Topiramate is an insulin-sensitizing compound in vivo with direct effects on adipocytes in female ZDF rats

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The present study was undertaken to determine whether Topiramate has insulin-sensitizing effects in female ZDF rats in vivo independently of decreased food intake or weight loss. The experiments were performed utilizing the hyperinsulinemic euglycemic clamp in conscious animals, combined with ex vivo and in vitro studies in skeletal muscle strips and isolated adipocytes. Our results demonstrate that Topiramate administration to insulin-resistant ZDF rats leads to insulin sensitization at the level of skeletal muscle and hepatic and adipose tissues, along with Topiramate showing direct insulin-sensitizing effects in adipose cells in vitro.

MATERIALS AND METHODS

Materials

Radioisotopes were purchased from ICN Radiochemicals (Costa Mesa, CA). All general reagents were purchased from Sigma Chemical (St. Louis, MO).

Animals Used

Eight-week-old female ZDF (Gmi-fafa) rats (Charles River) were selected for this study, because they display pronounced insulin resistance at this age and Topiramate treatment has been shown to lower blood glucose concentrations without appreciably large effects on body weight in this strain of rat. Rats (7 per group) were matched for weight, housed individually, and allocated to a control or a drug treatment group. Topiramate solutions were made up fresh each day by dissolving the powdered form of the drug in water. Rats in the fed state received Topiramate (100 mg/kg) in a volume of 3.6 ml by gavage one time daily for 7–9 days. The controls were given equal volumes of water at the same time every day (4:00 PM). In our preliminary in vivo studies (study 1), the body weights of control ZDF rats were equalized to those of Topiramate-treated animals by pair feeding. This was achieved by providing control rats with an equal amount of Purina lab chow (Rancho Cucamonga, CA) as consumed by Topiramate-treated rats each day. Food was temporarily removed from the cages at 8:00 AM (for 6 h) on days 4 and 7 so that basal blood glucose tests could be performed in 6-h-fasted rats. Food was returned to cages at 4:00 PM. Oral glucose tolerance tests (OGTTs) were performed with a 50% glucose solution (2 g/kg) in a volume of 3.6 ml by gavage one time daily for 7–9 days. The controls were given equal volumes of water at the same time every day (4:00 PM). In our second set of studies (study 2), we took advantage of the acute appetite-reducing effects of surgery, which occurred in (cannulated) ZDF rats on postsurgical days 1 and 2. Thus rats in study 2 underwent cather implantation before commencement of drug treatment, and 7–9 days later controls and Topiramate-treated rats achieved the same body weight without food restriction. Treated animals were administered Topiramate at the normal time on the day before clamping. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
local committee on animal care at the University of California, San Diego, approved the animal studies.

Surgery and Euglycemic Hyperinsulinemic Clamp Procedure

Rats were placed under single-dose anesthesia (42 mg/kg ketamine HCl, 5 mg/kg xylazine, 0.75 mg/kg acepromazine maleate) and cannulated with carotid artery cannulas for blood sampling and dual jugular vein cannulas for glucose and insulin infusions. Cannulas were tunneled underneath the skin, sutured to the outside, and encased in Silastic tubing (0.2 cm ID) for protection. Immediately after surgery, rats were provided with light warmth and permitted to recover. A euglycemic hyperinsulinemic clamp study was performed 7–9 days after cannulation. Six hours before the clamp procedure, food was withdrawn from the cages. Ninety minutes before clamp, animals were weighed and placed free in a modified metabolic chamber to acclimatize them to their surroundings. Basal samples were drawn at −60 and 0 min. A priming dose of 5 μCi of 0-[^3H]glucose (New England Nuclear, Boston, MA) was administered, and a tracer constant infusion of (0.167 μCi/min) was initiated at −60 min. After 60 min of tracer equilibration and basal sampling at time 0, glucose (variable infusion, 50% dextrose; Abbott Labs, Chicago, IL) and tracer plus insulin (25 mU·kg⁻¹·min⁻¹, Novlin R; Novo Nordisk, Copenhagen, Denmark) infusions were started simultaneously. Small blood samples (70 μl) were drawn every 10 min and immediately analyzed for glucose (YSI 2300 Glucose Analyzer; Yellow Springs Instrument, Yellow Springs, OH) to maintain the integrity of the clamp throughout the duration of the procedure. Larger blood samples (250 μl) were taken at basal and 120 min for determination of[^3H]glucose, cold glucose, insulin, free fatty acids (FFA), and Topiramate. All blood samples were immediately centrifuged, and plasma was stored at −80°C. A terminal dose of Nembutal (100 mg/kg iv) was administered after clamping to dissect perivisceral fat pads from euthanized rats. To obtain basal muscle and fat tissue preparations, a group of rats was allowed to recover from the clamp procedure by infusion of glucose, without insulin, for 30 min (to prevent hypoglycemia), allowing them to rest, and providing them with food and water, thereafter. Tissues of fully recovered rats were weighed and placed free in a modified metabolic chamber to scintillate vials and analyzed for 14C and 13H.

Determination of 2-Deoxyglucose Uptake in Isolated Adipocytes Ex Vivo

Adipocytes were isolated from periovarian fat pads, and 2-DOG uptake assays were performed. Dissected adipose tissue was minced with scissors and digested with 1 mg/ml collagenase (type II, Sigma) in a KHB solution plus 30 mM HEPES (pH 7.4, 38 mM mannitol, 4% BSA, 200 mM adenosine, and 2 mM pyruvate) by steady rotation (100 rpm/min) for 60 min at 37°C. The resulting cell suspension was filtered through a 440-μm nylon wire mesh (Small Parts, Miami Lakes, FL). Suspected cells were washed twice in BSA-free KHB (10 ml) by gentle agitation and floating. Afterward, cells were filtered three more times in similar fashion to remove unwanted debris. Cells were counted in a hemocytometer, diluted in KHB (+2.5% BSA), and allotted to round-bottom polypropylene tubes at a final concentration of 2 x 10⁶ cells/ml in each tube. Fat cells were preincubated in test tubes for 60 min at 37°C while being shaken gently. Cells were stimulated under these conditions with insulin (100 mU) and/or treated with Topiramate (44 μM) or left untreated (basal) for 30 min. [^3H]2-DOG uptake was assayed in basal and insulin-stimulated adipocytes by adding 1 nM[^3H]2-DOG (1.5 μCi) and 37 mM[^14C]mannitol (0.1 μCi) to the cell suspension for 30 min. Cellular reactions were terminated by pulse centrifugation of reaction buffers through 150 μl of silicone oil layers. Caps with overlying cell plugs were placed in scintillation vials and analyzed for[^14C] and 13H.

Calculations and Statistical Analysis

Hepatic glucose output (HGO) and glucose disposal rate (GDR) were calculated using Steele’s equation (12). Glucose infusion (GINF) rates were derived from glucose values obtained over the final half-hour by averaging a minimum of two successive points interspaced by 10 min. Data obtained from in vitro glucose transport experiments were analyzed using two-way analysis of variance. One- and two-tailed t-tests were used as appropriate for all other assays. All data are reported as means ± SE.

RESULTS

Blood Glucose Levels In 6-h-Fasted Noncannulated ZDF Rats

In preliminary in vivo experiments, a 100 mg/kg daily dose of Topiramate resulted in an initial decline in food intake lasting 24–36 h, with a concomitant decrease in body weight. As expected, average daily food intake calculated over 6 days was significantly lower in Topiramate-treated rats (15.5 g/day) compared with regular ad libitum-fed controls (30.7 g/day, P < 0.05), due to lower food consumption on days 1 and 2. After 36 h, Topiramate-treated rats demonstrated a return to normal food consumption levels and experienced a concomitant increase in body weight (Fig. 1A). Topiramate-treated rats gradually reached normal weights, reaching control values by 6–7 days (P < 0.05 vs. body weights on day 4). Figure 1B shows that, after a 6-h fast, blood glucose levels were significantly lower in Topiramate-treated rats compared with controls (P < 0.05). OGTTs were performed on day 16, and the results demonstrated that postchallenge glucose concentrations were markedly reduced in the Topiramate-treated rats (Fig. 1C). Thus these data indicate that Topiramate treatment reduces blood glucose concentrations in female ZDF rats independently of weight loss.
On the basis of these results, our protocol involved cannulation of rats (placement of indwelling jugular vein and carotid artery catheters) before initiating daily Topiramate gavage administration, since surgery independently suppresses appetite in rats for 1–2 days. As expected, both control and drug-treated groups experienced reductions in food intake lasting 1–2 days, recovering fully by day 6 of treatment. All groups of rats experienced the same reductions in body weight regardless of surgical state, with full weight regained by day 7. Basal blood glucose levels were significantly lower in Topiramate-treated animals compared with controls. Oral glucose tolerance tests were performed with a 50% glucose solution (2 g/kg), and results from control and Topiramate-treated ZDF rats are shown (data represent means ± SE; n = 5–6) and indicate lower glucose levels in Topiramate-treated rats compared with controls (P < 0.05). *P < 0.05 vs. rats on day 4. †P < 0.05 vs. concentrations of glucose in blood of pair-fed obese ZDF rats given placebo.

Hyperinsulinemic Euglycemic Clamp Studies

Table 2 shows some of the characteristics of Topiramate-treated ZDF and lean rats. Topiramate was detected in plasma of the drug-treated animals only. Basal glucose levels were lower in Topiramate-treated animals (P < 0.05), whereas insulin, leptin, and body weights were identical between groups. The hyperinsulinemic euglycemic clamp studies showed that Topiramate treatment led to a 30–40% increase in glucose infusion rate (G_{INF}; P < 0.05). None of the indexes of in vivo insulin sensitivity reported in Table 1 (glucose, insulin,
Table 1. Average food intake of the last 48 h, ovarian fat pad weight, and plasma adiponectin (ACRP30) in Topiramate- or placebo-treated ZDF rats carrying indwelling catheters

<table>
<thead>
<tr>
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<th>Controls</th>
<th>Topiramate Treated</th>
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<tbody>
<tr>
<td>Food consumption, g/day</td>
<td>26.5±1.2</td>
<td>24.8±1.8</td>
</tr>
<tr>
<td>Fat pad weight, g</td>
<td>7.96±0.64</td>
<td>9.33±0.30</td>
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<tr>
<td>Adiponectin, μg/ml</td>
<td>8.02±0.35</td>
<td>7.30±0.35</td>
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Values are means ± SE. ZDF, Zucker diabetic fatty rats.

leptin, or G_{INF}) were significantly altered in lean rats by Topiramate treatment.

Additional experiments were performed to distinguish between effects on glucose uptake and hepatic glucose production. This utilized the simultaneous infusion of [^{1}H]glucose to allow measurement of the rate of glucose disposal, as well as the ability of insulin to suppress hepatic glucose production. The results of these studies, shown in Fig. 2, demonstrate that Topiramate enhances insulin sensitivity for glucose disposal as well as for hepatic glucose production, resulting in an overall increase (∼35%) in total body insulin-stimulated glucose disposal rate (GDR), as well as a 40% increase in the ability of insulin to suppress hepatic glucose output (HGO) (P < 0.05). There was no significant effect of Topiramate on ISGDR or HGO in lean rats.

Serum FFA levels are another marker of in vivo insulin sensitivity. Insulin normally suppresses circulating FFA levels during the hyperinsulinemic clamp studies due to its antilipolytic effect in adipocytes. As seen in Fig. 2C, a greater decline in FFA levels occurred in the Topiramate-treated rats compared with controls (P < 0.05), whereas suppression of FFA levels in Topiramate-treated lean rats was the same as in controls.

Ex Vivo And In Vitro Studies In Muscle And Adipose Tissue

Skeletal muscle strips. To determine whether in vivo insulin sensitization of muscle caused by Topiramate treatment persisted ex vivo (i.e., once the tissue has been removed from the in vivo environment), we prepared soleus muscle strips taken from rats treated with Topiramate (7–9 days of dosing), as well as muscle strips from placebo-treated animals, and used them for assessments of basal and insulin-stimulated 2-DOG uptake in vitro.

As seen in Fig. 3A, muscle strips of ZDF rats displayed a 50% reduction in insulin-stimulated 2-DOG uptake in vitro compared with glucose uptake in lean rat muscle (P < 0.05). More importantly, ex vivo studies with muscle strips of Topiramate-treated rats demonstrated no increases in insulin sensitivity. If anything, there was a small decrease in the ability of insulin to stimulate glucose uptake in these muscle preparations compared with muscle strips of controls. Similar results were seen in experiments performed in hemidiaphragm muscle strips (data not shown). As seen in Fig. 3B, muscle preparations acutely exposed in vitro to Topiramate, before measurements of basal and insulin-stimulated 2-DOG transport, demonstrated no effect of drug treatment (for ≤90 min). These findings are consistent with the ex vivo studies and indicate that Topiramate does not have direct effects on skeletal muscle insulin action.

Isolated adipocytes. In addition to muscle strip experiments, we conducted ex vivo studies with adipose tissue. In one set of experiments, adipocytes were isolated from control and Topiramate-treated rats (7–9 days of dosing) and prepared for in vitro determination of basal and insulin-stimulated glucose transport. In contrast to the results with insulin-resistant skeletal muscle strips, the results of experiments conducted with insulin-resistant adipocytes were quite different. Adipocytes of Topiramate-treated ZDF rats showed an approximately twofold increase in insulin-stimulated glucose transport compared with cells from control ZDF rats (P < 0.05) (Fig. 4A). In vitro experiments using adipocytes obtained from untreated female ZDF rats showed that in vitro treatment with Topiramate for 30 min before insulin stimulation led to a marked increase (25–40%) in the ability of insulin to stimulate glucose transport in these cells (P < 0.05) (Fig. 4B).

DISCUSSION

Topiramate is a currently used antiseizure medication that can also lead to weight loss in humans as well as in experimental animals. In addition, recent studies have demonstrated that Topiramate treatment leads to a reduction in blood glucose

Table 2. Characteristics of female obese ZDF and lean rats carrying indwelling catheters and treated with Topiramate or placebo (water) for 7-9 days

<table>
<thead>
<tr>
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<th>ZDF Rats</th>
<th>Lean Zucker Rats</th>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Topiramate treated</td>
</tr>
<tr>
<td>Posttreatment body weight, g</td>
<td>242.4±6.0</td>
<td>246.1±9.3</td>
</tr>
<tr>
<td>Glucose concentration, mmol/l</td>
<td></td>
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<tr>
<td>Basal</td>
<td>10.70±0.49</td>
<td>9.29±0.49†</td>
</tr>
<tr>
<td>Clamped</td>
<td>9.77±0.39</td>
<td>9.58±0.49</td>
</tr>
<tr>
<td>Insulin concentration, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.00±0.09</td>
<td>1.00±0.11</td>
</tr>
<tr>
<td>Clamped</td>
<td>24.6±1.1</td>
<td>24.9±1.9</td>
</tr>
<tr>
<td>Leptin concentration, ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>13.49±0.82</td>
<td>14.75±0.94</td>
</tr>
<tr>
<td>Clamped</td>
<td>12.79±0.25</td>
<td>16.52±1.36</td>
</tr>
<tr>
<td>Topiramate concentration, mmol/l</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>16.6±1.2</td>
<td>22.0±0.81†</td>
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</table>

Values are means ± SE. G_{INF}, glucose infusion rate; ND, not done. *P < 0.01 vs. same measurement in all ZDF rats; †P < 0.05 vs. same measurement in ZDF controls; ‡P < 0.01 vs. same measurement in ZDF controls.
in genetically obese rodents, but whether this glucose-lowering effect is a result of weight reduction or an independent action of the drug has not been clarified. In the present studies, we find that Topiramate treatment leads to a reduction in glucose levels in female ZDF rats compared with weight-matched untreated animals. Based on these results, the major purpose of these studies was to assess the physiological mechanisms underlying this glucose-lowering effect. The central findings of the experiments are that Topiramate treatment leads to insulin sensitization and that this effect is exerted predominantly at the level of adipose tissue.

Hyperinsulinemic euglycemic glucose clamp studies were performed in treated and untreated insulin-resistant female ZDF rats as well as insulin-sensitive lean animals. The group of treated and untreated animals had comparable food intake and body weight, as described in MATERIALS AND METHODS. These studies demonstrated that Topiramate treatment led to a striking improvement in insulin sensitivity in the obese ZDF rats, as demonstrated by a 30–40% increase in the GINF rate necessary to maintain euglycemia in these animals. In addition, using tracer methodologies, we found a comparable 30–40% increase in ISGDR. Although it is possible that this increase in ISGDR reflects glucose uptake into adipose tissue, because a large component of total body ISGDR involves skeletal muscle glucose metabolism, these results raise the possibility that Topiramate treatment leads to an improvement in skeletal muscle insulin resistance. During these glucose clamp studies, we also measured the ability of insulin to suppress HGO. Suppression of HGO was impaired in the untreated obese ZDF rats compared with lean controls. We found that, compared with untreated animals, Topiramate-treated obese ZDF rats displayed enhanced insulin-mediated suppression of HGO, indicative of increased hepatic insulin sensitivity. Finally, during the insulin infusion, a marked decline in circulating FFA levels was observed, and this largely reflects the effect of insulin to suppress adipose tissue lipolysis. We found that the ability of insulin to suppress FFA levels was impaired in the untreated ZDF animals and that this defect was partially reversed in the treated animals, consistent with the improved adipose tissue insulin sensitivity.

Our studies also shed light on the primary tissue sites of action of Topiramate’s insulin-sensitizing effects. Thus, when isolated adipocytes and muscle strips from Topiramate-treated animals were studied ex vivo, we found that the insulin-sensitizing effect on insulin-stimulated glucose transport in muscle strips was no longer detectable, whereas enhanced insulin sensitivity in isolated adipocytes was fully present. Furthermore, when muscle strips and isolated adipocytes were prepared from nontreated insulin-resistant obese ZDF rats and then treated in vitro with Topiramate, drug treatment led to enhanced insulin-stimulated glucose transport in adipocytes but was without effect in muscle strips. Taken together, these results suggest that the primary tissue site of Topiramate action is adipose tissue. Topiramate might lead to enhanced insulin action in insulin-resistant adipocytes, and this, in some way, allows the adipocytes to “talk” to other insulin target tissues in vivo. This communication may involve an adipocytokine, or some other mediator, but is not preserved once the muscle tissue is removed from the in vivo environment. An alternate possibility is that Topiramate treatment leads to generation of a circulating factor, or some other signal, that sensitizes insulin

Fig. 2. Whole body insulin-stimulated glucose disposal rate (ISGDR), hepatic glucose output (HGO), and insulin’s ability to suppress plasma free fatty acid (FFA) concentration were determined with the euglycemic hyperinsulinemic clamp method. Topiramate treatment in vivo (7–9 days) led to an increase in total body ISGDR (A) and enhanced suppression of HGO (B) and FFA (C) in obese ZDF rats (n = 7), whereas drug treatment in lean animals (n = 4–6) was without effects on ISGDR, HGO, or FFA. Data are means ± SE in mg·kg⁻¹·min⁻¹. *P < 0.05 vs. insulin-stimulated levels of ISGDR, HGO, and FFA in lean rats. †P < 0.05 vs. insulin-stimulated levels of ISGDR, HGO, and FFA in ZDF controls.
target tissues, but the effects in adipocytes are more sustained (preserved ex vivo) than in muscle. However, the fact that in vitro treatment with Topiramate ameliorates insulin resistance in adipocytes but not muscle favors the first possibility.

Circulating FFAs that are released from adipocytes could act as a signal from adipose tissue to decrease insulin sensitivity in muscle or liver. Increased FFA levels can reduce insulin sensitivity in skeletal muscle and hepatic tissues in rodents (4, 7) and humans (1, 2). Topiramate treatment of obese ZDF rats led to a greater decline in circulating FFA levels during the hyperinsulinemic euglycemic clamp studies compared with untreated obese ZDF rats, indicating that Topiramate treatment improved insulin’s antilipolytic effects. Thus it is possible that Topiramate exerts its in vivo insulin-sensitizing effects through adipose tissue by promoting lower plasma FFA levels.

Topiramate appeared to be selective for insulin-resistant adipocytes, since drug treatment had no effect on insulin-sensitive cells. Evidence for this comes from several observations. First, Topiramate enhanced insulin’s antilipolytic effects in ZDF rats but did not improve FFA suppression in lean controls. Second, insulin-
resistant adipocytes of ZDF rats showed a marked improvement in insulin-stimulated glucose transport when assessed ex vivo, whereas cells from lean rats that were treated with the same dose exhibited no effect. Thus from these data it seems that Topiramate can function as an insulin sensitizer in fat cells only when they are already insulin resistant.

The EC50 of Topiramate in studies by Gustafson et al. (6) was lower than the plasma concentrations in our studies but comparable to what we used in vitro. Because Topiramate is an insulin sensitizer, we wondered whether it might be exerting effects through the peroxisome proliferator-activated receptor-γ (PPARγ). However, direct binding and transactivation assays, using a PPARγ GAL4 promoter, revealed no activity of this compound on the PPARγ receptor at concentrations as high as 100 μM. Furthermore, addition of Topiramate to preadipocytes had no effect to induce the PPARγ target gene AP2, nor was there any effect on adipocyte differentiation. Consequently, we conclude that Topiramate’s insulin-sensitizing effects are independent of the PPARγ mechanism.

In summary, our results indicate that Topiramate is a new drug that demonstrates insulin-sensitizing effects in vivo independently of weight loss. The combined in vitro and in vivo data suggest that Topiramate leads to in vivo insulin sensitization by directly enhancing insulin action in adipose tissue and that, to the extent that in vivo skeletal muscle insulin sensitivity was increased, this effect is secondary to the primary effects in adipocytes.

**GRANTS**

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