Mechanisms of acute cardiovascular response to periodic apneas in sedated pigs

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Chen, Ling, Anthony L. Sica, and Steven M. Scharf. Mechanisms of acute cardiovascular response to periodic apneas in sedated pigs. J. Appl. Physiol. 86(4): 1236–1246, 1999.—This study was designed to evaluate the importance of sympathoadrenal activation in the acute cardiovascular response to apneas and the role of hypoxemia in this response. In addition, we evaluated the contribution of the vagus nerve to apnea responses after chemical sympathectomy. In six pigs preinstrumented with an electromagnetic flow probe and five nonpreinstrumented pigs, effects of periodic nonobstructive apneas were tested under the following six conditions: room air breathing, 100% O2 supplementation, both repeated after administration of hexamethonium (Hex), and both repeated again after bilateral vagotomy in addition to Hex. With room air breathing, during the apnea cycle, there were increases in mean arterial pressure (MAP; from baseline of 108 ± 4 to 124 ± 6 Torr, P < 0.01), plasma norepinephrine (from 681 ± 99 to 1,825 ± 578 pg/ml, P < 0.05), and epinephrine (from 191 ± 67 to 1,245 ± 685 pg/ml, P < 0.05) but decreases in cardiac output (CO; from 3.3 ± 0.6 to 2.4 ± 0.3 l.min, P < 0.01) and cerebral sympathetic nerve activity. With O2 supplementation relative to baseline, apneas were associated with small increases in MAP (from 112 ± 4 to 118 ± 3 Torr, P < 0.01) and norepinephrine (from 675 ± 97 to 861 ± 170 pg/ml, P < 0.05). After Hex, apneas with room air were associated with small increases in MAP (from 103 ± 6 to 109 ± 6 Torr, P < 0.05) and epinephrine (from 136 ± 45 to 666 ± 467 pg/ml, P < 0.05) and decreases in CO (from 3.6 ± 0.4 to 3.2 ± 0.5 l.min, P < 0.05). After Hex, apneas with O2 supplementation were associated with decreased MAP (from 107 ± 5 to 100 ± 5 Torr, P < 0.05) and no other changes. After vagotomy + Hex, with room air and O2 supplementation, apneas were associated with decreased MAP (from 98 ± 6 to 76 ± 7 and from 103 ± 7 to 95 ± 6 Torr, respectively, both P < 0.01) but increased CO (from 2.7 ± 0.3 to 3.2 ± 0.4 l.min (P < 0.05) and from 2.4 ± 0.2 to 2.7 ± 0.2 l/min (P < 0.01), respectively). We conclude that sympathoadrenal activation is the major pressor mechanism during apneas. Cerebral sympathetic nerve activity does not reflect overall sympathoadrenal activity during apneas. Hypoxemia is an important but not the sole trigger factor for sympathoadrenal activation. There is an important vagally mediated reflex that contributes to the pressor response to apneas.

sleep apnea; catecholamines; hexamethonium; sympathetic nerve activity; vagotomy; hypoxemia

There is considerable interest in defining possible mechanisms underlying the links between sleep apnea and cardiovascular sequelae. One of the more frequently invoked mechanisms is an increase in sympathetic nerve activity (SNA). Clinical studies have demonstrated an elevation in plasma and urinary catecholamine levels and in muscle SNA in patients with sleep apnea syndrome (10). However, several questions need further clarification to evaluate the significance of these clinical findings. This is because of the presence of confounding variables, often uncontrolled for, in many clinical studies, which may influence SNA. For example, it is still unclear whether alterations in the sympathoadrenal system in patients are caused by periodic apneas or by other related factors such as age, obesity, salt intake, hypertension, and antihypertensive therapy (10). It has been suggested that during obstructive apneas there is a complicated interplay between several pathophysiological factors, such as large negative swings in intrathoracic pressure, hypoxemia, possibly hypercapnia, and arousals (4, 20). Recently, hypoxemia has been postulated to play an important role in acute cardiovascular responses to apneas (6, 8, 24). Moreover, hypoxia has been well documented to trigger autonomic reflexes, including chemoreceptor activation of carotid sinus as well as aortic and central chemoreceptors. Thus hypoxia is speculated to be a major trigger for the elevated sympathoadrenal tone during periodic apneas.

Reflex regulation carried by the vagus nerve may have an important role in determining the cardiovascular response to apneas. This includes vagal efferent and afferent activities. Enhanced vagal (parasympathetic) efferent discharge during apnea was reported to be responsible for decreased heart rate (HR) during the late apnea (17). Afferent input from aortic chemoreceptors and baroreceptors carried by the aortic nerve, which is associated with the vagus, could also be involved in circulatory regulation during apneas. Moreover, nonventilation during the apnea phase alternating with post-apneic hyperventilation may lead to cyclic changes in mechanoreceptor input of the lung and the chest wall. This may be associated with phase-related cardiovascular changes during apneas (40). In addition, mechanoreceptors located in the atria, ventricles, and great veins might also contribute to this response. A recent report describing obstructive sleep apneas in heart transplant recipients suggests that cardiac receptors play a role in the apneic pressor response (13). Many of these cardio-pulmonary receptors could send afferent traffic via the vagus nerve.

A sedated pig model of periodic apnea has been previously described from this laboratory (6–8). In this model, periodic nonobstructive apneas caused a large increase in mean arterial pressure (MAP) and decreases in cardiac output (CO) and stroke volume (SV). Although this suggests a pathophysiological role for
vasoconstriction, the pathways mediating vasoconstriction were not clarified. In the present study we investigated the importance of the sympathoadrenal response to apneas and the role of the vagus in this response. We hypothesized that 1) eliminating sympathoadrenal activation by autonomic ganglionic blockade would eliminate the acute cardiovascular response to periodic apneas, 2) hypoxemia is the major factor responsible for sympathoadrenal activation during periodic apneas, and 3) there is an important role for reflexes activated by vagal afferents in the cardiovascular response to apneas.

METHODS

The local Institutional Animal Care and Use Committee in accordance with National Institutes of Health guidelines approved all methods, protocols, anesthesia, and sedation involved in this study.

Preinstrumentation

Six female Yorkshire farm pigs (18–22 kg body wt) were preinstrumented with an electromagnetic flow probe that was used for later measurement of CO and SV during data collection. The methods have been reported in detail (6–8). Briefly, the animals were anesthetized using ketamine (20 mg/kg) and xylazine (2 mg/kg). Anesthesia was maintained using 1% halothane. Animals were intubated and mechanically ventilated through an endotracheal tube. Under sterile conditions, the chest was opened and a sterile square-wave electromagnetic flow probe (Biotronix) was placed around the ascending aorta (14–18 mm, depending on the size of the aorta). A catheter was placed into the left atrial appendage, filled with heparin, and plugged. The lead from the flow probe and the left atrial catheter were led out to a subcutaneous pocket. The thoracotomy was closed in layers. Penicillin, dihydrostreptomycin, and morphine sulfate were administered intramuscularly for antibiotic prophylaxis and pain control. The animals were allowed 7 days to recover from the surgery before data collection.

Data Collection in Sedated Animals

Data were collected from a total of 11 animals, including 5 nonpreinstrumented and 6 preinstrumented animals. The pigs were anesthetized using ketamine/xylazine, as described above. This produced 45–60 min of surgical plane anesthesia. Animals were intubated and mechanically ventilated for the duration of the study. Tidal volume was set to 10 ml/kg, and respiratory frequency was adjusted to yield an arterial PCO₂ of ~40 Torr.

Continuous sedation and paralysis. Approximately 30 min before the end of surgical plane anesthesia, a continuous intravenous infusion with a mixture of 0.9% alphaxalone and 0.3% alphadalone (Saffan, Pittman-Moore, Uxbridge, Middlesex, UK) was begun at 3–4 mg·kg⁻¹·h⁻¹. This level of sedation is sufficient to produce heavy sedation but not surgical plane anesthesia. Previous studies demonstrated that alphaxalone/alphadalone is associated with preservation of sympathetic reflexes and minimal ventilatory suppression compared with other anesthetic/analgescic agents (3). Once sedation was established and after all surgical manipulation, the animals were paralyzed by using cis-atracurium besylate (Nimbex, Glaxo Wellcome, Research Triangle Park, NC; 0.6 mg/kg bolus iv followed by 16 g·kg⁻¹·min⁻¹ continuous infusion). We are ensured of adequate sedation to eliminate pain or suffering under paralysis in a number of ways. 1) In studies in which animals are not paralyzed (6) but with the same apnea periodicity as in the present protocol (see below), no visible signs of suffering have ever been observed in this laboratory. 2) In previous studies in which apneas were produced with animals paralyzed and unparalyzed (6), there was no difference in baseline blood pressure or HR, suggesting no pain or suffering associated with paralysis and sedation. 3) In the present studies, there was continuous monitoring of blood pressure and HR before and after apneas were paralyzed in the presence of continuous sedation. No surges in blood pressure or HR were ever observed suggesting pain or suffering. 4) Surgical manipulation was done only during surgical plane anesthesia, and no surgical manipulation was done when animals were paralyzed and sedated. 5) In previous studies using a protocol similar to that used here (8), continuous monitoring of electroencephalogram before, during, and after apneas revealed no changes in amplitude or frequency that indicated arousal or suffering. 6) Heavy sedation was maintained continuously by continuous intravenous drip during all phases of the experiment when paralysis was induced. Animals were killed at the end of the experiments by bonus injection of Euthanasia-5 solution (0.3 ml/kg iv; Veterinary Laboratories, Lenexa, KS), which contains 5 g/ml pentobarbital sodium in 40% isopropyl alcohol and 2% propylene glycol.

Surgical preparation for data collection. Before any incisions were made, during surgical plane anesthesia, 2% lidocaine was infiltrated into the skin. A large-bore catheter via cutdown was placed in the femoral vein for administration of fluids and medications. A 7-F thermodilutor-tipped catheter was inserted into the femoral artery. It was advanced into the ascending aorta for measurement of blood pressure, collection of blood samples for blood gases and catecholamine levels, and calibration of the aortic flow probe in the preinstrumented animals. The cervical vagosympathetic trunks were isolated bilaterally and soaked in warm mineral oil. During surgical plane anesthesia the subcutaneous pocket in the preinstrumented animals was opened. The signals from the aortic flow probe were calibrated during the data collection phase by the thermodilution technique, in which iced saline was injected into the left atrium via the left atrial catheter and blood temperature was sampled via an aortic catheter. Airway pressure was measured via a lateral tap placed in the endotracheal tube.

Protocols. Periodic nonobstructive apnea was caused by turning the ventilator off at end expiration for 30 s (apnea phase) and on for 30 s (interpapnea interval). Thus an apnea-interapnea cycle was defined as 1 min. We tested the effects of apneas under the following conditions: 1) room air breathing (RA); 2) 100% O₂ administered to the entry port of the ventilator (O₂), 3) room air breathing after administration of hexamethonium dichloride (5 mg/kg; RBi, Natick, MA; HRA), 4) 100% O₂ breathing after hexamethonium (HRA), 5) breathing room air following addition of bilateral vagotomy after hexamethonium administration (VRA), and 6) 100% O₂ breathing after vagotomy and hexamethonium (VO₂). The order of control, hexamethonium, and hexamethonium and vagotomy could not be randomized and was thus performed sequentially. However, for any given condition, the order of RA vs. O₂ breathing was randomized.

A preliminary study was done to determine the appropriate dose of hexamethonium in pigs. Effects of periodic nonobstructive apneas under the RA condition were tested in two pigs (data not included in the present results) after three sequential bolus doses of hexamethonium (5, 10, and 20 mg/kg). There were no differences in the blood pressure, CO, and HR
responses to apneas among the three dose levels, suggesting that 5 mg/kg achieved maximum pharmacological effect.

Measurements were done at baseline, during the 12th apnea-interapnea cycle, and at recovery. "Baseline" indicates the period of ventilation after 20 min of stabilization under the experimental conditions but before any apnea intervention. "Recovery" is the same state as baseline after a 20-min stabilization following the end of the apnea intervention. One blood-gas sample from the 12th apnea-interapnea cycle was taken over a 10-s period beginning at 5 s before apnea termination and extending over the first 5 s of ventilation resumption. Blood samples for catecholamine measurements were drawn over a 10-s period immediately after blood-gas sampling. However, blood samples for catecholamines were not obtained after vagotomy. Hemodynamic parameters, airway pressure, and cervical SNA signals were digitized at a frequency of 128 Hz in 60-s epochs. Data were streamed through to a hard disk of a microcomputer using commercially available software (ACQ4600, Gould, Cleveland, OH).

Measurement of plasma catecholamines. A 3-ml sample of arterial blood was collected into precooled heparinized tubes to which 0.1 ml of sodium metabisulfite solution (380 mg/ml) was added. Samples were spun at 2,000 rpm in a refrigerated centrifuge for 20 min. One milliliter of plasma was drawn off and added to 0.1 ml of 4 M perchloric acid, spun at 3,000 rpm for 30 min, and decanted into plastic storage tubes. These were kept at −70°C for later measurement by a previously published HPLC technique (30). Measurements were performed in a commercial laboratory (Corning Labs, San Juan Capistrano, CA). Briefly, 20 µl of plasma was injected into the HPLC, and catecholamines were separated in a C18 column with a mobile phase of 85:15 H2O-methanol, 1% EDTA, 2 mM heptane sulfonic acid, and 1% acetic acid (pH 3.8) and a flow rate of 1 ml/min. Standards were included along with each assay. All measurements were done in duplicate.

Recordings of SNA. The right preganglionic superior cervical sympathetic nerve was isolated and removed from the vagosympathetic trunk caudal to the superior cervical ganglion. The nerve was verified by electrically stimulating the peripheral end of the nerve (25 Hz, 4.0-ms duration, 10-ms delay, 0.5 V) and observing dilation of the pupil. The central end of the nerve was desheathed and then placed on bipolar platinum electrodes in warm mineral oil. The signal was band-pass filtered between 0.1 and 2 kHz and then integrated using a moving time average integrator with a time constant of 0.1 s. Zero sympathetic activity was defined as the level of sympathetic activity obtained after the animal was killed after the central end of the nerve was cut. Integrated SNA was expressed as normalized to baseline for any given experimental condition.

Data analysis. Data were analyzed off-line using commercially available software (View II, Gould). Data during the 12th apnea-interapnea cycle were taken at specified times as follows: early apnea (first 5 s of apnea phase), late apnea (last 5 s of apnea phase), early interapnea (first 5 s of interapnea interval), and late interapnea (last 5 s of interapnea interval). Thus, each datum point represented a 5-s period. HR and MAP were measured from the blood pressure tracing. CO was defined as mean aortic flow. SV was measured on a beat-to-beat basis by integrating the stroke aortic flow. Systemic vascular resistance (SVR) was calculated as (MAP/CO) × 79.9 (dyn·s·cm−5).

Data were compiled and expressed as means ± SE. Student's t-test for paired variables with Bonferroni's correction when applicable was used for testing differences between baseline and recovery as well as between baselines of each pair of conditions. One-way ANOVA for repeated measures was used to test within-group changes between baseline and different points in the apnea-interapnea cycle as well as between early apnea and other points in the apnea-interapnea cycle. Two-way ANOVA was used for testing of differences between conditions. For the ANOVA, if significance was found, a Newman-Keuls procedure was used to analyze the differences between each pair of values. The null hypothesis was rejected at the 5% level.

RESULTS

Data for MAP, HR, and blood-gas tensions were collected from all 11 animals, including the 5 nonpreinstrumented and the 6 preinstrumented animals. Plasma catecholamines and cervical SNA were successfully collected from 10 animals, including the 5 nonpreinstrumented and the 5 preinstrumented animals. CO, SV, and SVR were collected only from the six preinstrumented animals.

Preparation Stability

There were no significant differences in any parameter at baseline between the nonpreinstrumented and the preinstrumented animals. Thus pooled data are presented. Moreover, at baseline there were no significant differences between any of the conditions for any parameter, except for CO (see Fig. 2) between HO2 and VO2 (3.83 ± 0.67 vs. 2.37 ± 0.16 l/min, P < 0.05) and between HO2 and VRA (3.83 ± 0.67 vs. 2.65 ± 0.29 l/min, P < 0.05). Finally, there were no significant differences between baseline and recovery in any parameters for any condition.

Blood-Gas Tensions

Table 1 demonstrates arterial blood-gas tensions. Under all conditions, apneas led to equal increases in arterial PCO2 and decreases in pH relative to baseline. In the three room air conditions, periodic apneas led to a similar degree of hypoxemia. In contrast, with O2 supplementation there was hyperoxia even during apneas.

Hemodynamics

Relative to baseline, MAP (Fig. 1) increased significantly at all points over the apnea-interapnea cycle for RA and O2 and only at late apnea for HRA. At late apnea, RA was associated with significantly (P < 0.05) greater percent increases in MAP from baseline (14.3 ± 2.7%) than with O2 (5.4 ± 1.9%) and HRA (6.5 ± 4.3%). On the other hand, compared with baseline, HO2, VRA, and VO2 were associated with significant decreases in MAP at all points over the apnea-interapnea cycle. With VRA the magnitude of the decrease in MAP was significantly (P < 0.001) greater than with VO2. For VRA, relative to baseline, MAP was lower during interapnea (early and late) than during early apnea (P < 0.05). For all other conditions, there were no differences between early apnea and other points in the apnea-interapnea cycle for MAP.

Relative to baseline, CO (Fig. 2) decreased significantly with RA at late apnea and early interapnea, as
well as at early interapnea with HRA. There were no significant changes in CO with O₂ and H₂O₂. In contrast to RA and HRA, relative to baseline, CO increased significantly with VRA at early apnea, late apnea, and late interapnea, as well as with VO₂ at early and late interapnea. With VRA, CO was higher at early apnea and late interapnea than early interapnea. There were no other significant differences between early apnea and late interapnea.

Fig. 1. Effects of periodic apneas on mean arterial pressure (n = 11). RA, room air breathing; O₂, breathing with 100% O₂ supplementation; HRA, room air breathing after hexamethonium; H₂O₂, breathing with 100% O₂ after hexamethonium; VRA, breathing room air following addition of bilateral vagotomy after hexamethonium; VO₂, 100% O₂ breathing vagotomy after hexamethonium; Apnea, 10 s of apnea with beginning at 5 s before ventilation resumption of 12th apneic-interapneic cycle. * P < 0.01 compared with baseline.

Fig. 2. Effects of periodic apneas on cardiac output measured from aortic flow probe (n = 6). See Fig. 1 legend for definition of abbreviations. Significantly different from baseline: * P < 0.05, ** P < 0.01.

Table 1. Effects of periodic apneas on arterial blood-gas tensions in sedated pigs

<table>
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<tr>
<th></th>
<th>RA Baseline</th>
<th>Apnea</th>
<th>Recovery</th>
<th>O₂ Baseline</th>
<th>Apnea</th>
<th>Recovery</th>
<th>HRA Baseline</th>
<th>Apnea</th>
<th>Recovery</th>
<th>H₂O₂ Baseline</th>
<th>Apnea</th>
<th>Recovery</th>
<th>VRA Baseline</th>
<th>Apnea</th>
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<th>VO₂ Baseline</th>
<th>Apnea</th>
<th>Recovery</th>
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<td>pH</td>
<td>7.43 ± 0.03</td>
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<td>7.42</td>
<td>7.43 ± 0.03</td>
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<td>7.28 ± 0.03</td>
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<td>PCO₂, Torr</td>
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<td>63.3</td>
<td>42.7 ± 3.3</td>
<td>41.8</td>
<td>41.4</td>
<td>42.9 ± 1.6</td>
<td>59.0</td>
<td>43.0</td>
<td>62.4 ± 4.1</td>
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<td>41.2</td>
<td>58.8 ± 2.9</td>
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<td>PO₂, Torr</td>
<td>99.2 ± 6.5</td>
<td>48.8</td>
<td>96.2 ± 3.3</td>
<td>336.4</td>
<td>324.4</td>
<td>90.3 ± 4.0</td>
<td>51.7</td>
<td>94.0</td>
<td>291.0 ± 6.5</td>
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<td>93.0</td>
<td>237.4 ± 6.5</td>
<td>105.5</td>
<td>363.2</td>
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Values are means ± SE; n = 11. RA, room air breathing; O₂, breathing with 100% O₂ supplementation; HRA, room air breathing after hexamethonium; H₂O₂, breathing with 100% O₂ after hexamethonium; VRA, breathing room air following addition of bilateral vagotomy after hexamethonium; VO₂, 100% O₂ breathing vagotomy after hexamethonium; Apnea, 10 s of apnea with beginning at 5 s before ventilation resumption of 12th apneic-interapneic cycle. * P < 0.01 compared with baseline.
other points in the apnea-interapnea cycle for any group.

With RA, HR (Fig. 3) was significantly \( (P < 0.01) \) faster at late interapnea than at baseline, early apnea, and late apnea. Moreover, with RA, at early interapnea, HR was significantly \( (P < 0.01) \) faster than at late apnea. With \( O_2 \), HR was significantly \( (P < 0.01) \) slower at late apnea than at baseline and early apnea. After hexamethonium pretreatment and after addition of vagotomy to hexamethonium, there were no significant within-group changes in HR.

Similar to CO, relative to baseline, SV (Fig. 4) decreased significantly at every point in the apnea-interapnea cycle for RA and at early interapnea for HRA. However, compared with baseline, SV significantly increased with VRA at early apnea and late interapnea. Compared with baseline, with \( V_2 \), SV decreased at all the points in the apnea-interapnea cycle. There was no significant change in SV with \( O_2 \) and \( H_2O \) compared with baseline. There were no significant changes in SV over the apnea-interapnea cycle compared with early apnea.

Relative to baseline, SVR (Fig. 5) increased significantly at all points in the apnea-interapnea cycle with RA and at early interapnea with \( O_2 \) but decreased significantly at all points in the cycle with VRA and \( V_2 \). There was no significant change in SVR with HRA and \( H_2O \). There were no significant changes in SVR relative to early apnea in the apnea-interapnea cycle.

Plasma Catecholamines

Relative to baseline, RA apneas were associated with significant increases in plasma norepinephrine and epinephrine (Fig. 6). Also, there were small but significant increases in norepinephrine with \( O_2 \) and a moderate increase in epinephrine with HRA. However, with \( H_2O \), neither dopamine level with any condition nor all the three catecholamines changed significantly during apneas relative to baseline.

Cervical SNA

RA was associated with significant decreases in normalized cervical SNA (Fig. 7) at early apnea and late apnea relative to baseline. With other conditions, normalized cervical SNA showed no significant change over the apnea-interapnea cycle relative to baseline.

DISCUSSION

Major findings in this study may be summarized as follows. 1) During apneas, sympathetic activation, as reflected in plasma catecholamine levels, was maxi-
mum under RA conditions. However, this was associated with a decrease in cervical SNA. Other conditions, namely O2 and HRA, were associated with less evidence of sympathetic activation, as reflected in catecholamine levels. 2) Hypoxemia appeared to be the predominant but not the sole factor associated with sympathetic activation during apneas. 3) Relative to baseline, MAP increased during apneas in the intact condition. This response was diminished but not totally abolished by eliminating hypoxemia (O2) or by ganglionic blockade alone (HRA), but it was reversed (decreased MAP during apneas) by the combination of hyperoxia and ganglionic blockade (HO2). Moreover, adding vagotomy to ganglionic blockade was associated with depressor effects during apneas with (VO2) or without O2 supplementation (VRA). In the ensuing discussion we consider these findings.

Experimental Preparation

The present model demonstrated good stability over time, as shown by the fact that there were no differences in any parameters between baseline and recovery with any condition. Furthermore, there are no significant differences in any parameters at baseline between conditions with and without O2 supplementation and between the nonpreinstrumented and the preinstrumented animals. Thus the previous instrumentation intervention did not affect cardiorespiratory response to apneas.

A distinguishing feature of obstructive apnea is the persistence of respiratory motoneuron output during the apneic period. In contrast, cessation of respiratory motoneuron output occurs during a central apnea. Thus our porcine model, in which respiratory muscles are paralyzed but respiratory motoneuron output presumably is intact (35), more closely represents neurological events occurring during obstructive apneas. We chose this paralyzed model of periodic apnea, since the pressor response in this model is greater than for a similar unparalyzed model (6), and presumably sympathoadrenal stimulation is maximized. We hoped therefore that perturbation of the system would yield large effects in the variables studied. By paralyzing the animals to create apnea, mechanoreceptor stimulation from intrathoracic pressure swings was minimized, thus controlling for a potentially confounding factor. The model also controlled for the confounding effect of arousal during apnea, since there are no changes in cortical state, as measured by electroencephalogram, suggestive of arousal (8). This model thus provided an opportunity to evaluate the role of metabolic (hypoxemia) and neuroendocrine factors not confounded by the additional effects of arousal and negative intratho-
ractic pressure. We note that other factors, including sleep state and postapneic hyperpnea, which could also influence the cardiovascular responses to apneas, are not simulated in this model.

Paralysis necessitates the use of positive-pressure mechanical ventilation, the opposite of normal breathing, where ventilation is generated by negative intrathoracic pressure. It might be thought that this would lead to unphysiological values at baseline. However, we (6) demonstrated that for this model there were no differences in any baseline hemodynamic values between normal spontaneous ventilation and mechanical ventilation before apnea onset.

Sympathoadrenal Activity

Numerous studies have reported an increase in peroneal muscle SNA and in plasma or urinary catecholamine level in sleep apnea patients (10, 29). However, confounding factors, including pathological states and medications that have effects on SNA, are often uncontrolled for in clinical reports (10). For example, patients with sleep apnea often have concomitant hypertension and/or congestive heart failure and may be on sympathetic blockers or other medications. In these cases, baseline sympathoadrenal tone may be expected to vary greatly. Furthermore, patient studies may be confounded by impaired renal catecholamine clearance (21, 44), which could lead to overestimation of sympathetic activity from catecholamine levels in clinical situations. Urinary catecholamine levels, while reflecting overall release (42), cannot be used to determine rapid changes that may occur during periodic apneas. The present study allowed us to test rapid changes in plasma catecholamine and cervical SNA after several cycles of periodic apneas in a well-controlled animal model. Clearly, sympathetic activation with apneas occurs with rapid onset, since we observed increased plasma norepinephrine/epinephrine levels within minutes of apnea onset.

Synamptically released norepinephrine spills over to the bloodstream. Hence, plasma norepinephrine levels are believed to reflect the level of sympathetic nerve activity in normal subjects (12) and in patients (5, 19, 20, 37). In the present study we recorded cervical preganglionic SNA. Unlike the peroneal nerve studies cited above, cervical SNA decreased during hypoxic apneas, despite increased plasma norepinephrine levels. One possibility for this discrepancy is that the sympathetic nervous system does not respond in an all-or-none fashion to apneas, and the central controller does not activate all sympathetic fields uniformly. Indeed, dissociation between sympathetic nerve firing rates in different areas of the body (16, 26) has been demonstrated. We also point out that we performed whole nerve recordings. Sympathetic fibers in the cervical sympathetic nerve innervate many nonvascular structures. It is possible that the cardiovascular component of the cervical sympathetic nerve did increase its activity but that the opposite effect in the noncardiovascular components overwhelmed the effects on the sympathetic component.

Our studies show that hypoxemia is an important but not the sole factor leading to increased plasma catecholamine levels, since small increases in norepinephrine were seen even with hyperoxia (Fig. 6). We observed an increase in plasma norepinephrine levels during hypoxia before ganglionic block (RA) and a smaller but still significant increase with hyperoxia before ganglionic block (O2). That no increase in plasma norepinephrine levels was seen after ganglionic block (HRA and HO2) most likely reflects the completeness of the block. The effects seen with hypoxia (RA) are consistent with previous studies showing a correlation between sympathoadrenal activation and the degree of hypoxia (11, 19, 28, 44).

The source of the small increase in norepinephrine during hyperoxic apnea is unclear. Hypercapnia may have contributed to increased sympathetic tone. On the other hand, hyperoxic apnea is essentially a breath-hold maneuver. In studies on normal subjects, breath-hold maneuvers have been shown to enhance muscle sympathetic tone even in the absence of hypoxia (25). This could be related to loss of afferent mechanoreceptor activity from the lungs or chest wall. Epinephrine levels increased only during hypoxia (RA and HRA), suggesting that adrenal stimulation does not depend on changes in afferent mechanoreceptor activity or hypercapnia but is a function only of hypoxia. Because secretion does occur after ganglionic blockade, the stimulus to adrenal medullary secretion is a direct effect of hypoxia on the adrenal medulla or residual preganglionic sympathetic activity. Hypoxia has been demonstrated to directly lead to catecholamine release from adrenomedullary cells (9, 23) and myocardium (33). Moreover, hexamethonium antagonizes nicotinic receptors but not muscarinic receptors that are located at the postganglionic neuron and the small intensely fluorescent cells in autonomic ganglia. In the presence of ganglionic blocking drugs, small intensely fluorescent cells may release catecholamines as their neurotransmitter (43). Furthermore, the postganglionic muscarinic receptor may take over the function of ganglionic neurotransmission normally carried out by the nicotinic receptor (43). Thus, in the presence of nicotinic ganglionic blockade, some muscarinic-mediated norepinephrine release could be possible.

Vagal-Related Pressor Response

Clearly, vagally carried traffic plays a role in modulating the pressor response to apneas. Loss of vagal activity in addition to sympathectomy led to a depressor response to apneas. In a recent study from this laboratory, vagotomy in the absence of any other manipulation abolished the pressor response to apnea (36). Which vagal afferents could be responsible for contributing to the sympathetic efferent activity and the pressor response during apneas? Although our results do not allow a definitive answer to this question, there are a number of possibilities. Sympathostimulatory vagal afferent traffic originates from a variety of sources. The aorta contains baroreceptors and chemoreceptors, which send inputs through the vagus via the
aortic nerve (25, 26). Afferent traffic from pulmonary mechanical receptors and central volume receptors, some of which is sympathostimulatory, is also carried by the vagus (1, 34). Finally, although chest wall sympathetic afferent fibers are carried mainly via spinal nerves, cardiovascular reflex effects arising from chest wall afferents are modulated by vagal afferent input as well (22). The present study does not allow us to differentiate among these possibilities. However, the sensitivity of the pressor response to hypoxia suggests that important vagal afferent activity leading to sympathetic stimulation is related to chemoreceptor activity, most likely aortic chemoreceptors. In the pig, these receptors send afferent traffic via the aortic depressor nerve, which accompanies the left vagus nerve and would have been sectioned during vagotomy.

Acute Cardiovascular Changes

HR. With RA, we observed a significantly higher HR during the interapnea phase than at late apnea. This observation is consistent with previous studies in apnea patients (45) and experimental animals (6, 8). Although we previously reported that HR was lower than baseline during apneas (6, 8), in other studies (7, 36) we observed HR to be greater or not different during apneas compared with baseline. The reasons for different HR responses to apnea in different studies are not clear. However, many factors could influence the HR response to apneas. For example, in those studies reporting HR decreases from baseline during apneas, animals were paralyzed with succinylcholine, whereas in studies, as in the present study, reporting HR increases relative to baseline during apneas, paralysis was produced using cis-atracurium. The well-known mild vagomimetic effects of succinylcholine might have led to an HR response during apneas that was different from baseline. However, in all studies, regardless of the HR during apnea relative to baseline, HR was consistently lower during apnea than at interapnea. This finding suggests that factors associated with the apnea or apnea termination are responsible for changes within the apnea-interapnea cycle. These factors have been discussed extensively (6, 27, 41). It is generally believed that the increase in HR after apnea termination is too rapid to be due to changes in blood-gas tensions. In human apnea, postapnea arousal contributes to increased HR. However, arousal is not a factor in this model. Others have concluded that stimulation of thoracic mechanoreceptors is responsible for increased HR during interapnea compared with apnea (6, 27, 41).

With O₂, even though HR was lower during apnea than at baseline, HR was also lower during early apnea than at interapnea. Thus, as with RA, during interapnea HR was greater than during early apnea. What is the reason for the finding that, with O₂, HR was lower during apnea than at baseline, whereas with RA, HR was not different from baseline (although lower than at interapnea)? One possible explanation could be the integration of sympathetic and parasympathetic responses during apnea. In the presence of hypoxia (RA), sympathetic responses are greater than with O₂. This could tend to drive HR up with RA compared with baseline. The tendency for increased HR could be counteracted by vagal influences during apnea. In the absence of hypoxia (O₂) the sympathetic response is less, and the residual vagal influences during apnea could lead to HR slowing compared with baseline.

Hexamethonium abolished the HR response to apneas with and without hypoxia. This suggests that HR changes are mediated by autonomic influences not by the primary effects of hypoxia. Our data do not allow us to distinguish between sympathetic withdrawal and vagal stimulation. However, other studies demonstrate that vagal efferent activity is largely responsible for HR slowing with apneas (17, 40).

SVR. The interaction of CO and SVR determines the MAP response to apneas. We view the effects of apneas on SVR as the result of the interaction of vasoconstrictor and vasodilator influences associated with apnea on the peripheral circulation. This is illustrated in the model presented in Fig. 8. Vasoconstrictor influences include hypoxic stimulation on the sympathoadrenal system, which are mediated via carotid (14) and aortic (2) chemoreceptors. Hypercapnic stimulation of sympathoadrenal tone, on the other hand, is primarily mediated by the central chemoreceptors (37). Other vagally carried afferents could be associated with vasconstriction. Hypoxia may be associated with stimulation of adrenal medullary or tissue production of epinephrine (see above). Decreased cardiac and/or respiratory mechanoreceptor activity during apneas could also lead to sympathoadrenal activation and vasconstriction.

On the vasodilator side are direct vasodilatory effects of hypoxia and hypercapnia acting on vascular smooth muscle. In addition to increasing vagal discharge to the heart, stimulation of the carotid body by hypoxia may increase vagal discharge to the peripheral circulation, leading to vasodilation (15). Although the relative strengths and interactions of these vasodilatory effects are not known, these effects could act additively to lead to vasodilation if unopposed by other factors leading to sympathetic vasoconstriction (below).

In the case of vagotomy plus hexamethonium, we observed vasodilation during hypoxic and hyperoxic apneas. With hypoxic apneas (VRA) the sum of direct vasodilatory effects of hypoxia and hypercapnia on peripheral vasculature, and the absence of sympathetic vasoconstriction to oppose these effects, leads to overall vasodilation. With hyperoxic apneas (VO₂), vasodilation was also seen, possibly secondary to the vasodilatory effects of hypercapnia. In our previous study (8) with a level of hypercapnia similar to that seen here, we observed a decrease in SVR of 300–500 dyn·s·cm⁻⁵ during apneas, similar to that observed in the present study with VO₂ conditions during apnea. Thus the degree of hypercapnia was sufficient to account for vasodilation with VO₂.

For hexamethonium alone (vagus intact), we observed no significant changes in SVR under any circum-
stances. This suggests that some vasoconstrictor activity persists that counteracts the vasodilator effects of hypercapnia (for HRA and HO2) and hypoxia (for HRA). With HRA, there was a moderate increase in epinephrine release during apneas. It is possible that the α-adrenergic effects of epinephrine counteracted the direct vasodilatory effects of hypoxia and hypercapnia.

Finally, with the intact vagus and sympathetic system, apneas led to increased SVR, the increase being greater with RA than with O2. We interpret this to mean that the vasoconstrictor effects associated with sympathetic stimulation during apneas overcame the direct vasodilator effects of hypoxia and hypercapnia. Sources of this stimulation include chemoreceptor stimulation from the carotid and aortic chemoreceptors (aortic nerve intact and sympathetic system intact) with RA and sympathetic stimulation via mechanoreceptor afferents even in the absence of hypoxia (O2 condition).

Others (18, 27) have also studied the effect of chemical sympathectomy on the pressor response to apneas. Given the above information concerning the complexity of the regulation of SVR and MAP during apneas as well as species differences, direct comparisons may be difficult. However, in sleeping dogs (27), administration of hexamethonium blocked the apneic pressor response and led to a depressor response after apnea termination (interapnea). Our data showing that hexamethonium almost completely blocked the pressor response to apneas are consistent with the notion that the sympathetic response to apneas is an important determinant of the pressor response. However, we did not observe a depressor response after apnea termination. Species and state difference (sleep vs. sedation) may be responsible for different findings. In the present study we observed a depressor response to apneas and during interapnea only after vagotomy was added to hexamethonium. Species differences could also explain these discrepancies if aortic chemoreceptor input was more active in pigs than in dogs or if pigs generated more epinephrine release from peripheral tissues than occurred in dogs. In humans undergoing Müller maneuvers (18), hexamethonium also blocked the pressor response. However, the sustained Müller maneuvers in humans are unlike obstructive apneas in a number of ways, including the fact that obstructive apneas are repeated, and hypoxia occurs. These additional stimuli, in addition to species differences, may have led to additional secretion of adrenally produced catecholamines in our model as opposed to the human study.

CO. As in our previous studies (6, 8), the present data support the notion that depression of CO during apneas was primarily determined by increases in left ventricular (LV) afterload. With RA apneas, CO and SV fell ~30% at late apnea from baseline, whereas LV afterload showed a marked increase as indicated by MAP and SVR. Furthermore, after hexamethonium and vagotomy, apneas, with and without hypoxemia (VRA and VO2), caused decreases in MAP and SVR, corresponding to an increase in CO and SV. VRA was associated with a greater decrease in LV afterload and a greater change in CO and SV than was associated with VO2. Thus we conclude that one major determinant of CO during apneas is LV afterload change produced by changes in peripheral circulatory mechanics.

The present study suggests a causal relationship between changes in sympathoadrenal tone and LV afterload during apneas. Diminution of apnea-related changes in sympathoadrenal tone by O2 supplementation or by hexamethonium leads to diminution of changes in LV afterload and CO. We propose that sympathoadrenal activation is the major mechanism mediating the adverse effects of apneas on LV afterload and subsequent decreased CO. Thus our data support our earlier conclusions. In normal hearts, adverse effects of apneas on CO are the result of neurophysiological effects of apneas, rather than the mechanical effects of decreased intrathoracic pressure on LV afterload. However, failing or myopathic hearts may be more sensitive to putative afterload effects of intrathoracic pressure than normal hearts.
Recently, Schneider et al. (32) studied cardiovascular changes with induced obstructive apneas in sleeping dogs. They observed an overall increase in CO during the apneic phase compared with the apneic baseline. The discrepancy between their study and the present study may be due to a number of factors. We view the effect of apneas on CO as the summation of a number of different influences. Increased arterial pressure tends to decrease CO because afterload increases. On the other hand, sympathetic stimulation and HR increases tend to increase CO. A number of different factors could explain the discrepancy between the study of Schneider et al. and the present study. 1) The sensitivity of the LV to increased afterload (arterial pressure) may be less in dogs than in our pigs, such that the sympathetic and HR changes predominate. Possible species differences in this regard are also illustrated in differences between CO responses to apnea. In humans, as in the present study (39), but unlike the dog study of Schneider et al., CO decreased during apneas. 2) Unlike sedated pigs, dogs exhibit a large degree of sinus arrhythmia during apneas and increases in HR during the apnea/interapnea phase (32). This could have led to an increase in overall CO. Indeed, Schneider et al. found that their changes in CO were largely HR mediated. These changes in HR may have overridden the detrimental effects of increased LV afterload on SV and CO in the study of Schneider et al.

Conclusions

This study demonstrates that sympathoadrenal activation is the major pressor mechanism during apneas. The sympathetic nervous system does not respond in a uniform manner during apneas, cervical sympathetic nerve activity being a poor correlate of overall sympathoadrenal activation. Furthermore, hypoxia is not the sole trigger mechanism activating the sympathoadrenal system during apneas. There is an important vagally mediated reflex that contributes to the pressor response to apneas. This may be a chemoreflex carried by the aortic nerve and/or a mechanoreflex. Changes in peripheral circulatory mechanics during apneas are the result of a complex series of interactions between mechanoreceptive and chemoreceptive reflex arcs and the local effects of hypoxia and possible hypercapnia. The resultant changes in peripheral circulatory mechanics determine the effect of apneas on LV afterload, which is the prime determinant of changes in CO during apneas.

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