Rapid Publications

Pirogliride: An Oral Hypoglycemic Drug Which Accelerates Glucose Usage and Insulin Secretion by Islets of Langerhans

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SUMMARY

Pirogliride potentiates glucose-induced insulin secretion from isolated islets. This effect is accompanied by a facilitated glucose metabolism. Pirogliride partially prevents the known inhibitory effects of mannoheptulose on glucose-induced secretion and utilization. Pirogliride might be useful not only to probe the underlying mechanism of stimulated insulin secretion but also in the therapy of those diabetics who have an impaired β-cell responsiveness to glucose. DIABETES 29:410–412, May 1980.

Impaired secretion of insulin in response to glucose appears to be a major defect in diabetes.[1–4] It is, therefore, a reasonable goal to develop therapeutic agents that stimulate insulin secretion by themselves or normalize the β-cells' response to glucose and possibly other physiologic stimuli as well. Orally effective sulfonylurea derivatives, which elevate serum insulin levels, have been used extensively to treat diabetics, but their safety and long-term efficacy has been questioned.[5] Recently, pirogliride [N-(1-methyl-2-pyrrolidinylidene)-N'-phenyl-1-pyrrolidinecarboximidamide] has been shown to be orally effective in producing hypoglycemia in experimental animals.[6,7] Pharmacologic studies with this drug suggested an involvement of the pancreatic β-cells, since the hypoglycemic effect was abolished in totally pancreatectomized dogs. We therefore decided to investigate whether pirogliride directly stimulates the secretion of insulin from islet tissue in vitro. The possible effect of pirogliride on glucose usage by isolated islets was also determined since the rate of glucose utilization by the tissue appears to control the rate of insulin secretion.[8–13] The results demonstrate that pirogliride (1) potentiates glucose-induced insulin secretion from isolated rat islets; (2) facilitates glucose metabolism by this tissue; and (3) partly overrides the effects of mannoheptulose, a seven-carbon sugar which in islets blocks glucose phosphorylation, glucose utilization, and glucose-induced insulin secretion.[14–17]

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MATERIALS AND METHODS

Islets of Langerhans isolated from the pancreases of male rats fed ad lib were used. The methodology for the islet isolation with collagenase, perifusion, and metabolic studies has been previously published.[10] Briefly, groups of 50 islets were usually perifused for 30 min with 2.75 mM glucose to establish basal secretory rates and then stimulated with 5.5 mM glucose in the presence or absence of various concentrations of pirogliride, mannoheptulose, tolbutamide, or combinations of these compounds. In one group of experiments, the inslets were first perifused for 30 min without added substrate and then for an additional 30 min with 10 mM fructose, with or without pirogliride. Insulin secretion was measured by radioimmunoassay using rat insulin as standard. After the perifusion, the metabolic capacity of these islets was determined by measuring the rate of ^3H_2O formation from [5-^{3}H] glucose or [5-^{3}H] fructose (purchased from New England Nuclear, Boston, Massachusetts). The composition of the medium used for studying insulin secretion in the perifusion system, and for measuring hexose metabolism in the subsequent incubations, was the same except that the radioactive tracer was included during the incubation. While this type of sequential analysis has its shortcomings,[19] this methodology has yielded useful information on the capacity of islets to utilize various substrates and how this relates to insulin release. Most importantly, the physiologic integrity of the islets can be established (by determining the islets' secretory response) before subjecting them to the metabolic analysis.

RESULTS

When the glucose concentration in the perifusion medium was raised from 2.75 to 5.5 mM, the secretion of insulin was increased two- to fourfold (Figure 1A). The addition of 100 μM pirogliride together with 5.5 mM glucose greatly en-

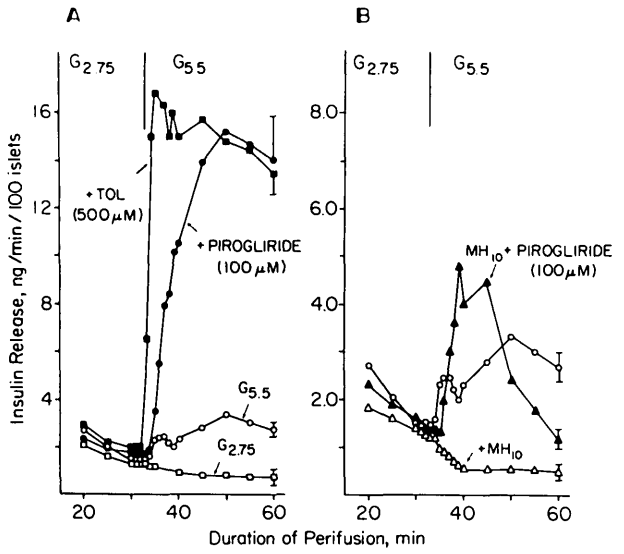

FIGURE 1. Effects of pirogliride, tolbutamide (TOL), and mannoheptulose (MH) on glucose-induced insulin release from isolated islets. (A) Groups of 50 islets were perifused for 30 min with 2.75 mM glucose. Islets were then exposed to 5.5 mM glucose alone (N = 8) or in combination with TOL (N = 3) or pirogliride (N = 7). (B) The islets were exposed to 10 mM MH with (N = 6) or without (N = 5) 100 μM pirogliride. The control response to 5.5 mM glucose is also shown for comparison. Note change in scale from (A) to (B).

hanced the secretion of insulin from islet tissue. Insulin secreted into the perifusion medium was significantly greater ($P < 0.05$, Student's t test) at all time points, except for the first minute of the response. With the drug present, insulin secretion was approximately fivefold greater than that observed with 5.5 mM glucose alone. In the absence of any added glucose, pirogliride at 50 or 100 μM failed to stimulate the secretion of insulin from islet tissue (N = 4 for each concentration, results not shown).

Experiments were conducted to compare pirogliride and the sulfonylurea tolbutamide. Although comprehensive dose-response studies were not performed, it was found that the insulin response to 5.5 mM glucose plus 500 μM tolbutamide resulted in a more pronounced first phase of insulin release when compared to that observed with 100 μM pirogliride (Figure 1A). The second phase release was similar with both drugs at the dosages used.

Mannoheptulose (MH) is a potent inhibitor of glucose-induced insulin secretion.[14–17] At 10 mM, MH completely blocked the insulin response to 5.5 mM glucose (Figure 1B). When 100 μM pirogliride was added together with MH, the β-cell response to glucose was partly preserved. Although insulin secretion diminished as the perifusion progressed, the total integrated insulin response to 5.5 mM glucose in the presence of 10 mM MH and 100 μM pirogliride (75 ± 10 ng/26 min/100 islets, N = 6) was comparable to that observed with 5.5 mM glucose alone (73 ± 12 ng/26 min/100 islets, N = 8).

Comparable experiments were performed with tolbutamide, glucose, and mannoheptulose. In the absence of any added glucose, 500 μM tolbutamide evoked a transient phase of insulin release (results not shown). The peak response occurred 3 min after stimulation and averaged 3.2 ± 0.3 ng/min/100 islets (N = 4) at this time point. Release quickly subsided to prestimulatory control values. In the presence of 5.5 mM glucose and 500 μM of the sulfonylurea, insulin release was greatly enhanced (see Figure 1A). The further addition of 10 mM MH to 5.5 mM glucose and 500 μM tolbutamide eliminated the potentiating effect of glucose and release was comparable to that obtained with tolbutamide alone. Peak release with this combination occurred 3 min after stimulation and averaged 3.0 ± 0.4 ng/min/100 islets (N = 4) at this time point.

Metabolic studies subsequently performed demonstrated that glucose utilization by islet tissue increased when the glucose concentration was elevated from 2.75 to 5.5 mM and that pirogliride significantly ($P < 0.05$) potentiated glucose usage at the latter concentration (Table 1, lines 3 and 4). No such acceleration of glucose metabolism was observed with 500 μM tolbutamide (Table 1, lines 5 and 6), although the insulin secretory response was comparable to that evoked by pirogliride. In addition to stimulating insulin secretion from mannoheptulose-blocked islets, pirogliride also significantly blunted the inhibitory effects of MH on glucose metabolism (Table 1, lines 7 and 8). In this group of experiments, the rates of glucose metabolism were measured in islets which were perifused with 2.75 mM glucose for 30 min and then removed for the metabolic analysis with the indicated additions. This was done because the insulin release due to this combination displayed kinetic characteristics that differed from those typical for glucose or glucose plus pirogliride. The insulin responses shown in Figure 1B were obtained in parallel experiments. In contrast to pirog-

TABLE 1
Effects of pirogliride, tolbutamide, and mannoheptulose (MH) on islet cell hexose metabolism

Substrate	N	Additions	Substrate usage, (pmol/islet) × h (mean ± SEM)
1. Glucose, 2.75 mM	8	—	32.0 ± 2.0
2. Glucose, 5.5 mM	8	—	52.2 ± 0.8
3. Glucose, 5.5 mM	3	Pirogliride, 50 μM	61.2 ± 0.9
4. Glucose, 5.5 mM	7	Pirogliride, 100 μM	66.6 ± 1.0
5. Glucose, 5.5 mM	3	Tolbutamide, 100 μM	50.3 ± 1.6
6. Glucose, 5.5 mM	3	Tolbutamide, 500 μM	50.0 ± 2.3
7. Glucose, 5.5 mM	5	MH, 10 mM	33.0 ± 2.0*
8. Glucose, 5.5 mM	6	MH, 10 mM + pirogliride, 100 μM	53.3 ± 2.0*
9. Glucose, 5.5 mM	4	MH, 10 mM + tolbutamide, 500 μM	31.5 ± 2.6*
10. Fructose, 10 mM	5	—	10.8 ± 0.3
11. Fructose, 10 mM	4	Pirogliride, 100 μM	10.4 ± 0.3

Batches of 50 islets were perifused for 30 min with 2.75 mM glucose and, in most cases, for an additional 30 min with the indicated substrate and additions (see Figure 1). In the fructose experiments, sugar was omitted from the initial 30 min period. After the perifusion, substrate usage was determined during an hour incubation with the stated additions.
* These groups of islets were perifused for 30 min with 2.75 mM glucose and then incubated with the stated additions to determine substrate usage.

liride, tolbutamide has no effect on the rate of glucose usage by mannoheptulose-treated islets (lines 7 and 9). Pirogliride did not alter the usage of 10 mM fructose by islet tissue (lines 10 and 11).

DISCUSSION

The data demonstrate that pirogliride is a potent potentiator of glucose-induced insulin secretion. Pirogliride is capable of attenuating mannoheptulose inhibition of glucose-induced insulin secretion. When compared with tolbutamide, a widely used hypoglycemic compound which is capable of weakly stimulating secretion in the absence of glucose,[20] pirogliride is effective only if glucose is present.

Perhaps the most intriguing aspect of the present report is the observation that pirogliride facilitated the utilization of glucose by islet tissue. While it might be argued that this facilitated metabolism results secondarily from an increased energy demand due to stimulated secretion, the results with tolbutamide hardly support this contention. In the presence of 5.5 mM glucose, insulin release was substantial with either 100 μM pirogliride or 500 μM tolbutamide. Only in the pirogliride-treated islets, however, is glucose usage increased. This, and the fact that pirogliride did not increase the usage of 10 mM fructose by islet tissue suggest, perhaps, that pirogliride is a specific accelerator of glucose metabolism.

While a considerable body of evidence has accumulated which supports the theory that glucose-induced insulin secretion is the result of glucose catabolism by the β-cell,[8–13] the precise nature of the intracellular trigger has, however, not been defined. The present report might be construed as evidence to support this theory. By accelerating the metabolism of glucose, pirogliride might amplify the metabolic signal normally produced by the hexose. There is evidence that pirogliride stimulates glucose oxidation of isolated muscle tissue in vitro.[21] The biochemical basis for this effect on glucose metabolism in both islet cells and peripheral tissue has yet to be elucidated.

Three pharmacologic actions of the drug fulfill the requirements of an ideal hypoglycemic agent: (1) effectiveness when administered orally, (2) potentiation of glucose-induced insulin release, and (3) direct activation of glucose metabolism. Future studies will have to be concerned with the biochemical mechanism of drug action, long-term efficacy, and with therapeutic safety.

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