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ORIGINAL ARTICLE

Effect of topiramate and its combination with SIB-1893 on body temperature in rats

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Abstract: This study was aimed at determining the effects of topiramate (TPM – a second-generation antiepileptic drug) administered alone and in combination with SIB-1893 (a selective non-competitive metabotropic glutamate subtype 5 [$mGlu_5$] receptor antagonist) on body temperature in freely moving rats. Temperature was monitored using programmed microchips, implanted subcutaneously in Wistar rats, at several time intervals: 0, 5, 10, 20, 30, 45, 60, 90, 120, 180, and 240 min after intraperitoneal administration of TPM, SIB-1893, and their combination. Statistical evaluation of data with two-way ANOVA with repeated measures of time revealed that SIB-1893 at a dose of 30 mg/kg, significantly decreased the body temperature in rats, at times ranging from 90-240 min after drug administration. In contrast, TPM at doses of 5 and 10 mg/kg, administered alone and TPM (10 mg/kg) in combination with SIB-1893 (30 mg/kg), did not significantly alter the body temperature in freely moving rats. Based on this preclinical study, one can conclude that TPM administered alone and in combination with SIB-1893 had no effect on body temperature in rats up to 240 min after intraperitoneal administration of the drugs.

Key words: topiramate, SIB-1893, temperature, telemetric temperature monitoring, rats

INTRODUCTION

Experimental evidence indicates that excitatory amino acids are involved in thermoregulation. It has been documented that N-methyl-D-aspartic acid (NMDA) increased temperature in rats [11], whereas some NMDA receptor antagonists, such as MK-801 and (±)-2-amino-5-phosphopentanoic acid ((±)-AP-5), reduced this increase [12]. In contrast, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (KA) exerted a biphasic effect on body temperature of experimental animals: short-lasting hypothermia followed by hyperthermia [21]. Likewise, some AMPA/KA receptor antagonists, such as NBQX, PNQX, and GYKI 52466, lowered the body temperature in experimental animals and produced hypothermia [10, 17, 18].

With regards to metabotropic ($mGlu_1$–$mGlu_8$) glutamate receptors, it has been reported that the selective $mGlu_1$ receptor antagonist – BAY 36-7620 induced a mild hypothermia in experimental rats [5]. Additionally, it has been found that MPEP, a selective non-competitive $mGlu_5$ receptor antagonist, significantly decreased temperature in rats [8]. In another study, MPEP and 2 other selective non-competitive $mGlu_5$ receptor antagonists (SIB-1893 and SIB-1757) have been reported to have no impact on body temperature in mice [2]. On the other hand, it has been reported that SIB-1893, in a dose-dependent manner, reduced body temperature in freely moving rats [3, 16].

Post-marketing reports clearly indicate that topiramate (TPM – a second-generation antiepileptic drug [AED]) evoked hypothermia in patients receiving valproate (VPA – a classical AED). It has been documented that hypothermia was reported either following the addition of TPM to an existing regimen of VPA or an increase in the TPM daily dose in patients on well-tolerated VPA therapy [14].

Considering the facts that SIB-1893 reduced body temperature in freely moving rats and that TPM enhanced the risk of hypothermia associated with VPA therapy in patients, it was of pivotal importance to evaluate the effects of TPM administered alone and in combination with SIB-1893 on body temperature in freely moving rats.

MATERIAL AND METHODS

Animals. Experiments were performed on adult male Wistar rats weighing 220-260 g. The animals were purchased from a licensed breeder (Dr. T. Górzkowska, Warsaw, Poland). The animals were kept in colony cages with free access to food and tap water, under standardized housing conditions (12 h of a light-dark cycle, stable temperature of 22 ± 1ºC, relative humidity 55 ± 5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 rats per group. All tests were performed between 08:00-15:00. Procedures involving animals and their care were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and Polish legislation on animal experimentation. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in this study were approved by the First Local Ethics Committee at the Medical University in Lublin.

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Drugs. SIB-1893 [(E)-2-methyl-6-(2-phenylethynyl)-pyridine] (Tocris Cookson Ltd., Bristol, UK), and TPM (Topamax®, Cilag AG, Schaffhausen, Switzerland) were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in 0.9% saline and administered intraperitoneally (i.p.) in a volume of 5 ml/kg body weight.

Measurement of body temperature. To prevent the effects of restraint stress and minimize handling associated with measuring of temperature in animals, the ELAMS (Electronic Laboratory Animal Monitoring System; BioMedic Data Systems Ltd., Seaford, UK – supported by the State Committee for Scientific Research KBN 6 P05F 022 20) was used to measure the body temperature in freely moving rats. This system consists of a desktop unit (DAS-5001, a portable data acquisition system), a probe attached to the desktop, and implantable microchips (IPTT-200; Implantable Programmable Temperature Transponder, BioMedic Data Systems Ltd, Seaford, UK). The transponders were programmed with identification numbers (ID) prior to implantation. The IPTTs contain an anti-migration device which immobilizes the transponder at the implantation (injection) site. Forty rats were subcutaneously (s.c.) implanted with transponders into the dorsal fat-pad. The implanted transponders were read by placing the probe within a distance of 5 cm and the ELAMS read both the temperature and ID of every rat. Animals were randomized into 5 groups (each group consisted of 8 rats) and administered with the vehicle, SIB-1893 (30 mg/kg), TPM (5 mg/kg), TPM (10 mg/kg), and the combination of SIB-1893 (30 mg/kg) with TPM (10 mg/kg). These drug doses were based on the results of our earlier studies [3, 4]. Temperature readings were taken repeatedly at various time points as follows: 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min after vehicle and drugs' administration. This experimental procedure has been described in detail in our earlier studies [15, 16].

Statistics. To confirm that the temperature in each group of rats was normally distributed we used the D'Agostino-Pearson K-squared omnibus normality test and Shapiro-Wilk normality test, which are specifically designed to detect departures from normality. In our study, the D'Agostino-Pearson K-squared omnibus normality and Shapiro-Wilk normality tests revealed that the temperatures measured in rats receiving vehicle, TPM (5 mg/kg), TPM (10 mg/kg), SIB-1893 (30 mg/kg) and the combination of SIB-1893 (30 mg/kg) with TPM (10 mg/kg), was normally distributed. Subsequently, two-way ANOVA with repeated measures of time tested the pattern of time-course data collected *via* ELAMS, using drugs as a between-subject factor, time intervals as a within-subject factor, and temperature as a dependent variable. The *post-hoc* Bonferroni's test was used to compare the temperature of rats administered vehicle with those injected with SIB-1893, TPM, and their combination. Statistical evaluation of data was performed using commercially available GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA, USA). Differences between the respective values were statistically significant at $P<0.05$.

RESULTS

Two-way ANOVA with repeated measures of time revealed that SIB-1893 at a dose of 30 mg/kg significantly reduced the body temperature in rats at 90-240 min after the drug administration. It was documented that SIB-1893 in the 90 min of the observation period decreased the temperature from 36.03 ± 0.19°C to 35.30 ± 0.16°C ($P<0.05$; Figure 1). Similarly, it was found that SIB-1893 at 120, 180 min, and 240 min of temperature monitoring decreased the body temperature in rats from 36.05 ± 0.19°C to 35.17 ± 0.16°C, 36.08 ± 0.18°C to 35.08 ± 0.15°C, and from 36.06 ± 0.17°C to 35.14 ± 0.15°C, respectively ($P<0.05$; Figure 1). In contrast, TPM administered at doses of 5 and 10 mg/kg for the whole time of temperature monitoring did not significantly alter the body temperature in rats, compared to the temperature of the control (vehicle-treated) animals (Figure 1). The combination of TPM (10 mg/kg) with SIB-1893 (30 mg/kg) also produced no significant decrease in the body temperature in rats (Figure 1). With two-way ANOVA with repeated measures on time it was found that the temperature in rats significantly decreased along with the time of measurement [$F(11,385) = 72.32$; $P<0.0001$]. It was noteworthy that the baseline temperature in rats measured on "time 0" (prior to injections of TPM, SIB-1893, and their combination) did not show variations among experimental groups (vehicle, TPM, SIB-1893 and their combination). Similarly, the temperature in rats did not differ significantly between the experimental groups at the same time of measurements [$F(4,385)=0.47$; $P=0.7544$]. The time course patterns for experimental groups (vehicle, TPM, SIB-1893 and their combination) were significantly different, as indicated by two-way ANOVA with repeated measures of time, revealing a significant interaction (treatment × time)-effect between experimental groups and time intervals [$F(44,385) = 7.04$; $P<0.0001$].

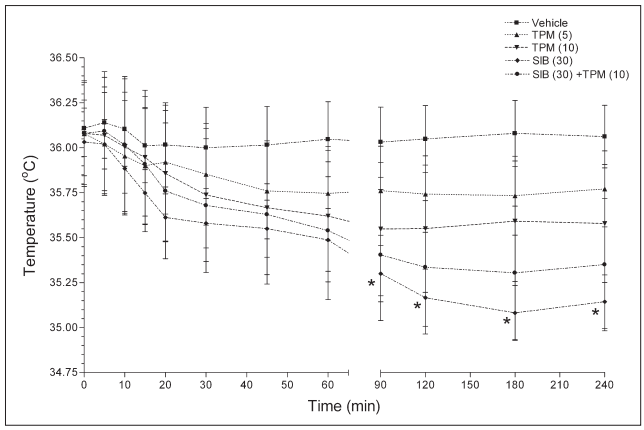

Figure 1. Effect of topiramate (TPM), SIB-1893, and their combination on body temperature in rats.
Data are expressed as means of temperature (in °C) ± SE (error bars) of 8 rats. SIB-1893 (30 mg/kg), TPM (5 mg/kg), TPM (10 mg/kg), the combination of TPM with SIB-1893 (SIB + TPM), and the equivalent amount of vehicle were administered i.p. at the time "0", considered as baseline (reference) time. Temperature was measured with microchips (implanted s.c. into the dorsal fat-pad of rats) at various time points, as follows: 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min after injection of the drugs or vehicle. The D'Agostino-Pearson K-squared omnibus normality and Shapiro-Wilk normality tests revealed that data were normally distributed. Changes in temperature related to the treatment and various time intervals were evaluated using two-way (treatment × time) ANOVA with repeated measures of time, followed by *post-hoc* comparisons *vs.* control using Bonferroni's correction. It is noteworthy that each experimental group under the factor 'treatment' had different subjects (rats), and this grouping factor did not involve any repeated measures. Conversely, individual rats within each factor "treatment" had repeated measurements taken at all intervals of time. Statistical evaluation of the data with two-way ANOVA repeated measures of time, followed by the *post-hoc* Bonferroni's test, revealed that rats receiving SIB-1893 (30 mg/kg) displayed a significant reduction in body temperature at 90, 120, 180, and 240 min post-dose. In contrast, TPM (5 mg/kg), TPM (10 mg/kg), and the combination of SIB-1893 (30 mg/kg) with TPM (10 mg/kg), displayed no significant changes in body temperature in freely mobile rats.

*$P<0.05$ *vs.* respective control temperature in vehicle-treated animals (Bonferroni's *post-hoc* test).

DISCUSSION

The results of this study clearly indicate that SIB-1893 administered systemically (i.p.) at a dose of 30 mg/kg significantly decreased body temperature in freely moving rats at 90-240 min post-dose. This finding is consistent with results described earlier in which SIB-1893 at 30 mg/kg in 90-180 min post-dose markedly reduced body temperature in freely moving rats subjected to the telemetric monitoring of temperature [16]. The presented study also indicated that TPM at a dose of 10 mg/kg (injected systemically either alone or in combination with SIB-1893) had no effect on body temperature in rats in the whole time of temperature monitoring (0-240 min after drug administration).

The presented results indicate that TPM combined with SIB-1893 alleviated the SIB-1893-induced reduction in body temperature in rats. Briefly, TPM diminished the hypothermic effects of SIB-1893 administered alone. Thus, the combination of both drugs (i.e., TPM and SIB-1893) did not significantly decrease the body temperature in rats. It seems that TPM inhibited a decrease in body temperature observed in animals after concomitant administration of SIB-1893. A similar situation was observed in our earlier study [150]. It has been found that OXC administered alone at a dose of 5 mg/kg had no impact on the body temperature in rats [15]. However, OXC (5 mg/kg) in combination with SIB-1893 (30 mg/kg) alleviated the hypothermic effect offered by SIB-1893 administered singly in freely moving rats [15].

Wile interpreting the results of this study, there is another fact worth mentioning. The experimental measures of body temperature in rats were performed at various time intervals after administration of the drugs; therefore, one could evaluate the time-course relationship of drugs and their corresponding changes in the body temperature in rats. Previously, it has been found that SIB-1893 (30 mg/kg) produced a significant decrease in body temperature in rats, showing simultaneously that the method for the evaluation of temperature in experimental rats using ELAMS, microchips and two-way ANOVA, was sensitive enough to detect any changes related to the hypothermic effect of SIB-1893 [15, 16].

In considering the molecular mechanisms of action of both drugs, the question arises whether TPM was able to prevent the reduction in temperature in rats receiving SIB-1893, or the observed effect for the combination of TPM with SIB-1893 was incidental and unrelated to the action of TPM *in vivo*. With respect to TPM, the drug possesses multiple biochemical/pharmacological mechanisms of action, including:

- inhibition of voltage-sensitive Na^+ channels [20];
- potentiation of GABA-mediated inhibitory neurotransmission through binding to a novel site on the $GABA_A$ receptors [22];
- blockade of the excitatory neurotransmission through a negative modulatory effect on Ca^{2+}-permeable AMPA and kainate subtypes of glutamate receptors [7];
- selective inhibition of GluR5 kainate receptors [9];
- inhibition of neuronal L-type high-voltage-activated Ca^{2+} channels [23];
- inhibition of $GABA_A$ receptor-mediated depolarizing responses enhancing the conductance of some types of K^+ channels [13];
- inhibition of the carbonic anhydrase isoenzymes (particularly, CA II and CA IV), and through the changes of pH, the modulation of voltage- and receptor-gated ion channels [6];
- phosphorylation of AMPA/kainate receptors and allosteric modulation of channel conductance [1, 19].

Thus, it seems that TPM reduces the release of glutamate and other excitatory amino acids from synaptic terminals. Since the body temperature in rats is controlled by excitatory amino acids (including glutamate) in the brain, SIB-1893 as a selective non-competitive $mGlu_5$ receptor antagonist, was unable to affect the decreased concentrations of glutamate evoked by TPM. Therefore, SIB-1893 could not significantly reduce the body temperature in rats. Although this hypothesis could readily explain the observed effects of SIB-1893, TPM and their combination on body temperature in freely moving rats, more advanced neurophysiological and experimental studies are required to elucidate the exact effects of these 2 drugs on body temperature.

In conclusion, the combination of TPM (10 mg/kg) with SIB-1893 (30 mg/kg) and TPM administered alone at doses of 5 and 10 mg/kg had no significant effects on the body temperature in freely moving rats up to 240 min after dosing. Only the administration of SIB-1893 (30 mg/kg) was associated with the reduction in body temperature in 90-240 min post-dose.

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