



## Prostaglandin involvement in hyperthermia induced by sleep deprivation: A pharmacological and autoradiographic study

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### ARTICLE INFO

#### Article history:

Received 19 June 2008

Accepted 9 December 2008

#### Keywords:

Cyclooxygenase-2 (COX-2) inhibitor

Sleep

Temperature

Hypothalamus

Rat

### ABSTRACT

**Aims:** Hyperthermia is a characteristic functional effect of sleep deprivation (SD). We hypothesize here that prostaglandin E2 (PGE2) could be involved in hyperthermia induced by sleep deprivation.

**Main methods:** To address this issue we examined the effects of a selective cyclo-oxygenase-2 inhibitor (COX-2) agent on hyperthermia induced by SD in rats. We also investigated binding to PGE2 receptors in hypothalamic brain areas of sleep-deprived rats using *in vitro* autoradiography. Male Wistar rats were deprived of sleep for 96 h using the platform technique. Sleep deprived and control groups received saline or Celecoxib (20, 30 and 40 mg/kg; p.o.) daily during the SD period. Colonic temperature was measured daily.

**Key findings:** Results indicated that core temperature of sleep-deprived rats that receiving saline increased from the first to the fourth day of SD compared to baseline and to the respective control group. However, the hyperthermia induced by SD was not blocked by COX-2 inhibitor at any dose. [<sup>3</sup>H]PGE2 binding did not differ significantly among the groups in any of a number of hypothalamic areas examined.

**Significance:** Although SD rats showed no response to the COX-2 inhibitor and no alterations in [<sup>3</sup>H]PGE2 binding, the possibility remains that other prostaglandin system and/or receptor subtypes may be altered by SD.

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### Introduction

Given the interactions that exist among temperature, thermoregulation, and sleep, hypotheses have been advanced about possible thermoregulatory functions of sleep. One of the first hypotheses on a thermoregulatory role of sleep (slow wave sleep) has been proposed by McGinty and Szymusiak (1990). Sleep deprivation (SD) procedure is one of the most used methods to explore the functions of sleep. Rats subjected to SD have consistently shown evidence of inability to retain body heat and elevated temperature set point which induces an increase in body temperature, an effect that in turn may develop a number of other functional consequences, and may be a factor in the lethality of prolonged SD. Body temperature increases by 0.5 to 1 °C above normal during the first days of SD and in a prolonged sleep deprivation protocol, mild hypothermia develops, which can worsen over a few days (Bergmann et al., 1989). Although it is clear that hypothermia follows hyperthermia, we do not know the reasons for this phenomenon and the mechanisms underlying either hyperthermia or hypothermia.

Accumulating evidence suggests that prostaglandins (PG) have numerous and diverse biological effects on a wide variety of phy-

siological activities, including a modulatory effect on both thermoregulation and sleep body temperature (Wolfe, 1982; Onoe et al., 1992). PGE2, the dominant form of endogenous E-series prostaglandins (PGE), is produced in the brain in response to exogenous and endogenous pyrogens and seems to be the crucial neuronal mediator of fever in the brain through an action on PG receptor-expressing neurons in the preoptic area (POA) of the anterior hypothalamus (Milton, 1982). Injections of PGEs into either the cerebral ventricle or the preoptic and hypothalamic area (POHA) evoke fever-like hyperthermia (Milton, 1982), and promote of wakefulness (Matsumura et al., 1989).

Both in hypothalamus and in cortex, PGE2 levels appear to reflect transitions between vigilance states. PGE2 levels are high in the middle of wakefulness, and then regularly drop before the onset of sleep; low levels persist during sleep then rise prior to the next period of wakefulness (Nicolaidis et al., 2001).

We have found that the hyperthermia induced by SD using the platform method can be partially blocked by sodium diclofenac, one of the major traditional nonsteroidal anti-inflammatory drugs (tNSAIDs) (Seabra and Tufik, 1993). In this procedure, rats are aroused from sleep when sleep-related atonia causes them to fall off the platform. The technique is based on the muscle atonia that accompanies paradoxical sleep (Jouvet et al., 1964). Along similar lines, Bergmann et al. (1993) reported that aspirin, a cyclooxygenase (COX-1) inhibitor, caused a fall in body temperature from the elevated level induced by SD (disk-over water method), suggesting involvement of PG in the hyperthermia due to prolonged wakefulness.

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We hypothesized that temperature changes after SD may be mediated by PGE<sub>2</sub>. To address this issue, we investigated the effect of selective COX-2 inhibitor agent, celecoxib, on hyperthermia induced by SD in rats. The density of PGE<sub>2</sub> binding sites in the brain using *in vitro* autoradiography was also investigated in sleep-deprived rats. We thought that measuring hypothalamic PGE<sub>2</sub> receptor binding would be important given that the hypothalamus is the thermostat of the body and since PGE<sub>2</sub> is an important sleep-promoting substance and the final mediator of fever.

## Materials and methods

### Animals

Experiments were conducted using male Wistar rats weighing 300–400 g housed at 21 ± 2 °C under a 12:12 h light–dark cycle (lights on at 07:00 AM) with free access to food and tap water throughout the study. The animals were bred and raised in the animal facility of the Department of Psychobiology. All procedures were carried out in accordance with the National Institute of Health (NIH) guidelines on animal care and were approved by the Ethical Committee of UNIFESP (CEP # 318/08).

### Sleep deprivation

Sleep deprivation was achieved using the multiple platform technique by placing the rats in a tank (22 cm long × 22 cm wide × 35 cm high) containing 18 platforms (7.0 cm in diameter), an arrangement that allowed them to move inside the tank, jumping from one platform to the other. The tank was filled with water to approximately 1 cm below the surface of the platforms. This method completely abolishes REM sleep and also reduces slow-wave sleep by approximately 30% (Machado et al., 2004). The animals were randomly assigned to two main groups: Control (CTR) – rats remained in their home-cages in the SD room; Sleep deprivation (SD) – rats were deprived of sleep for 96 h (4 days). Throughout the study both groups had free access to food and water. All rats were habituated to the platform technique for 1 h per day on the 2 days preceding the onset of the experiment.

### Temperature measurements

Colonic temperature was measured with a copper–constantan thermocouple. Each animal was picked up gently and held manually; the base of their tail was held lightly and the thermocouple, which had been lubricated with vegetable oil, was inserted into the rectum to a depth of 5 cm. The thermocouple was kept in place for 30 s, the time required for the digital thermometer to stabilize. Temperature measurements were performed once a day at 9:00 AM. To minimize core temperature changes due to handling, animals were habituated to this procedure for three days before the beginning of the experiment. The same procedure was conducted during 4 days of SD.

### Drugs

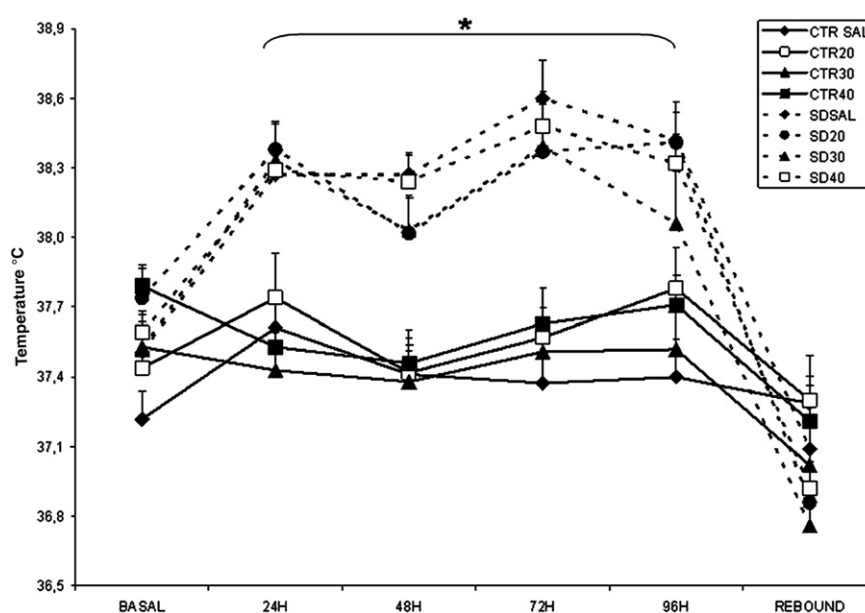
Celecoxib was purchased from PFIZER (Celebra<sup>®</sup>, São Paulo, Brazil), dissolved in 0.9% saline, and administered by oral gavage in a volume of 1 mL/kg. The solutions were prepared immediately prior to experiment.

### Experimental protocols

The effects of celecoxib (20, 30 and 40 mg/kg) or its vehicle (sterile saline 0.9%) on core temperature were evaluated in SD (*n*=40) and CTR (*n*=40) groups. During the baseline period (before SD started) and during the 4 days of SD the temperature was measured at 9:00 AM, and rats received drug or saline immediately after. On the last day of SD (96 h) and 24 h after the end of SD (rebound period – period that animals could sleep freely) only core temperature was measured and no drug or vehicle was administered. Animals were weighed once a day at the same time everyday.

### Autoradiography procedures

Separate groups of sleep-deprived (*n*=8) and CTR (*n*=7) animals were used for analyses of [<sup>3</sup>H]PGE<sub>2</sub> binding. At the end of 96 h of SD all rats were sacrificed by decapitation. Brains were rapidly removed frozen over dry ice and stored at –80 °C until used. Coronal cryostat sections (20 μm) were cut at –18 °C. PGE<sub>2</sub> receptors binding was measured with [<sup>3</sup>H]PGE<sub>2</sub> (Matsumura et al., 1992). Briefly, sections



**Fig. 1.** Colonic temperature measurements in control (CTR) and sleep deprived (SD) rats treated with celecoxib (20, 30 or 40 mg/kg) or vehicle (saline – SAL) during 96 h of SD and sleep rebound. Values are reported as mean ± SE. \* *p* < 0.0001, all sleep-deprived animals are different from respective basal value and CTR group.

were pre-incubated 4× in 150 ml of 50 mM Tris–HCl (pH 7.4) containing 0.1 M NaCl for a total of 60 min. The sections were then incubated with 20 nM [<sup>3</sup>H]PGE2 (PERKIN ELMER, Boston, MA – 200 Ci/mmol) for 30 min. Thereafter, they were rinsed in 4 sequential short (15 s each) dips in buffer, quickly dipped in distilled water, and air-dried. Nonspecific binding was obtained in adjacent sections by the addition of 100 μM unlabeled PGE2 to the incubation mixture. Once dried, the slides were tightly apposed to tritium-sensitive, Kodak Biomax film in the presence of calibrated standards and stored at 4 °C for 5 weeks. Densitometric analyses were performed using M2 MCID system (Imaging Research, St. Catharines, Ontario). Anatomical regions were defined according to the atlas of Paxinos and Watson (1997) and sampled without knowledge of group membership of the animals.

#### Data analysis

Core temperature data for the baseline phase was analyzed using a one-way ANOVA. Data for the sleep deprivation phase was analyzed with three-way repeated measures ANOVA, with group (CTR, SD), doses (20, 30, 40 mg/kg) and day as factors and data for the rebound phase were analyzed with a two-way ANOVA, with previous sleep deprivation condition (96 h). When warranted by significant interactions, post-hoc analyses were performed using the Tukey's test. Binding data were analyzed by one-way ANOVA for each brain region. Data are expressed as means±SE. A *p*-value ≤0.05 was considered statistically significant.

## Results

### Colonic temperature

Results are shown in Fig. 1. During baseline there were no significant differences among groups. A three-way ANOVA for the sleep deprivation period revealed a significant group effect  $F(1, 70)=74, p<0.00001$ , a significant day effect  $F(3,70)=31, p<0.00001$ , and a significant interaction between these factors  $F(3,70)=25, p<0.00001$ , but no effect of interaction between of the 3 factors (group, doses and day). Post-hoc analyses indicated that, as expected, the SD group that received vehicle presented significant increases in temperature compared both to baseline and to control group on each day of sleep deprivation ( $p<0.00001$ ). A two-way ANOVA indicated a significant group effect  $F(1, 76)=5.4, p<0.02$ , a significant day effects (96 h of SD and rebound period)  $F(1, 76)=185.6, p<0.00001$  and interaction between these factors  $F(1, 76)=54.73, p<0.00001$ . Post-hoc analyses indicated that at rebound time-point, the sleep-deprived animals shown significant reduction ( $p<0.00001$ ) on core temperature in comparison to the previous time point (96 h). No difference was observed between the sleep deprivation and control groups at the rebound period.

**Table 1**  
[<sup>3</sup>H]PGE2 binding to PGE2 receptors

	CONTROL (n=7)	SLEEP DEPRIVED (n=8)	<i>p</i>
<i>Anterior hypothalamic area</i>			
Lateral preoptic a.	1.888±0.122	1.840±0.111	0.773
Medial preoptic a.	2.105±0.117	2.057±0.124	0.786
Median preoptic n.	2.372±0.178	2.204±0.126	0.446
Ventromedial preoptic n.	2,317±0.114	2.276±0.150	0.839
<i>Posterior hypothalamic a.</i>			
Lateral hypothalamic a.	1.827±0.091	1.712±0.096	0.405
Supramammillary n., medial part	2.078±0.086	2.025±0.115	0.726
Supramammillary n., lateral part	2.040±0.105	1.987±0.083	0.695
Lateral mammillary n.	2.145±0.092	2.027±0.123	0.468
Medial mammillary n., medial part	2.190±0.109	2.121±0.127	0.689
Medial mammillary n., lateral part	2.089±0.091	2.032±0.109	0.697

Values are means±sem in pmol/g Tissue.

### [<sup>3</sup>H]PGE2 autoradiography

Results are shown in Table 1. The results indicated that [<sup>3</sup>H]PGE2 binding was not significantly altered by SD in any of hypothalamic areas examined.

## Discussion

The present study confirms previous observations that SD causes a sustained increase in body temperature, and found that SD-induced hyperthermia was not affected by COX-2 inhibition.

It is well known that the PGE forms the basis of an important pathway leading to increased set points in hypothalamic thermoregulation (for review see Lazarus, 2006). Sleep deprivation studies indicate that total SD in rats produces an elevation of the hypothalamic temperature set point (Prete et al., 1991). Seabra and Tufik (1993) previously found that hyperthermia induced by 96 h of SD was blocked by sodium diclofenac, a traditional nonsteroidal anti-inflammatory drug (tNSAID), but only partially. Similarly, Bergmann et al. (1993) reported a weak effect of aspirin (highly selective for COX-1) on control of temperature in sleep-deprived animals. Our study is partially consistent with these previous findings, inasmuch as a PG involvement in SD-induced hyperthermia could not be totally demonstrated. Interestingly, these different drugs (aspirin, celecoxib and sodium diclofenac) have distinct pharmacodynamic and pharmacokinetic characteristics, but have similar effects on the control of temperature in sleep-deprived animals. The tNSAIDs and coxibs (i.e. celecoxib) are 8- to 29- fold and 30 - to 433-fold more potent against COX-2 *in vitro*, respectively (Botting, 2006). These previous studies and the present results suggest that none of the available treatments can prevent the hyperthermia induced by the SD. The mechanisms by which SD affects the temperature are not completely understood, but the first 24 h of SD by platform method appear to be critical to alter the temperature regulation.

After the SD period ended (rebound time), core temperature was restored in all sleep-deprived groups. This observation is consistent with a thermoregulatory function for sleep, inasmuch as the recovery period after SD is marked by increased pressure to sleep (REM and Slow Wave Sleep – SWS). Machado et al. (2004) demonstrated that 24 h of undisturbed sleep after the end of 96 h of SD was enough for a complete recovery of both types of sleep.

It is possible that factors other than PG could be involved in the hyperthermia induced by SD. One possible stimulus for temperature increase in sleep-deprived rats is cytokine activity. Increased interleukin -1 (IL-1) concentrations after SD have been widely reported (Opp and Krueger, 1994; Taishi et al., 1998; Hu et al., 2003). Crucial involvement of IL-1 in the fever response to different exogenous pyrogenic stimuli is well recognized (Dinarello, 1984), and it is well established that PG and this cytokine can act on the brain, especially in the development of the fever response (Blatteis et al., 2005).

In the present study, quantitative receptor autoradiography revealed that PGE binding sites were not significantly affected by SD in hypothalamic regions. The absence of changes in PGE2 binding is in agreement with the pharmacological data. Thus, if SD alters the PGE2 system such alterations do not seem to occur at the level of altered receptor binding. However, it is also possible that changes in PGE2 binding occur early in the course of SD and later normalize in a similar manner, as the first 24 h of SD determines the induction of hyperthermia. It is also conceivable that binding changes would become detectable after 96 h of deprivation. This hypothesis could be tested by analyzing PGE2 receptor binding at earlier points during SD and after more prolonged periods of sleep deprivation.

Our results are in line with the observations of Gelir et al. (2005), in that no significant alterations in hypothalamic PG levels were observed after SD. The possibility remains that increased temperatures observed during sleep disruption might be due to alterations other PG

receptor subtypes. Ram et al. (1997) demonstrated a close relationship between the duration of SD and CSF levels of prostaglandins; these changes were similar for PGD, PGE, and PGF. These prostaglandins, mainly PGD and PGE, influence several biological phenomena, including sleep-wake activities (Hayaishi and Matsumura, 1995).

It is important to note that body temperature is influenced by many others physiological factors, including stress, behavioral state, locomotor activity, and biological rhythms, all of which are altered by SD condition. Several lines of evidence support the notion that SD is a stressful stimulus (Suchecki et al., 1998; Sgoifo et al., 2006). It has been argued whether the results of SD studies can be attributed to SD per se, or rather to stress-related factors derived from the SD procedure. SD itself can thus be considered a biological stressor given that sleep is essential for life and to health. It is therefore important to mention that we tried to minimize the stress of animals during the SD procedure and temperature assessments.

## Conclusion

The present findings provide pharmacological evidence that the selective COX-2 inhibitor celecoxib was ineffective for blocking hyperthermia induced by SD, suggesting that if the PG system is involved in hyperthermia, this is not mediated by PGE2 receptors. The absence of changes in PGE binding in the present study is in line with this possibility. The possibility remains that other PG receptor subtypes may be involved in this mechanism.

## Acknowledgments

The authors thank Karin Di Monteiro Moreira for her invaluable help in autoradiography study. The authors are also grateful to Diva Lima and Cristiano Resende for expert technical assistance. Research support was provided by FAPESP-CEPID (98/14303-3) and AFIP. D.C. Hipolide and S. Tufik received fellowships from CNPq.

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