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Effect of methyl palmoxirate, an oral hypoglycemic agent, on epinephrine-induced hyperglycemia in the rat

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Methyl palmoxirate (methyl 2-tetradecylglycidate), a potent specific inhibitor [1] of carnitine palmitoyl transferase (CPT), has been shown to be a hypoglycemic and hypoketonemic agent in diabetic animals [2–4] and man [5]. Evidence has been presented that the lowering of blood glucose results from inhibition of hepatic gluconeogenesis [2, 6, 7] and from stimulation of peripheral glucose utilization [2, 8, 9]. Since there is increasing evidence that the long-term complications of diabetes are related in part to poor glucose control [10, 11], there has been increasing effort to reduce the elevated blood glucose levels as close to the normal range as possible [12–14], but this approach may increase the risk of severe hypoglycemia [15]. Obviously, for any new oral hypoglycemic agent, such as methyl palmoxirate (MP), a similar concern exists. Counterregulatory hormones are known to play a critical role in defense against hypoglycemia. Therefore, it was of interest to determine whether a counterregulatory hormone, such as epinephrine, could elevate blood glucose in fed or fasted rats that had been treated with methyl palmoxirate.

Methods

Male Sprague–Dawley rats (200–280 g) from Charles River, maintained on Lab-Blox rat chow (Wayne), were used throughout. When required, they were fasted 18–24 hr prior to the epinephrine treatment.

Animals were dosed daily with MP or vehicle (0.5% methylcellulose). On the day of the study, all rats were bled from the tail vein for blood glucose determination, dosed, and 3 or 4 hr post dosing blood samples were again taken for measurement of glucose changes prior to and at 30, 60 and 90 min after administration of epinephrine bitartrate (Sigma Chemical Co., St. Louis, MO). Blood glucose was determined using Autoflo Glucose (Bio-Dynamics/bmc, Indianapolis, IN). In some experiments, animals were killed by decapitation immediately following the final blood sampling; heart, liver and gastrocnemii were removed for determination of glycogen [16]; and plasma samples were collected for analysis of free fatty acids (FFA) [16, 17]. In some cases, mitochondrial carnitine palmitoyl transferase was measured [1, 2] using a piece of the liver. The 3-mercaptopicolinic acid was obtained as a gift from Smith Kline & French Laboratories (Philadelphia, PA). 2,5-anhydro-*d*-mannitol was prepared at McNeil Pharmaceutical by Allen B. Reitz, and 5-methoxyindole-2-carboxylic acid was purchased from the Aldrich Chemical Co. (Milwaukee, WI).

For *in vitro* studies, hepatocytes from 24-hr fasted rats were isolated and incubated (30 min) under conditions reported elsewhere [6].

Results and discussion

As shown in Fig. 1, a dose of 83 μg/kg, s.c., of epinephrine produced a highly significant ($P < 0.01$) hyperglycemic response (approximately 100% increase of blood glucose), using either fed or fasted rats. As shown in Fig. 1, 3 days of once-a-day treatment with a therapeutic dose [2, 5] of MP (1.0 mg/kg, p.o.) did not diminish significantly this hyperglycemic effect of epinephrine. This dose of MP has been reported to lower plasma ketones and to inhibit liver mitochondrial CPT activity by >90% in fasted rats [18] and to increase plasma FFA levels [2, 3]. In the present study, the plasma FFA of the fasted rats increased 89% following MP treatment though, as reported previously [2, 3], MP did not elevate plasma FFA or lower blood glucose in fed rats (results not shown).

The dose of MP was then increased to produce near maximal decreases of plasma glucose and liver glycogen levels of fasted rats. With such a high dose, it was expected that a greater effect on the epinephrine response would be seen in the fasted state than in the nonfasted state, and this was found to be the case. In fasted rats where MP (2.5 mg/kg p.o./day for 3 days) lowered the baseline blood glucose 30% (Fig. 2) and decreased the heart and liver but not skeletal muscle glycogen levels (see inset), some significant, though small, attenuation of the epinephrine response was observed (Fig. 2). Using *ad lib.* fed rats, this dose of MP failed to significantly reduce liver or muscle glycogen levels or the hyperglycemic effect of epinephrine (results not shown).

Shikama and Ui [19], using intraperitoneally administered epinephrine, found enhanced incorporation of radioactively labeled gluconeogenic precursors into both blood glucose and liver glycogen of fasted nondiabetic rats. Other investigators have also reported [18, 20] increased deposition of liver glycogen in fasted rats following epinephrine injection. We also found that intraperitoneal administration of a high dose of epinephrine (0.2 mg/kg) to fasted rats resulted in a quantitatively small (compared to fed state) though significant increase in liver glycogen without altering the heart or skeletal muscle glycogen (results not shown). This epinephrine-induced increase of liver glycogen (level increased from 0.5 ± 0.6 in the controls to 4.2 ± 1.2 mg glucose/g liver at 90 min after epinephrine) was totally suppressed by 10 days of treatment with MP (2.5 and 5.0 mg/kg/day) even though the hyperglycemic effect of epinephrine was only partially inhibited. As

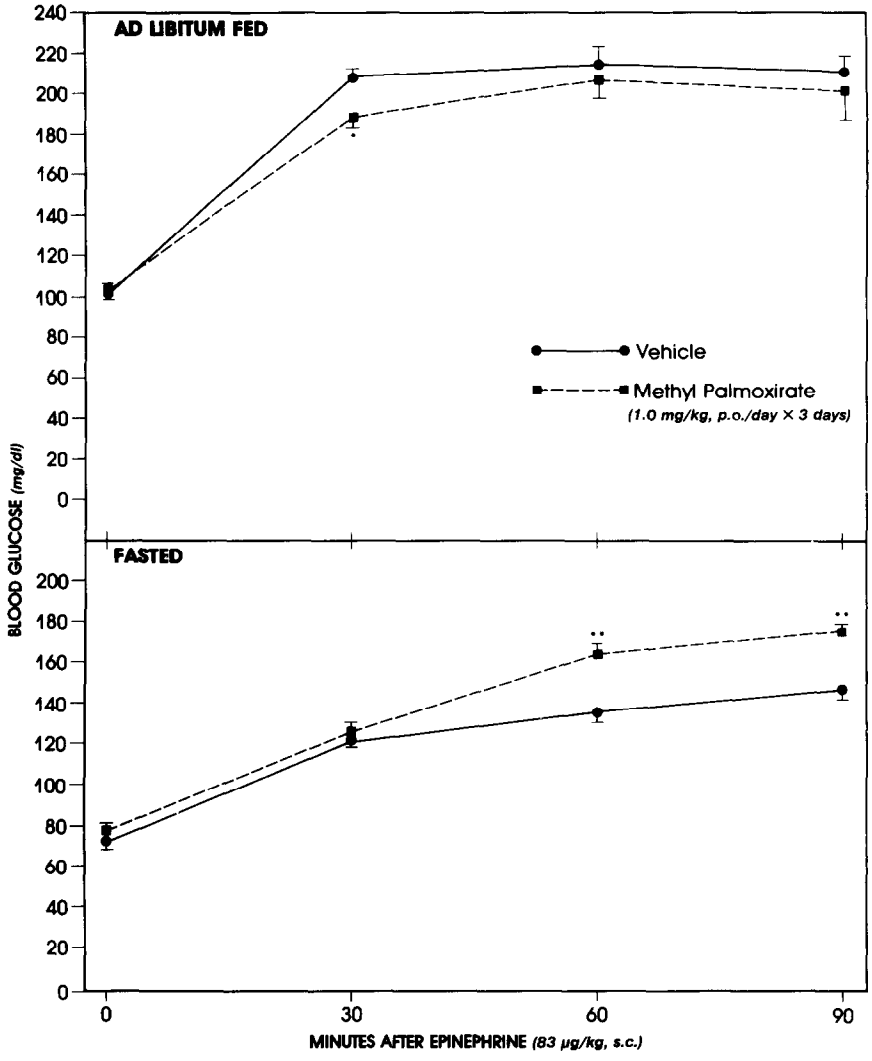

Fig. 1. Effect of methyl palmoxirate (1 mg/kg p.o./day × 3 days) on the blood glucose of fed and fasted rats following challenge with epinephrine. Fasted rats (five per group) received epinephrine (83 μg/kg, s.c.) 3 hr after the last dose of methyl palmoxirate or vehicle. Results shown are mean ± S.E.M. Statistical significance compared to vehicle-treated group was determined by Student's *t*-test. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

expected from previous studies [2, 21, 22], MP increased the plasma free fatty acids by 109–139%, and inhibited liver and diaphragm carnitine palmitoyl transferase activity by 83–92% and 50–63% respectively.

The epinephrine-induced hyperglycemia may be explained in terms of changes of metabolic activities that are induced by the catecholamine, i.e. the activation of hepatic glycogenolysis, enhanced gluconeogenesis, and the inhibition of peripheral glucose utilization directly or via suppression of insulin secretion. The relative contribution of these metabolic changes to the hyperglycemia probably depends on the metabolic and nutritional status of the animal. In fed rats, epinephrine has been reported to increase blood glucose partially through an increase of gluconeogenesis. This occurred, as in our study, without a decline of liver glycogen, and inhibitors of gluconeogenesis partially inhibit the rise of blood glucose [23, 24]. In fasting rats, the hyperglycemic effect of epinephrine is due predominantly to an enhancement of gluconeogenesis rather than from glycogen breakdown [19, 23–27], and the gluconeogenic inhibitors 5-methoxy-2-indole carboxylic acid, tryptophan, hydrazine, phenformin and 3-mercaptopicolinic acid [23, 25, 28, 29] totally suppress this hyperglycemic effect and the glycogen resynthesis. We have confirmed that 25 mg/kg, i.p., of 5-methoxy-2-indole carboxylic acid, 100 mg/kg, i.p., of tryptophan, and 75 mg/kg, i.p., of 3-mercaptopicolinic acid can totally abolish the hyperglycemic effect of epinephrine. We have made the same observation with the newly reported [30, 31] inhibitor 2,5-anhydro-*d*-mannitol (100 mg/kg, i.p.).

Also, epinephrine and norepinephrine stimulate gluconeogenesis *in vitro* in isolated hepatocytes [23, 24, 32, 33]. As shown in Table 1, 5 μM norepinephrine (a concentration producing a maximal stimulation) stimulated glucose production using isolated rat hepatocytes. Epinephrine also was effective (results not shown) but, since the effect was quantitatively smaller than with norepinephrine, we elected to use norepinephrine for these studies. As reported previously [6], addition of MP (Table 1) inhibited glucose production from lactate and pyruvate in the absence of the hormone; however, under these conditions, norepinephrine was still able to significantly

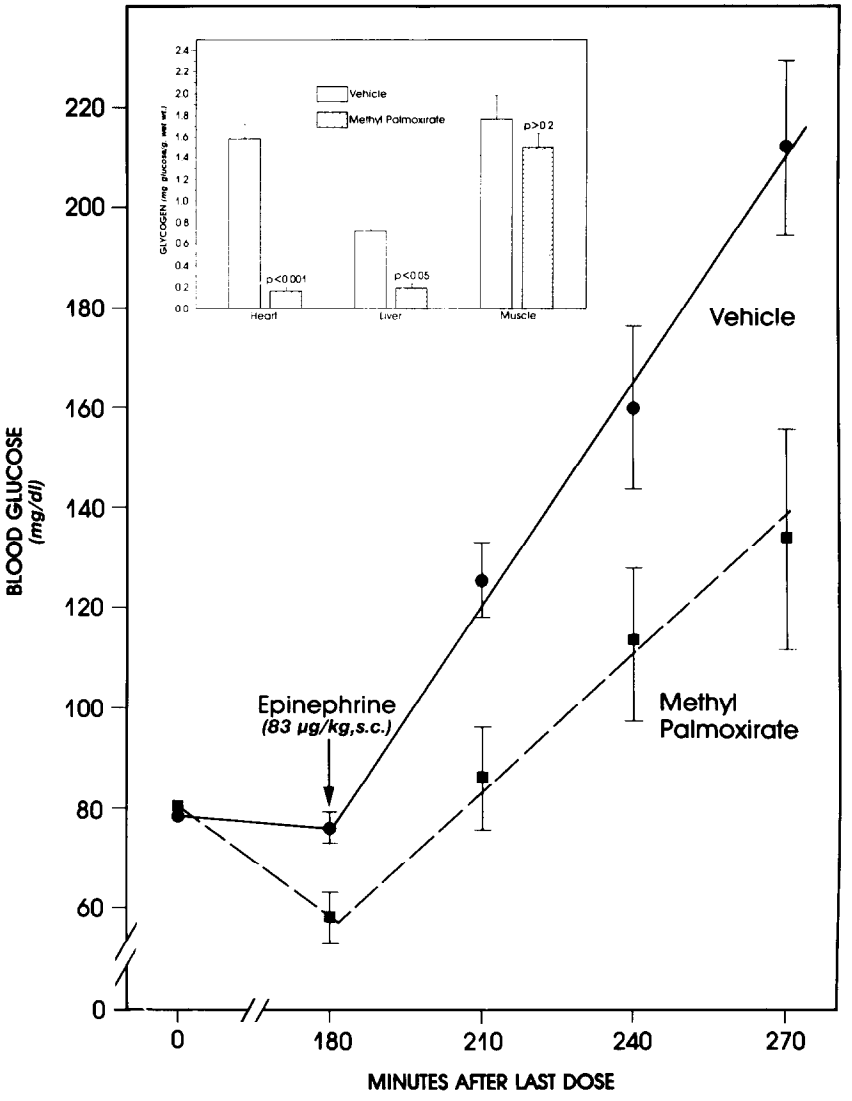

Fig. 2. Effect of methyl palmoxirate (2.5 mg/kg p.o./day × 3 days) on epinephrine-induced hyperglycemia in the fasted rat and tissue glycogen levels. Three hours after the last dose of methyl palmoxirate or vehicle, fasted rats (five per group) were bled from the tail vein prior to receiving the last dose of methyl palmoxirate or vehicle and again immediately prior to and at 30, 60 and 90 min after epinephrine (83 μg/kg, s.c.) administration. The drug or vehicle was given 3 hr prior to epinephrine. Tissue samples were taken 4.5 hr after the last dose of methyl palmoxirate or vehicle. Results shown are mean ± S.E.M. Statistical significance compared to vehicle-treated group was determined by Student's *t*-test.

stimulate gluconeogenesis by the same incremental amount seen in the absence of methyl palmoxirate. On the other hand, both 5-methoxyindole-2-carboxylic acid and 2,5-anhydro-*d*-mannitol produced an almost total block of the norepinephrine stimulation. The result with 2,5-anhydro-*d*-mannitol confirms the report of Riquelme *et al.* [31] and is shown in Table 1 for comparison. Thus, it appears that catecholamines can enhance hepatic glucose production *in vivo* and *in vitro* when long chain fatty acid oxidation is inhibited with MP. That the stimulation of gluconeogenesis by hormones is not dependent on the increased availability of fatty acids to the liver has been reported previously [34]. Furthermore, recent studies [35] in fasted nondiabetic and diabetic dogs have confirmed that 7 days of treatment with clinically effective doses of MP fails to impair the counterregulatory response (rise of blood glucose or increase in hepatic glucose production) to insulin-induced hypoglycemia (40–50 mg/dl). Also, we have found that the drug fails in isolated rat hepatocytes to inhibit the stimulation of gluconeogenesis produced by 10^{-6} M glucagon (unpublished results).

In summary, unlike results with more direct inhibitors of gluconeogenesis, prior treatment of fed or fasted rats for 3–10 days with the fatty acid oxidation inhibitor MP (1–10 mg/kg p.o./day) produced little, if any, decrease of the hyperglycemia following epinephrine. Furthermore, even though gluconeogenesis from 2 mM lactate and 0.5 mM pyruvate in isolated rat hepatocytes was inhibited 50–70% by methyl palmoxirate, norepinephrine still significantly stimulated gluconeogenesis by the same incremental amount seen in the absence of the drug.

Table 1. Effect of methyl palmoxirate (MP) and 2,5-anhydro-*d*-mannitol (AM) on norepinephrine-stimulated gluconeogenesis *in vitro* using isolated hepatocytes from non-diabetic rats

Additions	Norepinephrine concn (μM)	Glucose production* (nmoles/g/min)	Percent stimulation with norepinephrine
None	0.0	1036 ± 8	
None	5.0	1358 ± 82	31†
1 μM MP	0.0	601 ± 42 (42%)‡	
1 μM MP	5.0	837 ± 41	39†
10 μM MP	0.0	463 ± 33 (55%)‡	
10 μM MP	5.0	723 ± 19	56†
None	0.0	1237 ± 86	
None	5.0	1624 ± 16	31†
0.1 mM AM	0.0	868 ± 21 (30%)‡	
0.1 mM AM	5.0	781 ± 149	0
1.0 mM AM	0.0	459 ± 14 (63%)‡	
1.0 mM AM	5.0	470 ± 19	2

* Lactate (2 mM) and pyruvate (0.5 mM) were used as substrates. Values are means ± S.E.M.

† Significance was determined by Student's *t*-test: $P < 0.05$.

‡ Percent inhibition produced by drug in the absence of norepinephrine.

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