in the repaglinide-stimulated islets (2.07 ± 0.22 μU/islet/min; $n = 4$) than in the experiments conducted in the absence of the meglitinide analog (0.28 ± 0.04 μU/islet/min; $n = 8$). This difference is not owing solely to the higher secretory rate reached shortly after removal of D-glucose, in the experiments conducted in the presence, rather than absence of repaglinide. Indeed, the exponential line relating insulin release (R) to time (t) from min 50 to 69, according to the equation $R = R_o \cdot e^{-Kt}$, yielded a higher *K* value ($p < 0.001$) in the absence of the hypoglycemic agent (15.9 ± 0.8 10^{-2} min; $n = 8$) than in its presence (8.0 ± 0.6 10^{-2} min; $n = 4$).

Discussion

The present study confirms that the infusion of a hypertonic solution of D-glucose augments dramatically plasma insulin concentration even when the glycemia is only modestly increased *(8)*. Our results also reveal that the glucose-infused rats display a high sensitivity to stressful manipulations at the time of bleeding from the severed end of the tail and killing by decapitation, resulting in a marked decrease in plasma insulin concentration and paired insulin/glucose ratio, despite unchanged or barely increased glycemia. A comparable decrease in the insulinogenic index occurs, under the same experimental conditions, in hereditarily diabetic GK rats, but not in normal animals, suggesting an increased responsiveness of the B-cell to adrenergic agents in situations of sustained stimulation of insulin release by endogenous or exogenous D-glucose *(7)*.

The present observations also confirm that a transient and paradoxical stimulation of insulin release may occur in response to a decrease in D-glucose concentration in perifused islets from glucose-infused rats *(10)*. Such a phenomenon, which is currently ascribed to the accumulation of glycogen in the B-cell, was less pronounced in the present experiments than in a previous study otherwise performed according to a comparable design *(10)*. In this prior study, however, the glycemia was higher (15.9 ± 1.2 m*M*) and the duration of glucose infusion longer (3 d) than in the present work.

The major aim of this investigation was to explore the secretory response to repaglinide in the islets from glucose-infused rats. Despite the much higher rate of insulin release recorded in islets maintained at a high concentration of D-glucose (16.7 m*M*) throughout the isolation and perifusion procedures, repaglinide further augmented insulin output in the islets from glucose-infused rats. The drug-induced increment in hormonal release was, at least during the initial period of exposure to the meglitinide analog, as high as that normally recorded in islets from normoglycemic rats also perifused at a high concentration of D-glucose.

The preservation of a positive secretory response to repaglinide in the islets from glucose-infused rats was further documented in the experiments including a concomitant fall in the hexose concentration of the perifusate. Thus, under such conditions, the early repaglinide-induced increment in insulin output was as marked as that found in islets from glucose-infused rats perifused throughout in the presence of 16.7 m*M*, and actually three to four times higher ($p < 0.001$) than that recorded in islets from control rats also exposed throughout the 90 min of perifusion to the high concentration of the hexose. Moreover, even after 24 min of glucose deprivation, the output of insulin remained significantly higher in islets from glucose-infused rats when the experiments were conducted from the 46th to 70th min in the presence, rather than absence, of repaglinide. The latter finding sharply contrasts with the situation found in islets from normoglycemic rats, in which case repaglinide (10 μ*M*) fails to augment significantly basal insulin release *(1)*. Such contrasting results support the view that, in the glucose-infused rats, the stimulation of insulin secretion by repaglinide was probably favored by a mobilization of glucose phosphate esters from the endogenous stores of glycogen in the islets in response to the fall in extracellular D-glucose concentration *(10,11)*.

In conclusion, the present study documents that the secretory responsiveness to repaglinide is fully preserved in glucose-infused rats, considered as a model for the so-called phenomenon of B-cell glucotoxicity or glucose incompetence *(8,9)*. The maintenance of a positive insulinotropic action of repaglinide obviously contrasts with the paradoxical inhibition of insulin release caused by a rise in D-glucose concentration in either this and other experimental models of B-cell dysfunction *(8,12,13)* or noninsulin-dependent diabetic patients *(14,15)*. When considered together with recent studies on the immediate effects of repaglinide on insulin release in vivo *(3,4)* or in vitro *(1,2)* and on the long-terms effects of the meglitinide analog upon pancreatic islet function *(7)*, the present results thus reinforce the view that this novel insulinotropic agent may represent a useful tool for the treatment of type 2 diabetes mellitus.

Materials and Methods

Repaglinide (Karl Thomae, GmbH, Biberach, Germany) was provided by Novo Nordisk (Bagsvaerd, Denmark).

Twelve female Wistar rats (approx 2.5 mo old; Proefdierencentrum, Heverlee, Belgium) were divided into four groups of three animals each. During the whole period of experimentation, the animals had free access to water and food (AO3; Animalabo, Villemoissons-sur-Orge, France). In each group, the animals were anesthetized with ketamine (125 µg/g IP; Imalgen 500, Rhone-Mérieux, Lyon, France), and a catheter was introduced in the jugular vein. The animals were allowed to recover from surgery for 2–3 d. A sample of blood was then taken from the severed end of the tail (day 0) for the determination of plasma glucose *(16)* and insulin concentration *(17)*.

The infusion of glucose (1.67 *M*) was then started. The animals received 2.76 mL/h (or about 24 µmol/h/g body wt). At 48 h after the start of the infusion, a second blood sample was taken from the tail (day 2). The animals were then disconnected from the perfusion apparatus and sacrificed by decapitation. This procedure did not take more than 5 min. Blood was also collected at sacrifice.

Islets were isolated from the pooled pancreas of three rats by collagenase digestion *(18)*, the Hank's buffer being enriched with glucose 16.7 m*M*. From each preparation, three groups of 10 islets each were collected in 1 mL of phosphate buffer (0.1 *M*, pH 7.4) containing bovine serum albumin (0.5%, w/v) for determination of their insulin content *(18)*. Four groups of 100 islets each were placed in a perifusion chamber, and after an equilibration period of 30 min, the effluent medium was collected for measurement of insulin release *(2,19)*.

All results are presented as mean values (±SEM) together with the number of separate measurements *(n)*. The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

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