Original Articles

Hypolipidemic, Antiobesity, and Hypoglycemic-Hypoinsulinemic Effects of β,β′-Methyl–Substituted Hexadecanedioic Acid in Sand Rats

RUTH TZUR, GENE ROSE-KAHN, JONATHAN H. ADLER, AND JACOB BAR-TANA

Treatment of male sand rats kept on a balanced laboratory chow diet ad libitum with β,β′-tetramethyl–substituted hexadecanedioic acid (MEDICA 16) resulted in a hypolipidemic effect accompanied by an extensive reduction in adiposity, with a concomitant hypoglycemic-hypoinsulinemic effect. The overall effect was sustained as long as the drug was administered. The hypolipidemic effect of MEDICA 16 consisted of a 70 and 40% decrease in plasma triacylglycerols and cholesterol, respectively, and resulted from inhibition of liver lipogenesis and cholesterogenesis. Adipose reduction by MEDICA 16 treatment or calorie restriction consisted of a 75–90% decrease in the perirenal, omental, epididymal, and subcutaneous fat, with a 50% decrease in liver neutral lipids. The reduction in adiposity was accounted for by a respective decrease in the lipid content of individual adipocytes, with a concomitant decrease in the number of adipocytes of selected adipose tissues. The decrease induced in adiposity by MEDICA 16 treatment could not be accounted for by anorectic or cathartic effects of the drug. The hypoglycemic-hypoinsulinemic effect of MEDICA 16 consisted of amelioration of the tolerance of glucose with normalization of plasma insulin. It was accompanied by an eightfold increase in the number of insulin receptors in epididymal adipocytes, which was, however, counteracted by a decrease in their affinity for insulin. The receptor and postreceptor effects exerted by MEDICA 16 were similar to those of calorie restriction. The overall effect of MEDICA 16 in sand rats may reflect the pharmacological potential of MEDICA compounds in pathological hyperlipidemic-obesity-diabetic syndromes. *Diabetes* 37:1618–24, 1988

Long-chain fatty acids and their respective thioesters have repeatedly been reported to affect lipid metabolism by modulating key steps in lipid and lipoprotein synthesis and degradation. The modulating capacity of long-chain fatty acids, as opposed to their role as substrates, has initiated the design of nonmetabolic long-chain fatty acyl analogues to be exploited as hypolipidemic-antiobesity agents. β,β′-Methyl–substituted dicarboxylic acids (MEDICA) of C_{14}–C_{18} chain length [HOOC-CH_2-$C(CH_3)_2$-$(CH_2)_n$-$C(CH_3)_2$-CH_2-COOH, n = 8–12] appear to fulfill this role, with MEDICA 16 (n = 10) being the most potent of the concerned homologous series (1). Thus, the ω-carboxyl function interferes with the esterification of the dioic acid into lipid while still allowing for an ATP-dependent coenzyme A (CoA) thioesterification at either carboxylic end, and the β,β′-substitution prevents the β-oxidative catabolism of MEDICA compounds by either mitochondrial or peroxisomal systems. As a hypolipidemic drug in the rat, MEDICA 16 was found to potently inhibit liver ATP-citrate lyase, with a concomitant 80% inhibition of liver lipogenesis and cholesterogenesis (2). Inhibition of liver lipid synthesis resulted in a 60–70% decrease in plasma very-low-density lipoprotein (VLDL)-triacylglycerol and a 40–50% decrease in plasma VLDL-cholesterol under conditions of fat-free carbohydrate-rich feeding, where liver lipogenesis and cholesterogenesis constitute a major flux of liver lipid synthesis (1). MEDICA 16 was also found to act as a hypolipidemic effector under conditions of a balanced diet, which still allows for the production of lipoproteins from exogenous fatty acids and cholesterol (3,4). The 70% decrease in plasma chylomicrons-triacylglycerol observed under these conditions could be accounted for by an enhanced plasma catabolism of the triacylglycerol-rich lipoproteins due to a pronounced decrease in plasma apolipoprotein C III (3,4).

In the light of these features of MEDICA compounds, it became of interest to evaluate their potential as antiobesity agents in vivo in an animal model for obesity. The sand rat

From the Department of Biochemistry, Hebrew University–Hadassah Medical School, Jerusalem, Israel.

Address correspondence and reprint requests to J. Bar-Tana, Department of Biochemistry, Hebrew University Medical School, P. O. Box 1172, Jerusalem 91010, Israel.

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(*Psammomys obesus*) is the model of choice because of its spontaneous obesity and obesity-induced diabetes (5–12).

MATERIALS AND METHODS

Weaned male sand rats of the Hebrew University strain (13), weighing 66 ± 16 g, were either maintained on a laboratory chow diet (Amrod 935, Ambar, Hadera, Israel) fed ad libitum in the presence or absence of added 0.1% (wt/wt) of MEDICA 16 or were calorie restricted by being maintained on 4 g Amrod 935 per day supplemented ad libitum with fresh salt bush (*Atriplex halimus*) leaves. The Amrod 935 chow consisted of 11.7% (wt/wt) moisture, 50.4% (wt/wt) nitrogen-free extract (carbohydrate), 17.3% (wt/wt) protein, 4.0% (wt/wt) fat, 8.3% (wt/wt) fiber, and 8.1% (wt/wt) ash. The fresh salt bush consisted of 61.5% (wt/wt) moisture, 15.2% (wt/wt) nitrogen-free extract (carbohydrate), 5.0% (wt/wt) protein, 0.7% (wt/wt) fat, 9.9% (wt/wt) fiber, and 8.9% (wt/wt) ash. The animals were supplied with water ad libitum. The ad libitum chow consumption ranged from 10 ± 1 to 15 ± 1 g/day as a function of age. Animals maintained on calorie-restricted diet consumed 10–20 g/day of fresh salt bush leaves as a function of age. The overall calorie consumption of calorie-restricted animals was ~75% of the ad libitum chow ration. MEDICA 16 was administered by soaking the diet pellets in an ether solution of MEDICA 16 followed by exhaustive flash evaporation of the solvent.

Glucose tolerance was determined in nonfasting rats lightly anesthetized with 1% phenobarbital and subsequently injected with 100 mg glucose/100 g body wt i.p. in saline. Body temperature was maintained at 37°C by using heat lamps. Blood samples were collected from the retro-orbital venous plexus in heparin-coated capillary tubes 0, 1, 2, and 3 h after glucose loading. The blood samples were immediately cooled, centrifuged at 4°C, and plasma glucose was determined by the glucose oxidase method.

Plasma insulin, plasma glucose, and plasma lipids were determined in tail vein blood collected into heparinized capillary tubes or in blood from the abdominal vena cava of ether-anesthetized animals collected after they were killed. Plasma insulin was determined by a magnetic antibody immunoassay kit (Insulin Maia Kit, Serono, Coinsins, Switzerland). Plasma triacylglycerols and plasma cholesterol were determined enzymatically using Boehringer (Mannheim, FRG) kits 244473 and 172626, respectively.

^3H_2O incorporation into liver lipid in vivo was determined as previously described (1). Liver citrate, malonyl-CoA, and acetyl-CoA were determined as previously described (1).

Epididymal, perirenal, and omental adipocytes were prepared by collagenase type II treatment as described by Rodbell (14). To minimize cell disruption the tissues were digested for short incubation periods under conditions of gentle shaking. The diameter of the isolated fat cells was measured microscopically at ×400 with a micrometer fixed into the microscope eyepiece. Five replicate aliquot samples were used for each fat cell preparation, and ~100 cells were screened in each aliquot sample to yield the mean diameter ± SD for each adipocyte preparation. The lipid content per fat cell for each adipocyte preparation was calculated by regarding the fat cell as a sphere with a volume as determined above and filled with tripalmitin (density 0.86 g/ml). The number of adipocytes per adipose tissue was calculated by dividing the total lipid ester content of the tissue by the lipid content per fat cell (15). The total lipid ester content was determined by hydroxamate formation (16).

Insulin binding to epididymal adipocytes was determined under equilibrium conditions essentially as described by Gammeltoft and Gliemann (17). Thus, 2×10^5 cells were incubated in duplicate in 500 μl of Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) containing 20 mg/ml bovine serum albumin, 0.1 μCi [^{125}I]monoiodoinsulin (Nuclear Research Center, Negev, Israel), and 0.5–160 ng unlabeled porcine insulin. After incubation for 45 min at 37°C, a 200-μl aliquot of the cell suspension was transferred in duplicate to a plastic microtube containing 100 μl silicone oil (type 350, Merck, Darmstadt, FRG; sp gr 0.97 g/ml) and was centrifuged at maximal speed for 40–60 s in a Beckman 152 microfuge. The microtube was then cut through the silicone oil layer and the top and bottom layers were counted for radioactivity. Nonspecific binding was determined in the presence of an excess of unlabeled porcine insulin and was subtracted from the total bound count to yield the specific binding of insulin. Generally, the bound/free versus bound Scatchard plots were curvilinear, having an upward concavity with clearly distinguished high- and low-affinity sections. The apparent dissociation constant and the number of insulin receptors were derived by considering the high-affinity section of the Scatchard plot.

Glucose incorporation into adipose glycogen was determined by incubating a weighted piece (~150 mg) of the interscapular adipose tissue in 1.0 ml of KRB buffer (pH 7.4) containing 16 mM of [U-^{14}C]-D-glucose (New England Nuclear, Boston, MA; sp act 0.625 Ci/mol) in the presence or absence of porcine insulin. After incubation for 3 h at 37°C the tissue was washed in KRB buffer, dried, and extracted in isopropanol/heptane/1 N H_2SO_4 (40:10:2). The lipid-de-

TABLE 1
Antilipogenic-hypolipidemic effect of MEDICA 16 in sand rats fed ad libitum

	Untreated	MEDICA 16–treated
Plasma triacylglycerol (mg/dl)	205.0 ± 29.0 (6)	62.0 ± 15.0 (7)
Plasma cholesterol (mg/dl)	69.0 ± 7.0 (6)	39.6 ± 7.0 (7)
Liver lipids (μeq/g)	224.0 ± 24.0 (6)	133.0 ± 13.0 (7)
^3H_2O incorporation into liver lipids (μmol $^3H_2O \cdot g^{-1} \cdot 120\ min^{-1}$)		
Triacylglycerols	16.3 ± 4.4 (4)	5.1 ± 2.0 (4)
Phospholipids	4.4 ± 1.0 (4)	8.3 ± 1.9 (4)
3-β-Hydroxysterols	1.4 ± 0.2 (4)	0.4 ± 0.1 (4)
Total lipids	22.8 ± 4.4 (4)	14.5 ± 2.7 (4)
Liver citrate (nmol/g)	174.0 ± 25.0 (3)	69.5 ± 3.5 (3)
Liver acetyl-CoA (nmol/g)	132.0 ± 30.0 (3)	35.5 ± 8.0 (3)
Liver malonyl-CoA (nmol/g)	20.0 ± 3.0 (3)	5.0 ± 3.0 (3)

Values are means ± SD with number of animals in parentheses. Weaned male sand rats were treated by 0.1% (wt/wt) MEDICA 16 for 140 days as described in MATERIALS AND METHODS. Plasma triacylglycerol, plasma cholesterol, liver lipids, liver intermediate metabolites, and the incorporation of ^3H_2O into liver lipids were determined as described in MATERIALS AND METHODS. Liver weight at death was 7.6 ± 0.5 g (n = 6; 3.7% body wt) and 10.9 ± 2.0 g (n = 7; 7.0% body wt) for untreated and MEDICA 16–treated rats, respectively. All MEDICA 16–treated values significantly different ($P < .01$) from respective values of untreated rats.

TABLE 2
Adipose reduction by MEDICA 16 in sand rats

	Epididymal fat			Perirenal fat			Omental fat		
	Untreated	Calorie restricted	MEDICA 16 treated	Untreated	Calorie restricted	MEDICA 16 treated	Untreated	Calorie restricted	MEDICA 16 treated
Tissue weight (g)	3.8 ± 1.4	1.3 ± 0.3*	1.2 ± 0.5*	2.7 ± 1.3	0.7 ± 0.9*	0.5 ± 0.2*	1.7 ± 0.5	0.3 ± 0.1*	0.2 ± 0.1*
Tissue lipid content (mg/tissue)	1840 ± 583	522 ± 158*	315 ± 139*	1004 ± 601	273 ± 82*	85 ± 52*	449 ± 144	69 ± 39*	30 ± 20*
Adipocyte lipid content (μg/cell)	0.49 ± 0.13	0.15 ± 0.03*	0.13 ± 0.05*	0.66 ± 0.23	0.26 ± 0.04*	0.22 ± 0.05*	0.49 ± 0.28	0.17 ± 0.03*	0.11 ± 0.03*
Tissue cell number ($\times 10^{-6}$)	3.9 ± 1.2	3.5 ± 0.8	3.1 ± 1.3	1.5 ± 0.6	1.0 ± 0.2	0.4 ± 0.0*	1.1 ± 0.6	0.4 ± 0.2*	0.2 ± 0.2*

Values are means ± SD. Weaned male sand rats were maintained for 90–95 days on Amrod 935 fed ad libitum in the presence (n = 17) or absence (n = 19) of 0.1% (wt/wt) MEDICA 16 or were calorie restricted (n = 17) as described in MATERIALS AND METHODS. Total lipid content, adipocyte lipid content, and tissue cell number were determined for the epididymal, perirenal, and omental fats as described in MATERIALS AND METHODS. Tissue weight, tissue lipid content, adipocyte lipid content, and tissue cell number of the adipose tissues are combined from right and left pads.
*Significantly different ($P < .01$) from respective untreated value.

pleted tissue was then digested in 33% of boiling KOH for 20 min, and glycogen was precipitated by absolute ethanol at −20°C as described by Gutman et al. (18). The precipitate was dissolved in 0.5 ml H_2O and was counted in 40% Lumax in toluene.

Significance was analyzed by the Mann-Whitney U test.

MEDICA 16 was synthesized as previously described (1). Collagenase type II was from Sigma (St. Louis, MO). Crystalline porcine insuln was provided by Lilly (Indianapolis, IN).

RESULTS

Hypolipidemic effect. The overall hypolipidemic effect of MEDICA 16 in sand rats was essentially similar to that previously observed in albino rats (1,3) and consisted of a 70 and 40% decrease in total plasma triacylglycerol and cholesterol, respectively (Table 1). The observed hypolipidemic effect could be accounted for by 70% inhibition of liver lipogenesis and cholesterogenesis as determined by the incorporation of ^3H_2O into liver triacyglycerol–fatty acids and liver 3-β-hydroxysterols (Table 1), whereas the esterification of glycerol into neutral lipids in the presence of added palmitate remained unaffected (not shown). Inhibition of liver lipogenesis and cholesterogenesis could be ascribed to inhibition of liver ATP-citrate lyase (2) with a concomitant drastic reduction in liver acetyl-CoA and malonyl-CoA content (Table 1). However, in contrast with albino rats, where the content of liver citrate remained essentially unaffected under conditions of treatment with MEDICA 16 (1), the content of liver citrate in MEDICA 16–treated sand rats decreased twofold, although less remarkably than that of acetyl-CoA and malonyl-CoA. It is worth noting that the decrease in liver triacylglycerol and 3-β-hydroxysterol synthesis in MEDICA 16–treated sand rats was quite pronounced even when calculated on the basis of whole-liver fluxes, and in spite of the significant increase in liver weight of treated animals (Table 1).

Antiobesity effect. Treatment of sand rats fed ad libitum by MEDICA 16 resulted in an extensive decrease in the content of neutral lipids of the epididymal, perirenal, and omental fats (Table 2). Adipose reduction in MEDICA 16–treated sand rats was similar to that of calorie-restricted animals maintained on 70–80% of their ad libitum calorie ration. Thus, the final reduction in fat in MEDICA 16–treated rats amounted to 75, 92, and 93% for the epididymal, perirenal, and omental fats, respectively. In many cases the omental fat could hardly be detected in MEDICA 16–treated animals, whereas it could always be definitely recognized under conditions of calorie restriction. Adipose reduction by MEDICA 16 was primarily due to a 60–80% decrease in the neutral lipid content of individual adipocytes of the three adipose tissues studied, whereas the cell number was selectively affected. Thus, the perirenal and omental fats appeared to lose ~75% of their mature adipocytes in the course of MEDICA 16 treatment, whereas the number of cells in the epididymal fat remained essentially unaffected. Hence, the decrease in total lipid of the perirenal and omental fats could be accounted for by a decrease in the cellular lipid content of individual adipocytes as well as in the cell number of both tissues. On the other hand, the decrease in total lipid of the epididymal fat was not accompanied by a significant decrease in the number of epididymal fat cells and could be totally ascribed to the depletion of intracellular fat. The reduction in adiposity of MEDICA 16–treated sand rats was not only confined to the adipose tissues described but included subcutaneous adipose tissues (not shown) as well as fat deposits within splanchic organs, e.g., liver (Table 1).

Adipose reduction by MEDICA 16 was accompanied by a respective decrease in body weight that could be accounted for by the body composition of sand rats (13) and the antiobesity effect of the drug (Table 3). The weight-reductive effect of MEDICA 16 was somewhat more extensive than that effected by calorie restriction. The decrease in adiposity and body weight of MEDICA 16–treated sand rats could not be ascribed to a decrease in the net calorie intake as a result of either anorectic or cathartic effects of the drug. Thus, the daily ad libitum food consumption of MEDICA 16–treated sand rats was similar to that of untreated age-

TABLE 3
Weight reduction by MEDICA 16 in sand rats

Weight (g)	Untreated	Calorie restricted	MEDICA 16 treated
Initial	62 ± 11	66 ± 16	69 ± 16
Final	195 ± 16	171 ± 13	161 ± 14*
Gain	133 ± 19	105 ± 11	92 ± 18*

Values are means ± SD. Conditions as in Table 2.
*Significantly different ($P < .01$) from respective untreated value.

TABLE 4
Effect of MEDICA 16 on plasma glucose and insulin in sand rats

	Untreated	Calorie restricted	MEDICA 16 treated
Plasma glucose (mg/dl)	126 ± 38	95 ± 25	90 ± 18
Plasma insulin (μU/ml)	220 ± 63	52 ± 16*	48 ± 15*

Values are means ± SD. Conditions as in Table 2. Plasma glucose and insulin concentrations were determined as described in MATERIALS AND METHODS.
*Significantly different ($P < .01$) from respective untreated value.

matched animals kept under the conditions described in Tables 2 and 3 (10 ± 1 g chow/day for weaned animals weighing 70 g; 15 ± 1 g chow/day for older animals weighing 150–200 g). Similarly, the consistency of stool remained unaffected by MEDICA 16 treatment. Thus, the decrease in adiposity appeared to reflect a metabolic modality rather than a decrease in net calorie intake.

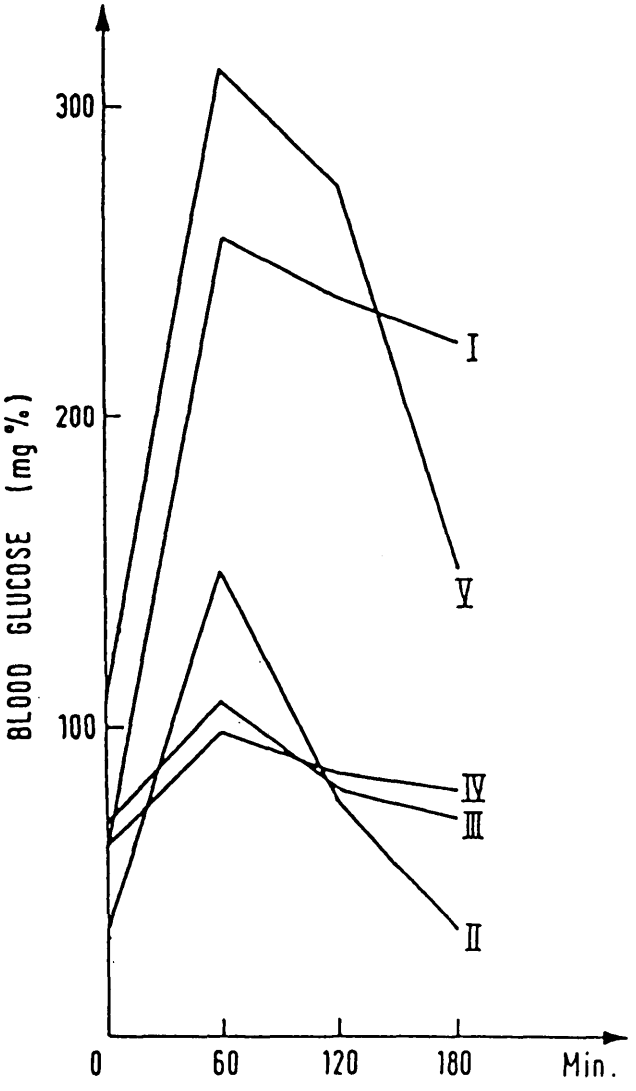

FIG. 1. Glucose tolerance in MEDICA 16–treated sand rats. Conditions as in Table 5. Glucose tolerance curves presented are those obtained with one representative animal (rat 3 in Table 5). I, pretreatment; II, 27th day of MEDICA 16 treatment; III, 71st day of MEDICA 16 treatment; IV, 34th day after cessation of MEDICA 16 treatment; V, 53rd day after cessation of MEDICA 16 treatment.

Hypoglycemic-hypoinsulinemic effect. MEDICA 16 treatment of sand rats maintained on laboratory chow ad libitum resulted in some decrease in plasma glucose, with a concomitant significant decrease in plasma insulin (Table 4). The plasma insulin level approached that of calorie-restricted animals and was in the range of that observed in normal albino rats of the Hebrew University strain. Moreover, the plasma insulin values in MEDICA 16–treated or calorie-restricted animals were within a limited range of 20–60 μU/ml, whereas the plasma insulin prevailing in untreated sand rats fed ad libitum varied within a broad range of 100–500 μU/ml.

Sand rats used in this study could be divided into two subgroups with respect to their glucose tolerance in response to an intraperitoneal glucose load. In ~75% of the animals the sum of the glucose values determined at 0, 1, 2, and 3 h after the injection of 100 mg glucose/100 g body wt i.p. was <400 mg/dl, in the range of that of calorie-restricted animals, whereas in ~25% of the population the sum amounted to 700–1000 mg/dl, thus defining a pathological tolerance pattern. The tolerance of glucose in sand rats selected for their decreased glucose tolerance was dramatically improved by MEDICA 16 treatment (Fig. 1; Table 5). Thus, as shown for the individual case of Fig. 1, the pathological glucose tolerance pattern of the untreated animal reverted back to normal after 27 days of treatment, and the normal pattern was sustained as long as treatment was maintained as well as during a 1-mo period after the suspension of the drug from the diet (Fig. 1; Table 5). In contrast to the improved glucose tolerance in sand rats treated by MEDICA 16, streptozocin-induced hyperglycemia in albino rats could not be improved by MEDICA 16 treatment. Thus, the plasma glucose in untreated streptozocin-induced diabetic and MEDICA 16–treated albino rats amounted to 610 ± 46 and 578 ± 13 mg/dl (means ± SD; $n = 6$), respectively, after 1 wk of treatment with 0.25% (wt/wt) of MEDICA 16 in the diet. Hence, the improved performance in MEDICA 16–treated sand rats required the presence of insulin and had to be ascribed to an improved peripheral handling of the glucose load.

The putative decrease in the peripheral resistance to insulin in MEDICA 16–treated sand rats was assessed by evaluating the number of insulin receptors in epididymal adi-

TABLE 5
Glucose tolerance in MEDICA 16–treated sand rats

Rat no.	Pretreatment	Treatment	Posttreatment
1	779, 1082	364	
2	947, 980, 958	442	
3	790	296, 329, 326	849
4	992	358, 336, 465	1049
5	867	434, 257, 388	470
6	717	555, 555	

Values are in milligrams per deciliter. Six sand rats were selected out of 25 animals for their distinctive pathological glucose tolerance and were treated with 0.1% (wt/wt) MEDICA 16 for 71 days. Pretreatment and treatment periods were followed by normal Amrod 935 diet (posttreatment). Each glucose tolerance test is represented by sum of glucose values (mg/dl) determined at 0, 1, 2, and 3 h. Duplicate or triplicate numbers refer to repeated glucose tolerance tests made during pretreatment or treatment periods.

TABLE 6
Epididymal insulin receptors in MEDICA 16–treated sand rats

	Untreated	Calorie restricted	MEDICA 16 treated
Insulin receptors/cell ($\times 10^{-4}$)	0.3 ± 0.1 (9)	2.4 ± 0.4 (11)*	2.6 ± 0.6 (8)*
K_d(M × 10^9)	0.4 ± 0.0	3.2 ± 0.8*	3.8 ± 0.3*

Values are means ± SD with number of animals in parentheses. Conditions as in Table 2.
*Significantly different ($P < .01$) from respective untreated value.

pocytes of untreated and MEDICA 16–treated animals. As shown in Table 6, MEDICA 16 treatment and calorie restriction resulted in eight- to ninefold increase in the number of insulin receptors per cell. The increase in insulin receptors effected by either MEDICA 16 or calorie restriction was, however, counteracted by a decrease in their affinity for insulin (Table 6).

Discrete postreceptor effects of MEDICA 16 were investigated by studying the incorporation of glucose into glycogen in the interscapular adipose tissue of sand rats maintained under the three intervention modes used (Table 7). Even in the absence of added insulin, the overall incorporation of glucose into adipose glycogen was increased fivefold, both in calorie-restricted and MEDICA 16–treated animals, compared with that of untreated rats fed ad libitum. Insulin was found to increase the incorporation rate of glucose by 20% in the treated and calorie-restricted animals, whereas it was essentially ineffective in untreated sand rats fed ad libitum.

DISCUSSION

Treatment of male sand rats kept on a balanced chow diet ad libitum with MEDICA 16 serving as a model compound for substituted long-chain β,β-dicarboxylic acids resulted in an extensive hypolipidemic effect accompanied by a reduction in adiposity, with a concomitant hypoglycemic-hypoinsulinemic effect. The overall effect was sustained as long as the drug was administered.

The hypolipidemic effect of MEDICA 16 in sand rats appears to be essentially similar to that reported in albino rats (1–3). Thus, in both species the hypolipidemic effect could be accounted for by inhibition of liver lipogenesis and cholesterogenesis as inferred from the incorporation of ^3H_2O into liver triacylglycerol–fatty acids and 3-β-hydroxysterol. Because the inhibition of the two synthetic pathways by MEDICA 16 was similar, it could be ascribed to inhibition of a metabolic step common to both. In the light of the established inhibition of liver ATP-citrate lyase by MEDICA 16 (2) and in line with the fourfold decrease in liver acetyl-CoA and malonyl-CoA content reported herein, the inhibition of liver lipogenesis and cholesterogenesis in MEDICA 16–treated sand rats is presumably accounted for by a crossover point at the ATP-citrate lyase step resulting in limitation of cytosolic acetyl-CoA for liver lipid synthesis.

The liver weight of MEDICA 16–treated sand rats was remarkably increased compared with that of untreated animals, in terms of absolute weight and relative to body weight (Table 1). The increase in liver weight was similar to that previously observed in albino rats treated with MEDICA 16 (19) and reflects the peroxisome proliferative capacity of the drug in rodents (19,20). The hypertrophic-hyperplastic effect initiated by MEDICA 16 acting as a peroxisome proliferator may also account for the observed increase in the relative amount of fatty acids channeled into phospholipids (21). Thus, the lipogenic flux culminating in phospholipids approached values of 19 and 57% of the total lipogenic flux in untreated and MEDICA 16–treated sand rats, respectively (Table 1).

The reduction in adipose fat in MEDICA 16–treated sand rats was characterized by its extensive scope and specificity with respect to the adipose tissues affected. Adipose reduction by MEDICA 16 was reversible, and elimination of the drug from the diet resulted in a rapid gain of adipose fat (R.T., unpublished observations). Because in sand rats, as opposed to albino rats, adipose tissue lipogenesis plays only a minor role in the overall synthesis of adipose fat (22), the inhibition of liver lipogenesis by MEDICA 16 presumably plays a causal role in adipose fat reduction by MEDICA 16. Furthermore, because adipose fat storage in sand rats is associated so much with liver triacylglycerol secretion (8) and the availability of plasma lipoproteins (22), the hypolipidemic effect induced by MEDICA 16 may be realized as the direct etiological cause for the adipose fat reduction observed. Moreover, as the K_m value of adipose lipoprotein lipase for plasma triacylglycerol is ~0.7 mM (23) and because the triacylglycerol concentrations prevailing in untreated and treated sand rats (2 and 0.7 mM, respectively) are in the range of the K_m value, the intravascular hydrolysis of plasma triacylglycerol by adipose lipoprotein lipase proceeds close to a first-order rate within the concerned range

TABLE 7
Glucose incorporation into adipose glycogen in MEDICA 16–treated sand rats

	Untreated	Calorie restricted	MEDICA 16 treated
Without insulin	208 ± 117 (3)	971 ± 512 (4)*	1117 ± 730 (5)*
With insulin	223 ± 79 (3)	1162 ± 833 (4)*†	1408 ± 860 (5)*†

Values are ng glucose · g^{-1} tissue · 3 h^{-1} (means ± SD), with number of animals in parentheses. Conditions as in Table 2, with and without 50 ng added insulin.
*Significantly different ($P < .01$) from respective untreated value.
†Significantly different ($P < .05$) from respective value without insulin.

of plasma triacylglycerol. Hence, the threefold decrease effected by MEDICA 16 in plasma triacylglycerol is expected to result in a respective decrease in the uptake of plasma lipids for adipose storage. It is worth noting that the overall activity of adipose lipoprotein lipase was found to remain unaffected in MEDICA 16–treated albino rats (R.T., unpublished observations).

The overall reduction effected by MEDICA 16 in adipose fat in the absence of either a concomitant decrease in net calorie intake or fat accumulation within splanchnic organs can only be made possible by expenditure of the calories spared from storage in adipose tissue. Indeed, MEDICA 16 treatment was recently observed to induce a 1.4-fold increase in the resting metabolic rate of albino rats (R.T., E. Smith, J.B.-T., unpublished observations), which may account for the overall energy expenditure of MEDICA 16–treated animals. This observed increase in basal oxygen consumption corroborates our previous results in which the irreversible disposal rate of glucose into carbon dioxide was found to be increased 1.3-fold in MEDICA 16–treated rats, and the observed increase could account for the glucose carbons spared from net lipid synthesis in the treated animals (1). The mechanism responsible for the MEDICA 16–induced increase in energy expenditure remains to be investigated.

Adipose reduction induced in sand rats by either MEDICA 16 treatment or calorie restriction differs from that induced by calorie restriction in albino rats with respect to the changes observed in the number of adipocytes of selected adipose tissues. Thus, calorie restriction of albino rats was repeatedly reported to leave the number of gonadal, inguinal, retroperitoneal, and subcutaneous adipocytes unchanged and to affect only their cellular lipid content (24,25). Similarly, the number of total adipocytes in lean sand rats was previously reported to remain unchanged under conditions of calorie-restriction–induced reduction in the total dissectable fat of obese animals (26). The epididymal tissue of our sand rats appears to follow the albino rat example (Table 2). On the other hand, the number of omental adipocytes was observed here to be significantly reduced by calorie restriction or MEDICA 16 treatment, and MEDICA 16 treatment also resulted in a significant and prompt reduction in the number of retroperitoneal adipocytes (Table 2). It is worth noting that the reduction in omental adipocytes observed here under conditions of calorie restriction could not be previously detected because the total dissectable fat rather than individual adipose tissues was subjected to analysis (26), and the mesenteric fat constitutes only a minor portion of the overall dissectable fat. Hence, in contrast with albino rats, in which in any given genetic setting the total number of adipocytes cannot be reduced once they are formed (27), the number of adipocytes in selected adipose tissues of sand rats may be modulated in both directions by either pharmacological or dietary means.

The hypoglycemic-hypoinsulinemic effect of MEDICA 16 was evaluated here in sand rats maintained at their normoglycemic-hyperinsulinemic stage (11,12) before the development of the fulminant ketotic-diabetic syndrome (6). In contrast with the previously reported lack of insulin receptors in isolated hepatocytes of sand rats (28), the epididymal insulin receptors could still be detected in the obese hyperinsulinemic animals studied. Furthermore, in line with previously reported observations made in other animal models of obesity (29–31), the peripheral resistance to insulin in untreated sand rats was characterized by a pronounced decrease in their epididymal insulin receptors compared with that of calorie-restricted animals. Note, however, that the peripheral resistance to insulin of the obese untreated sand rats could not be accounted for by reduction in their insulin receptors compared with that of calorie-restricted animals, because the eightfold decrease in the apparent number of insulin receptors observed in the obese animals was counteracted by an eightfold increase in their apparent affinity for insulin (Table 6). Similarly, the increase in the apparent number of insulin receptors in MEDICA 16–treated animals was counteracted by a respective decrease in their apparent affinity for insulin (Table 6). The observed increase in the apparent affinity for insulin in larger fat cells corroborates previous findings in Wistar rat epididymal adipocytes derived from aged animals (32) or by mesh filtration (33) but is, however, in contrast with that previously reported for Sprague-Dawley rat epididymal adipocytes (30). The difference could reflect a species-specific capacity for compensating for the decrease in the number of insulin receptors of larger fat cells by an increase in their affinity for insulin. The inverse relationship between the number of insulin receptors and their affinity for insulin could possibly be effected by the mode of embedding the insulin receptors in the plasma membrane as a function of cell size. Thus, a multimeric state of the insulin receptors resulting in a higher affinity for insulin with a concomitant masking of binding sites could perhaps be favored under conditions of an increase in the surface area of the plasma membrane.

By accounting for the apparent number of insulin receptors and their apparent affinity for insulin, the number of receptors actually occupied by insulin at the respective prevailing plasma insulin concentrations (Table 4) can be calculated to be 2100–2400 receptors per epididymal adipocyte for the untreated, MEDICA 16–treated, and calorie-restricted animals. The increase in glucose tolerance as well as in adipose glycogen synthase acitivity by either calorie restriction or MEDICA 16 treatment of obese sand rats, despite the similar occupancy of insulin-binding sites, may indicate that the diabetic pattern of obese sand rats and its reversion by calorie restriction or MEDICA 16 treatment presumably result from a modulation of postreceptor sites due to adipose reduction mediated by the two intervention modes. The sand rat follows in this respect previously reported animal model systems for obesity (29,31,34,35). Note, however, that the postreceptor effects of MEDICA 16 were still dependent on the availability of insulin, as MEDICA 16 could not replace insulin in streptozocin-induced diabetic albino rats. The relationship between the basal conditions set by dietary or pharmacological means and the action of insulin within the postreceptor domain still remains to be investigated.

The combined effects of MEDICA 16 in sand rats may reflect the pharmacological potential of MEDICA compounds in hyperlipidemic-obesity-diabetic syndromes.

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