Biochemical Pharmacology, Vol. 29, pp. 1421–1427.

0006-2952/80/0515-1421 $02.00/0

EFFECT OF THE ORAL HYPOGLYCEMIC AGENT, PIROGLIRIDE, ON GLUCONEOGENESIS

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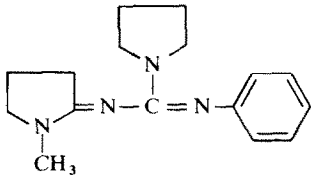
(*Received* 30 *March* 1979; *accepted* 19 *November* 1979)

Abstract—Pirogliride, a new orally active hypoglycemic agent, was shown to be an effective inhibitor of renal and hepatic gluconeogenesis in isolated rat kidney cortex slices, hepatocytes and perfused liver. The inhibition was concentration dependent (0.1–1.0 mM), with 1.0 mM pirogliride producing virtually total inhibition. The previously reported inhibitors of gluconeogenesis, cyclopropanecarboxylic acid and phenformin, were slightly less potent than pirogliride, while 3-mercaptopicolinic acid was at least ten times more potent. Unlike phenformin, pirogliride did not stimulate ketogenesis and did inhibit gluconeogenesis regardless of its apparent effects on the redox state. While the mechanism responsible for the inhibition of gluconeogenesis requires further study, several pieces of evidence point to a decrease of the ATP/ADP ratio, possibly secondary to an effect on the respiratory chain. Inhibition of gluconeogenesis, however, does not appear to be the primary mechanism responsible for the hypoglycemic action of pirogliride. While pretreatment of fasted rats with either 3-mercaptopicolinic acid or pirogliride produced hypoglycemia, only 3-mercaptopicolinic acid lowered the gluconeogenic capacity of kidney cortex slices incubated *in vitro* and inhibited the appearance of [^{14}C]glucose in the blood following intraperitoneal injection of [3-^{14}C]pyruvate.

Pirogliride [*N*-(1-methyl-2-pyrrolidinylidene)-*N'*-phenyl-1-pyrrolidine-carboximidamide] whose structure is shown below is a new oral hypoglycemic compound which differs structurally and mechanistically [1, 2] from those oral hypoglycemic agents currently being marketed, the sulfonylureas and the biguanides. The mechanism responsible for its blood glucose lowering action, however, is not understood completely.



The hypoglycemic activity of pirogliride in the fasting condition implies that it must either stimulate peripheral glucose utilization or inhibit hepatic or renal glucose output, directly or indirectly (i.e. through effects on metabolic hormone secretion). It has been shown that the suppression of glucagon [3] or pituitary and adrenal hormone secretion [1] is not responsible for the hypoglycemic effects of pirogliride; however, it may possess an insulin secretagogue action which might result in blood glucose lowering [3]. A non-pancreatic mechanism could also be attributed to pirogliride as a result of its ability to lower the blood glucose of rats made diabetic by the β-cell cytotoxin, streptozotocin. Pirogliride also produced a number of direct effects in isolated tissues, including the inhibition of lipolysis in rat epididymal fat pads [4] and the enhancement of glucose oxidation [5] in diaphragm muscle. Both of these actions could lead to stimulation of peripheral glucose utilization. As mentioned above, however, the hypoglycemic effect of pirogliride could also be the result of diminished hepatic or renal glucose production. Consequently, in the present report, we focused our attention on the effects of pirogliride on gluconeogenesis, which in the fasted state is the principal metabolic pathway involved in the maintenance of normoglycemia. *In vivo* experiments were conducted in addition to studies with the isolated perfused rat liver, hepatocytes, mitochondria and kidney cortex slices. For comparative pharmacological purposes, other hypoglycemic drugs which have been reported to inhibit gluconeogenesis were studied.

EXPERIMENTAL PROCEDURE

Materials

Chemicals and fat-free albumin were purchased from the Sigma Chemical Co., St. Louis, MO; cyclopropenecarboxylic acid from the Aldrich Chemical Co., Milwaukee, WI; and Dowex AG-501-X8 from Bio-rad Laboratories, Richmond, CA. Collagenase (150–200 units/mg) was obtained from the Worthington Biochemical Corp., Freehold, NJ, and all other enzymes from Boehringer–Mannheim Biochemicals, Indianapolis, IN. Radioactive glucose and pyruvate and the scintillation mixture, Aquasol, were purchased from New England Nuclear, Boston, MA. Phenformin and 3-mercaptopicolinic acid were gifts from Ciba-Geigy, Summit, NJ, and Smith Kline & French, Philadelphia, PA, respectively. Pirogliride (McN-3495) was synthesized at McNeil Laboratories, Fort Washington, PA, in the laboratory of Dr. C. R. Rasmussen [6].

Methods

Male albino Sprague–Dawley CD rats (200–280 g, Charles River Breeding Laboratories, Wilmington,

MA) were used throughout. For *in vitro* studies, rats were fasted for 48 hr. Methods for isolation and incubation of hepatocytes and for spectrophotometric determinations of acetoacetate, glucose, β-hydroxybutyrate, lactate and pyruvate have been reported previously [7]. Adenine nucleotides in neutralized perchloric acid extracts of hepatocytes were determined fluorometrically by the method of Williamson and Corkey [8]. The liver perfusion technique was a non-recirculating system similar to that described by Scholz *et al.* [9]. During the perfusion, the O_2 consumption of the liver was continuously monitored with a Clark-type electrode. The perfusion fluid was Krebs–Henseleit bicarbonate buffer [10], pH 7.4, saturated with an O_2/CO_2 mixture (95/5). All substrates and pirogliride were introduced into the perfusion fluid just prior to its entry into the liver.

Kidney slices. Kidneys were rinsed in cold saline, and slices (approximately 40 mg wet wt) were cut with a Stadie–Riggs slicer. For *in vitro* studies, two slices prepared from each kidney were randomly distributed to incubation vials and pre-incubated without substrate for 25 min at 37° in 4.0 ml of Krebs–Ringer bicarbonate buffer (KRB), pH 7.4, under $O_2 + CO_2$ (95 : 5). The slices were removed, gently blotted, and reincubated for an additional hour in 4.0 ml of KRB containing substrate and test compounds. When the animals were pretreated orally with drug or vehicle, duplicate kidney slices were incubated immediately without addition of drug. One slice from each animal was incubated in the absence of substrate to check for endogenous release of glucose. Following incubation, the slices were blotted and weighed, and samples (2 ml) of the incubation medium were analyzed for glucose by the glucose oxidase procedure (Glucostat, Worthington Biochemical Corp., Freehold, NJ).

Mitochondria. Isolation of rat liver mitochondria was accomplished by conventional differential centrifugation in a medium containing 70 mM sucrose and 220 mM mannitol. The final pellet was resuspended at a concentration of 20–30 mg/ml protein, as determined by the biuret method [11]. Rates of oxygen consumption were measured polarographically with a Clark oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, OH) at 25° in a buffer, pH 7.4, containing 120 mM KCl, 5 mM $MgCl_2$, 20 mM Tris–HCl, 5 mM KH_2PO_4, 1 mM L-carnitine, 0.5 mM EDTA and 0.5 mM maleate. The final concentrations of additions were as follows: 0.5 mM ADP, 0.75 mM Ca^{2+}, 5 mM succinate, 5 mM glutamate, 5 mM pyruvate and 1 mg/ml mitochondria.

Studies of gluconeogenesis in vivo. Rats received only water for 24 hr prior to experiments. Rates of gluconeogenesis were estimated by measuring the per cent conversion of [3-^{14}C]pyruvate into [^{14}C]glucose in the blood. [^{14}C]Pyruvate (0.5 M, 10 μCi/ml) was injected intraperitoneally in 0.5 ml of 0.2 mM HCl. Blood was drawn by cardiac puncture under light ether anesthesia 30 min later, and immediately deproteinized with $ZnSO_4$-$Ba(OH)_2$. The deproteinized extract was added to approximately 300 mg DOWEX-AG-501-X8 and mixed for 30 min. Radioactivity remaining above the settled DOWEX was assumed to be [^{14}C]glucose. Greater than 97 per cent [^{14}C]glucose and less than 5 per cent [^{14}C]pyruvate were recovered using this technique. Estimating the glucose space (ml) in the rat to be 30 per cent of the body wt in grams [12], the total glucose radioactivity was derived from the blood

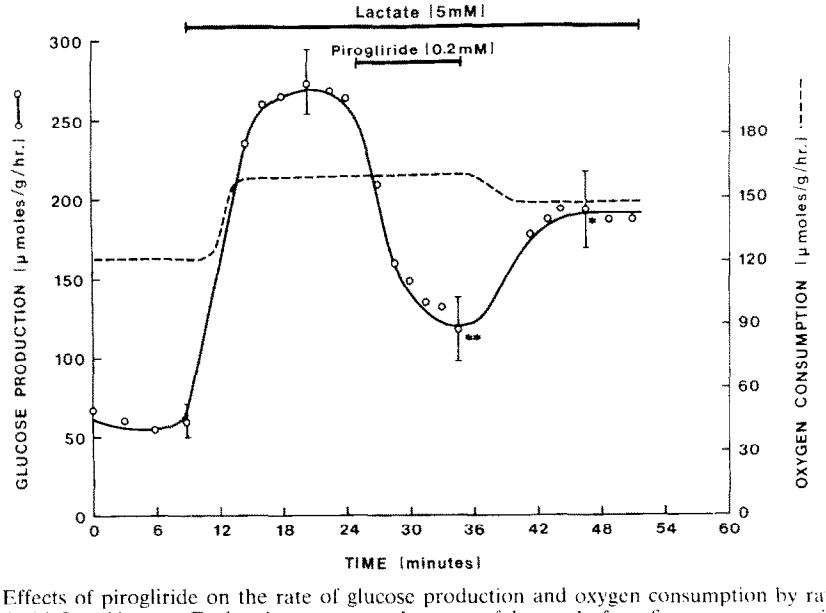


Fig. 1. Effects of pirogliride on the rate of glucose production and oxygen consumption by rat livers perfused with 5 mM lactate. Each point represents the mean of the results from five separate experiments. For clarity, only the standard errors for results 8–12 min into each new experimental condition are presented. The significance of results during and after perfusion with pirogliride, compared to the respective control, was determined by Student's *t*-test. Key: * $P < 0.05$, and ** $P < 0.01$.

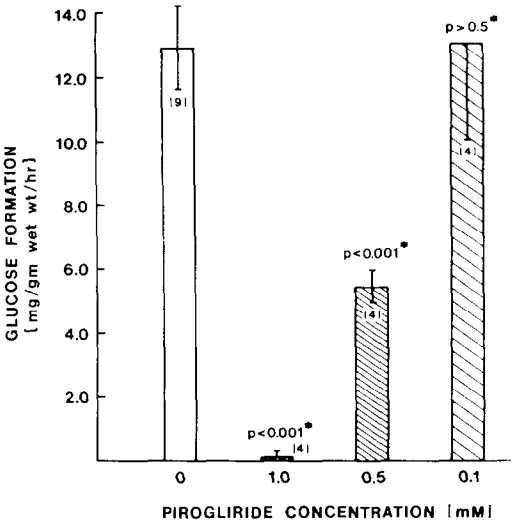


Fig. 2. Effects of pirogliride on gluconeogenesis of 48-hr fasted rat kidney cortex slices incubated *in vitro*. Significance, compared to controls, was determined by Student's *t*-test. Bars represent means ± S.E.M. Numbers in parentheses represent the number of replicate experiments.

level of [^{14}C]glucose. The total glucose radioactivity divided by the injected pyruvate radioactivity yielded the per cent conversion.

RESULTS

Gluconeogenesis in vitro

At 0.2–0.5 mM, pirogliride significantly ($P < 0.01$) inhibited glucose formation from lactate or fructose (5 mM), using isolated perfused livers from fasted rats. Oxygen consumption was increased slightly (4 per cent) but not significantly ($P > 0.05$). Results using 0.2 mM pirogliride and lactate are summarized in Fig. 1. The onset of the inhibitory effect on gluconeogenesis was immediate but was only partially reversible (37 per cent) when infusion of drug was stopped. Using fructose as substrate, 70 per cent of substrate-supported gluconeogenesis was recovered following drug removal. Acetoacetate and β-hydroxybutyrate release were also measured (not shown) but were not altered by pirogliride.

When pirogliride was added to kidney cortex slices *in vitro*, gluconeogenesis from either glutamate or alanine (10 mM) was inhibited. As shown in Fig. 2, with glutamate as substrate the inhibition was concentration dependent. For comparison, several reported hypoglycemic agents which inhibit gluconeogenesis [13–16] were also studied in kidney cortex slices. Figure 3 summarizes these data and illustrates the comparative potency of pirogliride. Cyclopropanecarboxylic acid and phenformin were slightly less potent than pirogliride, while 3-mercaptopicolinic acid was clearly at least ten times more potent.

Similar results were also obtained using hepatocytes from 48-hr fasted rats. Pirogliride inhibited gluconeogenesis in a concentration-dependent manner using concentrations in excess of 10^{-4} M. At 1.0 mM, pirogliride inhibited the glucose production 93–100 per cent using 10 mM lactate, pyruvate, alanine, fructose, dihydroxyacetone, glycerol or sorbitol as substrates. Glucose production in the absence of added substrates was also inhibited. This effect did not appear to be a general toxic effect of the drug since microscopic examination of cells treated with pirogliride, using exclusion of trypan blue, did not show differences from cells incubated in its absence. Again, 3-mercaptopicolinic acid was found to be more potent since it inhibited at concentrations as low as 0.005 mM.

While pirogliride inhibited gluconeogenesis from all precursors to an equal degree at 1.0 mM, at lower concentrations (0.1–0.2 mM) gluconeogenesis from lactate, pyruvate and alanine were inhibited to a greater degree than glucose formation from dihydroxyacetone, fructose, glycerol and xylitol. This finding is illustrated by the date in Table 1. Irrespective of the substrate used, however, ketogenesis and the β-hydroxybutyrate/acetoacetate (βOHB/AcA) ratio were not altered consistently, though at

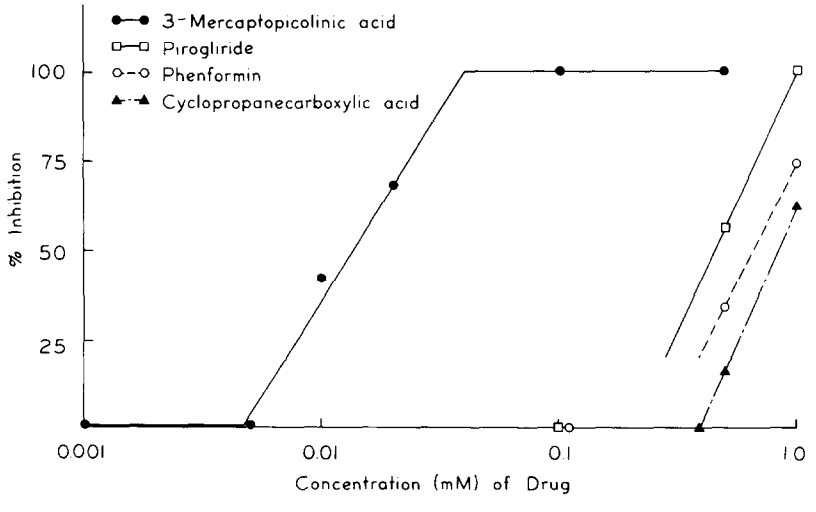


Fig. 3. Relative potencies of several hypoglycemic agents in inhibiting renal gluconeogenesis *in vitro*.

Table 1. Effects of pirogliride on the formation of glucose, lactate (Lac), pyruvate (Pyr), β-hydroxybutyrate (βOHB) and acetoacetate (AcA) in isolated rat hepatocytes incubated with various gluconeogenic substrates*

Substrate (1 mM)	Pirogliride (mM)	Glucose found (nmoles/g/min)	% Inhibition	Total Lac + Pyr (nmoles/g/min)	Lac/Pyr	Total βOHB + AcA (nmoles/g/min)	βOHB/AcA
D-Fructose	0	877 ± 30		174 ± 23	11.5	384 ± 15	0.32
	0.2	591 ± 40†	33	380 ± 30†	7.4	279 ± 5†	0.30
	0.5	161 ± 6†	82	385 ± 14†	9.0	350 ± 12	0.30
Pyruvate	0	597 ± 3		181 ± 13	16.0	345 ± 15	0.25
	0.2	222 ± 7†	63	427 ± 15†	10.0	322 ± 11	0.23
	0.5	87 ± 16†	86	281 ± 10†	22.2	439 ± 28	0.22
D-Alanine	0	478 ± 15		131 ± 10	6.5	412 ± 9	0.26
	0.2	96 ± 3†	80	219 ± 6†	3.6	337 ± 13†	0.24
	0.5	58 ± 1†	88	104 ± 4	17.5	473 ± 7†	0.24

* Pirogliride and substrates were added at the start of a 60-min incubation period. All results are the means ± S.E.M. of at least four experiments. Glucose production in the absence of any additions was 99 ± 3 nmoles/g/min.
† $P < 0.05$, significance compared to appropriate controls determined by Student's *t*-test.

0.2 mM total ketone production tended to be lower. On the other hand, the lactate/pyruvate (lac/pyr) ratio was lowered at 0.2 mM but was increased at 0.5 mM. Similar findings were also found using hepatocytes which were isolated from livers of streptozotocin diabetic rats. The above findings, however, contrast with our experience using phenformin. Phenformin, 1–2 mM, inhibited 50–70 per cent the rates of gluconeogenesis from fructose or pyruvate, but inhibited only weakly (20 per cent) at 0.5 mM. Ketogenesis was increased significantly and there was a concentration-dependent increase in the βOHB/AcA and lac/pyr redox couples. This stimulatory effect on ketogenesis and the increase of βOHB/AcA and lac/pyr have been reported previously with phenformin in perfused liver [17, 18].

The reasons for the greater inhibitory effects of 0.2 mM pirogliride on gluconeogenesis from substrates (pyruvate, alanine and lactate) that enter the pathway prior to pyruvate carboxylase are unclear. One possible explanation is that more energy in the form of ATP is needed for glucose formation from these substrates than from substrates which enter higher in the pathway. Thus, pirogliride might produce this differential sensitivity if it limited energy availability. Evidence for this hypothesis came from experiments measuring both glucose production and levels of adenine nucleotides in isolated rat hepatocytes. An example of the results using pyruvate as substrate is shown in Table 2. Pirogliride produced a concentration-dependent drop in ATP concentrations and the ATP/ADP ratio. At 0.5 mM there was also a significant increase in AMP concentration, while ADP concentrations were lowered from results obtained using 0.2 mM pirogliride. Similar effects on adenine nucleotides were also observed using fructose, alanine or no substrate.

Oxygen consumption by isolated rat liver mitochondria

Since the lowered hepatocyte ATP/ADP ratio produced by pirogliride might be due to an alteration of the respiratory chain, its effect on mitochondrial oxygen consumption was studied. A typical response of these polagraphic studies is shown in Table 3. The addition of 0.5–2.0 mM pirogliride to rat liver mitochondria increased the state 4 oxidation of succinate. The increase did not approach the state 3 rate of oxidation. Higher concentrations inhibited state 4 respiration. State 3 respiration was inhibited with concentrations of pirogliride in excess of 0.75 mM, with the mean K_I being approximately 1.3 mM. Sim-

Table 2. Effects of pirogliride on glucose production and levels of adenine nucleotides in isolated rat hepatocytes incubated with 1.0 mM pyruvate*

Pirogliride concn. (mM)	Glucose production (nmoles/g/min)	% Inhibition	AMP (nmoles/g)	ADP (nmoles/g)	ATP (nmoles/g)	ATP/ADP
0	330 ± 10		616†	2200 ± 80	5300 ± 360	2.41 ± 0.19
0.2	129 ± 3‡	61	750 ± 70§	3460 ± 70‡	3100 ± 300‖	0.89 ± 0.09‡
0.5	7.5 ± 4‡	77	1800 ± 120‖	2700 ± 360§	840 ± 310‡	0.31 ± 0.12‡

* Rats were fasted 48 hr before use. Results obtained after a 60-min incubation are expressed as means ± S.E.M. of three to four liver cell preparations.
† Single determination.
‡ $P < 0.001$, significance determined by Student's *t*-test.
§ $P > 0.05$, significance determined by Students *t*-test.
‖ $P < 0.01$, significance determined by Student's *t*-test.

Table 3. Effects of piroglitide on state 3 and state 4 respiration of isolated mitochondria from fed rats

Final concn. of piroglitide (mM)	O_2 consumption (μmoles/g/hr) Order of addition: Mitochondria	McN-3495-12	Succinate (5 mM)	ADP (50 μM)	% Inhibition*
0.0	28		55	325	
0.05	19	19	54	352	0
0.10	23	32	73	341	0
0.20	23	41	71	319	0
0.50	27	31	91	341	0
0.0	51	—	76	329	0
1.0	35	35	107	188	43
2.0	20	37	136	195	41
3.75	27	22	50	73	78
5.0	21	21	52	37	89
7.5	27	4	0	0	100
10.0	22	7	0	0	100

* Per cent inhibition of total state 3 respiration.

ilar changes in oxygen consumption under both state 3 and state 4 conditions were also seen using either pyruvate or glutamate as substrate. Furthermore, at concentrations where piroglitide inhibited state 3 respiration, it also inhibited the glutamate- or succinate-supported increase of oxygen consumption which occurred following addition of 0.75 mM $CaCl_2$ or the uncoupling agent, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone (FCCP).

Gluconeogenesis in vivo

Some attempts were also made to determine the significance of the inhibitory effect of piroglitide *in vitro* to its hypoglycemic effect *in vivo*. Kidney cortex slices, prepared from rats which had been treated with either piroglitide (100 mg/kg, p.o.) or 3-mercaptopicolinic acid (100–150 mg/kg, p.o.), were incubated in the absence of drug, using glutamate (10 mM) as substrate. The results were compared with results using animals which received the vehicle. Treatment of 48-hr fasted rats for 0.5, 1, 2 and 4 hr with piroglitide, while lowering blood glucose 25–50 per cent, failed to decrease the rate at which kidney cortex slices produced glucose when incubated *in vitro*. 3-Mercaptopicolinic acid, however, produced both hypoglycemia and inhibition of renal gluconeogenesis (approximately 50 per cent 2 hr after dosing). Similar findings were also made when conversion of injected [3-^{14}C]pyruvate to [^{14}C]glucose in the blood of fasted rats was used as the measure of *in vivo* gluconeogenesis. These results are shown in Table 4. Piroglitide failed to inhibit the conversion of [3-^{14}C]pyruvate to blood [^{14}C]glucose at a maximal hypoglycemic dose [1]; however, 3-mercaptopicolinic acid was effective.

Table 4. Effects of oral doses of piroglitide tartrate and 3-mercaptopicolinic acid on gluconeogenesis *in vivo**

Drug	Treatment: Min prior to [3-^{14}C]pyruvate injection	% Conversion $\bar{X} \pm$ S.E.M.	Mean % inhibition
Vehicle	30–120	23 ± 1	
Piroglitide	45	22 ± 2†	0
tartrate	90	21 ± 1†	0
(100 mg/kg, p.o.)	120	24 ± 2†	0
3-Mercaptopicolinic acid (150 mg/kg, p.o.)	30	4.3 ± 0.1‡	81

* Fasted rats (four per group) were injected with [3-^{14}C]pyruvate 30 min prior to being killed.

† $P > 0.05$, significance compared to vehicle-treated, determined by Student's *t*-test.

‡ $P < 0.001$, significance compared to vehicle-treated, determined by Student's *t*-test.

DISCUSSION

The new oral hypoglycemic agent, pirogliride, was found to produce a concentration-dependent inhibition of gluconeogenesis in rat kidney cortex slices, hepatocytes and perfused liver. This effect was not secondary to hypoxia because oxygen consumption in the perfused liver was not lowered, and inhibition of glycogenolysis seems an unlikely explanation because the glycogen content of kidney cortex slices is less than 0.01% [19]. Furthermore, the decrease of gluconeogenesis was not the result of inhibition of fatty acid oxidation since pirogliride did not inhibit ketogenesis in the perfused liver or hepatocytes. Similar to reports with several other hypoglycemic compounds (e.g. pent-4-enoic acid, hypoglycin, phenformin, 5-methoxyindole-2-carboxylic acid and diphenyleneiodonium), the inhibitory effect of pirogliride may be explained, at least partially, as being due to a depletion of ATP. As illustrated by the data in Table 2, pirogliride produced a marked concentration-dependent drop in hepatocyte ATP concentrations and the ATP/ADP ratio without altering the total pool of nucleotides. AMP concentrations were increased which also might contribute by inhibiting fructose diphosphatase. Since the theoretical ratio of high energy P_i required for glucose produced is greater (approximately 6 : 1) for gluconeogenesis from lactate, pyruvate and alanine than from fructose, dihydroxyacetone and xylitol (approximately 2 : 1), the observation that 0.2 mM pirogliride decreased the rate of gluconeogenesis from lactate, pyruvate and alanine to a greater degree than from the other substrates suggests that the inhibitory effect of pirogliride might be related to the energy requirements of gluconeogenesis. Thus, a causal correlation between a primary suppression of the ATP/ADP ratio and a secondary inhibition of gluconeogenesis by pirogliride may be assumed.

The mechanism responsible for the drop of ATP/ADP is unlikely to be a total block of glycolysis since the concentrations of lactate and pyruvate increased when gluconeogensis from fructose in hepatocytes was inhibited. Also, it is noteworthy that pirogliride inhibted gluconeogenesis regardless of its apparent effects on the redox state measured as the concentration ratios of lac/pyr and βOHB/AcA. These are considered [20, 21] to provide an indirect estimation of the free NADH/NAD^+ ratios of the cytoplasmic and mitochondrial compartments, respectively. At 0.2 mM, it decreased the lac/pyr and βOHB/AcA ratios slightly, while at 0.5 mM, the increase of the lac/pyr ratio was not accompanied by an increase of βOHB/AcA or ketogenesis. These results differ from those reported [17] for phenformin which inhibited mitochondrial respiration and caused the cytosolic and mitochondrial redox states in perfused livers to become more reduced. These effects were confirmed in our studies using isolated hepatocytes. Phenformin increased the lac/pyr and βOHB/AcA redox couples and increased ketogenesis while inhibiting gluconeogenesis.

The drop of ATP/ADP in hepatocytes, however, might be explained by the direct effect of pirogliride, as illustrated in Table 3, on mitochondrial respiration. At concentrations in excess of 0.75 mM, pirogliride inhibited state 3 or Ca^{2+}-stimulated respiration using glutamate and pyruvate, NAD^+-dependent substrates which donate electrons to NADH dehydrogenase, and succinate which reduces ubiquinone. Thus, a specific action on site I or site II phosphorylation can be excluded. Since high concentrations of pirogliride produced a comparable inhibition under state 4 and state 3 conditions, an effect on the respiratory chain rather than on the energy coupling sequence might be postulated. Also, on the basis of this hypothesis, it could be expected to decrease the rapid rate of oxygen uptake in the presence of an uncoupling agent and, indeed, this was found to be the case using FCCP.

It is unclear, however, why higher concentrations of drug were necessary to see an inhibition of respiration in mitochondria than the concentrations which lowered the ATP/ADP ratio in hepatocytes. Possibly a metabolite of pirogliride is produced by the hepatocytes, which is an even more potent inhibitor of respiration, or perhaps the slight stimulatory effect of the drug on state 4 respiration at lower concentrations is important. These and other possibilities require further study.

A variety of compounds possessing different mechanisms of action have been shown to inhibit gluconeogenesis [22]. However, a positive correlation between the inhibition of gluconeogenesis *in vitro* and the manifestation of this effect *in vivo*, as measured by hypoglycemia or isotopic techniques, has not always been readily demonstrable. For instance, the phenformin-induced inhibition of gluconeogenesis in the perfused liver [14, 15] is at variance with measurements of gluconeogenesis in normal and diabetic humans treated with phenformin [23, 24]. Therefore, it was important to evaluate whether pirogliride can inhibit gluconeogenesis *in vivo* and produce hypoglycemia in animal models where other inhibitors of gluconeogenesis are active. 3-Mercaptopicolinic acid, whose hypoglycemic activity has been attributed solely to inhibition of gluconeogenesis [11], was selected for comparison. While pretreatment of fasted rats with both 3-mercaptopicolinic acid and pirogliride produced hypoglycemia, only 3-mercaptopicolinic acid lowered the gluconeogenic capacity of kidney cortex slices incubated *in vitro* and inhibited the appearance of [^{14}C]glucose in the blood following intraperitoneal injection of [3-^{14}C]pyruvate. The reason for only mercaptopicolinic acid inhibiting *in vivo*, concomitant with a hypoglycemic effect, is unclear, but may be related to its greater potency of inhibition *in vitro*. We found, using both kidney cortex slices and hepatocytes, that it was approximately fifty times more potent than pirogliride. Furthermore, the concentrations of pirogliride which inhibited *in vitro* are somewhat high compared to its blood levels (10^{-5} M) as measured following administration of hypoglycemic doses (unpublished results). Thus, the above results have largely excluded an inhibitory effect on gluconeogenesis as being a primary mechanism of pirogliride's hypoglycemic effect.

Acknowledgements—We are indebted to Ms. Marie Gleason, Mr. Henry Fawthrop and Mr. Gary Bridi for their excellent technical assistance, and to Roseann Gramlich and Maria Castagno for help in the preparation of this manuscript.

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