Effects of Sinoaortic Denervation on Hemodynamic Parameters During Natural Sleep in Rats

Neide P. Silveira, PhD; Edson D. Moreira; Luciano F. Drager, MD, PhD; Gustavo J. J. Silva, PhD; Eduardo M. Krieger, MD, PhD

Hypertension Unit of the Heart Institute (InCor) do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil

Study Objectives: To analyze the role of arterial baroreflex on hemodynamic changes during synchronized and desynchronized sleep phases of natural sleep in rats.

Design: Experimental study.

Setting: Laboratory.

Participants: Seventeen male Wistar rats.

Interventions: No intervention (control, n = 8) or sinoaortic denervation (SAD, n = 9).

Measurements and Results: Sleep phases were monitored by electrocorticogram, and blood pressure was measured directly by a catheter in the carotid artery. Cardiac output, as well as total and regional vascular resistances, were determined by measuring the subdiaphragmatic aorta and iliac artery flows with Doppler flow probes, respectively. In contrast to the control group, the SAD group had a strong reduction in blood pressure (-19.9% ± 2.6% vs -0.7% ± 2.1%) during desynchronized sleep, and cardiac output showed an exacerbated reduction (-10.4% ± 3.5% vs 1.1% ± 1.7%). In SAD rats, total vascular resistance decreased during desynchronized sleep (-10.1% ± 3.5% vs -1.0% ± 1.7%), and the increase in regional vascular resistance observed in the control group was abolished (27.5% ± 8.3% vs -0.8% ± 9.4%).

Conclusions: SAD caused profound changes in blood pressure, cardiac output, and total vascular resistance, with a significant increase in muscle vascular resistance during synchronized sleep. Our results suggest that baroreflex plays an important role in maintaining the normal balance of cardiac output and total vascular resistance during sleep.

Keywords: Sleep, synchronized sleep, desynchronized sleep, sinoaortic denervation, blood pressure, total vascular resistance, cardiac output

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Address correspondence to: Eduardo M. Krieger, MD, PhD, Unidade de Hipertensão do Instituto do Coração (InCor) do HC-FMUSP, Av. Dr. Enéas de Carvalho Aguiar, 44 – 2º andar, bloco 2, sala 8, São Paulo – SP, Brazil, 05403-904; Tel: 55 11 3069-5048; Fax: 55 11 3069-5948; E-mail: edkrieger@incor.usp.br


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METHODS

Animals

We studied 17 male Wistar rats (≈ 250g) randomized into 2 groups: control (n = 8), and SAD (n = 9) animals. They were kept under a 12-hour light/dark cycle at a controlled ambient temperature (~22°C). Standard laboratory diet and water were available ad libitum. All experimental procedures were conducted in accordance with the guidelines for care and use of laboratory animals of the University of São Paulo, Brazil.

Surgical Procedures

Chronic Electrode Implantation for Electrocoritcogram Evaluation

Bipolar stainless-steel wire electrodes (100 μm in diameter) were implanted with the rats anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneal, Sigma Chemical Co., St. Louis, MO) in the frontal lobe, attached to the skull with methyl methacrylate. These procedures have been described elsewhere. The rats were allowed to recover for 2 weeks before the sleep session. The electrical activity recorded from this area allows discernment of the different phases and states of natural sleep.

Flow Probe Implantation

One week before the sleep session, the rats were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneal, Sigma Chemical Co., St. Louis, MO) and 2 pulsed Doppler flow probes (subminiature 20-MHz piezoelectric crystal transducer; Iowa Doppler Products, Iowa City, IA) mounted in a flexible silicone cuff (1.3-mm diameter) at a 45-degree angle were implanted and positioned around the subdiaphragmatic aorta and abdominal aorta, just before the bifurcation of the iliac arteries. The silicone cuff was secured around the vessel with suture, and the lead wire leading to the probe was then fixed to the body of the rat at several locations. Doppler flow probes were connected to a flow meter (model 545C-4, Iowa Doppler Products, Iowa City, IA) for aortic and hindquarter limb flow measurements. Mean aortic and iliac flow signals were generated by integrating both pulse-flow waveforms in an oscillographic recorder (Hewlett-Packard, model 7754B, Palo Alto, CA). Afterward, cardiac output was estimated by aortic blood flow (not taking into account the blood flow to the coronary bed), and both total vascular resistance and hindquarter limb resistance were calculated as the ratio between mean blood pressure and subdiaphragmatic aorta and abdominal aorta flows, respectively.

SAD Procedure

Forty-eight hours before the sleep session, the rats were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneal, Sigma Chemical Co., St. Louis, MO) and underwent SAD. The technique has been previously described by Krieger. Briefly, a 3-cm midline incision is made, and sternocleidomastoid muscles are reflected laterally to expose the neurovascular sheath.

The common carotid arteries and the vagal trunk are isolated, and the aortic depressor fibers, either traveling with the sympathetic nerve or as an isolated aortic nerve, are cut. The communicating branch of the aortic fibers is also sectioned. The third contingent of aortic baroreceptor fibers traveling with the inferior laryngeal nerve is interrupted by resection of the superior laryngeal nerve after the carotid bifurcations are exposed extensively for carotid stripping. In addition, the sinus nerve, all carotid branches, and the carotid body are also sectioned.

Immediately afterward, a catheter was implanted into the thoracic aorta via the right common carotid artery (PE-50, ID 0.58 mm, OD 0.965 mm, Biocorp, Huntingdale, Victoria, Australia) for direct blood pressure measurement. Polyethylene tubing was connected to a pressure transducer (Statham P23Dd; Hato Rey, Puerto Rico) to measure blood pressure in a multichannel oscillographic recorder (Hewlett-Packard, model 7754B, Palo Alto, CA) with the rats in a conscious state.

Both control and SAD groups were submitted to the same sequence of procedures before the sleep session: 14 days before the electrocoritcogram (ECoG), 7 days before flow probes, and 2 days before the implantation of catheter in both and denervation procedure in the SAD group. In the last procedure, the time of anesthesia (sodium pentobarbital, 30 mg/kg, intraperitoneal, Sigma Chemical Co., St. Louis, MO) was 15 to 20 minutes longer in the SAD group.

Hemodynamic Evaluation During Sleep Stages

Blood pressure, cardiac output, hindquarter limb blood flow, and ECoG were recorded for at least 5 hours. The animals were housed in individual cages before the sleep session. The electrodes, the blood flow probes, and the arterial catheter were plugged into a rotating connector in such a way that did not disturb the free-moving animals for the sleep-section measurements. The characterization of the different states of the wake-sleep cycle was done according to Roldan and Weiss and Timo-Iaria et al's description method. When awake, the rats were moving in the cage, lying quietly with closed eyes, or had low-voltage activity on the ECoG. In synchronized sleep, the rats adopted a curled position, breathing was deep and regular, and the ECoG showed slow waves of a high amplitude. The period of desynchronized sleep showed low-voltage fast activity lasting 1 to 3 minutes and usually appearing after several periods of slow-wave sleep. Moreover, the animals exhibited body relaxation, respiration became irregular, and jerks of different parts of the body occurred.

We measured the hemodynamic and the ECoG signals during the quiet awake condition just before the entrance into synchronized sleep. We considered as synchronized sleep, the period just before the desynchronized sleep. For data analysis, cardiac output and total and regional vascular resistances were evaluated by the mean percentage of changes in all episodes of synchronized sleep or desynchronized sleep. The relative variations in synchronized sleep were calculated considering the awake condition, and, in desynchronized sleep, the variations were calculated considering variations from both the awake condition and the synchronized sleep phase. The ECoG, blood pressure pulse, and aortic and iliac flow signals were recorded in a multichannel oscillographic recorder (Hewlett-Packard, model 7754B, Palo Alto, CA) for at least 5 hours using a very high compression rate.

Statistical Analysis

Data are expressed as mean ± SEM. Hemodynamic measurements during the different phases of natural sleep were compared by 2-way Analysis of Variance, followed by Tukey post-hoc analysis. A value of P < 0.05 was considered significant.

RESULTS

Awake Condition

Figure 1 exemplifies blood pressure, aortic and common iliac artery blood flows, and ECoG recordings in normal control (panel A) and SAD (panel B) rats evaluated during the awake condition and during synchronized and desynchronized sleep phases. After 48 hours of SAD, no significant change in the basal values of mean blood pressure was observed in the SAD animals compared with that in the normal control rats (116 ± 7 vs 106 ± 4 mm Hg, P = 0.81, for SAD and control groups, respectively).

Synchronized Sleep

The evaluation of all synchronized sleep episodes showed that control (136 episodes, average of 17 per animal) and SAD (109 episodes, average of 12 per animal) groups had similar and nonsignificant changes in mean blood pressure (Figure 2A) during the synchronized sleep phase, compared with the awake condition (control: 107 ± 4 vs 106 ± 4 mm Hg; SAD: 119 ± 7 vs 116 ± 7 mm Hg, P = 0.99, for SAD and control groups, respectively). The percentage change of values for awake and synchronized sleep for cardiac output (-0.7% ± 0.8% vs -3.0% ± 1.1%, P = 0.87, for SAD and control groups, respectively) and total vascular resistance (2.4% ± 1.5% vs 5.8% ± 3.2%, P = 0.82, for SAD and control groups, respectively) were similar in control and SAD rats, even when the change in SAD tended to be larger. In addition, the increase in regional vascular resistance (Figure 3C) during the synchronized sleep phase was not different between control and SAD groups (14.2% ± 2.8% vs 14.0% ± 3.8%, P = 0.99, for SAD and control groups, respectively).

Desynchronized Sleep

Considering the evaluation of all episodes of the desynchronized sleep phase observed in the control (82 episodes, average of 10 per animal) and SAD (71 episodes, average of 8 per animal) groups, a significant reduction was observed in mean blood pressure levels (-19.9% ± 2.6% vs -0.7% ± 2.1%; Figure 2B) only in the SAD group, compared with that in awake rats (control: 106 ± 4 vs 106 ± 4 mm Hg; SAD: 93 ± 6 vs 116 ± 7 mm Hg, P = 0.0324, for SAD and control groups, respectively). Cardiac output (Figure 3A) was reduced significantly only in the SAD group, compared with the changes observed in the control animals (1.1% ± 1.7% vs -10.4% ± 3.5%, P = 0.0412, for SAD and control groups, respectively). Similarly, SAD caused a significant reduction in total vascular resistance (Figure 3B) during the desynchronized sleep phase (-1.0% ± 1.7% vs -10.1% ± 3.5%, P = 0.0012, for SAD and control groups, respectively), compared with that in the control animals. In addition, the vasoconstriction observed in the hindquarter limb (Figure 3C) of the control animals was abolished in the SAD group (-0.8% ± 9.4% vs 27.5% ± 8.3%, P = 0.0306, for SAD and control groups, respectively) during the desynchronized sleep phase.

DISCUSSION

The main results observed in the present study are (1) cardiac output had a nonsignificant reduction during synchronized sleep and an exacerbated reduction during desynchronized sleep in SAD rats; (2) total vascular resistance did not change during the synchronized sleep phase and decreased significantly during desynchronized sleep in SAD rats; and (3) regional vascular resistance increased during both synchronized sleep and desynchronized sleep phases in the control animals but was abolished after SAD during the desynchronized sleep phase.

In the control group, our laboratory has previously described an increase in the blood pressure levels of con-
The increase in blood pressure was accompanied by reductions in iliac blood flow or vasoconstriction of the hindquarter vasculature and a vasodilation in viceria. Although blood pressure levels during the desynchronized sleep phase did not change significantly in the present study, we demonstrated an increase in the hindquarter limb peripheral arterial resistance in normal control rats during the desynchronized sleep phase. It was impossible to demonstrate whether the blood pressure changes during desynchronized sleep could be attributed to the stress induced by different surgical procedures, especially the instrumentation used for hemodynamic measurements, namely flow probes in different vessels (aorta and iliac artery), ECoG electrodes in the frontal lobe, and a catheter in the carotid artery. Nevertheless, all the animals were in good clinical condition 48 hours after the surgical procedures and did not exhibit apparent symptoms of pain.

After SAD, the blood pressure reduction observed during the desynchronized sleep phase has been reported by several studies in both the cat and rat. In the present investigation, the hypotension observed during the desynchronized sleep phase in SAD rats was due to a decrease in total vascular resistance and also in cardiac output. The decrease in total vascular resistance after SAD was probably due to a vasodilatation in renal/splanchinic areas because there were no observed changes in iliac vascular resistance. Accordingly, in other species, such as the cat, the decrease in blood pressure during the desynchronized sleep phase is caused by a reduction in total vascular resistance, explained, in part, by a vasodilatation in renal and mesenteric vascular beds. In addition, hypotension is accompanied by an intense reduction in heart rate. In pigs, Zinkovska et al demonstrated that the decrease in blood pressure during the desynchronized sleep phase is primarily caused by a significant reduction in total vascular resistance, without a change in cardiac output, although there is an increase in heart rate. The greater or smaller pattern of blood pressure changes during desynchronized sleep in various animals species (rats, cats, and pigs) should then be considered not only as the consequence of a more or less powerful central depressor influence, but also as a function of a more or less effective baroreflex buffering. The baroreflex probably has a greater capacity in rats than in cats to buffer the hemodynamic changes caused by the desynchronized sleep phase.

Recently, data were published in disagreement with the blood pressure changes during the desynchronized sleep phase described by us previously. In this study, Wistar rats underwent SAD procedures, and the blood pressure changes were evaluated during natural sleep 4 weeks after surgical procedures. Instead of showing a reduction in blood pressure during the desynchronized sleep phase in SAD rats, this study observed an increase in blood pressure values. The authors claim that 1 to 2 days was not enough time to recover from the surgeries and this timing could be influencing the blood pressure changes during sleep phases that we described. However, we have also shown a reduction in blood pressure levels during the desynchronized sleep phase in chronic SAD (10 days after the surgical procedure). After a 10-day period, both blood pressure and heart rate levels, in the awake condition, were not statistically different, compared with those in normal control animals, but the pattern of blood pressure changes during the sleep phases still persists (slight increase in mean blood pressure during synchronized sleep and marked decrease during desynchronized sleep). It should be stressed that, in the present study, an individual evaluation of the rats showed that 6 of 9 SAD rats had a reduction in blood pressure during the desynchronized sleep episodes, and only 1 animal had increased blood pressure values (data not shown).

Evidence in humans has shown the influence of the sympathetic discharge in the different phases of natural sleep. During the desynchronized sleep phase, sympathetic activity increases, compared with the synchronized sleep phase, becoming similar to that observed during the awake condition. Futuro-Neto and Coote conducted 1 of the most complete studies regarding autonomic nervous system influence on natural sleep in cats, by direct measurement of sympathetic nerve activity in the heart, kidney, gastrointestinal tract, pelvic viscera, and blood vessels of the skeletal muscle. During both spontaneously or induced (by physostigmine sulphate) desynchronized sleep, the fall in blood pressure was accompanied by reductions in sympathetic activity to the kidney and the heart, whereas the activity in sympathetic...
vasoconstrictor fibers in the muscle increased. In rats, Miki et al.\textsuperscript{34} and Nagura et al.\textsuperscript{35} have demonstrated that desynchronized sleep is accompanied by a rapid and sustained reduction of renal sympathetic nerve activity followed by a gradual increase in blood pressure. Conversely, the lumbar sympathetic nerve activity increases concomitantly with an increase in blood pressure during desynchronized sleep phase in rats.\textsuperscript{25} Recently, Kuo et al.\textsuperscript{36} performed a noninvasive analysis of the autonomic nervous system in Wistar rats and showed an attenuation of a low-frequency oscillatory component for the pulse interval during desynchronized sleep, which represents the sympathetic influence of heart rate variability. Moreover, the desynchronized sleep phase was characterized by an increase in parasympathetic influence to the heart, estimated by the high-frequency oscillatory component of pulse interval variability. Thus, the autonomic balance seems to be shifted toward a parasympathetic predominance during slow-wave sleep. The well-characterized predominance of parasympathetic activity during wakefulness decreases during desynchronized sleep and reaches minimal values in the synchronized sleep phase. These data suggest a different pattern of distribution of the sympathetic nervous system to the kidney, heart, and vessels during the desynchronized sleep phase.

Besides the involvement of baroreceptors, the chemoreflex may also have an influence on the control of the hemodynamic changes that have been described during sleep phases. Both afferent fibers from baroreceptors and chemoreceptors of the sinoaortic areas are disrupted during the SAD surgical procedure. The selective deafferentation of the carotid body chemoreceptors in cats exacerbates the fall in blood pressure observed during desynchronized sleep phase, whereas specific baroreceptor denervation does not modify the hypotensive effect.\textsuperscript{37, 38} In fact, we have previously demonstrated, by both sinus denervation and isolated carotid body artery ligation, that chemoreflexes exert a tonic and inhibitory influence on the sympathetic nervous system.\textsuperscript{39} In our previous work, we showed that basal blood pressure is reduced almost 10 mm Hg after selective chemoreflex ablation.\textsuperscript{39} These data suggest that the arterial pressure alteration produced by SAD in rats represents the net effect of the abolition of inhibitory (baroreceptor deafferentation) and excitatory (chemoreceptor deafferentation) influences on the arterial pressure.\textsuperscript{39} Moreover, the role of chemoreceptors on the neural control of the cardiovascular system during natural sleep is not precisely known, except in studies in cats.

We cannot also exclude humoral modulation at the central integration level in the reflex control of the cardiovascular system. Studies previously conducted in our laboratory suggest that the renin-angiotensin system could be involved in the baroreflex modulation of the cardiovascular changes that occur during natural sleep. Intracerebroventricular infusion of angiotensin II modifies the pattern of pressure changes during sleep phases in a way similar to that of SAD.\textsuperscript{21, 24} Angiotensin II increases blood pressure during the synchronized sleep phase and decreases it during the desynchronized sleep phase in normal control animals. In fact, it has been demonstrated by others\textsuperscript{40} that renin activity correlates with the sleep-associated variations in systemic blood pressure in normal rats (low levels of plasma renin activity in desynchronized sleep and high levels in synchronized sleep phase). In addition, our laboratory has investigated the role of the renin-angiotensin system in other experimental models. During natural sleep phases, rats with mild renal hypertension (1Kidney-1Claire) and normal activation of the renin-angiotensin system behave like normotensive rats, whereas, in those rats with severe hypertension accompanied by overactivity of the renin-angiotensin system, the patterns of blood pressure changes during the sleep phases are similar to those of SAD rats.\textsuperscript{22, 24} Taken together, these data suggest that renin-angiotensin system overactivity is altering the central integration of the baroreflex reflex during natural sleep in the rat.

One important contribution of the present study is the observation that arterial baroreflex controls regional blood flow, i.e., hind-limb quarter, during desynchronized sleep. In fact, SAD blunted the increase in muscle vascular resistance observed in normal control rats and not counteracting the vasodilatation of other regions, thus contributing to the larger reduction in the total vascular resistance. The increase in muscle vascular resistance in SAD rats actually reverted to a larger decrease during the desynchronized sleep phase.\textsuperscript{5} Considering the fact that we showed an exacerbation in blood pressure reduction during the desynchronized sleep phase after SAD, and that the decrease in blood pressure during desynchronized sleep is accompanied by an increase in baroreflex sensitivity,\textsuperscript{41, 42} our data suggest that the baroreflex is significantly involved in regulation of the cardiovascular system during sleep. Normal baroreflex function during natural sleep in the rat could be an important factor in preventing blood pressure from dropping to low values during

**Figure 3**—Changes in cardiac output (CO, panel A), total vascular resistance (TVR, panel B), and muscle vascular resistance (MVR, panel C) in normal controls (white bars and solid lines) and sinoaortic denervated (SAD) (black bars and dotted lines) rats during synchronized (SS) or desynchronized (DS) sleep. \*significant difference compared with that in the synchronized sleep phase in control animals, P ≤ 0.05. †significant difference compared with that in the desynchronized sleep phase in the SAD group, P ≤ 0.05.
desynchronized sleep, as has already stressed by Mancia and Zanchetti in studies in cats. Moreover, during desynchronized sleep episodes, the arterial baroreflex seems to be limiting the changes in total vascular resistance, mainly influenced by the vascular resistance to the hindquarter limb.

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