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Inhibition of Mitochondrial Carnitine Palmitoyl Transferase A *in Vivo* with Methyl 2-Tetradecylglycidate (Methyl Palmoxirate) and Its Relationship to Ketonemia and Glycemia[1] (42012)

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Abstract. The oral hypoglycemic agent, methyl 2-tetradecylglycidate (Me-TDGA), which inhibits *in vitro* mitochondrial carnitine palmitoyl transferase A (CPT-A) was used to study the relationship of CPT inhibition to changes in ketonemia and glycemia in normal and diabetic rats. After oral administration of Me-TDGA, the CPT activity of isolated rat liver mitochondria was substantially reduced with only the presumed outer enzyme fraction CPT-A released by digitonin treatment showing reduced activity. Mitochondrial fatty acyl–CoA synthetase was not inhibited. Oral doses of 0.1–2.5 mg/kg Me-TDGA produced both a dose-dependent lowering of plasma ketones and an inhibition of liver CPT. With single doses in excess of 2.5 mg/kg, po, heart and skeletal muscle CPT were also consistently inhibited. The effect on the liver enzyme persisted for at least 48 hr following 1 mg/kg, po, while the effect on ketones disappeared by 36 hr. The degree of inhibition of liver CPT produced by Me-TDGA was not altered by diabetes or the dietary state. At low doses (0.05–0.25 mg/kg, po), the most sensitive parameter was inhibition of hepatic CPT. Both plasma ketones and CPT were lowered with doses 10-fold less (0.1 mg/kg) than were required for blood glucose lowering, thus making Me-TDGA the most potent hypoketonemic compound known. In conclusion, inhibition of liver β-oxidation at the stage of CPT-A by Me-TDGA can explain the potent hypoketonemic effects of this compound in fasted normal and diabetic rats. Higher acute doses are needed for both inhibition of muscle CPT and lowering of blood glucose.

When long-chain fatty acids arrive in the liver, they are immediately esterified to coenzyme A (CoA). Since the CoA ester cannot cross the mitochondrial membrane, the fatty acyl moiety is transferred to carnitine by carnitine palmitoyl transferase A (CPT-A), which is located on the outside of the inner mitochondrial membrane. The carnitine ester then passes into the mitochondrion where esterification is reversed by carnitine palmitoyl transferase B. The resulting fatty acyl–CoA then undergoes β-oxidation, and the acetyl-CoA formed in excess of that required for the Krebs cycle is used in the synthesis of ketone bodies, acetoacetate, and β-hydroxybutyrate. In the transition from the fed state to states of rapid ketogenesis such as in fasting and diabetes mellitus, the mitochondrial CPT-A reaction from the above sequence has been postulated to be the primary control point (1); however, controversy still exists as to the precise way in which flux through this step is controlled (2–4). Furthermore, while the possibility that the ketogenic adaptation involves enhancement of CPT activity has been examined and increases have been found by some authors in fasting and diabetes (5–7), it is now generally believed that the marginal increases in the overall activity of this enzyme in ketogenic states do not correlate well with differences in rates of fatty acid oxidation (8, 9). Also, the switch-on of ketogenesis at birth seems to involve metabolic steps other than CPT (10). However, both pools of mitochondrial CPT (A and B) were not measured in all studies. Therefore, the precise temporal relationship *in vivo* for changes in ketogenesis and CPT activity has yet to be established.

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Recently, methyl 2-tetradecylglycidate (Me-TDGA; generic name: methyl palmoxirate), which has been shown to be an orally effective hypoglycemic and hypoketonemic agent in animals (11–13) and man (14, 15), was shown to produce irreversible, active-site-directed inactivation of CPT-A *in vitro* after first being converted to a CoA ester (13, 16). Thus after *in vivo* administration, this drug's effect on CPT-A should persist through the isolation procedure and enable one to compare the degree of enzyme inhibition to changes in blood levels of ketones and glucose. Such studies are reported here, in which the mitochondrial CPT activity of various tissues, blood glucose, and ketones of normal and diabetic animals were monitored following *in vivo* Me-TDGA treatment. Also, solubilization and fractionation of liver mitochondrial CPT activities have confirmed that CPT-A is the site of attack *in vivo* for Me-TDGA.

Methods. *Materials.* DL-[*methyl*-^{3}H] Carnitine (2 Ci/mmole) was obtained from Amersham and [1-^{14}C]-palmitate from New England Nuclear. Acetoacetate, β-hydroxybutyrate, L-carnitine, coenzyme A, digitonin, NAD^+, NADH, ATP, palmitoyl-L-carnitine, alloxan, and palmitoyl-CoA were purchased from Sigma. Trinder glucose oxidase kits for measurement of blood glucose and enzymes used in ketone analysis were obtained from Boehringer-Mannheim. Streptozotocin was purchased from Upjohn. All other chemicals were reagent quality or better.

Animals. The animals used for this work were male, albino, Sprague–Dawley rats (200–250 g) purchased from Charles River Breeding Laboratories and maintained prior to study on Lab-Blox rat chow (Wayne). Unless otherwise indicated, rats were fasted 48 hr prior to use.

Diabetic rats were prepared by injection of streptozotocin or alloxan and maintained as described previously (11). Rats selected for study had orbital sinus blood glucose levels in excess of 290 mg/dl. Rats were not given insulin for 48 hr nor food within 24–48 hr of an experiment.

Schedule-fed rats were allowed access to standard powdered laboratory chow for only 6 hr each day (9:00 AM to 3:00 PM). Following an adjustment period (1 week), rats which had adapted to the scheduled feeding were chosen for study.

General experimental procedure. Rats (four to six) were randomly assigned to groups for each treatment period and/or dosage group. Me-TDGA was suspended in 0.5% methylcellulose and was generally administered by oral gavage in a volume of 1.0 ml. When given intraperitoneally, a solution of drug complexed with bovine serum albumin was prepared as described previously (13) except that the drug was added to the albumin in *t*-butyl alcohol and this reagent was removed by lyophilization prior to dissolving the albumin drug complex for injection. At specified times after dosing, rats were sacrificed by decapitation and their blood was collected. Heparinized plasma was prepared immediately and stored at −20°C until assay. Livers were excised and mitochondria were isolated as described previously (13, 16); heart, diaphragm, and soleus muscle mitochondria were prepared similarly.

When schedule-fed rats were used, dosing was performed just prior to the feeding period (i.e., 9:00 AM) and the study was terminated 5 hr later after approximately 80–90% of the daily intake had been consumed.

Identification of inhibited enzyme by digitonin separation. Liver mitochondria from fasted rats were prepared 3 hr after dosing with 2 mg/kg of methyl palmoxirate or vehicle. CPT-A was then separated from CPT-B as described by Hoppel and Tomec (17) using digitonin at 0.12 mg/mg mitochondrial protein and a mitochondrial protein concentration of 50 mg/ml. At the suggestion of Dr. Hoppel, the success of this treatment was confirmed by showing that the mitoplasts lost the ability to oxidize palmitoyl-CoA while retaining that for palmitoyl carnitine; this was achieved using a mitochondrial chamber system similar to Hoppel and Tomec (17). For CPT measurement (see below), both fractions were adjusted to the same dilution fraction and sonicated twice for 15 sec at 4°C to ensure accurate measurement of mitoplast enzyme (CPT-B).

Analytical methods. Glucose measurements were determined directly on the plasma while total plasma ketones (β-hydroxybutyrate and acetoacetate) were measured on protein-free filtrates as described previously (11, 18). Mi-

tochondrial protein was determined by the Biuret method (19).

Mitochondrial carnitine palmitoyl transferase activity was measured by the formation of [^{3}H]palmitoyl carnitine as described previously (13) except that the preincubation period was eliminated and the final mitochondrial protein concentration was reduced to 0.5–1.0 mg/ml. Normally only two to three rats from each group were assayed for CPT activity. Mitochondrial fatty acyl-CoA synthetase was measured as [^{14}C]palmitoyl-CoA formation as described previously (18).

Statistical methods. Data were statistically analyzed using Duncan's multiple range test after one-way analysis of variance or, where appropriate, by Student's *t* test (20).

Results. *Inhibition of CPT A or B in vivo?* Previous *in vitro* studies (16) have identified Me-TDGA as a specific inhibitor of carnitine palmitoyl transferase A which is believed to be loosely bound to the outside of the inner mitochondrial membrane (1, 3, 8). To confirm this after *in vivo* treatment, fasted rats were orally dosed with Me-TDGA and 3 hr later their liver mitochondria were isolated. As shown in Fig. 1, only the outer enzyme CPT-A released by the digitonin solubilization procedure of Hoppel and Tomec (17) was inhibited while no effect was apparent in the mitoplast fraction which contains the inner enzyme, CPT-B. It should be noted that this technique does not produce an absolute separation of CPT-A and CPT-B, but rather results in two fractions "enriched" in one or the other enzyme. Our conditions favor the complete release of CPT-A (17, 21, 22). While this usually leaves little or no inhibitable enzyme in the mitoplast fraction, the soluble fraction almost certainly contains some CPT-B which is reflected by an apparent loss of inhibitability.

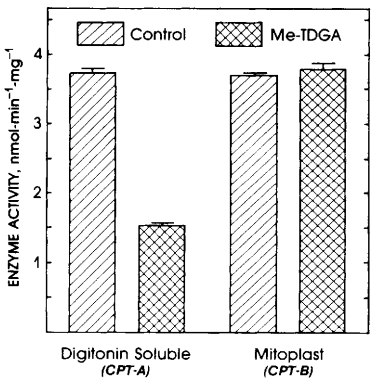

FIG. 1. Identification of carnitine palmitoyl transferase A as the enzyme inhibited after oral administration of methyl palmoxirate. The digitonin-released and mitoplast-associated liver mitochondrial CPT fractions were prepared from drug-treated and vehicle-treated rats. Results are presented as the means ± SE of triplicate determinations. The activity difference in the digitonin-released (CPT-A) fraction (2.24 nmole/min/mg) accounted for over 90% of that detected when the intact mitochondria of these drug-treated and vehicle-treated rats were compared (2.40 nmole/min/mg). The difference was significant by Student's *t* test ($P < 0.001$).

Tissue specificity. The magnitude of CPT inhibition in different tissues was studied after oral administration of Me-TDGA or its free acid to rats fasted for 48 hr. Results from several studies showed the liver to be the most sensitive of the tissues assayed while substantial and consistent inhibition of the heart, diaphragm, and soleus muscle CPT was seen only at the higher doses tested (>2.5 mg/kg, po). One study measuring effects only on liver, heart, and diaphragm muscle CPT is illustrated in Fig. 2. Results using streptozotocin diabetic rats were similar with liver, heart, and diaphragm CPT showing 66, 35, and 20% inhibition, respectively, following 10 mg/kg, po, of Me-TDGA.

Plasma ketones and liver mitochondrial carnitine palmitoyl transferase of fasted nondiabetic rats. Fasting of rats has been reported (23) to cause a prompt increase in the rate of hepatic ketogenesis with plasma concentrations peaking 12–24 hr into the fast. In our hands, fasting of rats for 24 or 48 hr resulted in peak plasma ketone concentrations. Under these conditions, as shown in Table I, oral administration of Me-TDGA produced a dose-dependent lowering of the plasma ketone levels with maximum effects occurring at 4–6 hr after dosing in four separate experiments. Treatment with higher doses (1–25 mg/kg, po) reduced plasma ketones 83–93% to the level observed in nonfasted vehicle-treated rats (0.24–0.39 m*M*). Interestingly, plasma ketones were significantly lowered with acute doses as low as 0.1–0.2 mg/kg, po (Table I), which is 10-fold less than acute doses required for blood glucose lowering (11, 13). In none of the

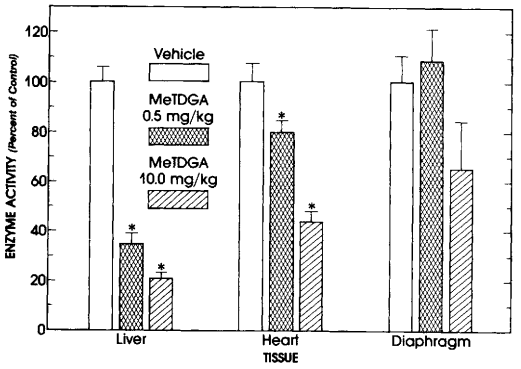

FIG. 2. Differential inhibition of mitochondrial carnitine palmitoyl transferase in various tissues after oral administration of methyl palmoxirate. Twenty-seven fasting rats were randomly assigned to three groups and orally dosed with vehicle or methyl palmoxirate at 0.5 or 10.0 mg/kg. Rats were sacrificed after 3 hr, and their tissues were excised. Prior to mitochondrial isolation, appropriate tissues were combined in threes. Enzyme activity was measured as described under *Methods* except that the carnitine concentration was increased to 2 m*M* for assay of heart mitochondria. After expression as nmole/min/mg, results were normalized to the vehicle-treated control for each tissue and are presented as percentage of control (mean ± SE, $N = 3$). Actual vehicle-treated enzyme levels were liver: 6.22 ± 0.37; heart: 13.00 ± 0.58; diaphragm: 1.85 ± 0.19 nmole/min/mg. An asterisk indicates statistical significance ($P < 0.01$) by the Duncan Multiple range test.

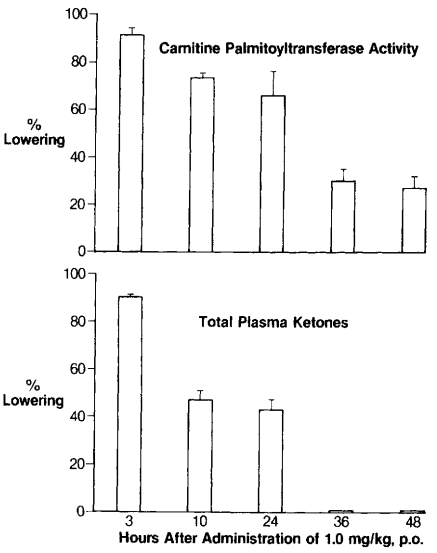

FIG. 3. Duration of the inhibitory effect of methyl palmoxirate on total plasma ketones and liver mitochondrial carnitine palmitoyl transferase. Fasted rats dosed with either vehicle or methyl palmoxirate (1 mg/kg, po) were sacrificed for determination of total plasma ketones and liver mitochondrial CPT. At each time point, results from drug- and vehicle-treated rats were compared to determine the percentage lowering which is presented here as means ± SE. All lowerings are statistically significant ($P < 0.05$) by Student's t test except those for ketones at 36 and 48 hr.

experiments performed which we report here was plasma glucose acutely lowered with doses of Me-TDGA less than 2.5 mg/kg, po.

In parallel with its effects on plasma ketones, oral dosing with Me-TDGA reduced the activity of liver mitochondrial carnitine palmitoyl transferase. As shown in Fig. 3, this effect persisted for at least 48 hr following

TABLE I. THE EFFECT OF METHYL 2-TETRADECYLGLYCIDATE (Me-TDGA) ON TOTAL PLASMA KETONES OF 48-hr FASTED NONDIABETIC RATS

Treatment	Dose (mg/kg, po)	Plasma ketones (m*M*) at hours after dosing							
		2		4		6		8	
Vehicle	—	2.14 ± 0.11[a]	—	2.53 ± 0.24	—	2.63 ± 0.08	—	2.41 ± 0.19	—
Me-TDGA	0.5	0.83 ± 0.12***	61%[b]	0.66 ± 0.15***	74%	0.73 ± 0.05***	72%	1.12 ± 0.15***	54%
Me-TDGA	0.2	2.35 ± 0.23	0	2.16 ± 0.49	15%	1.44 ± 0.20**	45%	1.85 ± 0.24	23%
Me-TDGA	0.1	2.42 ± 0.07	0	1.91 ± 0.20	25%	2.28 ± 0.11*	13%	2.49 ± 0.30	0
Me-TDGA	0.05	2.58 ± 0.26	0	2.06 ± 0.25	19%	2.26 ± 0.14	14%	2.73 ± 0.21	0

Note. Statistical significance was determined by the unpaired Student t test. The drug-treated groups were compared at each time point to their respective control group.

[a] Mean ± SEM of four per group.

[b] Percentage lowering from vehicle controls.

* $P < 0.05$

** $P < 0.01$.

*** $P < 0.001$.

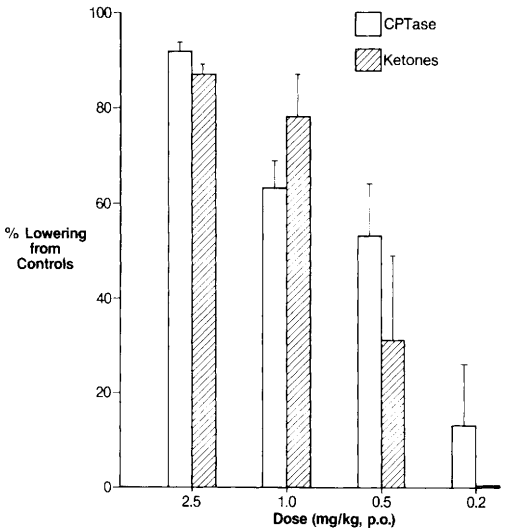

FIG. 4. Dose-dependent lowering of liver mitochondrial carnitine palmitoyl transferase activity and total plasma ketones of 24-hr fasted rats by methyl palmoxirate. Fasting rats were dosed with vehicle or methyl palmoxirate at 0.2, 0.5, 1.0, or 2.5 mg/kg, po, and sacrificed 3 hr later for determination of total plasma ketones and liver mitochondrial CPT. Percentage lowerings were calculated by comparison to control values obtained using vehicle-treated rats and are presented as means ± SE.

a single oral dose, while the effect on plasma ketones had normalized by 36 hr. Similar results were obtained using the free acid, TDGA. Consistent with findings *in vitro* (18), levels of liver mitochondrial palmitoyl-CoA synthetase were not altered at these clinically effective doses (14, 15).

As illustrated in Fig. 4, the magnitude of the suppression of both plasma ketones and liver mitochondrial carnitine palmitoyl transferase at 3 hr after dosing with Me-TDGA was dependent on the dose of drug administered. At lower doses, however, the most sensitive parameter of Me-TDGA action appeared to be inhibition of hepatic CPT. As shown in Table II, CPT was significantly inhibited 60% within 2 hr after 0.25 mg/kg, po, of Me-TDGA without a significant lowering of plasma ketones until the 5-hr time point. At the lower dose of 0.05 mg/kg, po, of Me-TDGA, only the enzyme activity was significantly inhibited.

Effect of feeding and diabetes. As shown in Table III, the rates of CPT activity measured on separate mitochondrial preparations from schedule-fed and fasted rats were not different and the degree of inhibition produced by Me-TDGA was not altered by the dietary state. For this study, the drug was given intraperitoneally so that feeding would not influence the absorption of the drug. However, virtually identical results have also been obtained giving 5 mg/kg orally to pair-fed or *ad libitum*-fed rats. As has been re-

TABLE II. THE TEMPORAL RELATIONSHIP OF THE INHIBITION OF LIVER MITOCHONDRIAL CPT TO THE LOWERING OF PLASMA KETONES FOLLOWING ADMINISTRATION OF LOW DOSES OF METHYL 2-TETRADECYLGLYIDATE (Me-TDGA) TO FASTED NONDIABETIC RATS

Dose Me-TDGA (mg/kg, po)	*N*	Hours after dosing		
		2	5	10
		Carnitine palmitoyl transferase activity (nmole/min/mg)		
Vehicle	2	2.51 ± 0.18[a]	2.54 ± 0.10	2.40 ± 0.15
0.25	2	0.99 ± 0.19***	0.65 ± 0.16***	1.16 ± 0.10***
0.05	2	1.88 ± 0.14*	2.22 ± 0.29	2.08 ± 0.08
		Total plasma ketones (m*M*)		
Vehicle	5	2.26 ± 0.20	2.58 ± 0.25	2.77 ± 0.23
0.25	5	1.85 ± 0.50	0.97 ± 0.33**	1.44 ± 0.14**
0.05	5	2.43 ± 0.10	3.13 ± 0.31	2.22 ± 0.43

Note. Statistical significance was determined using Duncan's multiple range test. The drug-treated groups were compared to their respective vehicle group at each time point.

[a] Mean ± SEM.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

ported previously (11), plasma glucose (results not shown) and plasma ketones (Table III) were found to be lowered by Me-TDGA treatment in the fasted but not the fed rats.

Also we found that making rats diabetic with the β-cell cytotoxins, streptozotocin and alloxan, did not alter the rates of liver mitochondrial CPT when compared to age-matched controls. Mitochondrial CPT activity for 24-hr fasted streptozotocin diabetic (blood glucose 308–427 mg/dl) rats ($N = 4$) was 3.41 ± 0.09 nmole/min/mg compared to 3.45 ± 0.13 for fasted nondiabetic controls. In diabetic rats, CPT activity was decreased 24, 62, 91, and 88%, respectively, at 3 hr after receiving 0.2, 0.5, 1.0, and 2.5 mg/kg, po, of Me-TDGA. The mean rate observed for liver CPT activity of alloxan diabetic rats ($N = 2$) was 3.00 ± 0.1 nmole/min/mg compared to 3.05 ± 0.21 for nondiabetic fasted controls. Alloxan diabetic rats dosed with 0.2, 0.5, 1.0, and 2.5 mg/kg, po, of Me-TDGA had this activity decreased by 54, 92, 90, and 92%, respectively, and as has been reported previously (11, 13), plasma ketone concentrations were brought into the range seen for non-fasted, nondiabetic controls.

Discussion. While β-oxidation of long-chain fatty acids is important for energy metabolism, this process assumes additional significance in the liver where rates of gluconeogenesis and ketogenesis appear to be determined by the concurrent rates of fatty acid oxidation (1, 24, 25). Therefore, the identification of the rate-limiting step(s) of fatty acid oxidation in liver and its control has been of considerable interest. In a series of excellent studies, McGarry, Foster, and associates have hypothesized a reciprocal coordination between *de novo* synthesis of long-chain fatty acids and fatty acid oxidation (1). The common link between these two processes has been suggested to be malonyl-CoA, which is a competitive inhibitor of CPT-A (26). It has further been suggested that hepatic CPT activity may control not only long-chain fatty acid oxidation and ketogenesis but also gluconeogenesis (18, 24, 27, 28). Therefore, the possible involvement of CPT-A in controlling the levels of glucose and ketones was evaluated *in vivo* using the oral hypoglycemic drug, Me-TDGA, the CoA metabolite of which is an extremely potent, specific active site-directed irreversible inhibitor of CPT-A *in vitro* (16). In brief, a linear relationship between the degree of enzyme inhibition with the drug and changes of either glucose or ketone lowering was not observed.

The "malonyl-CoA hypothesis" has been questioned mainly on the basis that CPT-A may not be rate-limiting and thus regulatory for ketogenesis (3, 29). The present studies do not clearly resolve this issue. In general, the results in Figs. 3 and 4 and Table I show a close parallelism between the reduction in enzyme activity and plasma ketones. However, as shown in Fig. 3, plasma ketones returned to elevated levels in fasted rats prior to total reversal of the inhibitory effect on the enzyme. This finding plus the observation (Table II) that only enzyme activity was

TABLE III. THE EFFECT OF ALBUMIN-BOUND Me-TDGA ON PLASMA KETONES AND LIVER MITOCHONDRIAL CARNITINE PALMITOYL TRANSFERASE (CPT) ACTIVITY IN FASTED AND FED RATS

Treatment	Nutritional state	Plasma ketones, m*M*	% Lowering	CPT activity nmole/min/mg	% Lowering
Vehicle	Fasted, 24 hr	1.49 ± 0.14[a]	—	2.50 ± 0.06[a]	—
Me-TDGA (5 mg/kg, ip)	Fasted, 24 hr	0.31 ± 0.02	79***	0.63 ± 0.10	75***
Vehicle	Fed	0.41 ± 0.08	—	2.51 ± 0.13	—
Me-TDGA (5 mg/kg, ip)	Fed	0.27 ± 0.03	34	0.49 ± 0.06	80***

[a] Mean ± SEM, $n = 4$. Animals sacrificed 5 hr after dosing with vehicle or Me-TDGA. Statistical significance determined using Duncan's Multiple range test.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

lowered at the lowest Me-TDGA doses could support the view that a small excess of CPT-A exists over that required for maximal flux of ketogenesis. However, this conclusion cannot be made with assurance since the drug's effect on ketone utilization and FFA uptake were not measured.

Generally it is believed (24, 25, 27) that the net rate of gluconeogenesis is proportional to the rate of fatty acid oxidation, and Me-TDGA, as well as other inhibitors of fatty acid oxidation (13, 18, 23, 27, 30), have consistently inhibited gluconeogenesis *in vivo* and *in vitro*. Therefore, we were at first surprised when the blood glucose of 48-hr fasted rats given low doses of Me-TDGA did not fall under conditions where hepatic CPT was inhibited and ketones were lowered by as much as 90%. McGarry and Foster (23) also noted a separation of FFA oxidation from gluconeogenesis in experiments where refeeding of fasted rats caused a 54% reduction in ketogenesis without altering the rate of gluconeogenesis. Therefore, our current belief is that, while the gluconeogenic capacity may be increased in fasting, it requires fatty acid oxidation only at higher levels of substrate usage. While further studies are needed to prove this point, we have found in isotopic labeling studies in diabetic dogs (31) that inhibition of hepatic glucose production with the drug seemed to contribute to glucose lowering only in animals having abnormally elevated rates of hepatic glucose production. In addition, epinephrine's hyperglycemic effect in fasted rats, which is mainly due to enhanced gluconeogenesis (32), and the counterregulatory increase of hepatic glucose production observed in fasted dogs in response to hypoglycemia (33), are not inhibited following 3–10 days of treatment with therapeutic doses of Me-TDGA.

Thus, the hypoglycemia observed in fasted rats following administration of single high doses (>2.5 mg/kg, po) of Me-TDGA appears to result from its well-established ability to stimulate peripheral glucose utilization (13, 31, 34). This conclusion is supported by the current observation that peripheral tissue (heart, diaphragm, and skeletal muscle) mitochondrial CPT activities were generally inhibited only with acute doses greater than 2.5 mg/kg, po. Why higher doses are required to inhibit the peripheral enzyme activity is unclear at this time. While greater availability to the liver from the orally administered drug might be expected, altered sensitivity of CPT-A from various tissues to Me-TDGA cannot be excluded since such tissue specific sensitivity differences have been reported using the competitive inhibitor, malonyl-CoA (35, 36).

Consistent with *in vitro* findings (16), administration of Me-TDGA to rats resulted in a substantial decrease of liver mitochondrial CPT activity which was shown to reflect a specific inactivation of CPT-A. The magnitude of enzyme inhibition was not clearly altered by either the dietary state or diabetes. In addition, the persistence of inhibition through the mitochondrial isolation procedure supports the view that inhibition is through formation of a stable, covalent bond. Turnbull *et al.* (37) have reported decreased ability to oxidize palmitoyl-CoA but not palmitoyl carnitine by liver mitochondria from rats treated for 3 months with a related compound, POCA, though direct measurements of CPT activity were not made. The specificity of Me-TDGA for inhibiting CPT-A is reflected in previous *in vivo* and *in vitro* studies using *db/db* mice (12), rat hepatocytes (10, 18), hemidiaphragms (34), soleus muscle (38), working perfused hearts (13), dog skeletal muscle (38), and guinea pig liver (30) in which Me-TDGA and its derivatives inhibited the oxidation of long-chain fatty acids but not short-chain fatty acids or palmitoyl carnitine. Furthermore, this explains why the drug's pharmacologic effects are reversed upon provision of the short-chain fatty acids (13, 30, 39). Thus, the pharmacologic action of Me-TDGA and related compounds may be totally explained as a consequence of the inhibition of fatty acid oxidation by Me-TDGA's intracellular CoA metabolite at the stage of CPT-A.

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