Lizard Lungs: CO_2-Sensitive Receptors in *Tupinambis nigropunctatus*

Mammals and birds increase ventilation when airway CO_2 concentration increases. Present theory regarding peripheral chemical regulation of respiration in mammals proposes that CO_2 and/or $[H^+]$ in blood or cerebrospinal fluid acts on peripheral chemoreceptors to cause ventilatory adjustments, which maintain CO_2 relatively constant in blood. However, additional chemoreceptors that help control breathing in birds have recently been found.

Using unidirectional ventilation and occlusion of pulmonary blood flow, PETERSON and FEDDE[1] localized the additional receptors to chickens' lungs. Single-fibre recordings have identified the vagus as the afferent pathway[2]. The avian pulmonary CO_2 receptor is not stretch sensitive[3], nor does it respond to hypoxia or drugs that stimulate mammalian carotid body receptors[2]; it appears to respond only to CO_2 changes in the intrapulmonary microenvironment of the receptor[3]. The action mechanism of CO_2 at the receptor level is not known nor have the receptors been identified morphologically.

It has previously been suggested that the ventilatory response of ammals to changes in inspired CO_2 could result, at least in part, from local action of CO_2 in the lungs[4]. However, that hypothesis was generally neglected in the enthusiasm generated by HEYMANS' work[5] on the chemoreceptor function of the carotid body. Recently, BARTOLI et al.[6] demonstrated a ventilatory response to changes in airway CO_2 in dogs (on cardiopulmonary bypass), which was independent of arterial CO_2 and abolished by vagotomy. Whether this response was prompted by pulmonary CO_2 receptors similar to those found in birds or to CO_2 sensitivity of other vagal lung receptors is not yet clear.

During experiments designed to evaluate the ventilatory response of the tropical lizard, *Tupinambis nigropunctatus* (common name, Tegu) to CO_2, we, as other investigators[7], observed a first breath response to changes in inspired CO_2. If this response is from pulmonary CO_2 receptors, it lends phylogenetic continuity to the existence of such receptors in mammals. It is first necessary, however, to determine whether or not the response in reptiles is mediated by lung receptors whose afferent pathway is in the vagus.

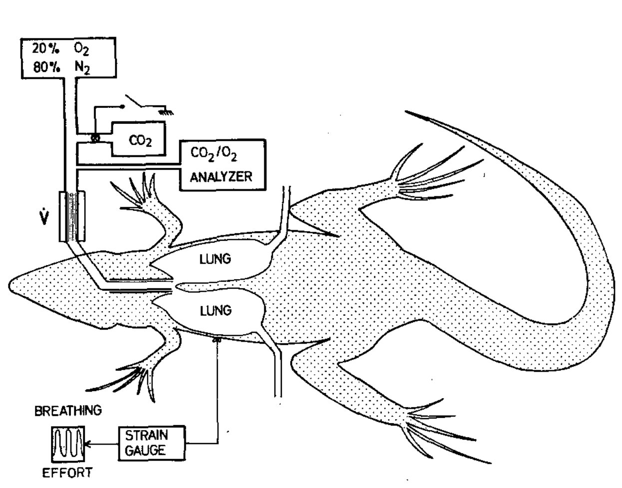

Fig. 1. Experimental arrangement to measure respiratory parameters during unidirectional ventilation of the Tegu. Unidirectional gas flow ($\dot{V}$) was measured with a pneumotachometer. CO_2 could be suddenly removed from the gas stream by closing a solenoid valve in the CO_2 line. Breathing effort and respiratory rate were measured with a strain gauge attached to the thoracic body wall.

Experiments were performed at a body temperature of 35 °C on 5 lizards weighing from 600 to 1600 g. They were anesthetized with 2% Halothane, and a brass cannula 10 cm long was inserted into the trachea to the bifurcation. A mid-ventral incision was made from the base of the neck to the caudal border of the sternum and extended laterally on both sides of the animal to a point just below the thoracic cage. The simple, sac-like lungs were opened at the caudal end and glass cannulae inserted and tied in place (Figure 1).

Humidified gas was passed unidirectionally into the tracheal cannula, through both lungs, and out to the atmosphere through the glass cannulae. Total gas flow, monitored by a pneumotachometer, was maintained at $1.0\ l \cdot min^{-1}$. Carbon dioxide was added to the gas stream to achieve the desired CO_2 concentration. Concentrations of CO_2 and O_2 in the ventilating gas were continuously monitored with an infrared CO_2 analyzer (Hartman and Braun, URAS 3) and a paramagnetic O_2 analyzer (Beckman E-2). A solenoid valve was placed in the CO_2 line so that the CO_2 in the gas stream could be suddenly removed. Time required for a CO_2-free gas front to reach the lungs after the solenoid valve was closed was about 0.3 sec. A strain gauge attached to the lateral thoracic wall measured respiratory frequency and breathing effort. Because the chest wall was open, the animal's breathing effort did not contribute to pulmonary ventilation.

Both vagi were exposed in the cervical region where thread loops were placed around them for easy access when vagotomy was desired. The 2 pulmonary arteries and the common pulmonary vein, dorsal to the apex of the heart, were dissected free, and thread loops were placed around them. Blood flow in both arteries and vein could be occluded by applying tension to the threads.

Halothane anesthesia at the surgical level completely abolished all visual evidence of breathing. It was, therefore, necessary during experiments to reduce the Halothane from 2% to between 0.2 and 0.5% to achieve respiratory responsiveness and, at the same time, maintain some analgesia.

The ventilatory movements of lizards differ somewhat from those of other air-breathing vertebrates. Respiratory depth and frequency vary from breath to breath. Breathing movements in the Tegu, as in several reptilian species, were triphasic: a short expiration, a long inspiration, then another short expiration[8]. Unidirectional ventilation had no observable effect on the triphasic breathing pattern.

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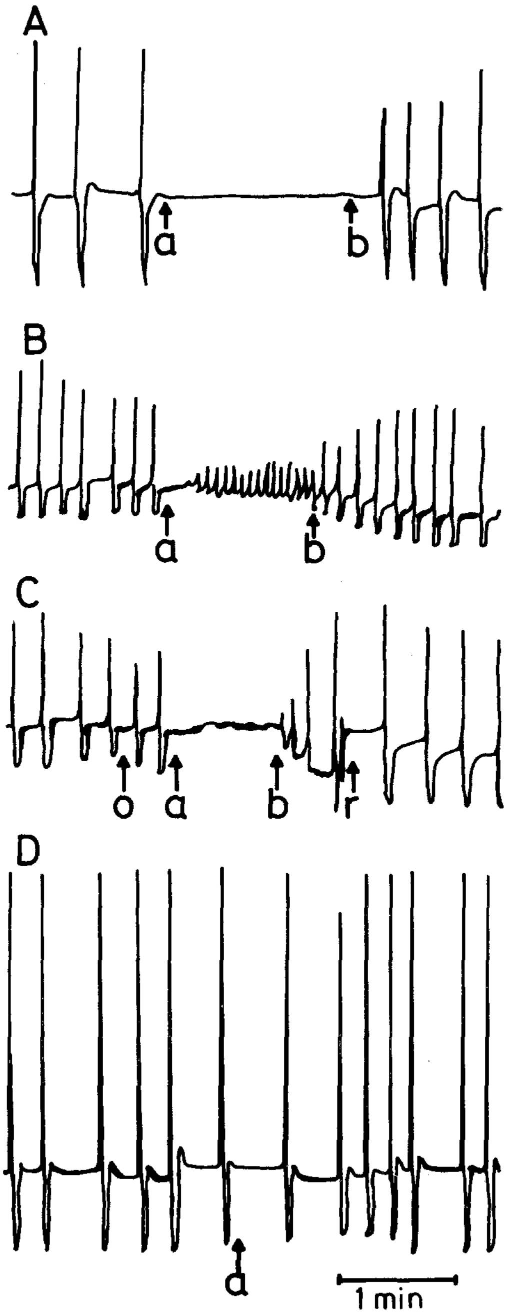

Fig. 2. Breathing response to sudden reduction in intrapulmonary CO_2 concentration. A) Apneic response during blood flow through the lungs; CO_2 reduced at (a) and returned (b). B) Increased respiratory rate and decreased breathing effort in response to reduction of intrapulmonary CO_2 during blood flow through the lungs; CO_2 reduced at (a) and returned at (b). C) Apneic response in the absence of blood flow through the lungs; blood flow occluded at (o), returned at (r); CO_2 reduced at (a) and returned at (b). D) Lack of breathing response to sudden reduction in intrapulmonary CO_2 concentration (a) after bilateral cervical vagotomy.

The steady-state ventilatory response of the lizards increased (primarily as a result of increased breathing effort rather than frequency) as the CO_2 concentration in the unidirectional ventilating gas stream increased. Either of two responses was always observed when CO_2 was suddenly removed from the unidirectional gas stream. Both responses were observed in each animal during different trials of CO_2 removal. Of the 62 trials on 5 animals, 45.2% were complete apneic responses (Figure 2A) and 54.8% responded by increasing respiratory rate and decreasing breathing effort (Figure 2B). The one response that occurred persisted throughout the period of CO_2 removal. When CO_2 was returned to the gas stream, the normal breathing pattern was re-established. Although we can offer no explanation for the different responses, we attribute both to the removal of CO_2 from the ventilating gas.

Even though the response was fast (from 0.8 to 1.4 sec after the CO_2-free gas entered the lung), it might have been mediated via extrapulmonary chemoreceptors responding to changes in blood P_{CO_2}. To determine if extrapulmonary chemoreceptors were involved, we conducted experiments in which CO_2 was removed from the ventilating gas after complete occlusion of all pulmonary blood flow. In some trials, as shown in Figure 2C, apnea occurred when CO_2 was removed from the ventilating gas even though there was no blood flow through the lungs. In other cases, a response similar to that shown in Figure 2B occurred. Thus, occlusion of pulmonary blood flow did not abolish the response to CO_2 removal. It seems unlikely, therefore, that the respiratory responses to reduction in intrapulmonary CO_2 concentration with lung perfusion result from CO_2 changes in systemic blood.

The rapid ventilatory response to changes in intrapulmonary CO_2 concentration that occurs in mammals and birds is abolished by vagotomy[1,6]. Bilateral cervical vagotomy in the Tegu completely eliminated the breathing response to CO_2 removal from the ventilating gas stream (Figure 2D).

Our results suggest a vagally-mediated, ventilatory response to changes in CO_2 concentration in the lungs of these lizards. The CO_2-sensitive receptors responsible for the response could not have been in the trachea because the cannula extended to the tracheal bifurcation. The lungs of the Tegu are rather simple sac-like structures without internal airways. Therefore, the receptors are apparently in the 2 short bronchi, the lung tissue, or both. Other investigators have postulated the existence of pulmonary CO_2 receptors in lungs of lizards[7], implying that they serve a primary chemoreceptor function. Although that hypothesis is attractive, our evidence is not sufficient to rule out the possibility that stretch or irritant receptors, which might also be CO_2 sensitive, were responsible for the ventilatory response to CO_2.

Zusammenfassung. In der Lunge von Eidechsen (*Tupinambis nigropunctatus*) wurden unter Anwendung von Einweg-Ventilationsmethoden CO_2-sensible Rezeptoren nachgewiesen (afferente Reflexbahn im N. vagus). Plötzliches Absenken der intrapulmonären CO_2-Konzentration löst innert 0,8 bis 1,4 sec einen von zwei Ventilations-Mechanismen aus (Atmungsstillstand oder reduzierte Atmungsleistung), wozu jedoch keine Blutdurchströmung der Lungenarterien und -venen notwendig ist.

R. N. GATZ[9], M. R. FEDDE[10] and E. C. CRAWFORD, JR.[11]

Department of Physiology, Max-Planck-Institute of Experimental Medicine, Hermann-Rein-Strasse 3, D–34 Goettingen (German Federal Republic, BRD),
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[9] Present address of R. N. GATZ: Battelle Memorial Institute, Medico-biological Research Group, Laboratory of Respiratory Physiology, 7, route de Drize, CH–1227 Carouge-Genève, Switzerland.

[10] Present address of M. R. FEDDE: Department of Physiological Sciences, Kansas State University, Manhattan, Kansas 66506, USA, Contribution No. 142, Dept. of Physiological Sciences, KAES, Manhattan, Kansas 66506, USA. This study was supported, in part, by a grant-in-aid from the Kansas Heart Association.

[11] Present address of E. C. CRAWFORD, JR.: School of Biological Sciences University of Kentucky, Lexington, Kentucky 40506, USA.