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Influence of oxcarbazepine and its combination with SIB-1893 on body temperature in rats

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Abstract: The aim of the study was to determine the effects of oxcarbazepine (OXC – a newer antiepileptic drug) administered alone and in combination with SIB-1893 (a selective non-competitive metabotropic glutamate subtype 5 [mGlu5] 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 and 180 min. after intraperitoneal administration of OXC, SIB-1893, and their combination. Statistical evaluation of data with two-way ANOVA with repeated measure on time revealed that SIB-1893 at a dose of 30 mg/kg significantly decreased the body temperature in rats at times ranging from 90-180 min. after drug administration. In contrast, OXC at a dose of 5 mg/kg, administered alone and in combination with SIB-1893 (30 mg/kg), did not significantly alter the body temperature in freely moving rats. Based on this pre-clinical study, one can conclude that OXC administered alone and in combination with SIB-1893 have no effect on body temperature in rats up to 180 min. after intraperitoneal administration of the drugs.

Key words: rats, oxcarbazepine, SIB-1893, body temperature, telemetric temperature monitoring

INTRODUCTION

Excitatory amino acids play an important role in various physiological and pathological phenomena in living organisms, interacting with 2 major groups of receptors: ionotropic (N-methyl-D-aspartic acid [NMDA], alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA], kainic acid [KA]) and metabotropic ($mGlu_1$–$mGlu_8$) glutamate receptors [1, 2]. Accumulating experimental evidence indicates that excitatory amino acids are involved in thermoregulation. It has been documented that NMDA increased temperature in rats [3], whereas some NMDA receptor antagonists, such as: MK-801 and (±)-2-amino-5-phosphopentanoic acid ((±)-AP-5) reduced this increase [4]. In contrast, AMPA and KA produced a biphasic effect on body temperature of experimental animals: short-lasting hypothermia followed by hyperthermia [5]. Likewise, some AMPA/KA receptor antagonists, e.g. NBQX, PNQX, and GYKI 52466 lowered the body temperature in experimental animals and produced hypothermia [6-8].

With regards to mGlu receptors, it has been reported that the selective $mGlu_1$ receptor antagonist - BAY 36-7620 induced a mild hypothermia in experimental rats [9]. Additionally, it has been found that MPEP, a selective non-competitive $mGlu_5$ receptor antagonist, significantly decreased temperature in rats [10]. 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 [11]. On the other hand, it has been reported that SIB-1893, in a dose-dependent manner, reduced body temperature in freely moving rats [12].

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The aim of this study was to evaluate the effects of oxcarbazepine (OXC – a second-generation antiepileptic drug) administered alone and in combination with SIB-1893 on body temperature in freely moving rats. OXC was administered intraperitonally (i.p.) at a dose of 5 mg/kg, and SIB-1893 injected at a dose of 30 mg/kg. These drug doses have been found to significantly reduce afterdischarge and seizure durations in amygdala-kindled rats [13]. Because the combination of OXC with SIB-1893 exerted a significant anticonvulsant effect in amygdala-kindled rats, and SIB-1893 by itself decreased the body temperature in rats, it was of pivotal importance to determine the effects of both drugs in combination 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 kept in colony cages with free access to food and tap water, under standardized housing conditions (12 h light-dark cycle, stable temperature 22 ± 1ºC for 24 h). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 rats. All tests were performed between 09.00-15.00. 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 of Lublin.

Drugs. SIB-1893 [(E)-2-methyl-6-(2-phenylethynyl)-pyridine] (Tocris Cookson Ltd., Bristol, UK), and OXC (Trileptal, Novartis Pharma AG, Basel, 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, Seaford, UK) 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, 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. Upon arrival, 32 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 each rat. Animals were randomized into 4 groups (each group consisted of 8 rats) and administered with a vehicle (1% solution of Tween 80 in 0.9% NaCl), SIB-1893 (30 mg/kg), OXC (5 mg/kg), and their combination (SIB-1893 + OXC). Temperature readings were taken repeatedly at various time intervals as follows: 0, 5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 min. after administration of the vehicle (1% solution of Tween 80 in 0.9% NaCl) and drugs.

Statistics. Two-way ANOVA with repeated measure on time tested the pattern of time-course data collected via ELAMS, using drugs as the 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 the vehicle with those injected with SIB-1893, OXC, 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 measure on time revealed that SIB-1893 at a dose of 30 mg/kg significantly reduced the body temperature in rats at 90-180 min. after drug administration. It was noted that at the 90 min. of the observation period SIB-1893 decreased the temperature from 35.95°C to 35.35°C ($P<0.05$; Table 1; Figure 1). Similarly, it was found that at 120 min., and 180 min. of the temperature monitoring, SIB-1893 decreased the body temperature in rats from 35.91°C to 35.17°C, and from 35.92°C to 34.99°C, respectively ($P<0.05$; Table 1; Figure 1). In contrast, OXC administered at a dose of 5 mg/kg for the whole time of temperature monitoring did not significantly alter the body temperature in rats, compared to the control (vehicle-treated animals) temperature (Table 1; Figure 1). The combination of OXC (5 mg/kg) with SIB-1893 (30 mg/kg) also produced no significant decrease in body temperature in rats (Table 1; Figure 1). With two-way ANOVA with repeated measure on time it was found that the temperature in rats significantly decreased along with the time of measurement [$F(10,280) = 30.16$; $P<0.0001$] (Table 1). It is noteworthy that the

Table 1 Effects of oxcarbazepine (OXC), SIB-1893, and their combination on body temperature in rats.

	Temperature (°C)			
Time	Vehicle	OXC (5 mg/kg)	SIB (30 mg/kg)	SIB + OXC
0	36.11 ± 0.21	36.08 ± 0.29	36.03 ± 0.20	36.08 ± 0.28
5	36.16 ± 0.20	36.10 ± 0.33	36.02 ± 0.20	36.10 ± 0.33
10	36.07 ± 0.20	36.02 ± 0.33	35.88 ± 0.21	36.05 ± 0.36
15	35.95 ± 0.22	35.96 ± 0.36	35.75 ± 0.21	35.91 ± 0.36
20	35.99 ± 0.23	36.05 ± 0.37	35.61 ± 0.20	35.80 ± 0.37
30	35.95 ± 0.24	36.09 ± 0.36	35.58 ± 0.21	35.68 ± 0.36
45	35.91 ± 0.23	36.08 ± 0.32	35.55 ± 0.20	35.63 ± 0.37
60	35.96 ± 0.22	36.08 ± 0.31	35.49 ± 0.21	35.62 ± 0.36
90	35.95 ± 0.24	36.09 ± 0.35	35.35 ± 0.20 *	35.68 ± 0.34
120	35.91 ± 0.22	36.08 ± 0.32	35.17 ± 0.20 *	35.63 ± 0.37
180	35.92 ± 0.20	36.01 ± 0.33	34.99 ± 0.21 *	35.62 ± 0.35

time: $F(10,280) = 30.16$ $P<0.0001$
treatment: $F(3,280) = 0.57$ $P=0.6387$
time × treatment: $F(30,280) = 8.06$ $P<0.0001$

Values presented as means (°C) ± SEM of 8 rats. Changes in temperature related to treatment. Various time intervals were evaluated using two-way (treatment × time) ANOVA with repeated measure on time followed by *post-hoc* comparisons versus control using Bonferroni's correction. Note: each experimental group under factor "treatment" had different subjects (rats), a grouping factor involving no repeated measurements. Inversely, individual rats within each factor "treatment" had repeated measurements taken at all time intervals.

F – F-statistics; P – probability.
* $P<0.05$ vs. respective control temperature in vehicle (1% solution of Tween 80 in 0.9% NaCl)-treated animals (Bonferroni's post-hoc test).

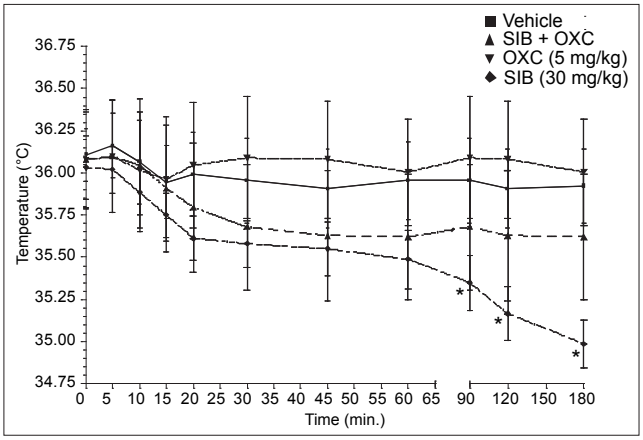

Figure 1 Effects of oxcarbazepine (OXC), SIB-1893, and their combination on body temperature in rats.
Data expressed as means of temperature (°C) ± SEM (as the error bars) of 8 rats. SIB-1893 (30 mg/kg). OXC (5 mg/kg). Combination of OXC with SIB-1893 (SIB + OXC), and the equivalent amount of vehicle (1% solution of Tween 80 in 0.9% NaCl) administered i.p. at time "0", considered as baseline (reference) time. Temperature was measured with microchips (implanted s.c. into dorsal fat-pad of rats) at various time intervals as follows: 0, 5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 min. after injection of drugs or vehicle (1% solution of Tween 80 in 0.9% NaCl). Statistical evaluation of data with two-way ANOVA with repeated measure on time, followed by post-hoc Bonferroni's test, revealed that rats receiving SIB-1893 (30 mg/kg) displayed a significant reduction in body temperature at 90, 120, and 180 min. post-dose. In contrast, OXC (5 mg/kg) and combination of OXC with SIB-1893 displayed no significant changes in body temperature.

* $P<0.05$ vs. respective control temperature in vehicle (1% solution of Tween 80 in 0.9% NaCl)-treated animals (Bonferroni's post-hoc test).

baseline temperature in rats measured on "time 0" (prior to injections of OXC, SIB-1893, and their combination) did not show variations among experimental groups (vehicle, OXC, SIB-1893, and their combination; Table 1). Similarly, the temperature in rats did not differ significantly between the experimental groups at the same time of measurements [$F(3,280)=0.57$; $P=0.6387$] (Table 1). The time-course patterns for experimental groups (vehicle, OXC, SIB-1893, and their combination) were significantly different, as indicated by two-way ANOVA with repeated measure on time, revealing a significant interaction (treatment × time)-effect between experimental groups and time intervals [$F(30,280) = 8.06$; $P<0.0001$] (Table 1).

DISCUSSION

The results obtained from 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 90-180 min. post-dose. This finding is consistent with results described earlier, reporting that SIB-1893 at 30 mg/kg in 90-180 min. post-dose markedly reduced body temperature in freely moving rats subjected to telemetric monitoring of temperature [12]. It was noteworthy that the reduction of body temperature in the rats was 0.81°C [12], whereas in the present study the body temperature in the rats decreased by 0.93°C. This study also indicated that OXC at a dose of 5 mg/kg (injected systemically either alone or in combination with SIB-1893) had no effect on body temperature in rats during the whole time of temperature monitoring (0-180 min. after drug administration). As presented in the Introduction, the dose of 5 mg/kg of OXC corresponded to the dose of the antiepileptic drug, which in combination with SIB-1893 (30 mg/kg) significantly suppressed afterdischarge and seizure durations in amygdala-kindled rats [13]. This is why OXC at the dose of 5 mg/kg was tested in this study. Results presented here demonstrate that OXC combined with SIB-1893 alleviated the SIB-1893-induced reduction in body temperature in rats. Briefly, OXC diminished hypothermic effects of SIB-1893 administered alone. Thus, the combination of both drugs (i.e., OXC and SIB-1893) did not significantly decrease the body temperature in the rats. It seems that OXC inhibited a decrease in body temperature observed in animals after concomitant administration of SIB-1893.

There is another fact worth mentioning concerning interpretation of the results of this study. The experimental measures of body temperature in the rats were performed at various time intervals after administration of the drugs; therefore, one could evaluate the time-course relationship of the drugs and their corresponding changes in the body temperature in the rats. It had been found previously that SIB-1893 (30 mg/kg) produced a significant decrease in body temperature in rats, and showed 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 [12]. It should be stressed that the measurement of body temperature was performed in naïve rats receiving OXC, SIB-1893, or their combination (at doses corresponding to those denoted previously in amygdala-kindled rats). No chronic electric stimulations or surgical brain operations were performed on rats subjected to the temperature monitoring. This was in order not to change their natural sensibility to temperature by electric current and/or infections, as well as not to destroy the brain set-point responsible for the control of temperature in animals. It is highly likely that procedures of implantation of bipolar electrodes to amygdala in the brains of rats, and chronic stimulations to evoke seizures in rats may impair the natural set-point in the brain and thus significantly affect the control of body temperature.

It is important to note that IPTT transponders can be easily implanted either subcutaneously (s.c.) into the neck region of rodents, or intraperitoneally (i.p.) into the abdominal region of the experimental animals [14]. However, the results presented by Kort *et al.* [14] revealed that temperatures taken either s.c. or i.p. did not differ significantly, although i.p. rather than s.c. better reflects the core temperature of the animals. On the other hand, the authors have documented that IPTT transponders placed s.c. were not influenced by differences in room temperature and were read more easily by the data scanner than implanted i.p. [14]. Thus, the s.c. site seems to be the preferred place of IPTT transponder implantation in rodents.

Considering the molecular mechanisms of the action of both drugs, the question arises whether OXC was able to prevent the reduction in temperature in rats receiving SIB-1893, or the observed effect for the combination of OXC with SIB-1893 was incidental and unrelated to the action of OXC *in vivo*. With respect to molecular mechanisms of action of OXC, the drug blocks voltage-dependent fast sodium channels and high-voltage-activated calcium channels in neurons [15]. Thus, OXC reduces the release of glutamate and other excitatory amino acids from the synaptic terminals [15]. As mentioned in the Introduction, the body temperature in rats is controlled by excitatory amino acids, including glutamate, in the brain. In this case, SIB-1893 – as the selective non-competitive $mGlu_5$ receptor antagonist – was unable to affect the decreased concentrations of glutamate evoked by OXC. Therefore, SIB-1893 could not significantly reduce the body temperature in rats pretreated with OXC.

With respect to SIB-1893, it is important to note that the agent also has some properties of the non-competitive NMDA receptor antagonist. In an *in vitro* study, it has been reported that SIB-1893 protected cultured rat cortical cells against glutamate- and NMDA-evoked neurotoxicity, suggesting that SIB-1893 blocked NMDA receptor-mediated neurotoxicity [16]. Thus, it seems that SIB-1893 produces its hypothermic effect *via* both, NMDA and $mGlu_5$ receptor-mediated antagonism. Since non-selective NMDA receptor antagonists produce a definite hypothermic effect in rodents [4], one can ascertain that both mechanisms (NMDA and $mGlu_5$ receptor-mediated antagonism) contribute to the reduction of body temperature in animals pretreated with SIB-1893. On the other hand, one can formulate an alternative hypothesis - ascertaining that SIB-1983 exerts its hypothermic effect in rodents only by blocking NMDA receptors in experimental animals, and the blockade of $mGlu_5$ receptors had no impact on the body temperature in animals. Because the above-mentioned hypotheses would readily explain the observed effects of SIB-1893, OXC, and their combination on body temperature in freely moving rats, more advanced neurophysiological and experimental studies are required to elucidate the exact effects of SIB-1893 on the body temperature in freely moving rats.

In conclusion, the combination of OXC (5 mg/kg) with SIB-1893 (30 mg/kg) and OXC administered alone at the dose of

5 mg/kg had no significant effects on the body temperature of freely moving rats up to 180 min. after dosing. Only the administration of SIB-1893 (30 mg/kg) was associated with the reduction in body temperature at 90-180 min. post-dose.

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