Topiramate effects lipolysis in 3T3-L1 adipocytes

GABRIELA POLTRONIERI CAMPAGNARO MARTINS1*, CAMILA OLIVEIRA SOUZA2*, SCHEROLIN DE OLIVEIRA MARQUES1, THAIS FERNANDES LUCIANO1, BRUNO LUIZ DA SILVA PIERI1, JOSÉ CÉSAR ROSA2, ADELINO SANCHEZ RAMOS DA SILVA3, JOSÉ RODRIGO PAULI4, DENNYS ESPER CINTRA4, EDUARDO ROCHETE ROPELLE5, BRUNO RODRIGUES5, FABIO SANTOS DE LIRA6 and CLAUDIO TEODORO DE SOUZA1

1Laboratory of Exercise Biochemistry and Physiology, Health Sciences Unit, University of Extremo Sul Catarinense, Criciúma, SC 88806-000; 2Immunometabolism Research Group, Institute of Biomedical Sciences, University of Sao Paulo, São Paulo, SP 05508-900; 3School of Physical Education and Sport of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, SP 14040-907; 4School of Applied Sciences, University of Campinas (UNICAMP), Limeira, SP 13484-350; 5Laboratory of Human Movement, São Judas Tadeu University, São Paulo, SP 03166-000; 6Immunometabolism Research Group, Department of Physical Education, University Estadual Paulista (UNESP), Presidente Prudente, SP 19060-900, Brazil

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Abstract. Studies have shown that topiramate (TPM)-induced weight loss can be dependent on the central nervous system (CNS). However, the direct action of TPM on adipose tissue has not been tested previously. Thus, the present study aimed to examine whether TPM modulates lipolysis in 3T3-L1. The 3T3-L1 cells were incubated in 50 µM TPM for 30 min. The β-adrenergic stimulator, isoproterenol, was used as a positive control. The release of lactate dehydrogenase, non-esterified fatty acid, glycerol and incorporation of 14C-palmitate to lipid were analyzed. The phosphorylation of protein kinase A (PKA), hormone-sensitive lipase (HSL), adipocyte triglyceride lipase (ATGL) and perilipin A, as well as the protein levels of comparative genetic identification 58 (CGI-58) were assessed. The levels of glycerol and non-esterified fatty acid increased markedly when the cells were treated with TPM. The TPM effects were similar to those observed in the cells treated with isoproterenol. The present results show that TPM increased the phosphorylation of pivotal lipolytic enzymes, which induced lipolysis in 3T3-L1 adipocytes, suggesting that this drug may act directly in the adipose tissue independent from its effect on the CNS.

Introduction

Obesity is associated with several chronic diseases. Environmental changes are not sufficient to control obesity; however, there is overwhelming evidence that certain pharmacological agents may act as therapeutic targets for obesity. One such agent is topiramate (TPM), initially used for epilepsy treatment and migraine prophylaxis. TPM is a glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptor agonist and enhances the inhibitory effects mediated by γ-aminobutyric acid (1). One of the side effects of TPM is weight loss, which makes this drug a possible option for obesity treatment. The central mechanisms suggested for TPM-induced weight loss include a reduction in energy efficiency, influence on the hypothalamus and alteration of neuropeptides (1). However, the direct action of TPM on adipose tissue, particularly on lipolysis, has not been observed.

Two essential enzymes for lipid hydrolysis are adipocyte triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). ATGL hydrolyzes triacylglycerols into a fatty acid and a diacylglycerol (2). To fully activate, ATGL must interact with its cofactor, comparative genetic identification 58 (CGI-58) (3). In adipocytes without lipolytic stimuli, CGI-58 is strongly bound to lipid droplets, interacting with perilipin A (2). However, perilipin A and CGI-58 dissociate when lipolytic activity is stimulated by the activation of β-adrenergic receptors, which causes an increase in the cyclic adenosine monophosphate and consequent activation of protein kinase A (PKA). This

Correspondence to: Dr Claudio Teodoro de Souza, Laboratory of Exercise Biochemistry and Physiology, Health Sciences Unit, University of Extremo Sul Catarinense, Criciúma, SC 88806-000, Brazil
E-mail: ctsouza@unesc.net

*Contributed equally

Abbreviations: ATGL, adipocyte triglyceride lipase; CGI-58, cofactor comparative genetic identification 58; CNS, central nervous system; HSL, hormone-sensitive lipase; LDH, lactate dehydrogenase; NEFA, non-esterified fatty acid; PKA, protein kinase A; TAG, triglycerides; TPM, topiramate

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*E-mail: ctsouza@unesc.net

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process releases CGI-58 to associate with ATGL. Perilipin A is a structural protein that coats lipid droplets and protects triglycerides (TAG) molecules from basal enzymatic hydrolysis (4). Additionally, in stimulated cells, perilipin A is phosphorylated and facilitates the HSL translocation from the fat vesicles surface with consequent access to its diacylglycerol substrate (5). Together, the phosphorylation of HSL and its translocation to the lipid droplets surface, coupled with the activation of ATGL by CGI-58, results in the hydrolysis of 90% of TAG.

TPM treatment has been shown to reduce adiposity in humans and rodents (6,7). TPM is assumed to act directly on lipolysis, however, this has not been explicitly described in vivo, once TPM acts on the central nervous system (CNS). Thus, in the present study, 3T3-L1 adipocytes were used and TPM was demonstrated to have a direct effect on lipolysis.

Materials and methods

Cell culture and measurement of lipolysis. 3T3-L1 cells were obtained from the American Type Culture Collection and cultured at 37°C in 5% CO₂ in Dulbecco’s modified Eagle’s medium supplemented with 25 mmol/l glucose, 1.0 mmol/l pyruvate, 4.02 mmol/l L-alanyl-glutamine and 10% fetal bovine serum (Gibco, New York, NY, USA). Cell differentiation began 24 h after confluency and occurred over 4 days in a medium containing 0.25 µM dexamethasone, 0.5 mmol/l 3-isobutyl-1-methylxanthine and 5 µg/ml insulin (Sigma, St. Louis, MO, USA). Following differentiation, the cells were cultured for 10 days in growth medium containing 5 µg/ml of insulin. Each parameter was evaluated using a 6-well aliquot from this culture. On day 10 after differentiation, the cells were incubated for 24 h in a medium containing 0.5% fetal bovine serum. Cells were treated with 10 µl of TPM (50 µM) or isoproterenol (20 µM) for 30 min. At the end of the incubation, the glycerol and non-esterified fatty acid (NEFA) contents were assayed in the incubation medium. The results of the blots are presented as direct comparisons of the area of the apparent bands in autoradiographs and quantified by densitometry using the Scion Image software (Scion Image software; Scion Corp., Frederick, MD, USA).

Statistical analysis. Bars represent 6 different experiments. Differences between the groups were evaluated using one-way analysis of variance followed by the Bonferroni post hoc test. P<0.05 was considered to indicate a statistically significant difference. The software used for data analysis was the Statistical Package for the Social Sciences (SPSS) version 17.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Effects of TPM on lipolysis in 3T3-L1 adipocytes. To evaluate TPM-induced physiological alterations in lipolysis, the glycerol and NEFA contents were assayed in the incubation medium. The contents of glycerol and NEFA in isoproterenol-treated cells were higher compared with the control group. Similar results were observed for the isoproterenol group (Fig. 1A and B). To analyze lipogenesis, insulin-induced [1-14C]-palmitate incorporation into lipids was assayed. These results show that the use of TPM decreased palmitate incorporation into lipids compared to the control group regardless of insulin stimulus (Fig. 1C). To evaluate whether TPM exposure effects cellular viability, the cytosolic enzyme LDH release was quantified. The analyzed groups did not demonstrate significant differences, suggesting that TPM was not cytotoxic for the times and dosages used (Fig. 1D).

Based on the current findings, we hypothesize that TPM treatment could increase the phosphorylation of the main lytic enzymes. Subsequently, the phosphorylation of PKAThr198, HSLSer563, ATGLSer406, and perilipin A, as well as the protein levels of CGI-58, PKAThr198, HSLSer563, ATGLSer406 and perilipin A were analyzed (Fig. 1E-J). TPM treatment led to a higher enzymatic phosphorylation compared to the control group. Therefore, this result may explain, at least
in part, why the glycerol and NEFA levels were increased. Notably, the analyzed protein phosphorylation and the CGI-58 protein levels were similar for cells treated with TPM or isoproterenol (Fig. 1E-J).

Discussion

As increased lipolysis and decreased lipogenesis cause fat loss in obese individuals, the present study tested whether the use of TPM increases lipolysis in 3T3-L1 cells. The 3T3-L1 cells treated with TPM presented high phosphorylation of lipolytic enzymes and subsequent lipolysis, without alteration of cell viability. In addition, the data showed that TPM modulated lipogenesis. A previous 6-month randomized human study indicated that TPM led to marked weight loss compared to the placebo (8). Another recent study showed that co-treatment with phentermine and TPM also induced weight loss in obese patients (9). A study in which animals were fed with a high fat diet showed that the concurrent TPM use at 50 mg/kg led to body weight reduction and insulin sensitivity improvement (10). Caricilli et al (11) observed that TPM improved insulin and leptin sensitivity in the hypothalamus of obese mice, which can contribute to the reduction of food intake and adiposity. However, these in vivo studies did not exclude the TPM effects on CNS.

In the present study, TPM treatment increased the release of NEFA and glycerol in the culture medium. In addition, decreased [14C]-palmitate incorporation was observed in adipocytes exposed to TPM. One of the proposed mechanisms for lipogenesis reduction can be the carbonic anhydrase enzyme inhibition, which performs the first step of de novo lipogenesis. Although this enzyme was not analyzed in the present study, a previous study suggested that TPM can inhibit the cytosolic and mitochondrial levels of this enzyme, leading to weight loss (12).
Subsequent to observing that NEFA and glycerol levels were changed, five molecules that have a crucial role in the triacylglycerol hydrolysis were analyzed. TPM treatment increased the phosphorylation of PKA, HSL, ATGL and perilipin A, as well as the protein levels of CGI-58 compared to control cells. However, the TPM-induced phosphorylation degrees were similar to those using isoproterenol. The present data did not allow the proposition of the mechanism responsible by the increase of these molecules induced by TPM. Taken together, these results demonstrate that TPM treatment led to lipolysis independently of its action on the CNS. These data increase the understanding of the processes involved on the TPM-induced weight loss and suggest a direct action of this drug on the adipose tissue.

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