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THE EFFECT OF METHYL-2-TETRADECYLGLYCIDATE (McNEIL 3716) ON HEART MITOCHONDRIAL METABOLISM IN RATS

E. BACHMANN, E. WEBER and G. ZBINDEN*

Institute of Toxicology, Swiss Federal Institute of Technology and University of Zürich, Schorenstrasse 16, 8603 Schwerzenbach, Switzerland

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Abstract—Methyl-2-tetradecylglycidate (MTG), one of a new class of hypoglycemic agents, given to healthy rats, prompted uncoupling of oxidative phosphorylation in heart mitochondria (measured *ex vivo*) without a concomitant effect on mitochondrial electron transfer reactions. At the same time heart creatinephosphate-kinase was inhibited and subsequently the semipermeability of the inner mitochondrial membrane was impaired as demonstrated by an influx of creatine. The triglyceride and total phospholipid content of heart tissue and its mitochondria showed a transient elevation. The hearts were enlarged, flabby and discoloured and had dilated ventricles. These effects could be the account of an adverse effect of MTG on the heart energy metabolism.

For a long time, diabetes mellitus was thought to be mainly a disorder of carbohydrate, and only secondarily a disturbance of fatty acid metabolism. However, the aberrations of carbohydrate metabolism could be reproduced in normal tissue by excessive oxidation of fatty acids [1–3], giving rise to the hypothesis of "Randle's glucose–fatty acid cycle". The mode of action of several hypoglycemic agents, i.e. hypoglycin, 4-pentenoic acid, (+)-decanoylcarnitine, and alpha-bromo fatty acids *in vitro* was found [4–6] to be the correction of excessive rate of long chain fatty acid oxidation, thus forcing increased use of glucose as energy source [7]. Lack of oral activity or toxicity prevented the therapeutic application of these agents [7, 8].

Methyl-2-tetradecylglycidate (McNeil 3716, MTG) was the result of a long search for fatty acid analogs that would be orally active inhibitors of long chain fatty acid oxidation and might, thus, be useful to correct hyperglycemia in diabetes mellitus [7, 8, 10]. It was expected that the drug would cause a rise in plasma free fatty acids (FFA), and stimulate lipid deposition in the liver and other organs. FFA are known to damage mitochondrial membranes and to uncouple oxidative phosphorylation [11]. Since the resulting interference with energy metabolism would be of particular concern for the cardiac function, we decided to study the effects of MTG on the mitochondrial metabolism of the rat heart.

MATERIALS AND METHODS

Female rats (Iva : SIV50) with a starting weight of 100–110 g were housed in Macrolone® cages with wood shavings as bedding. The animals had at all times free access to food (Kliba 343 rat pellets) and water. The environmental conditions were: room temperature 22 ± 1°, relative humidity 55 ± 10%, 12 hr light–dark cycle.

MTG was dissolved in Mazola corn oil and given twice daily (0800 and 1500 hr) per os, 5 days a week, for 4 weeks at doses of 2 × 10 mg/kg (LD) and 2 × 25 mg/kg (HD). The control group received 2 × 1 ml/kg/day of corn oil per os, representing the same volume of oil that was given to the drug-treated animals. Another control group received 2 × 1 ml/kg/day of 0.95% NaCl per os.

Twice a week, 17 hr after the second and eighth dose of the week, 3 rats per group were withdrawn at random. The animals were stunned and sacrificed by cervical dislocation. The hearts were excised immediately and placed into cold sucrose (0.25 M sucrose–0.01 M Tris–HCl pH 7.4) solution. Mitochondria were isolated from pooled organs by differential centrifugation following homogenization of the tissue in a Potter–Elvhjem glass homogenizer fitted with a Teflon pistill. Electron transfer reactions (oxygen consumption) coupled to oxidative phosphorylation of mitochondria was measured in a Gilson oxygraph (Gilson Medical Electronics, Middleton, WI, U.S.A.) equipped with a Clark electrode. Succinate, β-hydroxybutyrate, 2-oxoglutarate and DL-glutamate were used as substrates [12].

Creatinephosphate-kinase (E.C. 2.7.3.2.) and creatine content of heart mitochondria were also determined. The activity of creatinephosphate-kinase was measured according to the method of Jacobus and Lehninger [13]. Heart mitochondria were incubated with creatinephosphate and ADP in the presence of Mg^{2+} at 30° for 0–10 min. The reaction was stopped by adding aliquots of the incubation mixture to alpha-naphthol. The method of Eggleton *et al.* [14] was applied to determine the liberated creatine. Zero-time samples were used to measure the amount of creatine in the matrix of mitochondria.

* To whom correspondence should be addressed.

Table 1. The effect of MTG, given per os to rats, on the oxygen consumption and oxidative phosphorylation of heart mitochondria, measured *ex vivo*

Doses	Control (oxygen consumption nmoles/mg/min)				Corn oil 2 ml/kg/day (oxygen consumption nmoles/mg/min)				MTG 2 × 10 mg/kg/day (oxygen consumption nmoles/mg/min)				MTG 2 × 25 mg/kg/day (oxygen consumption nmoles/mg/min)			
	III	IV	RCI	ADP/O	III	IV	RCI	ADP/O	III	IV	RCI	ADP/O	III	IV	RCI	ADP/O
2	76	25	3.0	2.98	71	25	2.84	2.90	74	25	2.96	2.59	76	24	3.2	2.52
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	79	28	2.8	2.95	71	23	3.1	2.84	72	24	3.0	2.61	77	22	3.5	2.55
18	81	27	3.0	2.94	75	25	3.0	2.73	75	22	3.4	2.32	76	21	3.6	2.29
22	77	28	2.8	2.95	75	22	3.4	2.63	78	22	3.5	2.43	75	26	2.9	2.33
28	71	23	3.1	2.95	75	24	3.1	2.61	76	20	3.8	2.32	73	23	3.2	2.22
32	91	31	2.95	2.99	81	28	2.9	2.57	74	20	3.7	2.29	78	20	3.9	2.19
38	85	31	2.75	3.00	82	26	3.15	2.59	72	22	3.5	2.20	84	22	3.8	2.12

The data were obtained with β-OH-butyrate as substrate, but they are representative for all other substrates tested (succinate, 2-oxoglutarate and DL-glutamate). The results are averages of three independent determinations.
III and IV = state 3 and 4 respiration rates according to Chance.
RCI = respiratory control index.

Lipids were measured in tissue homogenate and in mitochondria. Phospholipids were determined according to the method of Jouanel *et al.* [15], using phosphatidylcholine as standard. For triglycerides the method of Kessler *et al.* [16] with triolein as standard was used. Protein determinations on aliquots of the probes were done by the Biuret method [17] using small amounts of deoxycholate to solubilize the samples.

All chemicals used were of reagent grade. Nucleotides, creatine, creatinephosphate and the lipid standards were purchased from Sigma Chemical Co., St. Louis, MO. MTG = Methyl-2-tetradecylglycidate (McNeil 3716) was a gift from Byk-Gulden Chem. Co., Konstanz, F.R.G.

RESULTS

The treatment with MTG was well tolerated. Weight gain was slightly reduced in the HD animals only. Heart mitochondria, isolated from rats treated with MTG, showed a dose-dependent, progressive uncoupling of oxidative phosphorylation. The results are summarized in Table 1. The most remarkable finding was the initial drop in ADP/O ratios after only two doses to a low of 80–85% of the controls. The final levels attained after 4 weeks of drug treatment were 75% for LD and 65% for HD, i.e. 15% and 25% respectively, below the effect of the corn oil vehicle. Electron transfer activities, state 3 and 4 respiration rates as well as the respiratory control index (RCI) were not affected in heart mitochondria throughout the 4 week treatment period. The data shown were obtained with β-hydroxybutyrate as substrate, but they are representative for all other substrates (succinate, 2-oxoglutarate and DL-glutamate) used.

Creatine content of heart mitochondria, expressed as percent of the saline controls, are shown in Fig. 1. The corn oil vehicle gave rise to a slight increase in creatine content (about 20%), whereas mitochondria from LD and HD animals contained progressively more creatine, reaching a level of 170% for the LD and 200% for the HD. The creatine content of heart mitochondria, isolated from the saline controls was $7.16 \pm 0.18 \times 10^{-6}$ M/mg protein (N = 14).

Mitochondria from control animals had a creatinephosphate-kinase activity of $9.19 \pm 0.72 \times 10^{-4}$ mole/min/mg protein (N = 140). The results obtained with the drug treated animals, expressed as percent of the saline controls, are presented in Fig. 2. Corn oil reduced the heart mitochondrial creatinephosphate-kinase by about 10%, a level reached in the second week of treatment and remaining constant thereafter. The LD animals showed progressive reduction in creatinephosphate-kinase activity, reaching a low of 75% of the controls after 4 weeks. In the HD group the enzyme activity fell quite rapidly to 70% after only 6 days of drug treatment, to remain at this level for the subsequent 3 weeks.

At autopsy yellow discoloration, suggesting fatty infiltration, was noticed in livers and kidneys. The hearts had a greyish and flabby appearance, the ventricles were dilated and the absolute and relative heart weights were increased (data not shown). The results of the analysis of total phospholipids are summarized on Table 2. No difference was seen

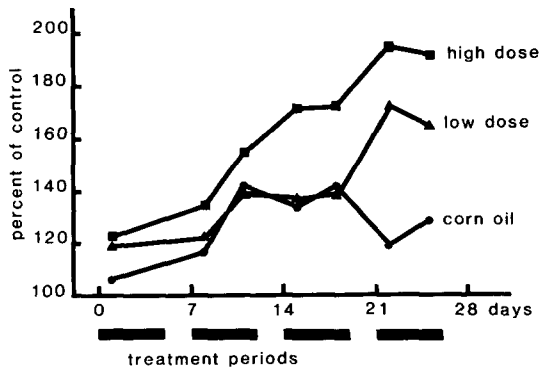

Fig. 1. The effect of MTG, given per os to rats, on the creatine content of heart mitochondria, expressed as percent of the saline controls = $7.16 \pm 0.18 \times 10^{-6}$ M/mg protein, N = 14.

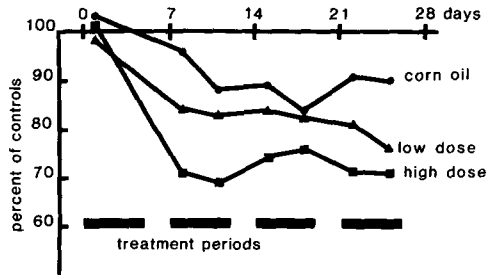

Fig. 2. The effect of MTG, given per os to rats, on the creatinephosphate-kinase of heart mitochondria (measured *ex vivo*). The activities were expressed as percent of the saline controls = $9.19 \pm 0.72 \times 10^{-4}$ mole/min/mg protein, N = 140.

throughout the whole period between the saline control and the group receiving corn oil.

Phospholipid levels in heart tissue increased immediately with both doses reaching 180% (LD) and 250% (HD) in the second week. During the third and fourth week the phospholipid levels returned gradually to (LD) or close to (HD) control levels. Heart mitochondrial phospholipid content followed the same pattern, but did not reach the same high levels measured in myocardial tissue.

Triglyceride concentration measured in heart tissue and heart mitochondria are shown in Table 3. Corn oil alone lowered the triglyceride level in heart tissue significantly to 55% of the saline controls, an effect not depending on time and the number of applied doses. In the MTG treated animals no consistent changes were seen. In the myocardial tissue triglyceride concentrations were higher than in the corn oil controls, but there was no clear-cut dose- and time-effect relationship. In mitochondria a moderate and transient decrease in triglyceride concentrations was noted.

DISCUSSION

MTG and related compounds [7, 19, 22, 23] represent a new class of hypoglycemic agents whose mechanism does not involve the pancreas. Their primary mode of action is inhibition of long chain fatty acid oxidation by competitive binding to CoA, and probably also gluconeogenesis, thus offering a promising new approach to the treatment of diabetes. Previous studies [19, 22, 23] showed a significant influence of these hypoglycemic agents only under conditions of diabetes.

Table 2. The effect of MTG, given per os to rats, on the content of total phospholipids in heart tissue (homogenate) and its mitochondria, measured as phosphatidylcholine

TOTAL PHOSPHOLIPIDS measured as phosphatidylcholine	HEART tissue		mitochondria	
	μg/mg protein			
Controls N=14 (NaCl + corn oil	0.216±0.032		0.420±0.022	
	LD	HD	LD	HD
2 doses	0.328	0.263	0.584	0.445
8 doses	-	-	-	-
12 doses	0.389	0.540	0.567	0.580
18 doses	0.339	0.339	0.537	0.651
22 doses	0.233	0.259	0.483	0.500
28 doses	0.216	0.263	0.550	0.643
32 doses	0.210	0.251	0.441	0.504
38 doses	0.218	0.266	0.445	0.504

Table 3. The effect of MTG, given per os to rats, on the content of triglycerides in heart tissue (homogenate) and its mitochondria, measured as triolein

TRIGLYCERIDES measured as triolein	HEART tissue		mitochondria	
	μg/mg protein			
NaCl controls N=7	6.588±0.366		19.716±0.889	
corn oil N=7	3.630±0.71		21.370±2.07	
	LD	HD	LD	HD
2 doses	6.27	5.00	20.10	27.08
8 doses	-	-	-	-
12 doses	13.90	5.89	21.72	22.33
18 doses	7.18	5.51	18.33	16.34
22 doses	7.12	4.72	17.75	18.94
28 doses	7.42	8.18	16.92	15.37
32 doses	12.48	8.94	17.86	18.50
38 doses	7.91	6.86	21.36	21.90

Giving MTG to healthy animals, progressive uncoupling of oxidative phosphorylation of heart mitochondria was observed. This disturbance of energy producing processes was probably the reason for the subsequent loss of semipermeability of the mitochondrial inner membrane, measured as the inability of the organelles to exclude creatine. The *in vitro* data of Tutwiler *et al.* [7] indicate that MTG does not inhibit the oxidation of short chain fatty acids, nor does it interfere with citric acid cycle activities. This is in accordance with observations made in this study, showing that electron transfer activities of heart mitochondria were not affected throughout the treatment period, using succinate, β-hydroxybutyrate, 2-oxoglutarate or gluatamate as substrates.

The drug not only interfered with the conservation step of the energy generated in the form of high-energy-bond formation (ATP), but also with the transfer reactions for these high-energy bonds through the mitochondrial membrane to the creatine in the cytosol as seen in the inhibition of the creatine-phosphate-kinase.

The inhibition of these enzymatic steps, both part of the high-energy-bond formation and transfer to the place of use (myofibrillar contraction) is likely to result in a reduction of the available energy. Whether this was serious enough to cause significant impairment of cardiac function, is difficult to evaluate. However, the flabby appearance of the hearts (loss of muscle tone), the enlargement of the organ and the dilatation of the ventricles are ominous signs, whose toxicological significance requires further investigation.

The content of triglycerides and total phospholipids increased not only in liver and kidneys (data not

shown) but also in the heart, although only in a transient fashion. There was no concomitant increase in plasma FFA (data to be published). Thus, it is unlikely that the FFA were responsible for the functional changes of the heart mitochondria. It is conceivable that MTG or a metabolite had a direct effect on heart mitochondrial membranes and the enzymes located there.

Previous studies [19, 22, 23] showed a significant metabolic influence of this class of hypoglycemic agents only under conditions of diabetes or fasting, which are accompanied by an elevation of plasma FFA. No effects were seen in normal, fed animals. In contrast, the changes in heart mitochondrial function seen in this study were obtained with normal animals, having free access to regular rat diet and water at all times, and with drug doses equal or only slightly higher than those needed to induce hypoglycemia in diabetic animals. Thus, they are not a consequence of the therapeutically desirable shift in carbohydrate and fatty acid metabolism, but must be regarded as a direct and toxicologically relevant effect on heart energy metabolism.

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