Breathing and upper airway CO2 in reptiles: role of the nasal and vomeronasal systems

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Breathing and upper airway CO_2 in reptiles: role of the nasal and vomeronasal systems

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COATES, E. LEE, AND GARY O. BALLAM. *Breathing and upper airway CO_2 in reptiles: role of the nasal and vomeronasal systems.* Am. J. Physiol. 256 (Regulatory Integrative Comp. Physiol. 25): R91–R97, 1989.—The ventilatory response of the garter snake, *Thamnophis sirtalis*, to 2% CO_2 delivered to the upper airways (UA) was measured before and after the olfactory or vomeronasal nerves were transected. The UA (nasal cavities and mouth) were isolated from the gas source inspired into the lungs by inserting an endotracheal T tube into the glottis. CO_2 was administered to the UA via a head chamber. The primary ventilatory response to UA CO_2 was a significant decrease in ventilatory frequency (f) and minute ventilation. The decrease in f was caused by a significant increase in the pause duration. Tidal volume, expiratory duration, and inspiratory duration were not altered with UA CO_2. The f response to UA CO_2 was abolished with olfactory nerve transection, whereas vomeronasal nerve transection significantly increased the magnitude of the f depression. These results indicate that CO_2-sensitive receptors are located in the nasal epithelium and that the olfactory nerves must be intact for the UA CO_2 f response to be observed. In addition, the vomeronasal system appears to modulate the ventilatory response to UA CO_2.

control of breathing; olfaction; snakes; upper airway carbon dioxide receptors; ventilatory inhibition

A VENTILATORY RESPONSE to upper airway (UA) CO_2 has been reported for amphibians [*Rana catesbeiana* (27)] and several species of reptiles [*Uromastix aegyptius* (29), *Epicrates striatus* (8); and *Tupinambis nigropunctatus* (2, 3, 7)]. The response is characterized by a decrease in ventilatory frequency (f) and minute ventilation ($\dot{V}E$). Generally, tidal volume (VT) does not significantly change during elevated UA CO_2. In the tegu lizard (3), *T. nigropunctatus*, the decrease in f with elevated UA CO_2 was caused by a significant increase in pause duration (TP) and minor increases in expiratory (TE) and inspiratory duration (TI). The study of the tegu lizard (3) is the only study to date to examine the effect of UA CO_2 on these ventilatory parameters.

Two previous studies were designed to establish the specific location and innervation of the UA CO_2-sensitive receptors: the first in the bullfrog, *R. catesbeiana* (27), the second in the tegu lizard, *T. nigropunctatus* (7). Sectioning both the olfactory and trigeminal nerves of the bullfrog abolished the depression in $\dot{V}E$ normally observed with elevated UA CO_2. However, the ventilatory inhibition remained when the rami frontalis of the trigeminal nerves was sectioned. These results suggest the existence of a CO_2-sensitive afferent pathway from the nasal mucosa via the olfactory nerves. Unfortunately, the CO_2 concentrations (5.7 and 14%) used in the bullfrog study (27) may have been high enough to function as a nonspecific noxious stimulus. In the second study, which used the tegu lizard (7), transection of the olfactory peduncle abolished the ventilatory response (f decreased) to 2% UA CO_2. Loss of the UA CO_2 ventilatory response with olfactory peduncle transection indicated that the CO_2-sensitive receptors were located in the nasal sensory epithelium or the vomeronasal organ and not in the nasal respiratory epithelium, which is sparsely innervated by the trigeminal nerves. Because olfactory peduncle transection interrupted afferent traffic originating in both the olfactory and vomeronasal epithelia, it could not be ruled out that the UA CO_2 ventilatory response originated in the vomeronasal epithelium.

The objectives of the present study were to describe the ventilatory response of the garter snake to UA CO_2 and to determine the role of the olfactory and vomeronasal systems in this response. In the first part of the study the ventilatory response to UA CO_2 in the garter snake was described in terms of the ventilatory parameters [TE, TI, TP, total breath duration (TT), f, VT, and $\dot{V}E$] measured in the tegu lizard (3). Based on results from the studies mentioned previously, the first hypothesis formulated was UA CO_2 causes a decrease in f and $\dot{V}E$, an increase in TP and TT, and no change in TE, TI, or VT. A description of the UA CO_2 ventilatory response of the garter snake was also a necessary prerequisite of the second part (nerve transection experiments) of this study.

In the second part of the present study the role of the olfactory and vomeronasal systems in the ventilatory response to UA CO_2 in the garter snake was determined by independently transecting the olfactory and the vomeronasal nerves and comparing the pre- and postsurgery ventilatory responses. Based on the results from the studies using the bullfrog (27) and the tegu lizard (7) two additional hypotheses were formulated: *1*) the ventilatory response to UA CO_2 in the garter snake originates from receptors located in the nasal epithelium innervated by the olfactory nerves, and *2*) intact vomeronasal nerves are not required to observe a ventilatory response to UA CO_2.

METHODS

Animals. Forty Canadian garter snakes, *Thamnophis sirtalis*, male and female, weighing from 22.3 to 232.5 g (mean: 75.3 g) were purchased from an animal supplier. The snakes were housed individually in clear plastic cages containing paper towel flooring and a water dish. The cages were kept in a room with a temperature of 26°C and a 12-h light-dark cycle. The snakes were fed goldfish weekly.

Gas delivery system. During the experiments, the snakes were loosely taped to a restraining platform without restricting breathing movements. The UA were isolated from the lungs and trachea by inserting an endotracheal T tube into the glottis (Fig. 1). To reduce irritation caused by the endotracheal T tube, lidocaine ointment (Fougera) was applied to the outside of the endotracheal T tube before insertion. Humidified, warmed, fresh air was delivered at 500 ml/min into one arm of the T tube. The air was warmed by blowing it through heated copper tubing. To heat the copper tubing, resistor wire (4 Ω/ft) was coiled around the tubing and connected to a power supply (Lambda, model LPD-422A-FM). The temperature of the air exiting the copper tubing, measured downstream from the T tube, was 26°C. To humidify this air a syringe pump slowly delivered water droplets to a gauze plug located in the airstream preceding the copper tube. Excess water from the copper tubing was collected in a downstream water trap. From this gas source entering the T tube the snake could inspire warmed, humidified, fresh air into the lungs. The other arm of the T tube was connected to a short piece of tubing that conducted expired gas and excess fresh air away from the snake. A pneumotachometer, Validyne pressure transducer (model MP45-1), and a Validyne demodulator system (model MC1-3) were connected to the tubing leaving the endotracheal tube. The output of the demodulator was zeroed with the resistance adjustment at the bias flow of 500 ml/min. Inspiratory and expiratory flows were derived from the pneumotachograph. Inspiratory and expiratory flows were calibrated by delivering or removing a known volume of air at a constant rate from the airstream by means of an in-line calibration syringe. With the endotracheal T tube in place, CO_2 could be elevated in the UA independent of the fresh air inspired into the lungs. An air-CO_2 mixture was delivered to the UA at 1 l/min by means of a head chamber. The head chamber was made by cutting a small plastic bottle (200 ml) in half longitudinally. During the experiment, the chamber was placed over the snake's head and taped to the restraining platform. The gas concentrations entering the head chamber were regulated by a control valve and flowmeter in series with the solenoid valve. Downstream from the addition of CO_2 to fresh air a second control valve and flowmeter maintained the flow of gas entering the head chamber constant (MKS Instruments).

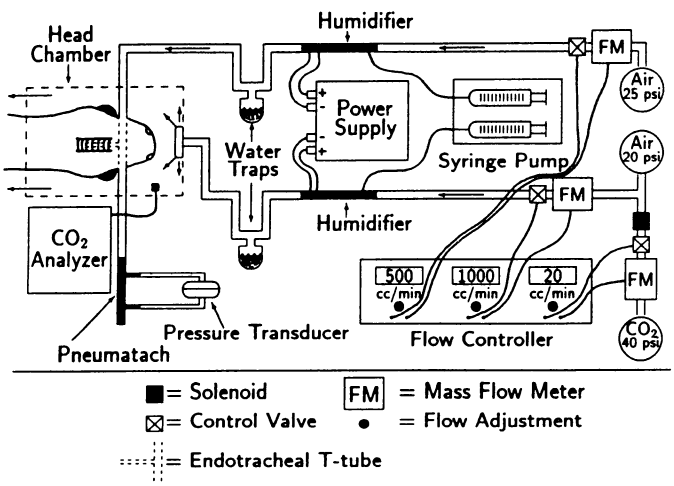

FIG. 1. Schematic diagram of gas delivery to lungs and head chamber.

To measure the CO_2 concentration entering the head chamber a small-bore polyethylene tube was inserted into the head chamber and connected to a respiratory mass spectrometer (Medspect II, Chemetron). The mass spectrometer (sampling rate = 15 ml/min) was calibrated to room air and a gas of known CO_2 concentration (5.0%).

Protocol of presurgery ventilatory response to UA CO_2. Initially, fresh air was delivered to the head chamber for 1 h, allowing the snake to become accustomed to the experimental apparatus. Most snakes developed a regular breathing pattern after 10–15 min of restraint. Those snakes that did not develop a regular breathing pattern after 1 h were not used. After 1 h, the solenoid valve was opened and the control valve adjusted to deliver a 2.0% CO_2-air mixture for 4 min followed by 8 min of fresh air. This regime was continued until three CO_2 delivery sequences were administered. Inspiratory and expiratory airflows and UA CO_2 concentrations were recorded on magnetic tape (3968A Instrumentation tape recorder, Hewlett-Packard) and on a four-channel pen-recorder (Recorder 2400S, Gould) before, during, and after the addition of CO_2 to the head chamber. For analysis of breath-to-breath changes, the data stored on tape were converted from an analog-to-digital signal using a Labmaster A-D converter and transferred to a microVAX computer. The data were computer analyzed using a digital wave analysis program developed at the Lovelace Medical Foundation, Albuquerque, NM. This program simultaneously displayed the head chamber CO_2 and airflow waveforms. To derive TE, TI, TP, TT, and VT, markers were manually placed on the ventilatory airflow signal. The equations used to determine these parameters are shown in Fig. 2.

Changes in breathing patterns during UA CO_2 were determined by analyzing the breaths 1 min before the addition of CO_2 to the head chamber (zone 1), 1 min after CO_2 onset (zone 2), 1 min before the termination of CO_2 in the head chamber (zone 3), and 1 min after the termination of CO_2 in the head chamber (zone 4) (Fig. 3). Breaths occurring in zone 1 were used as the control breaths for analysis. Breathing pattern changes occurring in zone 2 were defined as the transient UA CO_2 response, whereas changes occurring in zone 3 were defined as a steady-state UA CO_2 response.

Ten of the 40 snakes were not used in the study. Five of these snakes were too small for the endotracheal T tube to be functional, i.e., the inside diameter (0.86 mm)

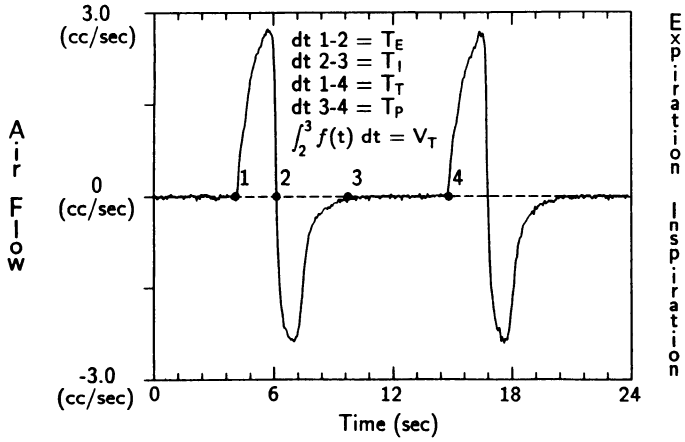

FIG. 2. Digitized tracing of airflow during 2 breathing cycles. Each breath is initiated with an expiration (upward deflection) followed by an inspiration (downward deflection) and a pause. TE, expiration duration; TI, inspiration duration; TP, pulse duration; TT, total breath duration; and VT, tidal volume calculated from inspiration. Bias airflow = 500 ml/min.

of the largest tracheal tube that could be inserted into the glottis of these snakes was too small and frequently became obstructed with mucus. The best results were obtained when endotracheal tubes with inside diameters of 1.19 or 1.57 mm were used. Four of the 10 snakes never became accustomed to the restraint or the endotracheal T tube and one snake died of unknown causes before surgery.

Surgery. The remaining 30 snakes were randomly divided into four surgical groups: sham olfactory nerve lesion, sham vomeronasal nerve lesion, olfactory nerve lesion, and vomeronasal nerve lesion. Each snake was weighed and anesthetized with 17 mg/kg body wt methohexital sodium (Brevital sodium, Eli Lilly) before surgery (30). There were no fatalities caused by the anesthesia or the various surgical procedures.

The surgical techniques used in the present study were developed and described in detail elsewhere (23). For olfactory nerve lesions, two holes were drilled in the skull above the anterior ends of the main olfactory bulbs (MOB). The anterior portion of the MOB was then lifted by the dura and severed with microdissecting scissors. Sham olfactory nerve lesions were made by drilling holes above the MOB and tearing the dura.

For vomeronasal nerve lesions, a single hole was drilled above the anterior ends of the accessory olfactory bulbs (AOB), located 1 mm posterior to the anterior border of the frontal scale. With the aid of a dissecting microscope, the vomeronasal nerves were sectioned with fine microdissecting scissors at the point where the nerves travel medial to the MOB. Sham vomeronasal nerve lesions were made by drilling a single hole in the skull above the AOB and tearing the dura.

After surgery, Gelfoam (Upjohn) was placed over the exposed nervous tissues, 5% lidocaine ointment was applied to the skin surrounding the wound, and the hole in the skull was sealed with bone wax. Seventy-two hours after surgery the snakes were tested again for a ventilatory response to UA CO_2 using the methods and protocol previously described.

Verification of lesions. After testing, the snakes were killed with 0.4 ml intraperitoneal T-61 euthanasia solution (Hoechst-Roussel) and decapitated. The heads were fixed in 10% buffered Formalin for 24 h and transferred to a formic acid decalcifying solution (25% formic acid with 7.5% sodium citrate). After 5 days in the decalcifying solution, the heads were neutralized for 24 h in 5% sodium sulfate. The heads were then trimmed by removing the lower jaw and the roof of the skull and stored in 10% buffered Formalin until they were infiltrated and embedded in paraffin. After embedding, the heads were cut in 5-μm horizontal sections. Every other section was deparaffinized in xylene, hydrated, stained with cresyl violet acetate (9), counterstained with light green, dehydrated, and mounted.

The lesions were verified by independent observations of two people. The type of surgery and the grouping of the snakes were unknown to either observer. In two cases, attempts to lesion the olfactory nerves resulted in partial nerve lesions. Results from these animals were not used. In two of the snakes, attempts to lesion the olfactory or vomeronasal nerves left the nerves entirely intact. These snakes were placed in the corresponding sham surgery group.

Statistical methods. For statistical analysis of the presurgery ventilatory response, a mean for each ventilatory parameter was calculated for each zone from the three CO_2 sequences. Means of the zones were compared using the repeated measures analysis of variance procedure. If a significant difference occurred between any of the means, a two-tailed Bonferroni t test was performed. In five snakes, the presurgery response to UA CO_2 was so strong that no breaths occurred during zones 2 or 3. In these cases it was not possible to determine TE, TI, TP, TT, VT, or $\dot{V}E$. Therefore the results from these five snakes were not included in the analysis of the presurgery UA CO_2 ventilatory response.

To determine whether a significant postsurgery ventilatory response occurred, the f during zone 1 was compared with the f during zone 2 in the four surgical groups before and after the various surgical procedures. Comparisons were made using a two-tailed Student's paired t test. This test was also used to make paired comparisons of f during zone 1 before and after surgery to determine whether f was altered after nerve transections. Differences at the 5% level or less of confidence were considered significant. Values in the text and in Table 1 are means ± SD.

RESULTS

Ventilatory pattern. Figure 3 illustrates the ventilatory pattern of the garter snake *T. sirtalis* breathing through an endotracheal T tube. These breaths are representative of the typical breathing pattern observed in all the snakes tested (n = 25). Each breathing cycle was initiated with an expiration followed by an inspiration and a pause. The means of the ventilatory parameters during the control breaths (zone 1) are listed in Table 1. $\dot{V}E$ during each zone was calculated for each snake by multiplying f and VT.

Presurgery ventilatory response to UA CO_2. The ventilatory response of the garter snake to UA CO_2 was

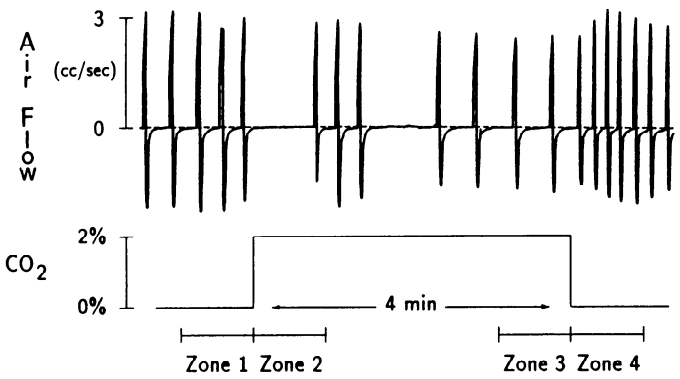

FIG. 3. Typical ventilatory response of garter snake to 2% upper airways (UA) CO_2. Each zone = 1 min. Breaths occurring during zone 1 are control breaths. Breaths occurring during zone 2 are transient response to UA CO_2, whereas breaths occurring during zone 3 represent steady-state response. Bias airflow = 500 ml/min.

characterized by a significant increase in TP, which caused a decrease in *f* and a subsequent decrease in $\dot{V}E$ (Fig. 3 and Table 1). These responses were usually initiated within one breath after the addition of CO_2 to the head chamber (zone 2; Fig. 3). Additionally, many of the snakes exhibited a hyperpnea that occurred within one breathing cycle after the termination of CO_2 to the head chamber (zone 4; Fig. 3).

Table 1 summarizes the changes in the respiratory variables with UA CO_2. TP increased 129.7% from zone 1 to zone 2 and 109.8% from zone 1 to zone 3. However, there was no significant difference in TP between zones 2 and 3 or between zone 1 and 4. TE and TI did not change significantly during UA CO_2. Because TE + TI + TP = TT, changes in TT with UA CO_2 paralleled those observed for TP. With the onset of UA CO_2, *f* decreased 51.4% from zone 1 to zone 2 and 45.9% from zone 1 to zone 3. There was no significant difference between the *f* during zone 2 and the *f* during zone 3. Likewise, the *f* during zone 1 was not significantly different from the *f* during zone 4. VT did not significantly change during UA CO_2. Therefore changes in $\dot{V}E$ during UA CO_2 paralleled those observed for *f*.

Postsurgery ventilatory response to UA CO_2. To determine whether the ventilatory response to UA CO_2 was present with the olfactory or vomeronasal nerves transected, *f* was measured before and after nerve transection. Ventilatory frequency was chosen in this case because a decrease in *f* was one of the primary ventilatory responses to UA CO_2 in the garter snake. Figure 4 is a representative example of the ventilatory response to 2% UA CO_2 before and after the vomeronasal and olfactory nerves were transected. In Fig. 4, the top two tracings and bottom two tracings were from two different snakes. The *f* response to UA CO_2 was not abolished when the vomeronasal nerves were transected, i.e., *f* was depressed to the same or greater extent as before transection. After the olfactory nerves were transected, the *f* response to UA CO_2 was abolished. The general biphasic pattern of breathing (expiration followed by inspiration) of the control breaths (zone 1) did not change after either olfactory or vomeronasal nerve lesions. There was no significant difference in the *f* of the breaths during zone 1 before (2.7 ± 0.5) and after (3.0 ± 1.3) olfactory nerve transection or before (4.1 ± 1.3) and after (3.3 ± 0.8) vomeronasal nerve transection. Likewise, there was not significance in any of the other ventilatory parameters (TE, TI, TP, TT, VT, and $\dot{V}E$) during zone 1 before and after surgery.

The combined *f* response to 2% UA CO_2 of all the snakes in each surgical group is illustrated in Fig. 5. The *f* response is depicted as the *f* during zone 2 divided by the *f* during zone 1 and expressed as a percentage. There was a significant decrease in the *f* response to UA CO_2 in all the groups before surgery. After sham olfactory and sham vomeronasal nerve lesions there was still a significant *f* response to UA CO_2, and this response was not significantly different from the *f* response of the presurgical groups. Figure 5 also illustrates that the *f* response to UA CO_2 was abolished when the olfactory nerves were transected, i.e., there was a significant difference between the *f* response before (77%) and after (19%) olfactory nerve transection. However, the 19% decrease in *f* from zone 1 to zone 2 observed after the olfactory nerves were transected was not significant. With vomeronasal nerve transection the *f* response to UA CO_2 was intensified. The *f* depression after the vomeronasal nerve was transected (89%) was significantly greater than the *f* depression before the vomeronasal nerves were transected (63%).

DISCUSSION

Ventilatory patterns. Reptiles are often described as periodic breathers (1, 12, 21, 26). Periodic breathing is characterized by brief episodes of ventilation followed by

TABLE 1. *Summary of changes in respiratory variables during 2% UA CO_2*

Variables	Zone 1	Zone 2	Zone 3	Zone 4
TE, s	2.2±0.6	2.0±0.7	2.2±0.6	2.1±0.6
TI, s	3.1±1.2	2.6±0.8	2.9±0.9	2.8±0.8
TP, s	17.8±8.4	40.9±16.4*†	37.4±14.7*†	14.6±8.5
TT, s	22.8±9.6	45.2±16.6*†	42.4±14.8*†	19.2±8.6
f, breaths/min	3.3±1.3	1.6±0.9*†	1.8±0.8*†	4.1±1.9
VT, ml BTPS·breaths^{-1}·g^{-1}	0.030±0.001	0.028±0.015	0.027±0.014	0.029±0.016
$\dot{V}E$, ml BTPS·min^{-1}·g^{-1}	0.09±0.04	0.05±0.03*†	0.05±0.03†‡	0.11±0.06

Values are means ± SD; n = 25. Zone 1, 1-min period before 2% CO_2 was delivered to head chamber; zone 2, 1-min period after CO_2 was added to head chamber; zone 3, 1-min period before CO_2 was terminated in head chamber; zone 4, 1-min period after CO_2 was terminated in head chamber; TE, expiratory duration; TI, inspiratory duration; TP, pause duration; TT, total duration; *f*, respiratory frequency; VT, tidal volume; $\dot{V}E$, minute ventilation. Significant differences between zones 1 and 2 or between zones 1 and 3; * $P < 0.001$; ‡ $P < 0.01$. Significant differences between zones 4 and 2 or between zones 4 and 3: † $P < 0.001$.

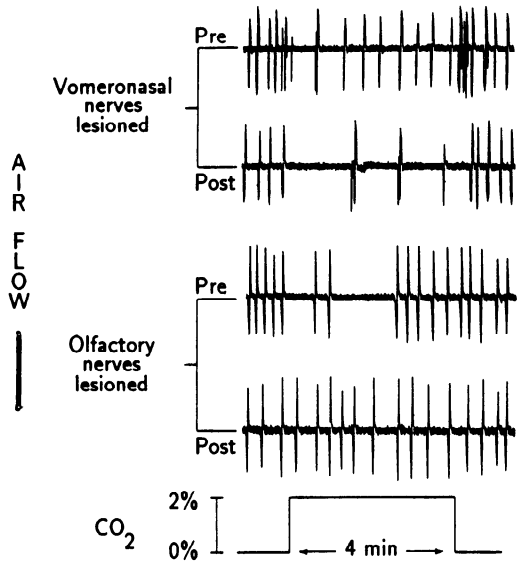

FIG. 4. Ventilatory response to 2% upper airways (UA) CO_2 before and after vomeronasal nerves were transected and before and after olfactory nerves were transected. Top 2 tracings are from a representative snake in vomeronasal nerve lesion group, whereas bottom 2 tracings are from a representative snake in olfactory nerve lesion group. Abscissa = time; ordinate = airflow. Bias airflow = 500 ml/min.

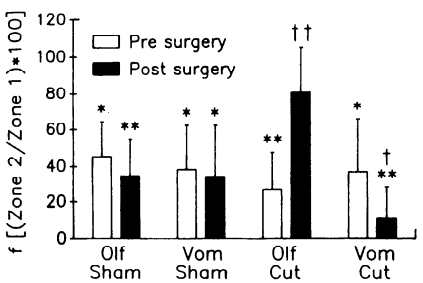

FIG. 5. Combined ventilatory response before and after surgery of all snakes in each surgical group. Olf sham, sham olfactory nerve lesion (n = 7); vom sham, sham vomeronasal nerve lesion (n = 6); olf cut, olfactory nerve lesion (n = 7); vom cut, vomeronasal nerve lesion; f, respiratory frequency. Ordinate = f in zone 2 compared with f in zone 1 (expressed as a percentage). Bars, SD. * $P < 0.01$; ** $P < 0.001$ (for comparisons of f during zone 1 to f during zone 2). † $P < 0.05$; †† $P < 0.01$ (for comparisons of pre- and post-f response).

long periods of apnea. However, many investigators have reported ventilatory patterns characterized by a regular frequency and short apneic periods between every breath (2–4, 7, 20, 28). Ventilation in garter snakes appears to follow this latter pattern. In the present study, and in a study by Bartlett and Birchard (4), *T. sirtalis* was found to breathe at a constant frequency during prolonged undisturbed air breathing. Similarly, Hicks and Riedesel (20) observed that the garter snake, *T. elegans*, exhibited regular patterns of ventilatory movements during daylight periods. In all of these examples the experimental temperature was at room temperature (20–23°C) or near the snakes preferred body temperature of 26.5°C (5).

Although the variability of the reptilian ventilatory pattern makes it difficult to make valid generalizations, the pattern of the individual breath appears to be consistent throughout the reptilian order (11). The typical breath is biphasic, initiated with an active expiration followed by an active inspiration followed by a pause that continues until the initiation of the next breath. All the snakes tested in the present study exhibited a biphasic pattern of ventilation (Figs. 2 and 3). On several occasions however, an expiration without an apparent active inspiration was observed. This pattern occurred exclusively during UA CO_2 (zones 2 and 3) and has not been reported in other studies using reptiles.

In addition to the breathing pattern, the values for the ventilatory parameters (f = 3.31 ± 1.29 breaths/min; V_T = 3.0 ± 1.4 ml/100 g; $\dot{V}_E$ = 9.4 ± 4.0 ml·min^{-1}·100 g^{-1}) found in this study were similar to those found by Bartlett and Birchard (4) in *T. sirtalis* [f = 2.95 (calculated from V_T and $\dot{V}_E$); V_T = 2.0 ± 1.2; $\dot{V}_E$ 5.9 ± 3.7]. The minor differences could be due to the sizes of the snakes used [23.4–232.5 g in this study; 23–58 g for *T. sirtalis* (4)] or methodology. The values for f, V_T, and $\dot{V}_E$ reported by Bartlett and Birchard (4) were obtained from garter snakes in which the UA were not bypassed with an endotracheal T tube. In the same study Bartlett and Birchard (4) reported that bypassing the UA with an open tracheal T tube led to an increase in f in three of five snakes. They did not report values for f, V_T, or $\dot{V}_E$ when the UA where bypassed.

Ventilatory response to UA CO_2. Although several studies have reported a ventilatory response to UA CO_2 in reptiles (2, 3, 7, 8, 29), evidence for an UA CO_2 ventilatory response in the garter snake, *T. sirtalis*, is reported for the first time in the present study. However, a preliminary report of this response in *T. sirtalis* has appeared elsewhere in abstract form (6). The ventilatory response to UA CO_2 of *T. sirtalis* was characterized by a significant decrease in f and $\dot{V}_E$ with no apparent change in V_T. This was similar to the response of the tegu lizard to UA CO_2 (3, 7). Also, like the tegu lizard (3), the steady-state response (zone 3) was as great as the transient response (zone 2). These results indicate that the receptors mediating the UA CO_2 response in both the garter snake and the tegu lizard do not adapt within the time frame used in these experiments (4 min). Results from a preliminary experiment conducted in this laboratory on one tegu lizard have shown that the steady-state decrease in f after 40 min of 3% UA CO_2 was as great as the transient response. The garter snake has not been tested in experiments of this duration. It is difficult to characterize the response of the UA CO_2 receptors in experiments of this duration because other chemoreceptors may attenuate or amplify the UA CO_2 response. If the arterial P_{CO_2} or acid-base balance of reptiles is precisely controlled, then it is expected that the depression in $\dot{V}_E$ caused by UA CO_2 would be overridden when the systemic or central CO_2-H^+ chemoreceptors were stimulated by increasing CO_2 concentrations. That is, the duration or the strength of the UA CO_2-induced f depression may be dependent on the rate of production of metabolic CO_2 and the sensitivity of the arterial or central CO_2-H^+ chemoreceptors. The study by Bartlett and Birchard (4) may provide an example of this interaction between the UA CO_2 receptors and the arterial or central chemoreceptors. They found that 2 or 4% inspired CO_2 caused an immediate transient inhibition of breathing lasting 4–5 min. This response was probably partially induced by the stimulation of UA CO_2 receptors. However, it is

possible that intrapulmonary CO_2 chemoreceptors could cause this response (10). Because the CO_2 was inspired into the lungs of the garter snake in the study by Bartlett and Birchard (4) the contributions of intrapulmonary chemoreceptors to the response could not be distinguished from the contribution of the UA CO_2 receptors. At 7 min the breathing rate was approximately equivalent to the rate of the pre-CO_2 breaths. Thereafter, f and VT slowly increased until the inspired CO_2 was terminated. At this point, a transient increase in $\dot{V}E$ was often observed. This increase in $\dot{V}E$ was probably caused by the removal of the UA CO_2-induced inhibition. Once this inhibition was removed, the unmasked response (increase in $\dot{V}E$) induced by the stimulation of the arterial and central chemoreceptors was observed. Until experiments are designed to eliminate the contribution of arterial, pulmonary, and central chemoreceptors on ventilation, it will be difficult to accurately define the characteristics of the UA CO_2-sensitive receptors. Likewise, the presence of UA CO_2 receptors makes it difficult to accurately characterize the arterial, pulmonary, and central chemoreceptors' responses to inspired CO_2.

The decrease in f with UA CO_2 in this study was caused by an increase in TP. Because neither TE nor TI were significantly altered with UA CO_2, the changes in TT paralleled those observed for TP. These changes were also consistent with the changes reported for the tegu lizard (3), with the exception that a small but statistically significant ($P < 0.05$) increase in TI during 2% UA CO_2 was observed in the tegu lizard.

Based on the present study and the study on the tegu lizard (3), it appears that a diverse group representing the class Reptilia exhibit the presence of UA CO_2 receptors that respond to UA CO_2 with a decrease in f and $\dot{V}E$ caused by an increase in TP. A study on the bullfrog (27) indicates that the receptors may also be found in some amphibians.

The function of the UA CO_2 receptors is unknown. It has been suggested that the UA CO_2 receptors may sample the CO_2 concentrations in burrows to determine the presence of either prey or predators (2). Alternatively, the receptors may be part of a ventilatory control loop, functioning to maintain ventilatory efficiency (7), i.e., the UA CO_2-induced ventilatory depression may be a way of maintaining a significant gradient between expired and inspired CO_2 concentrations. In addition, it has been suggested that the UA CO_2 receptors sample the CO_2 concentrations in an expiration and alter the ventilatory pattern of later ventilatory cycles (2). Further discussions on some of the possible functions of the UA CO_2 receptors appear elsewhere (3, 7).

Olfactory and vomeronasal nerve transection. In the second part of this study the olfactory and vomeronasal nerves were independently transected to determine the contribution of these sensory systems to the UA CO_2 response. The garter snake was chosen as the experimental animal because the anatomy of its olfactory and vomeronasal systems has been described in detail (14, 15). Previous studies (7) that attempted to localize the UA CO_2 receptors have not differentiated the role of the olfactory system and the vomeronasal systems in the UA CO_2 response or have failed to recognize the possible contribution of the vomeronasal system (27). In the past, the vomeronasal system has been often overlooked because it was thought to be functionally auxiliary to the olfactory system.

Winans and Scalia (31) were the first to demonstrate the complete separation of the main olfactory system from the vomeronasal system in rabbits. This discovery led to the dual-olfactory system hypothesis, which states that two parallel nonconverging olfactory pathways can be traced from the olfactory and vomeronasal epithelia to the hypothalamus and thalamus. Today this hypothesis is widely accepted, in part because of anatomic and behavioral studies using reptiles. These studies have shown that the two systems are parallel and nonoverlapping (13, 15), have different end points (14), that the vomeronasal system is not functionally auxiliary to the main olfactory systems, and the vomeronasal system is not redundant in its transmission of information to the central nervous system (17–19, 23, 24). In studies that selectively lesioned the olfactory and vomeronasal nerves it was found that aggregative (19), courtship (24), feeding (17), and prey trailing (23) behaviors in garter snakes require an intact vomeronasal system but not an intact olfactory system.

The primary difference between these two sensory systems is thought to be in the nature of the stimuli by which they are affected. Substances detected by the vomeronasal organ are usually high-molecular-weight compounds that are nonvolatile and contain proteins. The olfactory stimuli are primarily volatile low-molecular-weight compounds (16). The finding in this study, as presented below, that CO_2 stimulates receptors located in the nasal epithelium is consistent with the above-mentioned observation that low-molecular-weight compounds selectively activate the olfactory sensory system.

Results from the nerve lesion experiments of this study show that CO_2-sensitive receptors are located in the nasal epithelium of the garter snake. This conclusion was made based on the observation that the f response to UA CO_2 was abolished with olfactory nerve transection. This observation supports the hypothesis that the CO_2-sensitive receptors are located in the nasal epithelium and that the olfactory nerves must be intact for the UA CO_2 response to be observed. These results are consistent with those reported on the tegu lizard (7), where olfactory peduncle transection abolished the f depression normally observed with UA CO_2. However, in that study (7) olfactory peduncle transection severed nerve tracts traveling from both the AOB and MOB. Therefore the relative contribution of the olfactory or vomeronasal system to the UA CO_2 response could not be determined. The present study further defines the UA CO_2 response as originating in the olfactory system.

The observation that the f response to UA CO_2 was enhanced after vomeronasal nerve lesions was not predicted. It was hypothesized that the f response to UA CO_2 would not be significantly different before and after the vomeronasal nerves were transected. This hypothesis was based in part on reported observations that low-molecular-weight volatile compounds, similar to CO_2,

generally stimulate receptors located in the olfactory system and not in the vomeronasal system. In addition, because the two systems are thought to be completely separate, with no overlap of function, it was predicted that only olfactory nerve transection would effect the UA CO_2 response. Results from the present study indicate, however, that the vomeronasal system may interact with the olfactory system to determine the UA CO_2 response. Because transection of the vomeronasal nerves increased the response to UA CO_2, it is hypothesized that the vomeronasal system normally exerts an inhibition on the olfactory-mediated UA CO_2 response. This inhibition could be mediated via CO_2 receptors located in the vomeronasal organ or by tonic modulation of the UA response originating from the vomeronasal organ.

The interaction between the olfactory and vomeronasal systems of the garter snake may take place in the hypothalamus. The olfactory system of the garter snake projects to the medial and lateral mammillary nuclei of the hypothalamus, whereas the vomeronasal system projects to the ventromedial nucleus of the hypothalamus (14). There is no evidence for anatomic connections between the olfactory or vomeronasal systems before the hypothalamus (13–15). In mammals, there is anatomic (22) and physiological (25) evidence for a convergence of the olfactory and vomeronasal pathways in the amygdaloid. Licht and Meredith (25) reported that single units recorded from the posteromedial cortical nucleus of the amygdaloid responded to electric stimulation of either the vomeronasal organ (vomeronasal nerve bundle) or the MOB. They also recorded several single units in the amygdala that were driven by the stimulation of one system and suppressed by stimulation of the other system.

Further anatomic and physiological studies are needed to determine whether these two sensory systems interact in the amygdala or hypothalamus of reptiles. In addition, studies need to be designed to determine whether central pathways convey environmental CO_2 information from the amygdala or hypothalamus to the respiratory centers.

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