Additive effects of leptin and topiramate in reducing fat deposition in lean and obese ob/ob mice

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Abstract

The objective of the present study was to investigate the effects of the antiepileptic drug topiramate (TPM) on components of energy balance in lean and obese (ob/ob) mice in the presence or absence of leptin. Lean and ob/ob mice infused with either leptin or phosphate-buffered saline were treated with TPM for 7 days. TPM was mixed into the diet and administered at a dose of 60 mg/kg/day, whereas leptin was infused at the rate of 100 μg/kg/day using osmotic minipumps, which were subcutaneously implanted in the interscapular region. Food intake and body weight were monitored throughout the study. Body composition was measured prior to and following treatment with TPM and leptin, using dual-energy X-ray absorptiometry (DEXA). Glucose (glucose oxidase method) and insulin (radioimmunoassay) were also determined. TPM and leptin significantly reduced body weight gain, food intake and body fat gain in obese mice. The effects of TPM and leptin on fat gain were also statistically significant in lean animals. There was no interaction of TPM and leptin on the energy balance variables, the effects of the two substances being additive instead. Leptin abrogated hyperinsulinemia in obese mutants whereas TPM did not alter insulin levels in either lean or obese mice. The combination of leptin and TPM led to the normalization of glucose levels in obese mice.

Our study demonstrates an effect of TPM in leptin-deficient animals, which suggests that TPM does not require the presence of leptin to exert its effect. They also show that the effects of leptin and TPM can be additive. The treatment with leptin in ob/ob mice neither accentuated nor blunted the effect of TPM on energy balance.

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1. Introduction

Topiramate [TPM; 2,3:4,5-bis-O-(1-methyl-ethylidene)-β-D-fructopyranose sulfamate] is a monosaccharide derivative currently indicated for the treatment of epilepsy, which is under assessment for the treatment of other neurological and affective disorders, including migraine [1], bipolar disorder [2,3] and binge eating disorder [4]. It exerts numerous neurobiochemical effects that determine its anticonvulsant and mood-stabilizing properties. TPM has been reported to exhibit inhibitory actions on (1) GABA transmission [5–8], (2) AMPA/kainate receptors and (3) voltage-dependent sodium and calcium channels [9–14]. Evidence currently accumulates to suggest that TPM interacts with or alters the phosphorylation level of substrate proteins (Shank, R., personal communication), which could constitute a common mechanism underlying all the actions of the drug on ligand- and voltage-gated ion channels. TPM has also been reported to have inhibitory effects on carbonic anhydrase isoenzymes CA-I, CA-II and CA-IV [15], which could also account for some of the effects of TPM on the GABA and AMPA/kainate receptors [16].

In contrast with most anticonvulsant or mood-stabilizing drugs, TPM induces noticeable weight loss, which have been reported in numerous clinical studies [17,18], including investigations done in obese patients with binge eating disorders [4], in depressed obese patients [19] and in subjects exhibiting weight gain while being treated for affective disorders [20,21]. Recently, investigations in laboratory rodents have clearly demonstrated the ability of TPM to reduce body weight and body fat gains in lean and obese laboratory rodents [22–25]. The reduction in fat gain following TPM is due to reduced food intake and increased energy expenditure. TPM seems to have the ability to reduce energetic efficiency and has therefore been
reported to reduce energy gain even without reducing energy intake [23].

The mechanisms whereby TPM affects the regulation of energy balance have yet to be delineated. Recently, TPM has been reported to markedly reduce leptin levels in female rats [25]. In the latter, leptin reduction after administration of TPM appears larger than that predicted by the effects of TPM on fat mass, raising the possibility that TPM could alter leptin secretion, metabolism or requirement. The adipocyte-derived hormone leptin is secreted in proportion to the fat mass and has been reported to decrease food intake and increases thermogenesis in leptin-sensitive animals [26].

The present study was aimed at investigating the effects of TPM on components of energy balance in lean and obese (ob/ob) mice in the presence and absence of leptin. The ob/ob mouse is leptin deficient but apparently very sensitive to the metabolic action of exogenously infused leptin [27–29]. In addition to being massively obese, the ob/ob mouse is hyperglycemic, hyperinsulinemic and hypertriglyceridemic [30,31] and thereby constitutes a helpful model to investigate the effects of TPM on glucose and lipid metabolism abnormalities.

2. Materials and methods

2.1. Animals, diet and design

Obese (ob/ob) and lean female C57BL/6J mice aged 6−7 weeks were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All mice were cared for and handled according to our institutional animal care committee. The animals were housed individually in cages and fed ad libitum with a pelleted stock diet (Charles River Rodent Diet, distributed by Ralston Products, Woodstock, Ontario, Canada). They were subjected to a 12:00:12:00 dark–light cycle (lights on between 0700 and 1900 h) and kept under an ambient temperature of 25 ± 1 °C.

2.2. Experimental design and treatments

Lean and obese mice were assigned to a 2 (Hormone: vehicle and leptin) by 2 (Drug: untreated and TPM) factorial design. Eight groups were thus formed: lean-vehicle-untreated (n = 14), lean-vehicle-TPM (n = 14), lean-leptin-untreated (n = 14), lean-leptin-TPM (n = 14), obese-vehicle-untreated (n = 11), obese-vehicle-TPM (n = 12), obese-leptin-untreated (n = 11), obese-leptin-TPM (n = 13). TPM was mixed with a ground pelleted stock diet, which was thereafter pelleted again once TPM was mixed into it. TPM was administered at a dose of 60 mg/kg/day. The drug was kindly provided by Johnson & Johnson Pharmaceutical Research and Development (J&JPRD, Spring House, PA, USA). Recombinant mouse leptin (Peprotech Canada, Ottawa, Ontario, Canada), at a dose of 100 µg/kg/day, and PBS (vehicle), were infused using Alzet osmotic minipumps (Model 1007D, ALZA, Palo Alto, CA, USA). The 1007D pumps, which deliver their content at a flow rate of 0.5 µl/h for 7 days, were subcutaneously implanted in the interscapular region under isoflurane anesthesia.

2.3. Body weight, food intake and body composition measurements

Body weight was monitored every day. Food intake was also measured by subtracting the amount left uneaten by mice from the amount provided to the animals. Food spillage was accounted for in the food intake measurements. Body composition was measured at the beginning and end of the treatment period by dual-energy X-ray absorptiometry (DEXA), using a mouse densitometer (PIXImus, Lunar, Madison WI, USA). Previous data have shown that the DEXA-estimated values of fat and lean masses are strongly correlated with their chemical values [32]. At the time of killing (between 0800 and 1000 h), mice were anesthetized with an intraperitoneal injection of 0.4 ml/kg of a ketamine (20 mg/ml):xylazine (2.5 mg/ml) mixture.

2.4. Plasma glucose, insulin and triglycerides measurements

Blood was harvested by cardiac puncture, centrifuged (1500 × g, 15 min at 4 °C), and the separated plasma was stored at −70 °C until later biochemical measurements. Plasma glucose concentrations were determined with a glucose oxidase method using an automated glucose analyzer (Beckman Instruments, Palo Alto, CA). Plasma triglycerides were assayed by an enzymatic method using a reagent kit from Roche Diagnostics (Laval, Quebec, Canada). The triglyceride assay allowed for correction for free glycerol. Plasma insulin levels were determined by radioimmunoassay using a reagent kit from Linco Research (St. Charles, MO, USA) with a rat insulin standard.

2.5. Statistical analyses

A 2 × 2 factorial analysis of variance (ANOVA) was used to determine the main and interaction effects of Hormone (vehicle, leptin) and Drug (untreated, TPM) in lean and obese mice. When appropriate, a posteriori comparisons were performed using the Bonferroni/Dunn procedure. Simple regression analyses were performed to establish associations between variables of interest.

3. Results

Body weight and food intake were monitored every day throughout the period of treatment. The gain in body weight and the total amount of food ingested are illustrated in Fig. 1. TPM and leptin reduced body weight gain (Fig. 1a) and
food intake (Fig. 1b) in both lean and obese animals. The effect of leptin appeared particularly strong in obese mice, which exhibited a marked \( (P < .001) \) weight loss and a noticeable \( (P < .001) \) reduction in food ingestion following the administration of the hormone. In lean mice, the effects of TPM on body weight gain (\( P = .071 \)) and food intake (\( P = .066 \)) were close to statistical significance. In either lean or obese mice, there was no interaction of TPM with leptin on body weight gain and food intake; the effects of the hormone and the drug were additive instead.

Body fat and body lean gains were determined by subtracting the lean and fat masses measured at the end of the treatment period from the estimations obtained at the beginning of the trial (Fig. 2). Fat and protein masses were determined in vivo by DEXA. TPM and leptin significantly reduced fat gain in lean and obese animals, the effect being apparently much stronger in obese than in lean animals. There was no interaction of TPM and leptin on fat gain, regardless of the phenotype. It is noteworthy that the effect of TPM on fat gain even added to that of leptin, which was already very pronounced. In contrast to leptin, TPM did not significantly retard lean mass deposition. The reduction in lean mass in obese mice following leptin potentially resulted from the strong effect of leptin on food intake. Changes in

![Fig. 1. Main and interaction effects of TPM and leptin on body weight gain (panel a) and food intake (panel b) in lean and obese mice treated with TPM and leptin. Each bar represents individual means of 13 mice ± standard error. A 2 \times 2 factorial ANOVA was used to determine the main and interaction effects of hormone (vehicle, leptin) and drug (untreated, TPM) in lean and obese mice.](image1)

![Fig. 2. Main and interaction effects of TPM and leptin on body fat gain (panel a) and lean mass gain (panel b) in lean and obese mice treated with TPM and leptin. Each bar represents individual means of 13 mice ± standard error. A 2 \times 2 factorial ANOVA was used to determine the main and interaction effects of hormone (vehicle, leptin) and drug (untreated, TPM) in lean and obese mice.](image2)
body weight gain following TPM and leptin were strongly predicted by changes in fat gain ($r = 0.90$, $P < 0.0001$).

The effects of TPM and leptin on glucose and insulin levels are illustrated in Fig. 3. Leptin abrogated hyperinsulinemia in obese mice whereas it was without effect in lean animals (Fig. 3a). TPM was in contrast ineffective in reducing insulin levels in either obese or lean mice. Leptin also significantly reduced triglyceride levels in obese animals (data not shown). Neither leptin nor TPM alone affected glycemia, whereas their combination brought glucose levels in obese mice down to lean values (Fig. 3b). Neither leptin nor TPM affected glycemia in lean animals.

4. Discussion

This study demonstrates the ability of TPM to reduce body weight and fat gains in lean and $ob/ob$ mice. The effect of the drug was stronger in obese than in lean animals. In addition, the effects of TPM were not prevented or potentiated by leptin, which markedly reduced fat gain in $ob/ob$ mice in consonance with previous studies [29]. At the doses used in this study, the effects of TPM and leptin on body weight and fat gains were cumulative. The present study also confirms the ability of leptin to abolish hyperinsulinemia in the $ob/ob$ mouse [33].

The present results represent the first data describing the effects of TPM on body composition in mice. They are consistent with previous findings emphasizing the ability of TPM to reduce energy deposition in the rat [22–25]. The reducing effect of TPM on body weight gain was largely explained by a reduction in fat gain. This is in line with previous data that have also demonstrated that the effects of TPM are predominantly exerted on fat mass [22–25]. The present data also confirmed the ability of leptin [27–29] to reduce body weight and fat gains in lean and obese mice. The reducing effect of TPM and leptin on fat gain was accompanied by a food intake reduction, which undoubtedly accounted for a large part of the effects of the treatments on fat and protein gains. Both leptin [27–29,34] and TPM [23] have been reported to alter energy balance by reducing food ingestion and increasing energy expenditure. The hypophagic effects of TPM and leptin appeared clearly stronger in obese animals than in lean mice. Obese $ob/ob$ mice have been reported to be particularly sensitive to the action of leptin. It is, however, premature to postulate that obese animals are also more sensitive to the action of TPM, as $ob/ob$ mice received, in absolute terms, more TPM than lean mice.

In contrast to leptin, which abrogated hyperinsulinemia in obese mice, TPM did not reduce insulin levels in either lean or obese mice. The effect of leptin on insulin secretion could have resulted in large part from the marked effect of the hormone on fat gain and food intake. The effects of TPM on fat gain and food intake were, on the other hand, not as marked as those of leptin and might not have been sufficient to correct hyperinsulinemia in obese mice. TPM and leptin, when combined together, normalized glycemia in obese mice, possibly because of the marked effect of the treatment on food intake and fat gain, which are known determinants of glycemia.

The effects of TPM on food intake and fat gain were additive to those of leptin. This was seen even in obese mice, in which the hormone produced very strong reductions in food intake and fat gain. TPM and leptin do not likely affect energy metabolism through similar pathways, which could partly explain their additive effects on energy balance variables. Leptin exerts several central actions
stimulates proopiomelanocortin production [37] and strongly inhibits the synthesis of the orexigenic peptides neuropeptide Y [38] and agouti-related protein [39]. On the other hand, there is no indication that TPM can affect the expression of these peptides. There is evidence to suggest that TPM can even induce the genes encoding neuropeptide Y [22] and agouti-related protein (Richard, D., unpublished results) in the arcuate nucleus, possibly as a compensatory mechanism to blunt its catabolic effects. TPM has also been demonstrated, similar to leptin [40,41], to down-regulate CRF expression in the paraventricular hypothalamic nucleus [22] and consequently to diminish the activity of the pituitary–adrenal axis [22]. The delineation of the mechanisms whereby TPM affects energy balance remains a very complex and challenging task because of the heterogeneous biochemical/pharmacological actions of the drug. One would predict from the stimulatory effect of TPM on the GABA system a stimulating effect on body fat gain comparable to that led by other antiepileptic drugs such as valproate and benzodiazepines, which enhance the activity of the GABAa receptor, or vigabatrin, which inhibits the breakdown of GABA [42–44]. Conversely, it appears that antiepileptic drugs that inhibit the activity of glutamatergic receptors could promote weight loss [43].

The present results demonstrate the ability of TPM to reduce body weight gain, food intake and body fat gain in lean and obese mice. They also show that the effects of TPM and leptin on energy balance variables are additive, and that leptin can abolish hyperinsulinemia in obese mutants, whereas TPM did not alter insulin levels in either lean or obese mice. Finally, the findings also demonstrate that the combination of leptin and TPM led to the normalization of glucose levels in obese mice. In conclusion, this study provides evidence that TPM can reduce food intake and fat gain in lean and obese (ob/ob) mice and that the presence of leptin in ob/ob mice neither accentuated nor blunted the effects of TPM on energy balance.

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