Cardiovascular responses to nonrespiratory and respiratory arousals in a porcine model

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Launois, Sandrine H., Nathan Averill, Joseph H. Abraham, Debra A. Kirby, and J. Woodrow Weiss. Cardiovascular responses to nonrespiratory and respiratory arousals in a porcine model. J Appl Physiol 90: 114–120, 2001.—Spontaneous and provoked nonrespiratory arousals can be accompanied by a patterned hemodynamic response. To investigate whether a patterned response is also elicited by respiratory arousals, we compared nonrespiratory arousals (NRA) to respiratory arousals (RA) induced by airway occlusion during non-rapid eye movement sleep. We monitored mean arterial blood pressure (MAP), heart rate, iliac and renal blood flow, and sleep stage in 7 pigs during natural sleep. Iliac and renal vascular resistance were calculated. Airway occlusions were obtained by manually inflating a chronically implanted tracheal balloon during sleep. The balloon was quickly deflated as soon as electroencephalogram arousal occurred. As previously reported, NRA generally elicited iliac vasodilation, renal vasoconstriction, little change in MAP, and tachycardia. In contrast, RA generally elicited iliac and renal vasoconstriction, an increase in MAP and tachycardia. The frequent occurrence of iliac vasoconstriction and arterial pressure elevation following RA but not NRA suggests that sleep state change alone does not account for the hemodynamic response to airway occlusion during sleep.

sleep apnea; swine; regional blood flow; vascular resistance; blood pressure

METHODS

Animals

Fourteen 4-wk-old Yorkshire female pigs (mean initial body weight = 10.9 ± 0.5 kg) were included in the study. Seven were excluded because of postoperative complications, poor acclimatization to study conditions, or technical failure. We therefore report the results in only 7 pigs. The study period lasted 3–5 wk. At the end of the study, animals were killed with a lethal injection of thiopental sodium. The absence of renal or iliac iatrogenic stenosis was ascertained by postmortem inspection.

The protocol was approved by the Animal Care Committees of the West Roxbury VA Medical Center and of the Beth Israel Deaconess Medical Center.

Instrumentation

Abdominal instrumentation. Ultrasonic flow probes (Transonic Systems) were placed, through a ventral midline laparotomy under general anesthesia, around a renal artery and around the common trunk or a branch of an iliac artery, as previously described (27). A catheter was inserted in the aorta for blood pressure measurements. The flow probes and catheter were passed subcutaneously to exit from the back of the animal.

Sleep electrode instrumentation. Approximately 1 wk after the first surgical procedure, electrodes were implanted, following a procedure described previously (34). Briefly, electroencephalogram (EEG) and electroocculogram stainless steel electrodes were implanted through the frontal sinus under general anesthesia. Electromyogram electrodes were placed

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in a neck muscle through a small skin incision. Electrodes were attached to a small 9-pin connector that was secured to the skull with methyl methacrylate bone cement (Surgical Simplex P, Howmedica).

Tracheal balloon catheter. One to two weeks after sleep electrode placement, the animal was sedated, and anesthesia was maintained with isoflurane in O2 through a tightly sealed snout mask. The trachea was exposed through a 4-cm cervical incision, and a small hole was made between the second and third tracheal rings. An 8-Fr occlusion balloon catheter (Meditech) was inserted through the hole and placed above the carina. The catheter was secured to adjacent muscles, exited through the incision, and secured to the skin at the back of the neck.

Protocol

During the postsurgical recovery period, animals were trained to be handled and to sleep under experimental conditions. Sleep was undisturbed until the study period started.

Measurements. Vigilance state was determined by EEG, electroocculogram, and electromyogram signals and behavior (34, 36). Arousal was defined as an abrupt increase in EEG frequency, lasting three or more seconds (2). HR was derived from surface electrocardiogram via a cardiotachometer in five animals and from beat-to-beat arterial blood pressure in two animals. Arterial blood pressure was measured by connecting the indwelling arterial catheter to a pressure transducer referenced to heart level. MAP was recorded in five animals. Beat-to-beat arterial blood pressure was recorded in two animals, and MAP was calculated using appropriate software (Advanced Codas, Datqa Instruments). Iliac and renal flow probes were connected to a dual-channel flowmeter (Transonic Systems). Mean iliac and renal blood flows (IBF and RBF, respectively) were recorded. Iliac and renal vascular resistances were calculated as the ratio of MAP over regional blood flow. Tracheal pressure was monitored by filling the tracheal catheter with sterile saline and connecting it to a pressure transducer. Because of movement artifacts and baseline shifts, the signal could not be quantified reliably. HR, MAP, and regional flows were calibrated at the beginning and end of each experimental session. In the first four pigs, signals were recorded on a paper chart recorder, at a speed of 1 mm/s for later analysis. In the remaining three animals, signals were acquired through an external analog-to-digital board (DI 220, Datqa Instruments) and appropriate software (Windaq/200, Datqa Instruments) at a sampling rate of 250 Hz. Data were stored on a portable personal computer and were later analyzed using commercial analysis software (DADiSP, DSP and Advanced Codas, Datqa Instruments). In two animals, arterial blood gases were measured (ABL5, Radiometer) during wakefulness and NREM sleep, and immediately after 2 RA for the first animal and 3 RA for the second.

Experimental protocol. Experimental sessions began after the pig had recovered from sleep electrode placement (1–3 days). During the first sessions, only NRA were recorded. An arousal was classified as spontaneous (SPA) if the investigator failed to detect an external stimulus within the 60 s preceding the arousal. Acoustic arousals (AA) were provoked with an unquantified acoustic stimulus (loud metallic noise) and tactile arousals (TA) were provoked by using a 1-ml icy mist on the ear or by touching the animal’s back. Acoustic and tactile stimuli were applied after at least 1 min of sleep was observed. After tracheal catheter placement, RA were added to the experimental sessions. RA were produced by manually infating the occlusion balloon over 1–3 s with a predetermined amount of air. The inflation was silent, and the length of the catheter allowed the investigator to stand several feet away from the animal while inflating the balloon. Typically, the inflation procedure did not arouse the pig. Inflation of the balloon was not timed to the respiratory cycle. Inflation was maintained until an EEG frequency shift was visually detected. The balloon was then deflated rapidly. The number of occluded breaths varied between 2 and 5. Provoked nonrespiratory and respiratory stimuli were presented randomly. SPA occurred during all sessions. All events, interventions and postural changes were noted by the investigator. Experimental sessions took place between 0900 and 1400 and lasted 1.5–3 h. Each animal was studied on several occasions.

Data Analysis

Prearousal baselines. Before each arousal, cardiovascular variables were measured every 5 s for at least 1 min of NREM sleep, and a mean value was obtained for each NREM segment.

Arousals. To be considered for analysis, arousals had to be preceded by at least 1 min of NREM sleep. Arousals occurring after <1 min of sleep were not analyzed. Immediately after EEG changes, measurements were made every second for 15 s after the arousal. Variations in hemodynamic parameters were expressed as a percentage of the baseline value. Approximately 30% of the arousals were not analyzed because of artifacts, preceding sleep durations <1 min, poor signal quality, or insufficient chart annotation. Based on our previous results, which showed no statistical difference in the pattern or amplitude of the cardiovascular response (27), we grouped AA, TA, and SPA under the term NRA. A total of 13.6 ± 3.8 NRA per animal and 4.3 ± 1.7 RA per animal were analyzed. For each animal, we calculated a mean value of hemodynamic parameters for each arousal type.

In two animals, technical failure of one of the probes (the iliac flow probe in animal 7 and the renal flow probe in animal 6) did not allow complete regional flow pattern analyses. In animal 5, poor electrocardiogram quality did not allow HR determination.

Two-group comparisons of arterial blood gases and maximum cardiovascular responses to arousal were performed using a two-tailed Student’s t-test. Two-group comparisons of mean cardiovascular response at each second after EEG arousal were performed using a repeated-measures ANOVA. Statistical analysis was performed with Statview 4.5 for Macintosh. Results are reported as means ± SE. P ≤ 0.05 was considered statistically significant.

RESULTS

Arterial Blood Gases

During quiet wakefulness, arterial Po2 (PaO2) was 83.6 ± 1.4 Torr, arterial PCO2 (PaCO2) was 34.2 ± 2.4 Torr, and arterial O2 saturation (SaO2) was 96.9 ± 0.1%. Values did not change significantly during NREM sleep. Arterial blood gases measured after arousals induced by airway occlusion showed a significant decrease in PaO2 and SaO2 and an increase in PaCO2 (Fig. 1).

Cardiovascular Response to Arousal From NREM Sleep

During NREM sleep, in the five animals in which both RBF and IBF data were available, the most com-
mon hemodynamic response pattern induced by NRA was characterized by iliac vasodilation and renal vasoconstriction, accompanied by mild tachycardia and no change in MAP (Figs. 2A and 3). In contrast, the most common hemodynamic response pattern induced by RA was characterized by iliac vasoconstriction, with more profound renal vasoconstriction, mild tachycardia, and an increase in MAP (Figs. 2B and 3). Approximately one-half of the NRA and two-thirds of RA elicited the regional blood flow responses described above. Other patterns of regional vasoreaction were observed, as detailed in Table 1.

For the entire group, mean maximum changes from baseline after NRA and RA were significantly different for iliac vascular resistance (−29.9 ± 2.5 vs. 25.9 ± 18%, respectively; \( P = 0.02 \)) and renal vascular resistance (11.1 ± 6.2 NRA vs. 29.0 ± 9.3% RA; \( P = 0.01 \)), whereas the difference in maximum mean blood pressure response did not reach significance (2.8 ± 3.3% for NRA vs. 12.8 ± 3.2% for RA; \( P = 0.07 \)). Postarousal tachycardia was similar for both NRA and RA (21.8 ± 3.0 vs. 21.1 ± 6.5%, respectively; \( P = 0.9 \)).

**DISCUSSION**

To our knowledge, this report is the first to compare regional hemodynamic response patterns to NRA and RA in any species. We hypothesized that arousal would elicit a cardiovascular defense reaction, regardless of the stimulus causing the arousal. However, we found that, in this porcine model, NRA and RA from sleep elicited different cardiovascular responses. As previously reported, NRA were accompanied by renal vasoconstriction and iliac vasodilation, with minimal blood pressure changes. In contrast, RA elicited renal and iliac vasoconstriction, with significant increase in MAP. With both types of arousal, however, other hemodynamic patterns were also recorded.

The fact that arousals after obstructive apneas are accompanied by a surge in blood pressure is well documented in human patients (12, 20). The mechanism of

![Fig. 1. Arterial blood gases before airway occlusion (Pre AO; open bars) and on release of AO (Post AO; solid bars) performed during non-rapid eye movement (NREM) sleep. PaO2, arterial P O2; SaO2, arterial O2 saturation; PAO2, arterial PCO2.](http://jap.physiology.org/)

![Fig. 2. Polysomnographic tracings from 1 animal during NREM sleep illustrating the most common hemodynamic responses to spontaneous or nonrespiratory (A) and respiratory (B) arousals. Arrows indicate arousals. Time scale is 1.2 s per division. EEG, electroencephalogram; ABP, arterial blood pressure; IBF, iliac blood flow; RBF, renal blood flow; Ptrach, tracheal pressure.](http://jap.physiology.org/)
this surge, however, remains unclear but may be important to understand why occlusion sleep apnea patients develop diurnal hypertension. In this study, as well as in previous reports, a porcine model of sleep apnea was used to characterize the hemodynamic response to arousal (34, 27). Obstructive apneas during sleep in a conscious animal model can be created by occluding the trachea (6, 25, 31, 34) or by using a tight-fitting mask to collapse the upper airway (5). Although the latter method mimics spontaneous obstructive sleep apnea more closely, the former method interferes less with sleep and, therefore, is widely used.

By using a balloon catheter to occlude the trachea, we obtained obstructive apneas of variable duration, between 2 and 5 breaths. These occlusions were somewhat shorter than apneas created in dogs (9, 31) and reported in humans (20) but were similar to tracheal occlusions in newborn piglets (5, 6), suggesting that interspecies differences in arousal latency may exist. Despite their short duration, the apneas produced in our porcine model simulated obstructive apneas with regards to inspiratory efforts and arterial blood gas abnormalities (Figs. 1 and 2).

Some studies suggest that arousals that terminate an obstructive apnea and arousals that are elicited by an acoustic tone induce comparable blood pressure responses. Ringler et al. (35) recorded similar blood pressure and HR responses in apneic patients after an obstructive apnea and after an acoustic tone. Using vibrations or acoustic tones to elicit arousals in healthy subjects, investigators were able to record significant blood pressure increases (17, 29). Furthermore, Ali et al. (1) recorded a blood pressure response after arousals caused by periodic leg movements. However, previous studies in this model suggest that arousal type

Table 1. Distribution of hemodynamic response patterns

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Percentage of NREM Arousals</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>NRA</td>
</tr>
<tr>
<td>IVD-RVC</td>
<td>49.1 ± 5.6</td>
</tr>
<tr>
<td>IVD-RVD</td>
<td>36.4 ± 9.5</td>
</tr>
<tr>
<td>IVC-RVC</td>
<td>6.7 ± 3.3</td>
</tr>
<tr>
<td>IVC-RVD</td>
<td>2.7 ± 2.0</td>
</tr>
<tr>
<td>No change</td>
<td>0</td>
</tr>
<tr>
<td>IVD</td>
<td>4.1 ± 3.0</td>
</tr>
<tr>
<td>IVC</td>
<td>1.1 ± 1.1</td>
</tr>
<tr>
<td>RVD</td>
<td>0</td>
</tr>
<tr>
<td>RVC</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 pigs. NRA, nonrespiratory arousals; RA, respiratory arousals; IVD and RVD, iliac and renal vasodilation, respectively (vascular resistance decreased 5%); IVC and RVC, iliac and renal vasoconstriction, respectively (vascular resistance increased 5%).
modulates the blood pressure response. We showed a substantial and consistent blood pressure response to airway occlusions (34) but not after SPA, AA, or TA (27). The present study confirms that, in this model, NRA and RA elicit different blood pressure responses. By simultaneously monitoring IBF and RBF and MAP, we were able to ascribe this difference to distinct regional hemodynamic response patterns.

Despite some overlap, the response patterns to NRA and RA were different. The design of the present study was not aimed at explaining these differences, as we hypothesized that NRA and RA would elicit similar responses. Therefore, we can only speculate on the mechanism(s) responsible for our findings. Differences in chemostimulation, respiratory pattern, tracheal stimulation, and arousal may explain these findings. As we demonstrated in two pigs, tracheal occlusions resulted in substantial changes in arterial blood gases (Fig. 1). The integrated cardiovascular response to acute or short-term hypoxia is complex, and experimental studies aimed at characterizing this response have yielded conflicting results. Severe local hypoxemia produces marked vasodilation in some beds (coronary and cerebral) but little or none in others (limb and renal) (15). Similarly, systemic hypoxemia has a differential effect on muscle and renal beds (vasoconstriction) and on coronary, intestinal, and cutaneous beds (vasodilation) (16, 22, 44). The response is mediated by peripheral chemoreceptor stimulation and sympathetic activity (16, 19, 22) and can be modulated by the effects of hyperventilation on vascular resistance. An overall increase in total peripheral resistance was reported in some studies (16, 22, 26, 44), whereas others showed overall vasodilation (8, 45) or no change (30). Furthermore, the presence of hypercapnia and acidosis potentiates the effects of hypoxemia (32, 44). Dissociated vascular tone responses, differences in hypoxia severity and duration, presence or absence of concomitant hypercapnia, potential effects of anesthetic agents, species differences, and modulation of the response by pulmonary mechanoreceptors may explain why conflicting effects of hypoxemia on total vascular resistance have been reported. It is likely that systemic arterial blood gas changes contributed to the pattern of muscle and renal vasoconstriction seen in pigs after RA. The importance of this contribution, however, has yet to be established.

Strong inspiratory efforts were generated by the animal during the tracheal occlusion and abruptly decreased on arousal. This abrupt release could contribute to the pressor response associated with the end of the apnea by allowing increased venous return. However, a recent study in normal subjects suggests that the blood pressure response to voluntary apneas during wakefulness is not mediated through mechanical influences (24).

Release of tracheal occlusion was accompanied by a brief period of hyperventilation, which typically did not occur after NRA, although it was common to observe an augmented breath on arousal. Vascular response to lung inflation has been well studied in conscious and anesthetized dogs and is characterized by generalized vasodilation that is proportional to lung volume changes and is normally compensated by baroreceptor reflex activation (3, 13, 14, 37). One study in anesthetized dogs showed that the vasoconstriction elicited in some vascular beds by chemostimulation may be attenuated or reversed in the presence of lung inflation (13). In conclusion, the circulatory effects of hyperventilation are unlikely to contribute significantly to the RA hemodynamic responses in this model.

Inflation of the tracheal balloon may have stimulated tracheal receptors. In spontaneously breathing, anesthetized cats, mechanical tracheal stimulation caused a modest increase in MAP, with a concomitant increase in cerebral sympathetic activity discharge (42). In anesthetized rabbits, tracheal stimulation with smoke induced renal sympathetic activation, independent of baroreceptor, chemoreceptor, or pulmonary stretch receptor stimulation (33). It is, therefore, possible that mechanical tracheal stimulation contributed to sympathetic activation and vasoconstriction in visceral beds and, therefore, to the blood pressure increase seen after RA.

Finally, central nervous system activation may have been different after tracheal occlusion with asphyxia and after SPA or less threatening stimuli, such as noise and touch. The time course of the cardiovascular response was remarkable in that the circulatory changes consistently occurred immediately after the disruption of sleep, with little or no change during the occlusion itself. Admittedly, the occlusions were short, and many of the factors discussed above could require several seconds before reflexively affecting the vascular tone. Nevertheless, it is striking that blood flow and arterial pressure did not change before the arousal. This observation suggests that differences in the “arousal state” may have contributed to differences in the cardiovascular responses after NRA and RA. Neurophysiological events associated with sudden sleep disruption are still poorly understood, and definitions of arousals rely on crude EEG parameters (2). Studies in humans suggest that the degree of cortical activation, as measured by EEG analysis, is correlated to the amplitude of the blood pressure response (17, 39). Several experimental studies have demonstrated cortical modulation of cardiovascular reactions. In cats, stimulation of specific cortical sites can modulate the hemodynamic response elicited by amygdala stimulation (41), and emotions influence the cardiovascular response to external stimuli (28). In dogs and humans, there is ample data on the effects of emotions such as fear, anxiety, and anger on the cardiovascular and autonomic nervous systems (7, 38, 40, 43).

We previously attributed variability in the hemodynamic response to NRA to circadian influences, variable stress levels in the animals, and minor changes in vigilance after sleep disruption, in the absence of a better understanding of the central nervous system pathways that generate cardiovascular responses to sleep disruption (27). Tracheal pressure recordings revealed another factor that could influence this variabili-
ity. Some, but not all, NRA were followed by an augmented breath and a central apnea (Fig. 2A). It is, therefore, conceivable that hypocapnia occurred after some NRA. There was however, no relationship between the postarousal ventilatory pattern and the pattern of hemodynamic response. The MAP response to NRA, for the group, was minimal, as previously reported (27). Individual blood pressure responses, however, were variable (no change, increase, or decrease), presumably in response to variable balance between muscle vasodilation and renal vasoconstriction. Although the hemodynamic response was less variable after RA, three patterns of regional blood flows were recorded (Table 1). In addition to the factors mentioned above, variability in postarousal hyperventilation and chemostimulation are likely to have played a role. However, we could not correlate hemodynamic responses and PaO2, because blood pressure, and, hence, regional vascular resistances, could not be monitored while blood gas samples were obtained.

Animal and human studies suggest that the acute blood pressure response to obstructive sleep apnea is mediated by sympathetic nervous system activation (18, 21, 24). In humans and other species, NRA are also accompanied by sympathetic nervous system activation (25). However, in the present study, we demonstrated that the cardiovascular response was different after NRA and RA. Modulation of sympathetic nervous system activation in response to different stimuli could account for the different muscle vascular responses to NRA and RA observed in juvenile pigs. Indeed, dissociated activation of vascular beds in response to an external stimulus is well established in cats and dogs (4, 11, 28).

In conclusion, in a porcine model of sleep apnea, NRA and RA elicited different acute hemodynamic responses. This finding is in contrast with data from humans, in which the acute blood pressure response to NRA and RA is similar (1, 17, 35) but does explain why repeated RA produce chronic hypertension in dogs whereas repeated NRA do not (10).

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