Asian Journal of Medicine and Health

5(2): 1-8, 2017; Article no.AJMAH.34428

Leptin and GABA Interactions on Body Temperature of Rats with Experimental Model of Obesity

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Authors' contributions

This work was carried out in collaboration between all authors. Author MHH designed the study, performed the statistical analysis, wrote the protocol and managed the literature searches. Authors EDB and RPN managed the analyses of the study. Author KSY supervised experimental work and wrote the final version of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJMAH/2017/34428
Editor(s):
(1) Maria Manuel Azevedo, Department of Microbiology, Faculty of Medicine, University of Porto, Porto, Portugal.
Reviewers:
(1) Jude Nnabuife Egwurugwu, Imo State University, Owerri, Nigeria.
(2) Wagih Mommtaz Ghannam, Mansoura University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/19635

Original Research Article

Received 27th May 2017
Accepted 10th June 2017
Published 21st June 2017

ABSTRACT

Leptin as well as gamma-aminobutyric acid (GABA) are involved in the regulation of feeding behavior and energy balance. The present study determined the effect of leptin on the core body temperature in rats with experimental model of obesity, as well as the changes of body temperature in obese rats after systemic (i.p.) administration of $GABA_B$-agonist baclofen and $GABA_B$-antagonist CGP35348, applied separately or in combinations with leptin. The results suggested significant changes in the effects of substances on rats with experimental model of obesity in comparison with their effects on rats with normal weight. Leptin produced significant long lasting hyperthermia, the hyperthermic effect of $GABA_B$-antagonist CGP35348 was relatively short lasting, while the $GABA_B$-agonist baclofen caused longer lasting hypothermia. The hyperthermic effect of leptin didn't appear when applied in combination with baclofen. There wasn't synergism in hyperthermic effect of leptin and $GABA_B$-antagonist applied simultaneously. Our results provide new point of view on the complex interactions between thermoregulation and energy homeostasis.

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Keywords: Leptin; GABA; body temperature; obesity; interaction.

1. INTRODUCTION

Leptin, the adipose tissue-derived hormone, is involved in the regulation of feeding behavior and energy balance. Leptin is a polypeptide hormone which is produced by adipocytes in proportion to their content of triglycerides. Its function is primarily associated with the regulation of body weight, food intake and energy expenditure. Leptin regulates energy balance largely through isoform B leptin receptor-expressing neurons (LepR neurons) in the brain. Leptin activates one subset of LepR neurons (leptin-excited neurons) while inhibiting the other (leptin-inhibited neurons) [1].

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system. Experimental data suggest involvement of GABAergic mechanisms in the regulation of body temperature. GABA, acting at central $GABA_B$ receptors, plays a physiological role in the control of feeding behavior [2].

Previous results demonstrated that leptin mainly acts on GABAergic neurons to reduce body weight, and that leptin activates proopiomelanocortin neuron activity by reducing GABA release onto these neurons, suggesting a body weight-promoting role for GABA released from leptin-inhibited neurons [3]. Several years ago, a strong GABAergic modulation of leptin was postulated. The data show a strong association between leptin levels and doses of GABA-mimetic active substance [4]. The results indicate that $GABA_B$-R1 subunit is constitutively expressed by adipocytes to primarily regulate leptin expression at the transcriptional level through a mechanism not relevant to the function as a partner of heterodimeric assembly to the functional $GABA_B$-receptor [5]. The investigations have shown that administration of baclofen, a $GABA_B$-receptor agonist, significantly decreased both body weight and waist circumference, and the serum leptin levels were also decreased significantly by the treatment [6].

Animal studies have shown that GABA as well as GABA receptor agonists produce hypothermia, while GABA receptor antagonists cause hyperthermia [7,8]. Our previous investigations on rats with normal weight have shown lack of synergism in hyperthermic effects of leptin and $GABA_B$-antagonist CGP 35348. The effect of combination was lower than the effects of substances applied separately. Neither hyperthermic effect of leptin nor hypothermic effect of $GABA_B$-agonist baclofen occurred when leptin was applied just prior to baclofen [9].

The aim of the present study was to investigate the effects of leptin on the core body temperature in rats with experimental model of obesity, as well as the interactions between leptin and $GABA_B$-agonist and antagonist on this level.

2. MATERIALS AND METHODS

2.1 Substances

Leptin (OB) Rat Recombinant (Sigma, Germany), CGP 35348 (Sigma, Germany) and Baclofen (Sigma, Germany) were used in this study. The doses used were defined by literature data, as well as own previously made experiments. The substances were administered systemic (intraperitoneally, i.p.) in a volume 0.2 ml/100 g body weight and compared with control group treated with the same volume of saline (NaCl).

2.2 Experimental Animals

The experiments were carried out on sexually mature male Wistar rats, divided into 6 groups of 6-7 rats each. Experimental animals were maintained on a standard 12 h light/dark cycle and allowed food and water *ad libitum*. The animals additionally received cafeteria diet for one month before the experiment. Cafeteria diet reflects more accurately the variety of delicious, high-calorie food, prevalent in Western society and is widely used to induce obesity in rodents [10]. Individual weights were measured daily. We used Lee index in order to determine obesity in rats [10].

In the handling and care of all animals, the International Guiding Principles for Animal Research were strictly followed.

2.3 Monitoring of Body Temperature

All experiments started at 10 a.m. and were conducted at ambient temperature 22 ± 1°C. Temperature was measured with thermistor probes (TX8) inserted rectally to a depth of 6 cm and monitored on multichannel recorder Iso-Thermex 16 (Columbus Instruments, USA). The initial temperature of the animals was determined, and then checked at 30-min

intervals until 150 min after substances injection. The movements of the rats were slightly restricted, as previously described by Rosow et al. [11].

2.4 Data Analysis

The results were expressed as delta (Δ) values (average changes in temperature compared to the initial one) (mean Δ values ± S.E.M.) and analyzed with two-way analysis of variance, as well as graphically presented by SigmaPlot. For statistical significance a Student's t-test was used.

3. RESULTS

3.1 Effects of Leptin, Baclofen and CGP35348 on the Core Body Temperature in Rats with Experimental Model of Obesity

Systemic (i.p.) administration of leptin in a dose 0.5 mg/kg on rats with experimental model of obesity produced significant long lasting hyperthermia that occurred at 30th, 60th, 90th and 120th min after application. The hyperthermia was with maximum response on the 60th min after application (Fig. 1).

$GABA_B$-receptor antagonist CGP35348 (5 mg/kg i.p.) caused increase in body temperature of rats with experimental model of obesity significant at 30th and 60th min after leptin injection (Fig. 1).

Injection of $GABA_B$-receptor agonist baclofen in a dose 5 mg/kg i.p. caused significant decrease in core body temperature in rats with experimental model of obesity between 30th and 120th min. The hypothermia was with maximum response on the 90th min after application (Fig. 1).

3.2 Interactions between Leptin and Baclofen on the Core Body Temperature in Rats with Experimental Model of Obesity

Administration of baclofen (5 mg/kg i.p.) just prior to leptin (0.5 mg/kg i.p.) on rats with experimental model of obesity altered the effects on body temperature of both leptin and baclofen. The

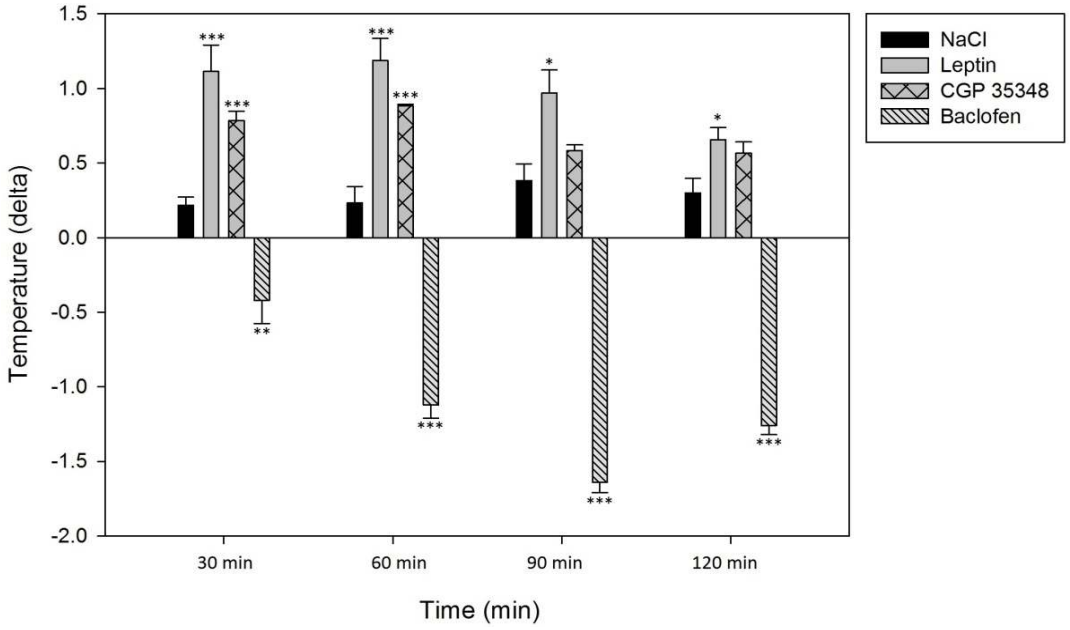

Fig. 1. Effects of intraperitoneal (i.p.) injection of leptin, baclofen and CGP35348 applied separately on the core body temperature in rats with experimental model of obesity

Mean changes (temperature delta℃) after i.p. administration of leptin (0.5 mg/kg), baclofen (5 mg/kg) and CGP35348 (5 mg/kg). Leptin produced significant long lasting hyperthermia, the hyperthermic effect of $GABA_B$-antagonist CGP35348 was significant at 30th and 60th min after leptin injection, while the $GABA_B$-agonist baclofen caused longer lasting hypothermia.

*Significant differences in comparison with control group (NaCl): *P < 0.05, **P < 0.01, ***P < 0.001*

hyperthermic effect of leptin didn't appear when applied in combination with baclofen. In the opposite, hypothermic effect of combination was observed. The hypothermic effect of baclofen applied alone was significantly higher ($P<0.05$) between 60th and 120th min, in comparison with the effect of combination baclofen and leptin (Fig. 2).

3.3 Interactions between Leptin and CGP35348 on the Core Body Temperature in Rats with Experimental Model of Obesity

Administration of CGP35348 (5 mg/kg i.p.) just prior leptin (0.5 mg/kg i.p.) caused higher hyperthermic reaction in comparison with CGP35348 (5 mg/kg i.p.) applied alone. The effect of CGP35348 is statistically significant lower at 30 min after injection ($P<0.01$) in comparison with hyperthermic effect of combination CGP35348 and leptin (Fig. 3).

3.4 Comparison of the Effects of Leptin, Baclofen and CGP35348 on the Core Body Temperature in Rats with Experimental Model of Obesity and Normal Weight

There was a trend of decreasing in hyperthermic effect of leptin on rats with experimental model of obesity in comparison with hyperthermic effect produced by leptin on rats with normal weight. However, the hyperthermia in obese rats was longer lasting (between 30th and 150th min after application) in comparison with hyperthermic effect of leptin on rats with normal weight, where the effect was significant only at 30th and 60th min after leptin injection (Fig. 4A). The hypothermic effect after injection of combination of baclofen and leptin on rats with experimental model of obesity was higher and longer lasting (between 30th and 120th min), while hypothermic effect of combination of baclofen and leptin on rats with normal weight was lower and significant only between 60th and 90th min after injection (Fig. 4B). The hyperthermic effect of combination of

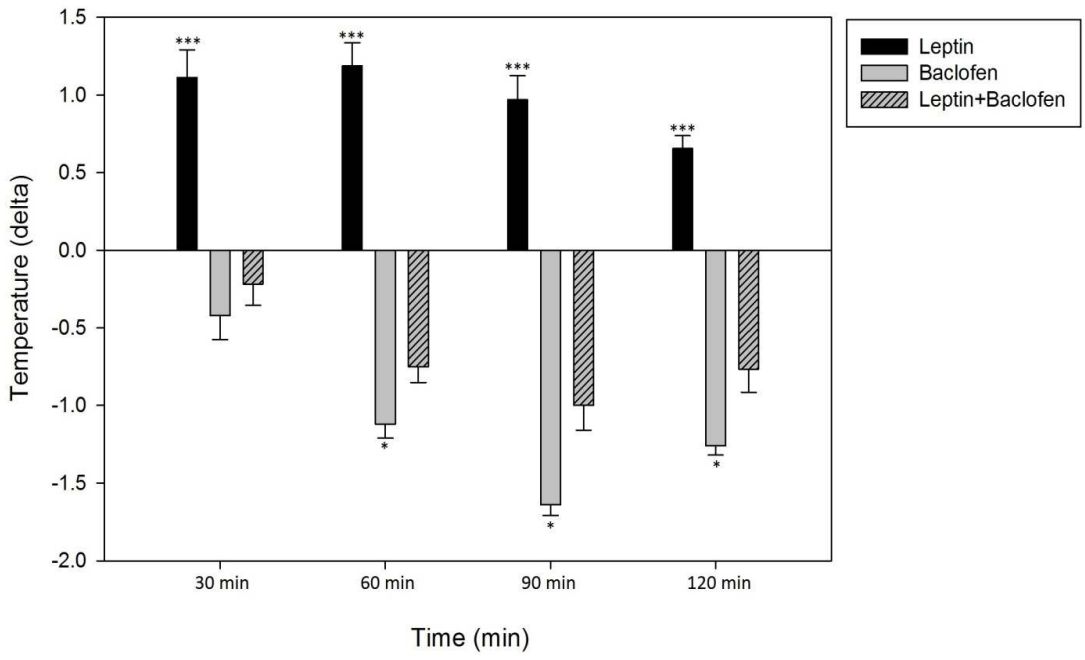

Fig. 2. Effect of Intraperitoneal (i.p.) Application of Leptin and Baclofen Separately and in Combination on the Core Body Temperature in Rats with Experimental Model of Obesity

Mean changes (temperature delta℃) after i.p. admin istration of leptin (0.5 mg/kg) and baclofen (5 mg/kg) separately and in combination. The hyperthermic effect of leptin didn't appear when applied in combination with baclofen. In the opposite, hypothermic effect of combination was observed.
*Significant differences in comparison with combination: *P < 0.05; ***P < 0.001*

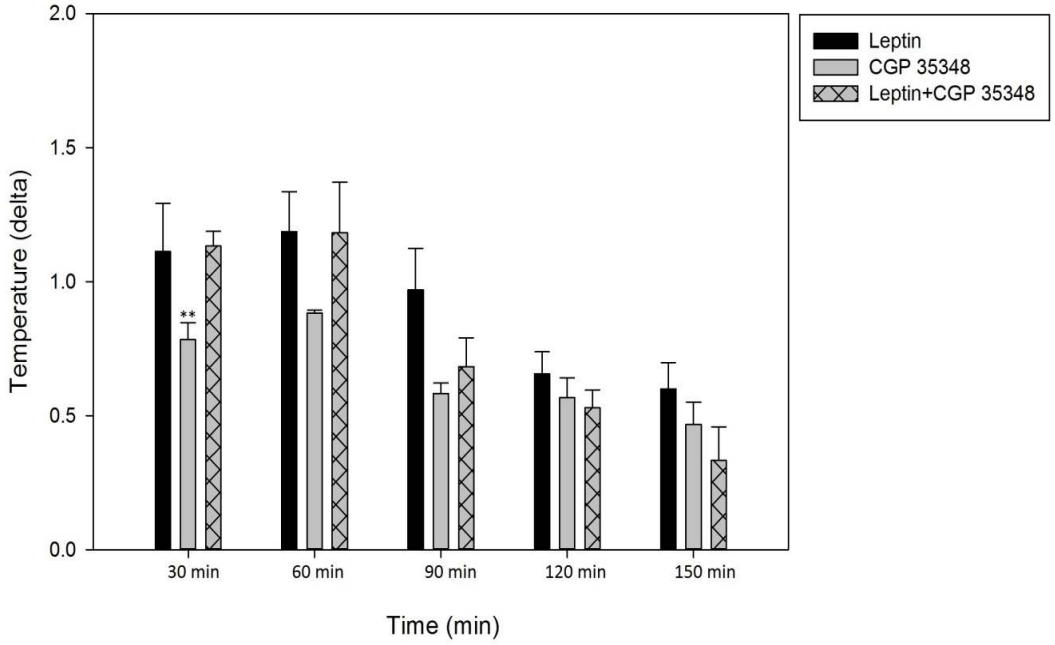

Fig. 3. Effect of intraperitoneal (i.p.) application of leptin and CGP35348 separately and in combination on the core body temperature in rats with experimental model of obesity

*Mean changes (temperature delta℃) after i.p. admin istration of leptin (0.5 mg/kg) and CGP35348 (5 mg/kg). Administration of combination (CGP35348 and leptin) caused higher hyperthermic reaction in comparison with CGP35348 applied alone (significant lower at 30 min after injection). Significant differences in comparison with combination: **P < 0.01*

CGP35348 and leptin on rats with experimental model of obesity was lower and statistically significant only at 30th and 60th min after injection, while hyperthermic effect of combination CGP35348 and leptin on rats with normal weight was higher and long lasting (between 30th and 150th min) (Fig. 4C).

4. DISCUSSION

In our results, systemic administration of leptin induced statistically significant hyperthermia in rats with experimental model of obesity. There was a trend of decreasing in hyperthermic effect of leptin on rats with experimental model of obesity in comparison with hyperthermic effect produced by leptin on rats with normal weight. The feeding of rodents with high-calorie diet leads to obesity, resulting in hyperleptinemia. The failure of elevated leptin levels to suppress feeding and mediate weight loss in common forms of obesity defines a state of so-called leptin resistance (attenuation of leptin signaling). LRb (the long isoform of leptin receptor) signaling via STAT3 (Signal Transducer and Activator of Transcription 3) and a number of other pathways is required for the totality of leptin action. Regulation of body weight, food intake and energy expenditure by leptin is well demonstrated in humans and rodents lacking a functional form of leptin, resulting in serious obesity and increased appetite [1,12]. Interestingly, treatment with recombinant leptin resulted in normalization of body weight, appetite, metabolic and neuroendocrine functions in these cases [13]. Administration of recombinant leptin to obese subjects with hyperleptinemia did not decrease body weight and appetite [14].

It has been found that leptin acts on the brain to increase postprandial heat production in skeletal muscle of sheep through altered mitochondrial function, indicative of adaptive thermogenesis [15]. The control of brown fat cell development and activity is physiologically ensured by the sympathetic nervous system (SNS), which densely innervates brown adipose tissue (BAT). SNS-mediated thermogenesis is largely governed by hypothalamic and brainstem neurons. With regard to energy balance, the leptin-melanocortin pathway appears to be a

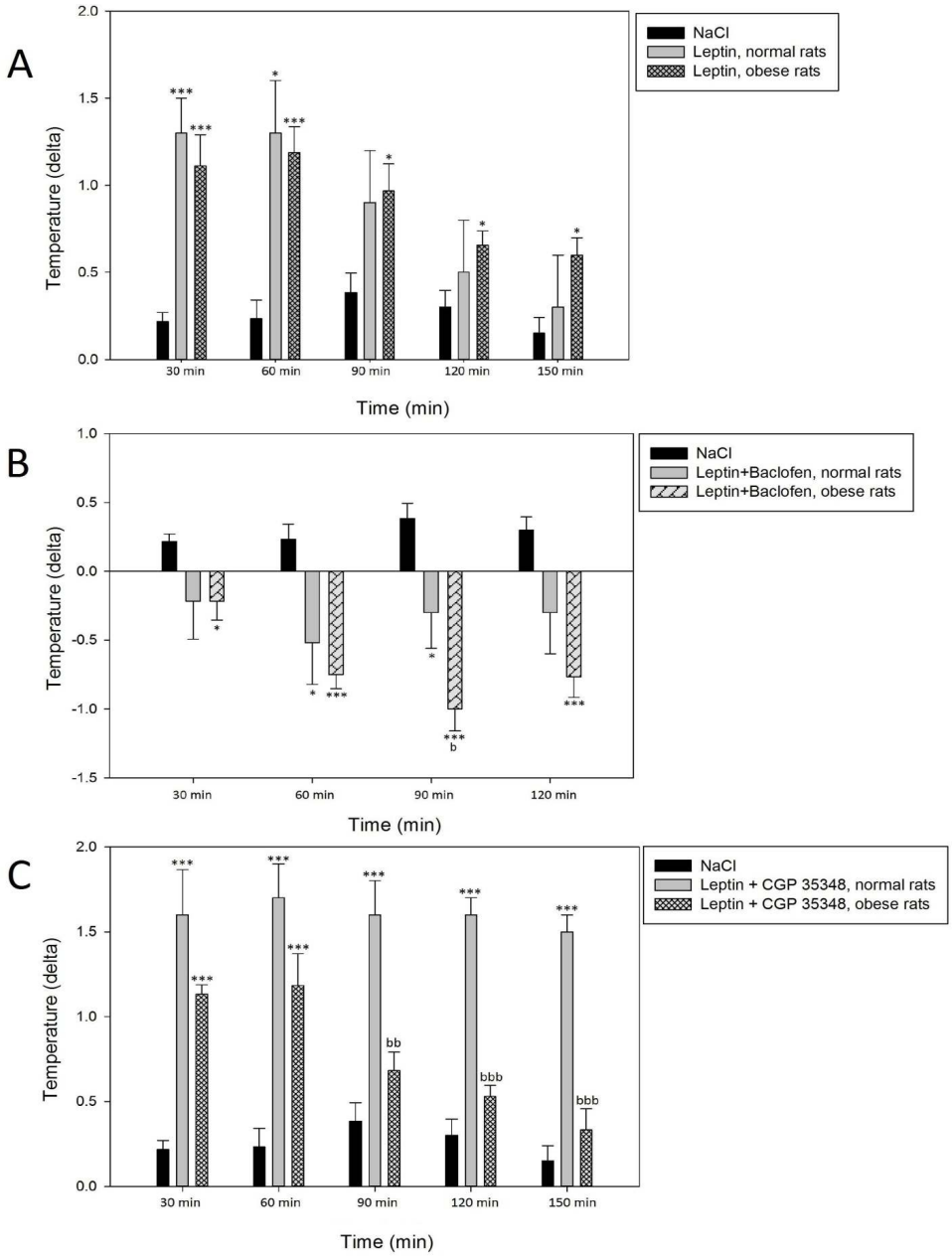

Fig. 4. Effects of intraperitoneal (i.p.) injection of leptin (A), combination of leptin and baclofen (B) and combination of leptin and CGP35348 (C) on the core body temperature in rats with normal weight and experimental model of obesity

Mean changes (temperature delta℃) after i.p. admin istration of leptin (0.5 mg/kg), baclofen (5 mg/kg)+ leptin (0.5 mg/kg) and CGP35348 (5 mg/kg)+leptin (0.5 mg/kg) on rats with normal weight (normal rats) and rats with experimental model of obesity (obese rats).

*Significant differences in comparison with control group (NaCl): *P < 0.05,**P < 0.01, ***P < 0.001.*

Significant differences in comparison with the group of rats with normal weight (normal rats): ^{b}P <0.05, ^{bb}P <0.01, ^{bbb}P <0.001

major factor in controlling brown adipocyte thermogenesis. The involvement of this homeostatic pathway further supports the role of the brown adipocyte in energy balance regulation [16]. Bechtold et al. [17] have found that altered thermoregulation in Gpr50(-/-) mice is associated with attenuated responses to leptin and a suppression of thyrotropin-releasing hormone.

In our results, systemic administration of $GABA_B$-antagonist CGP35348 induced statistically significant hyperthermia in rats with experimental model of obesity, while $GABA_B$-agonist baclofen induced a decrease in body temperature. The effects of baclofen and CGP35348 on body temperature were less pronounced in rats with experimental model of obesity compared to rats with normal weight. No synergism was observed in the hyperthermic effect of combining leptin and $GABA_B$-antagonist. In the combined administration of baclofen and leptin, a fall in body temperature was observed and the effect was less pronounced than that of the baclofen applied alone. There are many evidences about interactions of leptin and GABA on different levels. Recent experimental studies have shown the involvement of $GABA_B$ receptor function in regulation of body temperature, feeding behavior and energy expenditure. It has been shown that the vast majority of leptin's antiobesity effects are mediated by GABAergic neurons. Leptin, working directly on presynaptic GABAergic neurons, reduces inhibitory tone to postsynaptic POMC (proopiomelanocortin) neurons. As POMC neurons prevent obesity, their disinhibition by leptin action on presynaptic GABAergic neurons probably mediates, at least in part, leptin's antiobesity effects [18]. It has been shown that leptin inhibited norepinephrine (NE) efflux from the hypothalamus in a dose-dependent manner. The results demonstrate that leptin could act directly on the hypothalamus to inhibit NE efflux through GABA. It was concluded that leptin could probably produce its central and neuroendocrine effects by modulating NE and GABA levels in the hypothalamus [19].

5. CONCLUSION

There was significant changes in the effects of leptin, $GABA_B$-antagonist and $GABA_B$-agonist on the level of thermoregulation in rats with experimental model of obesity in comparison with their effects on rats with normal weight. Our results provide new point of view on the complex interactions between thermoregulation and energy homeostasis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

ACKNOWLEDGEMENTS

This work was supported by Grant 2016 of the Council for Medical Sciences, Medical University of Sofia under Contract № 2/2016.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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