Effects of vagotomy on cardiovascular response to periodic apneas in sedated pigs

D. SLAMOWITZ, L. CHEN, AND S. M. SCHARF
Pulmonary and Critical Care Division, Long Island Jewish Medical Center, Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, New York 11042

Slamowitz, D., L. Chen, and S. M. Scharf. Effects of vagotomy on cardiovascular response to periodic apneas in sedated pigs. J. Appl. Physiol. 86(6): 1890–1896, 1999.—There are few studies investigating the influence of vagally mediated reflexes on the cardiovascular response to apneas. In 12 sedated preinstrumented pigs, we studied the effects of vagotomy during apneas, controlling for apnea periodicity and thoracic mechanical effects. Nonobstructive apneas were produced by paralyzing and mechanically ventilating the animals, then turning the ventilator off and on every 30 s. Before vagotomy, relative to baseline, apnea caused increased mean arterial pressure (MAP; +19 ± 25%, P < 0.05), systemic vascular resistance (SVR; +33 ± 16%, P < 0.0005), and heart rate (HR; +5 ± 6%, P < 0.05) and decreased cardiac output (CO) and stroke volume (SV; −16 ± 10% P < 0.001). After vagotomy, no significant change occurred in MAP, SVR, and SV during apneas, but CO and HR increased relative to baseline. HR was always greater (~14%, P < 0.01) during the interapneic interval compared with during apnea. We conclude that vagally mediated reflexes are important mediators of the apneic pressor response. HR increases after apnea termination are related, at least in part, to nonvagally mediated reflexes.

apnea; vagus; hemodynamics

Obstructive sleep apnea is a relatively common clinical condition in which upper airway collapse and cessation of airflow produce an acute cardiovascular response, including elevations in blood pressure as well as changes in heart rate and ventricular function (3, 24, 26). In general, cardiovascular variables fluctuate during the apnea-interapnea periods because of mechanical alterations and reflex changes in autonomic tone. Reflex effects leading to changes in autonomic tone may be activated through 1) changes in thoracic mechanics from obstructed respiratory efforts (4), 2) changes in blood-gas tensions (hypoxia and/or hypercapnia) (12, 18), and 3) postapneic arousals. These factors ultimately culminate in an increase in sympathoadrenal tone (9, 13), which is associated with a pressor response during the apnea and postapnea periods. Studies to date have focused mainly on the relative effect of these physiological alterations with regard to the overall cardiovascular response to apneas. However, few studies have examined specific autonomic pathways mediating this response. In particular, little is known about the relative contribution of vagal traffic in the modulation of the sympathetic response. The vagus nerve could play a prominent role in the cardiovascular response to apneas because of afferent and/or efferent autonomic signals carried within the nerve. Sensory afferent signals from aortic receptors (baroreceptors and/or chemoreceptors) or mechanoreceptors carried via the vagus could be responsible for the augmentation in sympathetic tone during apneas (1, 25). In addition, mechanoreceptor afferents carried via the vagus could increase sympathetically mediated responses through an arousal mechanism (11, 14). On the other hand, parasympathetic efferent signals carried via the vagus nerve could modulate sympathetic activation by decreasing heart rate and lowering blood pressure (3). Thus the vagus could play an important role in mediating the cardiovascular apneic response through both efferent and afferent mechanisms.

In this study, we used a previously developed porcine model of periodic apnea (4) to test the effects of vagal traffic disruption on the acute cardiovascular response to apnea. Previous studies using this model, in which apneas are produced in preinstrumented, sedated, and paralyzed pigs, demonstrated an increase in sympathoadrenal tone as well as a pressor response during the apnea-interapnea cycle (4, 7) compared with preapnea baseline. The model was chosen for this study because of its ability to control for confounding variables such as apnea periodicity, blood-gas and thoracic mechanical alterations during apnea, and acute arousal effects postapnea. We examined the role of vagal traffic in modulating the pressor response associated with apneas by performing bilateral cervical vagotomy. We hypothesized that, because known vagal afferents associated with chemoreceptor (aortic) stimulation are sympathoexcitatory, vagal section would blunt the pressor response to apneas. On the other hand, sympathoinhibitory fibers are also carried by the vagus. If the importance of these were greater than that of sympathoexcitatory fibers, then vagotomy could even have the opposite effect.

METHODS

This study consisted of two phases: a sterile instrumentation phase and a data-collection phase. The local Institutional Animal Care and Use Committee, in accordance with National Institutes of Health guidelines, approved all methods, including protocols, anesthesia, and data collection. Instrumentation phase. Twelve conditioned female Yorkshire farm pigs, weighing 16–22 kg, were fasted for 12 h before surgery. Surgical plane general anesthesia was established using ketamine (20 mg/kg) and xylazine (2 mg/kg im). The animals were intubated and mechanically ventilated by using a tidal volume of 12 ml/kg and a respiratory rate of 8750-7587/99 $5.00 Copyright © 1999 the American Physiological Society http://www.jap.org

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15 breaths/min. Anesthesia was maintained by administration of halothane (0.5–0.75%) in an enriched oxygen mixture (35–45%). The expiratory port from the anesthesia machine was vented, and all gases were collected by a scavenging system. Heart rate (HR), electrocardiogram (lead II), and oxygen saturation (ear oximeter) were continuously monitored. The animals were placed in the right lateral decubitus position, and 40 mg succinylcholine were administered intramuscularly. By using sterile surgical techniques, an incision was made in the sixth left intercostal space. The fifth rib was cut at or near the sternum to expose the heart and great vessels, and the pericardium was widely excised. After the ascending aorta was carefully separated from the pulmonary artery, a sterile square-wave electromagnetic flow probe (Biotronix) was placed around the ascending aorta (size 14–18 mm, on the basis of aortic diameter). The pericardium was then loosely approximated, and the wire from the flow probe was placed in a sterile plastic bag. After the bag was closed with a rubber band, it was brought through the chest wall and placed in a subcutaneous pocket. A 7-F chest tube was then placed percutaneously, and the chest incision was closed in three layers. The chest was then evacuated by using negative pressure, and the chest tube was removed. The wound was then covered with cellophane sterile gauze, and the anesthesia was discontinued. The animals were allowed to awaken and were extubated once they exhibited strong negative pressure, and the chest tube was removed. The chest was then placed percutaneously, and the chest incision was closed in three layers. The chest was then evacuated by using negative pressure, and the chest tube was removed. The wound was then covered with cellophane sterile gauze, and the anesthesia was discontinued. The animals were allowed to awaken and were extubated once they exhibited strong spontaneous ventilation and chewing movements. The animals were then placed in an individual pen and were allowed to recover. Treatment with penicillin G and benzathine (66,000 U/kg im) during surgery and the following day was administered to prevent wound infection. Postsurgery, morphine sulfate (5 mg im) was given every 6 h during the first 24 h for pain control.

Data-collection phase. Five days postsurgery, the animals were anesthetized with ketamine and xylazine as above. This induced 45–60 min of surgical plane anesthesia, during which time study instrumentation was accomplished and all surgical manipulation was done. In addition, before any incision was made, 2% lidocaine was infiltrated locally. All surgical manipulation was done. In addition, before any surgical plane anesthesia, a continuous drip (0.9% alphaxalone-alphadolone iv; 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 have demonstrated that alphaxalone-alphadolone is associated with preservation of sympathetic reflexes and minimal ventilatory suppression compared with other anesthetic-analgesic agents (2, 10). Once sedation was established, the animals were paralyzed by using d-suxamethonium (0.6 mg/kg bolus) followed by a 16 µg·kg⁻¹·min⁻¹ infusion. We were assured of adequate sedation to eliminate pain or suffering under paralysis in a number of ways. 1) In studies in which animals are not paralyzed (4), but apneas are produced with the same 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 in animals both paralyzed and unparalyzed (4), there was no difference in baseline blood pressure or HR, again suggesting that there was no pain or suffering associated with the paralysis and sedation. 3) In the present study, there was continuous monitoring of blood pressure and HR before and after animals were paralyzed, in the presence of continuous sedation. No surges of blood pressure or HR suggestive of pain or suffering were ever observed. 4) Surgical manipulation was only done during surgical plane anesthesia. No surgical manipulation was performed when animals were paralyzed and sedated without surgical plane anesthesia. 5) In previous studies using a protocol similar to that in this study (6), continuous monitoring of electroencephalogram before, during, and after apneas revealed no changes in amplitude or frequency that would indicate arousal or suffering. 6) Heavy sedation was maintained by continuous intravenous drip during all phases of the experiment when paralysis was induced. Animals were euthanized at the end of the experiments by injection 0.3 ml/kg of Euthanasia-5 solution (Veterinary Laboratories, Lenexa, KY), containing pentobarbital sodium (5 g/ml) in 40% isopropyl alcohol and 2% propylene glycol.

Experimental protocol. While the animals were under continuous mechanical ventilation and sedation as described above, periodic apneas were produced by turning off the ventilator for 30 s at end expiration (apnea phase) and then on for 30 s (interapnea phase). Thus a complete apnea-interapnea cycle was defined by 1 min. After an initial baseline period of 20 min, five apnea-interapnea cycles were performed. The animals were then allowed to recover for 15 min. For each condition, measurements were taken at baseline (mechanical ventilation, no apneas), after 5–7 apnea-interapnea cycles had passed from a single apnea-interapnea cycle, and then during recovery (mechanical ventilation, same as baseline conditions). Bilateral cervical vagotomies were then performed on the previously tagged vagus nerves. The above protocol was then repeated. Thus each animal served as its own control.

Data acquisition and calculations. Cardiovascular hemodynamic data recordings were taken at baseline, during the fifth apnea-interapnea cycle, and at recovery both pre- and postvagotomony. Blood-gas data were taken from a sample collected after 5 s of the fifth apnea-interapnea cycle 5 s before apnea termination until 5 s into the interapnea interval. Comparison of “baseline” and “recovery” data allowed for assessment of time-related changes. Data variables collected, including HR, MAP, instantaneous CO, and SV, were sampled at a frequency of 200 Hz in 60-s epochs and streamed through a microcomputer hard disk by using commercially available software (ACQ4600, Gould, Cleveland, OH).

Data were analyzed off-line by using commercially available software (View II, Gould). The apnea portion of the apnea-interapnea cycle was defined as the last 5 s of the apnea phase, and the interapnea portion of the apnea-interapnea cycle was defined as the last 5 s of the interapnea interval. HR and MAP were measured from the blood pressure tracing, and CO was measured from aortic flow. Sys-
temic vascular resistance (SVR) was calculated as 
\[ SVR = \frac{MAP \times 79.9}{P_{CO_2}} \] (dyn·s·cm⁻²).

Statistical analysis. Data were compiled and expressed as means ± SE and percent change ± SE from baseline. Statistical significance between the apnea-interapnea points and recovery period compared with baseline for the control and vagotomy condition was assessed by using a one-way analysis of variance for repeated measures. If significance was found, a post hoc analysis (Newman-Keuls procedure) was used to determine the source of significance. Two-way analysis of variance with planned comparisons was used to test for differences between control and vagotomy for baseline, apnea, and interapnea.

RESULTS

Figure 1 demonstrates the changes in blood-gas tensions for the control and postvagotomy condition. There were no significant differences in \( P_{O_2} \), \( P_{CO_2} \), or pH between baseline and recovery for each condition. As expected, during apneas \( P_{O_2} \) fell significantly, \( P_{CO_2} \) rose significantly, and pH fell significantly compared with baseline in both conditions (\( P < 0.05 \)). Although there was no significant difference in \( P_{O_2} \) during apnea between conditions, \( P_{CO_2} \) during apnea was slightly but significantly greater in the control condition compared with the vagotomy condition. Accordingly, pH was slightly but significantly less during apnea for control than after vagotomy.

Acute hemodynamic effects are shown in Figs. 2–6. For each condition (control, vagotomy) there was no significant difference between baseline and recovery. Thus the stability of the preparation over time was validated. In the control condition, MAP (Fig. 2) increased significantly relative to baseline during apnea and interapnea. SVR (Fig. 3) behaved similarly to MAP, with a significant (\( P < 0.05 \)) increase relative to baseline in the control condition during apnea but not postvagotomy. The overall difference between control and vagotomy was significant (2-way ANOVA), the point of significance being during apnea. Figure 4 shows that CO decreased significantly relative to baseline in the control condition during apnea (\( P < 0.05 \)). However, postvagotomy CO actually increased significantly relative to baseline during apnea and interapnea postvagotomy. The overall difference (2-way ANOVA) between control and vagotomy was also significant (\( P < 0.01 \)). A significant decrease in SV (\( P < 0.05 \)) relative to baseline was observed during apnea and interapnea in the control but not the vagotomy condition (Fig. 5). During apnea, SV was significantly lower (\( P < 0.01 \)) in control vs. vagotomy. Finally, HR (Fig. 6) increased significantly (\( P < 0.05 \)) relative to baseline during...
interapnea in the control condition and during both apnea and interapnea postvagotomy. In the control condition the increase in HR was significant between interapnea and apnea (P < 0.05), but after vagotomy the difference in HR between apnea and interapnea was not significant. The difference in HR at apnea was significant between control and vagotomy conditions. Finally, the difference in baseline HR between control and apnea was significant.

DISCUSSION

In this porcine model of periodic apnea, vagotomy substantially altered the hemodynamic response to apneas during both the apnea and interapnea period. Postvagotomy the pressor response during the apnea-interapnea period was blunted, and there were corresponding modulation in the responses of SVR, SV, and CO. In contrast, the HR response during the apnea and interapnea periods was augmented postvagotomy. The ensuing discussion will consider the study results with respect to the limits of the experimental preparation and presently available literature.

Experimental preparation. A distinguishing feature of obstructive apnea is the persistence of respiratory motoneuron output during the apnea 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 is presumably intact (20), neurologically simulates events occurring during obstructive rather than central apneas and is not to be equated with central apneas. This paralyzed model of periodic apnea was chosen because the pressor response in this model is greater than for a similar nonparalyzed model (4). We hoped, therefore, that perturbation of the system by vagotomy in this model would yield large effects in the variables studied. Because the animals were paralyzed, effects of swings in intrathoracic pressure during apnea on venous return and cardiac function were not present.
potentially confounding factor was controlled for. However, it must be remembered that, in terms of mechano- and proprioceptor sensory neuron feedback, the model resembles central rather than obstructive apneas. The model also controlled for the confounding factor of arousal during apnea. In previous studies, no changes in cortical state suggestive of arousal as measured by electroencephalography were found in this model (6). Finally, it might be thought that baseline hemodynamics with mechanical ventilation would be different from those with spontaneous ventilation and that conclusions about the hemodynamic response to apneas drawn from paralyzed apneas may not apply to baseline conditions for obstructive breathing (spontaneous ventilation). However, Chen and Scharf (4) reported that baseline hemodynamic variables were the same in this model between spontaneous and mechanical ventilation.

In this model of nonobstructive apneas, there are few hemodynamic differences between early interapnea and end apnea (4, 6, 7), most likely because there is no evidence for arousal at apnea termination (6). In addition, there are few hemodynamic differences between late interapnea and early apnea. Thus we chose to analyze the changes at end apnea and end interapnea in addition to baseline and recovery because this gives a good representation of changes over the apnea cycle.

Although we tried to control for blood-gas tensions by setting the apnea-interapnea period at 1 min for both control and vagotomy, P\textsubscript{C\textsubscript{0}}\textsubscript{2} was slightly but significantly greater during apnea in the control condition compared with postvagotomy. We have no explanation for this finding but assume that it is related to changes in dead space after vagotomy, which can influence airway tone. However, we doubt that this difference affected the study findings. In previous studies (6) using similarly sedated, paralyzed, and mechanically ventilated animals, hypercapnia caused sympathoexcitation (increased MAP and CO) associated with vasodilation (decreased SVR possibly associated with hypercapnia). When these variables in our study during apnea in control (higher P\textsubscript{C\textsubscript{0}}\textsubscript{2}) are compared vs. vagotomy, only MAP behaved in a similar fashion, whereas CO decreased and SVR increased. Furthermore, in the previous study, an elevation in P\textsubscript{C\textsubscript{0}}\textsubscript{2} of 20 Torr produced an increase in MAP of ~10 mmHg (6). In the present study, MAP increased by 20 mmHg in the face of a P\textsubscript{C\textsubscript{0}}\textsubscript{2} increase of only 8 Torr. Thus we believe that the difference in MAP during apneas was too great to be accounted for on the basis of increased P\textsubscript{C\textsubscript{0}}\textsubscript{2}. However, we cannot entirely rule out a role for central chemoreceptor stimulation accounting for part of the difference between control and vagotomy.

**Hemodynamic responses during apneas: vagotomy.**

The hemodynamic response during the apnea-interapnea period in the control condition included an increase in MAP and SVR, a decrease in CO and SV, and an increase in HR relative to the baseline. The increase in MAP and SVR during apnea and interapnea compared with baseline in the control (prevagotomy) condition is similar to the hemodynamic response seen in humans (8, 21) and observed in previous animal studies (4, 6). We have previously explained the decrease in CO and SV observed during apneas on the basis of increased LV afterload (increased MAP). The present data are consistent with this explanation because the decrease in CO and SV during apneas was in fact seen only when MAP increased.

Although this laboratory previously reported a decrease in HR relative to baseline associated with apneas (4, 6), other studies from this laboratory (5, 7) have also seen HR to increase during apneas compared with baseline. However, in all studies, no matter what HR does during apnea compared with baseline, during apneas HR has always been less than during interapnea (4–7). In this regard, the model mimics clinical findings (16).

The reasons for different HR responses reported during apneas and interapneas compared with control in different studies are not clear. However, many factors could influence the HR response. For example, in those studies reporting a decrease in HR relative to baseline during apneas, animals were paralyzed with succinylcholine, whereas in studies reporting increased HR relative to baseline during apneas, paralysis was produced by using cis-atracurium. The well-known mild vagal mimetic effects of succinylcholine might have led to a different HR response during apneas relative to baseline. However, the fact that HR consistently increases during interapnea compared with apnea (regardless of the relationship of HR to preapnea baseline) suggests that factors associated with apnea termination are responsible for this rise in HR. One such factor could be stimulation of pulmonary stretch receptors postapnea. It has been previously reported (4, 15, 22) that HR increases immediately at apnea termination (interapnea period) with resumption of breathing movements. This increase is too rapid to be due to changes in blood-gas tensions, and therefore stimulation of pulmonary stretch receptors has been postulated to be the causal factor. On the other hand, with the resumption of breathing, mechanoreceptors in the chest wall could also be activated. Our data show that HR increased at interapnea compared with apnea, postvagotomy as well as prevagotomy. Presumably, vagal section eliminated pulmonary stretch receptor input. Thus these findings suggest that nonvagal receptors, possibly chest wall mechanoreceptors, play a role in the HR increase seen postapnea.

**Hemodynamic responses: effects of vagotomy.**

Our data clearly demonstrated blunting of the pressor response (MAP) postvagotomy. Instead of decreasing during apnea as it did prevagotomy, CO actually increased during apnea postvagotomy. This increase in CO may have been related, in part, to the effects of apneas on HR.

Postvagotomy, baseline HR was elevated as expected because vagal tone was lost. During apneas postvagotomy, there was a significant increase in HR during apnea relative to baseline. We believe it likely that the augmentation of HR during apneas relative to baseline postvagotomy is due to an increase in sympathetic...
sympathetic activity could continue while activity to sympathetic activity. Thus it is possible that cardiac continued vasoconstriction clearly attested to increased sympathetic nerve during apneas, despite the fact that demonstrated diminished activity in the cervical sympathetic during apneas, observed prevagotomy, could be due to decreased sympathetic tone (related, at least in part, to reflex effects of hypoxia), which could lead to increases in venous return (23) and increase HR and possibly myocardial contractility. In this study, an increase in MAP was prevented (postvagotomy), allowing mechanisms leading to CO augmentation to predominate. This resulted in an increase in CO postvagotomy. Because changes in myocardial contractility have not been observed during apneas in this model (4, 5), we believe that increased HR and probably venous return (23) are the factors responsible for the increase in CO during apneas postvagotomy when increased LV afterload is not observed.

Mechanism for effects of vagal section on pressor response. Our data demonstrate that the pressor response to apneas in this model is dependent to a large extent on the vagus nerve. Sympathetic activation during periodic apneas is thought to occur from reflex effects elicited from hypoxia, possibly hypercapnia and mechanical alterations (6, 9, 12, 13, 18). These physiological alterations cause activation of peripheral and central sensors, including baroreceptors, chemoreceptors, and mechanoreceptors. Afferent information from these receptors is carried to the central nervous system and ultimately leads to the cardiovascular response. Thus the blunting of the pressor response seen in this study is likely due to loss of the vagal afferent input responsible for eliciting an increase in sympathetic efferent activity. The absence of increased SVR during apneas postvagotomy suggests that vagal nerve traffic prevented sympathetically mediated vasoconstriction. This finding suggests that, at least in pigs, carotid chemosensitive or baroreceptors may not be as important as aortic receptors in the peripheral sympathetic activation associated with apnea.

The absence of SVR response to apneas postvagotomy might appear to be at odds with the continued HR response to apneas. If, as suggested previously, there is less sympathetic efferent activity postvagotomy, how can we explain the increased HR during apneas on the basis of continued sympathetic activity? First, it is possible that the sympathetic response is not an all-or-nothing response. Chen et al. (7) previously demonstrated diminished activity in the cervical sympathetic nerve during apneas, despite the fact that continued vasoconstriction clearly attested to increased sympathetic activity. Thus it is possible that cardiac sympathetic activity could continue while activity to peripheral vasculature is diminished during apneas postvagotomy. On the other hand, it is possible the increased HR during apneas postvagotomy could be the result of epinephrine release during apneas. Consistent with this, Chen et al. demonstrated that epinephrine release occurred during apneas in response to hypoxia even after chemical sympathectomy.

Which vagal afferents could be responsible for eliciting the sympathetic efferent activity 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 both baroreceptors and chemoreceptors, which send inputs through the vagus via the aortic nerve (1, 17). Although, in humans, most carotid baroreceptor and chemoreceptor afferent information travels via the sinus nerve and the glossopharyngeal nerve, the sinus nerve does not send several branch fibers via the vagus (25). However, these fibers should be intact in our study postvagotomy because vagal section was performed below the carotid bifurcation. Because the baroreceptors are sympathoinhibitory unless unloaded by a fall in pressure, these are likely of little importance. Afferent traffic from pulmonary mechanical receptors and central volume receptors, some of which is sympathostimulatory, is also carried by the vagus (1, 19). 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 (14). Our results suggest that afferent information from the above-mentioned receptors is important mediators in the cardiovascular response during the apnea-interapnea period and should be explored further.

In summary, vagotomy substantially blunted the pressor response observed with periodic paralyzed apneas. The effects seen postvagotomy may have been due to disruption of vagally mediated inputs from aortic chemosensitive or baroreceptors, central volume receptors, or thoracic mechanical receptors. Further investigation into vagal mechanisms leading to the pressor response during apneas seems warranted.

Address for reprint requests and other correspondence: S. M. Scharf, Pulmonary and Critical Care Division, Long Island Jewish Medical Center, New Hyde Park, NY 11040.

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