Total Sleep Deprivation Alters Endothelial Function in Rats: A Nonsympathetic Mechanism

Fabien Sauvet, MD, PhD¹; Geneviève Florence, DVM, PhD¹; Pascal Van Beers, AS¹; Catherine Drogou, MS¹; Christophe Lagrume, AS¹; Cyrielle Chaumes, AS¹; Sylvain Ciret, AS¹; Georges Leftheriotis, MD, PhD^{2,3}; Mounir Chennaoui, PhD¹

¹Armed Forces Biomedical Research Institute (IRBA), Brétigny-sur-Orge, France; ²University of Angers, Angers, France; ³Unité mixte Centre National de la Recherche Scientifique (CNRS) 6214 - Institut National de la Santé et de la Recherche Médicale (INSERM) 771, Angers, France

Study Objectives: Sleep loss is suspected to induce endothelial dysfunction, a key factor in cardiovascular risk. We examined whether sympathetic activity is involved in the endothelial dysfunction caused by total sleep deprivation (TSD).

Design: Two groups: TSD (24-h wakefulness), using slowly rotating wheels, and wheel control (WC).

Participants: Seven-month-old male Wistar rats.

Interventions: Pharmacological sympathectomy (reserpine, 5 mg/kg, intraperitoneal), nitric oxide synthase (NOS) inhibition (*N*^G-nitro-L-arginine, 20 mg/kg, intraperitoneally 30 min before experiment) and cyclooxygenase (COX) inhibition (indomethacin, 5 mg/kg, intraperitoneally 30 min before experiment).

Measurements and Results: In protocol 1, changes in heart rate (HR) and blood pressure were continuously recorded in the sympathectomized and non-sympathectomized rats. Blood pressure and HR increased during TSD in non-sympathectomized rats. In protocol 2, changes in skin blood flow (vasodilation) were assessed in the sympathectomized and non-sympathectomized rats using laser-Doppler flowmetry coupled with iontophoretic delivery of acetylcholine (ACh), sodium nitroprusside (SNP), and anodal and cathodal currents. ACh- and cathodal current-induced vasodilations were significantly attenuated after TSD in non-sympathectomized and sympathectomized rats (51% and 60%, respectively). In protocol 3, ACh-induced vasodilation was attenuated after NOS and COX inhibition (66% and 49%, respectively). Cathodal current-induced vasodilation decreased by 40% after COX inhibition. In TSD compared to WC a decrease in ACh-induced vasodilation was still observed after COX inhibition. No changes in SNP- and anodal current-induced vasodilation were detected.

Conclusion: These results demonstrate that total sleep deprivation induces a reduction in endothelial-dependent vasodilation. This endothelial dysfunction is independent of blood pressure and sympathetic activity but associated with nitric oxide synthase and cyclooxygenase pathway alterations.

Keywords: Acetylcholine, arterial pressure, current-induced vasodilation, endothelial dysfunction, iontophoresis, skin blood flow, sodium nitroprusside, sympathectomy, vascular reactivity

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INTRODUCTION

Sleep loss resulting from increased shift work and various other challenges imposed by modern society¹ may represent a serious threat to health. Decreased sleep time is notably associated with the development of cardiovascular diseases including stroke, coronary events, sudden cardiac death, and hypertension.²⁻⁴ In a recent study, we showed that 29 h of continuous wakefulness is sufficient stress to trigger a decrease in endothelial-dependent cutaneous vasodilation⁵ in healthy humans. Other authors have confirmed this endothelial dysfunction in healthy subjects after acute sleep restriction.⁶⁻⁹ Endothelial dysfunction is already known to be a key factor in the pathophysiology of cardiovascular disease.¹⁰ The effect of total sleep deprivation (TSD) on the endothelial function of rodents has never before been published to the best of our knowledge.

Among the possible mechanisms linking sleep disorders to cardiovascular diseases are activation of the sympathetic nervous system suspected of inducing an increase in blood

pressure¹¹ and an endothelial dysfunction⁶ that may trigger the cardiovascular events more frequently observed in chronic short sleepers (< 6 h)¹² or after an acute lack of sleep.^{13,14} Indeed, many studies have observed that chronic sleep disorders or acute sleep restriction are associated with activation of the sympathetic nervous system, increased blood pressure, and baroreflex sensitivity changes in both humans^{6,11,15} and rodents.¹⁶ However, the effects of acute TSD on sympathovagal balance are less clear. Although some studies showed increased sympathetic activity and heart rate (HR),¹¹ others reported no significant changes in resting HR, increased arterial pressure, and a reduction in muscle sympathetic nerve activity.¹⁷⁻¹⁹ Moreover, we observed that endothelial dysfunction appears before the increase in sympathetic activity and blood pressure⁵ during 40 h of wakefulness (i.e., TSD) in healthy humans. In other words, the available data still do not provide a clear picture regarding the role of sympathetic activation in the development of endothelial dysfunction during sleep loss.

Because the skin is readily accessible in humans and rodents, it provides an appropriate site to assess peripheral microvascular endothelial function with noninvasive methods.^{20,21} In particular, vascular response to ACh administration, a substance that induces cutaneous vasodilation via endothelium-dependent releases of a variety of vasoactive substances such as nitric oxide (NO), prostacyclin (PGI₂) and endothelium-derived hyperpolarizing factors (EDHF), is correlated with cardiac and large-vessel endothelial function.²²⁻²⁴ Currently,

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Dr. Fabien Sauvet, NCO / Unité Fatigue et Vigilance, French Armed Forces Biomedical Research Institute, Institut de recherche biomédicale des armées (IRBA), AP 73, 91223 Brétigny sur Orge, France; E-mail: fabien.sauvet@irba.fr

low ACh-induced cutaneous vasodilation is used as a surrogate marker of cardiovascular risk.²⁵

The purposes of this study were (1) to characterize the effect of TSD (24 h of wakefulness) on blood pressure and HR in sympathectomized and non-sympathectomized rats; (2) to assess the effect of TSD and sympathectomy on cutaneous endothelial-dependent vasodilation; and (3) to evaluate the effects of TSD on the NO and PGI₂ metabolic pathways implicated in endothelial-dependent vasodilation.

METHODS

Animals

Experiments were performed with male Wistar rats (250-350 g, 7 to 9 w old, Janvier laboratory, Le Genest-St-Isle, France). Procedures for the maintenance and use of the animals were carried out in accordance with French regulations and approved by the institutional ethics committee for animal experimentation.

On arrival the rats were housed five per cage in a constant environment (temperature: $22 \pm 2^\circ\text{C}$; relative hygrometry: $50 \pm 15\%$) and a 12/12 light/dark cycle (300 Lux) with lights on from 07:00 to 19:00. Animals had access to standard rodent pellets and water *ad libitum*. All animals were given at least 1 w before the experiment to acclimatize to the environment. The rats were then randomly distributed into two groups: TSD (total sleep deprivation) or WC (wheel control). Each rat's weight was recording daily in both groups (TSD and WC).

Total Sleep Deprivation

Rats were singly housed throughout the TSD experiments in activity wheels (Lafayette Instruments, Lafayette, IN, USA) customized as previously described and validated.²⁶ Briefly, these are large motorized stainless-steel activity wheels that allow free access to food and water. In the 5 days preceding the TSD experiment the rats were habituated to the activity wheel with a 30-min session at 09:00 and 15:00 every day. TSD (09:00-09:00) was produced by the rotation of the activity wheel, programmed on a schedule of 3 sec "on" at a speed of 3 m.min⁻¹ and 12 sec "off". Similar protocols have previously been shown to result in wakefulness for more than 93% of a 24-h period.²⁶ In our experimental condition, we observed that the animals had the following sleep pattern: $97.1 \pm 2.5\%$ of wakefulness, $2.8 \pm 2.1\%$ of non-rapid eye movement (NREM) sleep and $0.1 \pm 0.1\%$ of rapid eye movement (REM) sleep.

To control the nonspecific effects of the activity wheel (i.e., locomotor activity and restraint) a WC group was used. These rats were housed in the wheel moving for 36 min in every 3-h period for a 24-h period (09:00-09:00). The speed of the wheel was the same as that of the TSD group. In this group the rats experienced the same amount of locomotor activity as in TSD (≈ 800 m/24 h) but they had the opportunity to enter and maintain deep sleep ($56.7 \pm 4.5\%$ of wakefulness, $37.0 \pm 3.1\%$ of NREM sleep and $6.3 \pm 2.1\%$ of REM sleep). We confirmed that no differences in endothelial function parameters were observed between the WC and control cage (CC) groups (see supplemental material for CC condition values). In the CC condition, rats were housed in the rodent room during all the experiments.

Telemetry Surgical Implantation

Telemetry transmitters, for the recording of arterial blood pressure, electrocardiogram (ECG) and body temperature (Tco), were chronically implanted in rats (C50-PXT, Data Sciences Int., St Paul, MN, USA). We used a surgical procedure that guarantees high-quality recordings in rats, even during physical activity.¹⁶ Briefly, the rats were anesthetized with isoflurane [5% (induction) / 1-2%, oxygen (maintenance)] and buprenorphine (0.05 mg/kg, subcutaneously, 20 min before surgery). The transmitter catheter was inserted into the abdominal aorta, just proximal to the iliac bifurcation. The two electrodes for ECG recording were fixed to the dorsal surface of the xiphoid process and the anterior mediastinum, close to the right atrium. The transmitter body was introduced into the abdominal cavity and attached to the abdominal wall with a 3-0 prolene suture. Subsequently, the rats were injected with a prophylactic antibiotic (benzathine benzylpenicilline, Extencilline®, 60,000 UI, intramuscularly) and received postoperative analgesic treatment for 3 days (carprofen, 4 mg/kg subcutaneously per day).²⁷ After surgery, the rats were singly housed and allowed to recover for 14 days.

Endothelial Function Assessment

Two days before the experiment, a depilatory lotion was used to remove the rats' hair from the skull and along the back to avoid any skin irritation at the time of the study. For the experiment, the animals were tranquilized (phenobarbital sodium, Nesdonal, 40 mg/kg intraperitoneally) and placed in the prone position in a warm ($30 \pm 0.5^\circ\text{C}$) incubator to maintain a stable rectal temperature throughout the experiment (ML295/R Homeothermic Controller, ADInstruments Ltd, Oxford, UK).^{21,28,29} Skin blood flows (SkBf) were assessed using laser-Doppler flowmeters (PF 5001 Master Periflux, Perimed, Sweden) and heat-controlled (33°C) laser-Doppler probes (PF 481-1, Perimed) coupled with iontophoresis devices. After gentle cleaning with an alcoholic solution, the probes were positioned on the hairless skin of the back. Iontophoresis is an established method in which an applied electric field is used to facilitate the cutaneous penetration of vasoactive drugs.²⁰ Combined with laser-Doppler measurements, iontophoresis allows the assessment of a drug's effect on SkBf. All drugs for iontophoresis were dissolved in deionized water and placed in the 1.2-cm² sponge of the iontophoretic drug-delivery electrode, which also contained the laser-Doppler probe at its center. A battery-driven power supply provided constant anodal or cathodal currents for drug delivery. All drugs were delivered using a 30 sec continuous current (100 μA , 0.4 mC).

Each rat was implanted with four iontophoresis/laser-Doppler probes for delivery of an acetylcholine chloride solution (ACh, 5.5 mM, Sigma-Aldrich CO, St. Louis, MO, USA.), a sodium nitroprusside solution (SNP, 67 mM, SERB laboratory, Paris, France), and the carrier alone (deionized water, in the other two probes) in order to control the effect of anodal and cathodal current-induced vasodilation.²⁸ ACh delivered with an anodal current induces endothelium-dependent vasodilation (Figures 1 and 2). The nitric oxide (NO) donor SNP delivered with a cathodal current induces an endothelium-independent vasodilation. Although the current applications are not painful, it is generally admitted that iontophoretic current-induced

vasodilation relies on an axon reflex due to excitation of cutaneous nociceptors by the current.³⁰ Moreover, among the various observations regarding current-induced vasodilation, it has been reported that cathodal current induces a vasodilation dependent on cyclooxygenase-1 (COX-1) activation both in rats²⁸ and in humans.³⁰

SkBF signals were digitized (500 Hz sampling frequency) using a computerized acquisition system (PowerLab, ADInstruments Ltd). Data collection started with a 10-min control period before the current stimulations began and was continued for 20 min. SkBf is expressed in arbitrary perfusion units (PU). Recordings started only if the SkBf control values were between 20 and 100 PU.

Systolic and diastolic arterial pressure (SAP and DAP) was noninvasively assessed with a pulse transducer and a pressure cuff system placed on the tail (ML125/R NIBP system, ADInstruments Ltd). They were determined before iontophoresis and at the end of the experiment.

Sympathectomy

In some experiments sympathectomy was induced by injection of reserpine (5 mg/kg intraperitoneally per day) on 2 consecutive days: 24 h and 1 h before the experiment.³¹ The reserpine was dissolved in 2% ascorbic acid in a 0.9% saline solution (solvent).³¹ Similarly, the non-sympathectomized rats received an equivalent volume of solvent (intraperitoneally). Such a chronic sympathectomy is reported not to alter the blood pressure and HR baseline levels but to markedly enhance blood pressure variability.^{31,32} At the end of the experiment, the efficacy of the sympathectomy was checked by the attenuated response to an injection of a sympathomimetic agent (tyramine, 250 µg/kg intravenously).³³ With tyramine, mean arterial pressure (MAP) increased by 13 ± 2 mmHg in the sympathectomized rats versus 57 ± 4 mmHg in non-sympathectomized rats ($P < 0.01$).³³ All chemicals were obtained from Sigma-Aldrich CO, St. Louis, MO, USA.

Protocols

Three protocols were performed with different sets of animals.

In protocol 1, we assessed the effect of sleep deprivation on subsequent recovery of the daily rhythm of blood pressure, HR and Tco. Fourteen days after surgery, data from the sympathectomized and non-sympathectomized rats ($n = 6$ per condition) were continuously recorded the day before (baseline), during TSD and WC, and the following day (Dataquest ART Acquisition program, Data Science Int., St. Paul, MN, USA). The ASCII files were sent to Labchart (ADInstruments Ltd) for post-acquisition analysis.

In protocol 2, we examined the effect of TSD on vascular reactivity (ACh-, SNP-, anodal current- and cathodal current-induced vasodilation) in sympathectomized and non-sympathectomized rats ($n = 8$ per group).

In protocol 3, a pharmacological approach was used to identify the involvement of the underlying signaling pathways implicated in cutaneous vascular reactivity. Vascular reactivity was evaluated in rats ($n = 6$) previously treated (30 min before

the experiment) with a saline solution (Control), a nonspecific COX inhibitor (INDO, indomethacin, 5 mg/kg intraperitoneally, Sigma-Aldrich CO, St. Louis, MO, USA.), a nonspecific nitric oxide synthase (NOS) inhibitor (L-NNA, N^G-Nitro-L-arginine, 20 mg/kg intraperitoneally, Sigma-Aldrich), and a combination of INDO + L-NNA.^{28,29}

Data Analysis

In protocol 1, arterial pressure, ECG, and Tco signals were analyzed offline using Labchart software (ADInstruments Ltd). Average R-R interval (RR, ms) and HR (bpm), SAP (mmHg), DAP (mmHg), MAP (mmHg), and Tco were quantified and expressed as the mean value of successive 3-h periods.

In protocols 2 and 3, SkBf signals were averaged every 10 sec to reduce the effects of instantaneous variability due to vasomotion. Baseline values were calculated as the mean of the 2-min control period before applying the current. SNP-, ACh-, cathodal- and anodal-induced vasodilation responses were reported as peak changes (increase in SkBf from baseline, as a % of baseline) in response to the iontophoretic delivery. In order to evaluate the effect of ACh and SNP without any influence of the current on the vascular structure, the vasodilations observed with anodal or cathodal currents were subtracted from ACh and SNP values, respectively.

Statistics

In protocol 1, differences between TSD and WC animals were evaluated using a two-way analysis of variance: “time of day” (repeated factor, 12 per day, i.e., 230 degrees of freedom) and “experimental situation” (non-repeated factor, three levels: TSD and WC, i.e., 10 degrees of freedom). If appropriate, we compared the values measured at the same time of day using a *post hoc* Bonferroni test. We had previously confirmed that the distribution of values followed a normal law.

In protocol 2 and 3, differences between TSD and WC and between the sympathectomized and non-sympathectomized rats were evaluated using a Kruskal-Wallis one-way analysis of variance on ranks followed by a between-groups pairwise Mann-Whitney rank sum test where appropriate. All statistical analyses were made using Statistica (StatSoft, Maisons-Alfort, France). Data are reported as the mean $\pm$ standard error of the mean (SEM).

RESULTS

Protocol 1: Effect of TSD on Arterial Pressure, HR, and Tco

In non-sympathectomized TSD rats, we observed an increase in MAP (Figure 3) and SAP between 09:00 and 18:00 compared to WC (interaction between the “time of day” and the “experimental situation”, $F(10, 230) = 97.2$, $P < 0.01$ for MAP and $F(10, 230) = 99.7$, $P < 0.01$ for SAP). For HR, a significant interaction between the “time of day” and the “experimental situation” was detected ($F(10, 230) = 102.7$, $P < 0.01$). HR in non-sympathectomized TSD rats was higher compared to WC between 09:00 and 18:00. No significant increase in TSD was noticed during the recovery day. Tco in non-sympathectomized TSD rats was greater than in WC rats between 09:00 and 12:00 (interaction between the “time of day” and the “experimental situation”, $F(10, 230) = 57.2$, $P < 0.05$).

In sympathectomized rats, no significant difference in SAP, DAP, MAP, and HR between the groups was evidenced whatever the day. Tco was higher between 09:00 and 13:00 in the TSD group compared to WC (interaction between the “time of day” and the “experimental situation”, $F(10, 230) = 53.5$, $P < 0.05$).

Protocol 2: TSD Effect on Cutaneous Vasodilation

The iontophoretic delivery of ACh increased SkBf from baseline in non-sympathectomized ($64.3 \pm 8.2\%$) and in sympathectomized rats ($52.7 \pm 5.6\%$, $P = 0.12$; Figure 4). In TSD compared to WC, we observed a reduced ACh-induced vasodilation in both non-sympathectomized ($31.7 \pm 5.5\%$, $P < 0.05$) and sympathectomized rats ($28.3 \pm 6.1\%$, $P < 0.05$). Sympathectomy induced a decrease in ACh-induced vasodilation in WC rats ($60.2 \pm 7.6\%$ versus $50.2 \pm 4.6\%$, $P < 0.05$).

No significant difference in anodal current-induced vasodilation was observed between sympathectomized ($10.8 \pm 4.2\%$, in WC) and non-sympathectomized rats ($6.6 \pm 3.8\%$, in WC) or between TSD and WC.

Regarding the SNP-induced vasodilation, no differences were observed between non-sympathectomized ($40.2 \pm 5.1\%$ for WC) and sympathectomized rats ($38.7 \pm 4.3\%$ for WC) or between TSD and WC groups.

The cathodal current-induced dilation was less in TSD ($8.6 \pm 1.3\%$) compared to WC ($20.3 \pm 5.0\%$, $P < 0.05$). We showed no significant changes in WC and TSD in non-sympathectomized compared with sympathectomized rats.

No significant differences were observed in the basal SkBf (57.4 ± 12.1 PU) or MAP (88 ± 5 mmHg) between TSD and WC or between sympathectomized and non-sympathectomized rats. As expected, the effect of tyramine on MAP was higher in non-sympathectomized compared to sympathectomized rats (54 ± 4 mmHg versus 13 ± 1 mmHg in WC, $P < 0.05$ and 53 ± 3 mmHg versus 14 ± 3 mmHg for TSD, $P < 0.05$).

Protocol 3: TSD Effect on Endothelial-Dependent Vasodilation Metabolic Pathways

The inhibition type (sympathectomized and non-sympathectomized) and groups (WC and TSD) did not significantly change the basal SkBf (mean value 55.3 ± 10.5 PU) or the MAP (mean 85 ± 5 mmHg), except the L-NNA treatment that induced an increase in MAP (mean value $30 \pm 8\%$) showing the efficiency of the NOS inhibition.

The ACh-induced vasodilation ($70.7 \pm 7.6\%$) was almost abolished after NO synthase inhibition ($7.7 \pm 2.5\%$, $P < 0.001$) and was reduced after COX inhibition ($40.8 \pm 7.7\%$, $P < 0.01$; Figure 5). In TSD after COX inhibition compared to WC, we observed that the decrease in ACh-induced vasodilation persisted ($15.1 \pm 5.3\%$ in TSD, $P < 0.05$), whereas no significant changes were observed after NOS inhibition and COX + NOS inhibition.

No significant differences in anodal current- ($10.9 \pm 4.5\%$) and SNP- ($33.0 \pm 7.5\%$) induced vasodilation were observed after COX, NOS, or COX + NOS inhibition or between TSD and WC.

The cathodal current-induced vasodilation ($43.3 \pm 7.5\%$) decreased after COX inhibition ($6.5 \pm 4.8\%$, $P < 0.01$), whereas no difference was observed after NOS inhibition. Moreover, the cathodal current-induced vasodilation was also lower in TSD when compared to WC after NOS inhibition ($32.5 \pm 6.9\%$,

$P < 0.05$), whereas no significant differences were detected after COX or COX + NOS inhibition.

DISCUSSION

In this study we observed that 24 h of continuous wakefulness (i.e., TSD) induced a transient increase in blood pressure and HR in rats. These changes were related to an increase in sympathetic activity that disappeared the day after TSD. We also showed for the first time that TSD is sufficient stress to trigger a decrease in endothelium-dependent vasodilation in rodents. Moreover, our results demonstrate that this endothelial dysfunction is a sympathetic-independent mechanism because it is still obvious after pharmacological sympathectomy. Finally, we showed that the endothelial dysfunction induced by TSD is related to an alteration of both the COX-1 and NOS pathways.

Sleep Deprivation Alters Endothelial Function

In this study on rats we demonstrated for the first time that 24 h of wakefulness alter ACh-induced vasodilation, a sign of

endothelial dysfunction.²³ This result confirms previous studies showing endothelial dysfunction after acute sleep deprivation^{5,9,34} or acute sleep restriction⁶⁻⁸ in healthy humans. Indeed, we showed a significant effect of sleep deprivation on endothelium-dependent vasodilation in TSD rats compared to WC groups. Moreover, as previously described in humans³⁴ we showed no significant effect of TSD on endothelium-independent vasodilation as assessed with SNP delivery. These results support the view that acute TSD in rats is sufficient stress to trigger an alteration of the endothelial-dependent vasodilation that is a sign of endothelial dysfunction.

A Sympathetic Independent Mechanism

According to previously published studies of rats and humans, 24 h of wakefulness induce a transient increase in blood pressure and HR that did not persist the day following TSD.^{16,35} These changes were not observed in sympathectomized rats. On the contrary, it has been reported that 24 h of paradoxical sleep deprivation do not induce significant

increases in HR and MAP (96 h are necessary).³⁶ So, a decrease in total sleep time produces an increase in sympathetic activity during the wakefulness period, leading to a transient rise in blood pressure and HR. Sympathetic activity enhancement has been involved in the decrease of endothelial-dependent vasodilation³⁷ and suspected to link sleep deprivation and endothelial dysfunction.⁶ However, these results show for the first time that endothelial dysfunction induced by TSD persists in sympathectomized rats. Thus, we may conclude that the initial endothelial dysfunction observed after 24 h of wakefulness is a sympathetic independent mechanism. This is an important result for understanding the kinetics of the vascular alterations observed after sleep loss.

However, we cannot exclude that a persistent increase in sympathetic activity would favor endothelial dysfunction during repeated TSD or prolonged sleep restriction. Indeed, Amir et al.³⁴ and Wehrens et al.³⁸ showed that endothelial dysfunction after a night shift was greater in subjects with a longer history of night-shift duty and was associated with an increase in sympathetic activity. Moreover, Sgoifo et al.¹⁶ demonstrated that sleep deprivation changes baroreflex sensitivity. In their study, animals were exposed to an acute restraining stress and sleep deprivation induced a blunted parasympathetic antagonism following sympathetic activation, together with an increased

susceptibility to cardiac arrhythmia. So, when TSD is associated with stress, we cannot exclude that a large increase in sympathetic activity could be related to endothelial dysfunction.

Metabolic Pathways Implicated

Our results confirm previous findings^{28,30} that concluded that ACh-induced vasodilation is almost abolished after NOS inhibition and is reduced after COX inhibition in rat skin, thus demonstrating the major role of NO in the *in vivo* endothelium-dependent vasodilation and the significant participation of prostaglandins.^{28,30} Moreover, although current-induced vasodilation (CIV) exists at both anodal and cathodal electrodes, we corroborate previous findings in rats²⁸ that CIV amplitude is almost three times greater at the cathode (35%) than the anode (10%) for the same charge. Our results also confirm that indomethacin (COX inhibitor) delivery decreases cathodal CIV by almost 80%, proving the major contribution of the COX-1 pathway in cathodal CIV, as previously reported in humans³⁰ and rats.²⁸

In this study, we found that after COX inhibition (i.e., with only the NO pathway available) there was less ACh-induced vasodilation in TSD rats compared to WC. Our results suggest for the first time that in rodents 24 h of wakefulness alter the NO pathway that is the most important endothelium-dependent

vasodilation pathway. However, because we also observed that TSD decreased cathodal-induced vasodilation, we conclude that acute TSD also decreases the COX-1 dependent vasodilation pathway. Our results demonstrate that acute TSD alters cutaneous endothelial function, and in particular both the NOS and COX-1 pathways. However, the evidence suggests that there is considerable “cross talk” between NO and prostaglandins biosynthetic pathways.³⁹ The effect of PGI₂ is closely related to NO effects because PGI₂ potentiates NO release, and in turn NO potentiates the effect of PGI₂ on vascular smooth cells via a cyclic AMP turnover pathway.⁴⁰

Many authors have observed that sleep deprivation in rodents changed COX-1 and NOS bioavailability in several brain regions and showed a decrease in cortical neural NOS activity during partial sleep deprivation and subsequent 24-h recovery.^{41,42} Moreover, 5 days of TSD inhibited neuronal NOS activity in the cardiovascular afferent neurons (i.e., nodose neurons) of adult rats.⁴³ Because NO may play an inhibitory role in the regulation of sympathetic tone in the cardiovascular afferent neurons, these authors related the decrease in NO production to the increased sympathetic activity observed during sleep disorders. Furthermore, systemic injection of an NOS inhibitor suppressed sleep rebound induced by sleep deprivation in rats, in particular NREM sleep rebound.⁴⁴ Others showed that paradoxical sleep deprivation and TSD (72 h) are associated with oxidative stress in the cortex and hippocampus and the protective effect of an antioxidant treatment on behavior and memory.^{45,46} It is well established that the NO production

may play a role as a signaling molecule in the homeostatic regulation of sleep, and that TSD alters NOS activity.^{44,47} In addition, sleep deprivation also inhibits COX activity in the cardiovascular afferents of adult rats.⁴³

Sleep deprivation adversely affects several systems that could change vascular response. In particular, many studies have demonstrated that TSD is associated with low-grade systemic inflammation with increased levels of tumor necrosis factor (TNF- α)⁴⁸, interleukin-6⁴⁹ and C-reactive protein⁴⁹ that are suspected to induce endothelial dysfunction and impair NO availability by a COX-2 dependent pathway finally leading to increased production of oxidative stress.⁵⁰ The vascular reactivity of skin to ACh is significantly reduced during low-grade chronic inflammation.⁵¹ Furthermore, an increase in endothelin-1 (the main endothelial vasoconstrictive hormone) plasma levels has been observed after 96 h of partial sleep deprivation in rats⁵² and 27 h of wakefulness in humans.⁵³ Endothelin-1 is secreted in particular during sympathetic activation or inflammation.⁵⁴

Limitations

Although our results are consistent with previous findings in humans, they were obtained in tranquilized rats. Sodium pentobarbital was used because it does not alter the COX and NOS portions of ACh vasodilation in rodents,⁵⁵ although it modifies the EDHF pathway that also contributes to ACh-dependent vasodilation.^{29,55} However, the physiological role of EDHF is more important in older rodents in which the age-related decrease in NO availability is in part compensated with

an EDHF-dependent backup.²¹ Previous findings in humans showed that TSD induces a greater increase in SAP and MAP in the elderly than in young adults,⁵⁶ so it would be pertinent to study the specific effect of TSD on the EDHF pathway.

The pharmacological sympathectomy induced by reserpine appears to be incomplete after 24 h of TSD or WC because there remained a small increase in MAP (< 15 mmHg versus > 50 mmHg in non-sympathectomized rats) that could be explained by the half-life of the reserpine-induced sympathectomy (about 20–24 h).^{31–33} Longer subchronic sympathectomy methods would need to be used for sleep deprivation/restriction protocols of longer than 24 h.

CONCLUSION

These original results demonstrate that 24 h of wakefulness in rats induce a decrease in endothelial-dependent vasodilation that is not related to changes in blood pressure or sympathetic activation. Our findings also suggest that this endothelial dysfunction is associated with a decrease in the activity of both the NOS and COX-1 pathways.

ABBREVIATIONS

ACh, acetylcholine chloride
CC, control cage group
CIV, current induced vasodilation
COX, cyclooxygenase
DAP, diastolic arterial pressure
EDHF, endothelium-derived hyperpolarizing factors
HR, heart rate
INDO, indomethacin, an inhibitor of cyclooxygenase 1 & 2
L-NNA, N^G-Nitro-L-arginine, an inhibitor of nitric oxide synthase
MAP, mean arterial pressure
NO, nitric oxide
NOS, nitric oxide synthase
PU, perfusion units
SAP, systolic arterial pressure
SkBF, skin blood flow
SNP, sodium nitroprusside
Tco, body temperature
TSD, total sleep deprivation (i.e., 24-h of continuous wakefulness)
WC, 24-h wheel control group

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SUPPLEMENTAL MATERIAL

Table S1—Blood pressure, heart rate, and body temperature values in non-sympathectomized and sympathectomized rats in the control cage experimental condition

	Non-sympathectomized		Sympathectomized	
	Light ON	Light OFF	Light ON	Light OFF
SAP, mmHg	110.87 ± 4.42	122.19 ± 5.23 ^a	105.56 ± 2.60	106.36 ± 2.70 ^b
DAP, mmHg	82.05 ± 3.23	83.35 ± 3.43	84.77 ± 2.13	82.37 ± 2.16
MAP, mmHg	93.32 ± 3.18	99.96 ± 3.11 ^a	93.70 ± 2.31	93.70 ± 2.36 ^{a,b}
HR, bpm	335.12 ± 15.08	371.41 ± 19.47 ^a	310.73 ± 21.88 ^b	305.00 ± 22.84 ^a
Tco, °C	37.35 ± 0.37	37.90 ± 0.29 ^a	37.33 ± 0.29	37.88 ± 0.15 ^a

Values are mean ± standard error of the mean. n = 6. ^aDifference between light ON and light OFF period (P < 0.05). ^bDifference between non-sympathectomized and sympathectomized (P < 0.05). DAP, diastolic arterial pressure; HR, heart rate; MAP, mean arterial pressure; SAP, systolic arterial pressure; Tco, body temperature.

Table S2—Effect of sympathectomy on endothelial function in the control cage experimental condition

Change in SkBF after application of:	Non-sympathectomized	Sympathectomized
ACh, % baseline	64.1 ± 8.8	52.7 ± 5.1 ^a
Anodal current, % baseline	10.8 ± 4.2	6.6 ± 3.8
SNP, % baseline	41.6 ± 6.1	31.8 ± 7.2
Cathodal current, % baseline	22.6 ± 5.9	15.4 ± 3.0

n = 8. Values are mean ± standard error of the mean. Maximal relative increases in skin blood flow (SkBF, % baseline) were calculated after acetylcholine (ACh), anodal current, sodium nitroprusside (SNP) and cathodal current application. ^aSignificant differences with non-sympathectomized (P < 0.05).

Table S3—Effect of nitric oxide and cyclooxygenase on endothelial function inhibition, in the control cage experimental condition

Change in SkBF after application of:	Control	COX inhibition	NOS inhibition	COX + NOS inhibition
ACh, % baseline	70.6 ± 7.6	40.8 ± 7.7 ^a	7.7 ± 2.2 ^a	2.6 ± 2.1 ^a
Anodal current, % baseline	10.9 ± 4.5	8.7 ± 3.2	7.9 ± 5.3	9.3 ± 6.2
SNP, % baseline	33.0 ± 7.5	31.0 ± 8.7	33.8 ± 5.2	32.0 ± 8.2
Cathodal current, % baseline	43.3 ± 6.5	6.5 ± 4.7 ^a	35.8 ± 3.2	4.9 ± 6.2 ^a

n = 8. Values are mean ± standard error of the mean. Maximal relative increases in skin blood flow (SkBF, % baseline). Maximal relative increases in skin blood flow (SkBF, % baseline) were calculated after acetylcholine (ACh), anodal current, sodium nitroprusside (SNP) and cathodal current application. Values were observed in rats treated with saline solution (Control) or indomethacin [Cyclooxygenase (COX) inhibition] or L-NNA [Nitric oxide synthase (NOS) inhibition] or with a combination of indomethacin and L-NNA [COX + NOS inhibition]. ^aSignificant differences versus control (P < 0.05).