EVIDENCE OF A FUNCTIONAL ROLE IN LUNG INFLATION FOR THE BUCCAL PUMP IN THE AGAMID LIZARD *UROMASTYX AEGYPTIUS MICROLEPIS*

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Summary

This study has demonstrated that the agamid desert lizard *Uromastyx aegyptius microlepis* ventilates its lungs both with a triphasic, thoracic aspiratory pump and by gulping air, using a buccal pump. These two mechanisms never occur simultaneously because bouts of buccal pumping are always initiated after the passive expiration that terminates a thoracic breath. Lung inflation arising from thoracic and buccal ventilation was confirmed by direct recording of volume changes using a whole-body plethysmograph. This observation was further confirmed by mechanical separation of the inflationary pressures associated with these two breathing mechanisms, enabling the effects of lung inflation on buccal breathing to be observed. This revealed that the buccal pump is influenced by a powerful Hering–Breuer-type reflex, further confirming its role in lung inflation. Bilateral thoracic vagotomy tended to increase the variance of the amplitude and duration of the breaths associated with the aspiration pump and abolished the effects of lung inflation on the buccal pump. *Uromastyx* has vagal afferents from pulmonary receptors that respond to changes in lung volume and appear not to be sensitive to CO_2. This study describes two lung-inflation mechanisms (an amphibian-like buccal pump and a mammalian-like aspiration pump) in a single extant amniote, both of which are subject to vagal feedback control.

Key words: Reptilia, lizard, *Uromastyx microlepis*, buccal pump, vagotomy.

Introduction

There has been a great deal of confusion concerning the respiratory mechanisms of reptiles. Lizards, in common with all other reptiles (except some crocodilians), lack a diaphragm. However, unlike modern amphibians, they do have ribs, and it has long been considered that reptiles aspirate air into the lungs using intercostal muscles acting on the rib cage, rather than using positive-pressure buccal pumping as amphibians do (e.g. Bellaires, 1970). In many species (including lizards), each thoracic respiratory cycle has three components: expiration (stage I)–inspiration–expiration (stage II) (Boelaert, 1941; Templeton, 1960; Templeton, 1964; Templeton and Dawson, 1963; Gans, 1970), but in some reptiles (e.g. crocodilians) the respiratory movements are diphasic (Gans and Hughes, 1967; Naifeh et al., 1970a). Both expiration (stage I) and inspiration are active processes, but in some reptiles head or body plethysmography has revealed that the final expiratory stage (expiration-II) in the breathing cycle is passive (Boelaert, 1941; Templeton and Dawson, 1963; Gans and Hughes, 1967; Gaunt and Gans, 1969; Naifeh et al., 1970a; Naifeh et al., 1970b; Rosenberg, 1973; Gans and Clark, 1976).

In addition to thoracic, aspiratory breathing, many species of reptile show conspicuous buccal or gular movements. These have been considered not to ventilate the lungs or tracheobronchial dead space because the glottis was reportedly closed during these movements (Hansen, 1941; McCutcheon, 1943). Instead, these gular movements or buccal oscillations were thought to be used for thermoregulation, for olfaction or as part of a behavioural repertoire such as courtship or threat displays. Electroencephalographic (EEC) recordings taken from the olfactory bulbs demonstrated activity synchronous with the gular movements, suggesting that they constitute an olfactory mechanism (Agassiz, 1857; Boelaert, 1941; McCutcheon, 1943; von Saalfeld, 1934; Smith, 1946). In addition, throat inflation and hissing is used during courtship and defence behaviour (Deban et al., 1994; Bels et al., 1995). However, a more recent study, using X-ray imaging of the varanid lizard *Varanus exanthematicus*, revealed that, during recovery from exercise, the animals used gular pumping in addition to the costal pump, with between one and five gular pumping movements following each costal inspiration. These gular pumping movements clearly caused lung inflation, with caudal translation of the visceral mass (Brainerd and Owerkowicz, 1996). Subsequently, these lizards were shown to employ gular pumping when walking,

thus overcoming a supposed mechanical constraint on active lung ventilation during exercise (Owerkowicz and Brainerd, 1997). These reports were the first seriously to challenge the traditional view that only aspiratory pumping is utilised by amniotes.

The present study used classic physiological techniques to investigate the role of buccal pumping in the agamid lizard *Uromastyx aegyptius microlepis*. Early claims that gular movements contributed to respiration were discredited because no reliable pressure/flow or electromyographic measurements were available. A number of problems are encountered when research is conducted on the gular pump. First, visualisation of the glottis during gular pumping is difficult because opening the mouth abolishes the pumping mechanism (Al-Ghamdi, 1995). Second, electrical recordings from small laryngeal muscles may be contaminated with signals from larger neighbouring muscles. Third, thoracic impedance measurements will not detect the filling of air sacs, which project abdominally to the pelvic girdle. These problems were obviated in Brainerd's studies (Brainerd and Owerkowicz, 1996; Owerkowicz and Brainerd, 1997) by using X-ray fluoroscopy, and more directly avoided in the present study by performing a dual tracheostomy and directly measuring the positive inflationary pressures of the gular pump from the rostral tracheal cannula. This proved that the gular movements did not simply represent buccal oscillations, but that the glottis was open during the buccal pumping episodes and that strong inflationary pressures could be delivered to the lung sacs. This technique has been briefly reported (Al-Ghamdi et al., 1995).

Once it had been established that the buccal pump of *Uromastyx* could direct positive pressure pulses to the subglottal airways, our aims in this study were to compare the functioning of the buccal pump in lung inflation with that of the aspiration pump and to investigate the role of vagal feedback on the control of both motor mechanisms. This was achieved by recording lung inflation directly, using whole-body plethysmography, and by testing the effects of lung inflation on the buccal pump before and after bilateral thoracic vagotomy.

Materials and methods
Animal maintenance

Fourteen agamid lizards *Uromastyx aegyptius microlepis* Blanford 1874 of either sex (420–1133 g) were used in this study. Having obtained CITES clearance and satisfied import controls (Gardner et al., 1993), the animals were collected from Saudi Arabia during the spring and summer of 1991, 1992 and 1993 around the city of Rhiadh and transported to Birmingham, where the experiments were conducted. The animals were kept in two large cages (1.8 m×1.8 m×1.2 m high), the floor of which was covered with fine silver sand. In each cage, there were two basking areas heated by 240 W pig-rearing lamps and two long-tube lamps, one a 40 W True Light Powder Twist ultraviolet (UV lamp), and the other a 40 W (UVB/UVA lamp). Both long-tube lights were run simultaneously to ensure the correct quantities of UV/UVA/UVB light. The lighting was kept on for 12 h per day (from 08.00 to 20.00 h), allowing the lizards to come out of their natural torpor when the daytime temperature under the lamps reached an ambient 30–32 °C. By mid-afternoon, they basked in an ambient temperature ranging between 45 and 47 °C. At night, the lamps were switched off, and ambient temperature dropped to approximately 18–20 °C. Relative air humidity ranged between 54 and 65 %. Food (vegetables and fruits) was provided daily and live adult locusts two or three times per week. Details of the animal husbandry involved in the maintenance of wild-caught uromastids have been reported previously (Gardner et al., 1993). Animals that were gaining or holding weight were used in the experiments.

Animal preparation

Each animal was fasted for 24 h then anaesthetized with 30 mg kg^{-1} intraperitoneally of sodium pentobarbitone (Sagatal, May & Baker Ltd). The level of anaesthesia was monitored by assessing withdrawal reflexes to pinch or pressure applied to the ventral surface of the tail; supplementary doses of anaesthetic were administered as required. A rectal probe (Digitron instrumentation 3200K) was inserted through the cloacal opening to measure rectal temperature, and a lamp was used to warm the animal. Blood pressure was recorded in three lizards, *via* a polyethylene tube (0.5 mm diameter, Ponex code 800/1101140/100) inserted into the femoral artery and connected to a pressure transducer (PDCR 75/2 S/N 100; Druck Ltd) and amplified (Oscillograph, Washington; 400 MD 2C). In another group of animals (*N*=5), a dual tracheostomy was performed, a polyethylene cannula (1.5–2.0 mm inner diameter) was inserted caudally towards the lungs and a second cannula (of the same diameter) was inserted cranially towards the larynx (Fig. 1B). Care was taken to avoid damage to the recurrent laryngeal nerves and associated blood vessels. Tracheal pressure signals were transduced and amplified using the same system employed for blood pressure recording.

In a third group of animals (*N*=6), bilateral thoracic vagotomy was performed by making an incision in the mid-line of the chest for 2–3 cm, then carefully deflecting the viscera to reveal the course of the main vagus nerve beneath the heart. The nerve was sectioned, taking care that both cardiac and laryngeal vagal branches were left intact. The chest was then closed and sutured, and the lung-inflation experiment was repeated (see below).

Recording EMGs and ECGs

Electromyograms (EMGs) were recorded from the sphincter colli and geniohyoid muscles in the neck region. Bipolar copper (0.2–0.3 mm in diameter) or stainless-steel (0.03 mm diameter; Johnson Matthey Metals Ltd) wire electrodes were inserted into the belly of the appropriate muscle. The EMG associated with activity in the intercostal muscles was recorded by placing two precordial leads on either side of the chest

Fig. 1. Schematic diagram of the preparation used to measure lung ventilation and associated variables in *Uromastyx aegyptius microlepis*. (A) Whole-body plethysmograph. The syringe was used for injecting calibration volumes into the plethysmograph. PT-1, port for arterial blood pressure; PT-2, port for ventilatory pressures; bipolar leads were used to record electromyographic activity (EMG) from the geniohyoid, sphincter colli and intercostal muscles. (B) Connections to the tracheotomised preparation that separated the buccal and thoracic pumps, enabling the effects of lung inflation on buccal ventilation to be measured. Valves are numbered 1–5. The buccal pump could be isolated by turning valves 2 and 3, in which case the buccal pressure was directed to the underwater seal or the pressure transducer. A pressure pulse of room air was created by adjusting the depth of a tube under water between 2.5 and 7.5 cmH_2O

($1\,cmH_2O=98.1\,Pa$) and delivered to the lower airways by first turning valve 1 and then valves 3 and 4 simultaneously. In the same manner, a pressure pulse of CO_2-rich air was created and delivered by turning valve 1 and then valves 3 and 5 simultaneously.
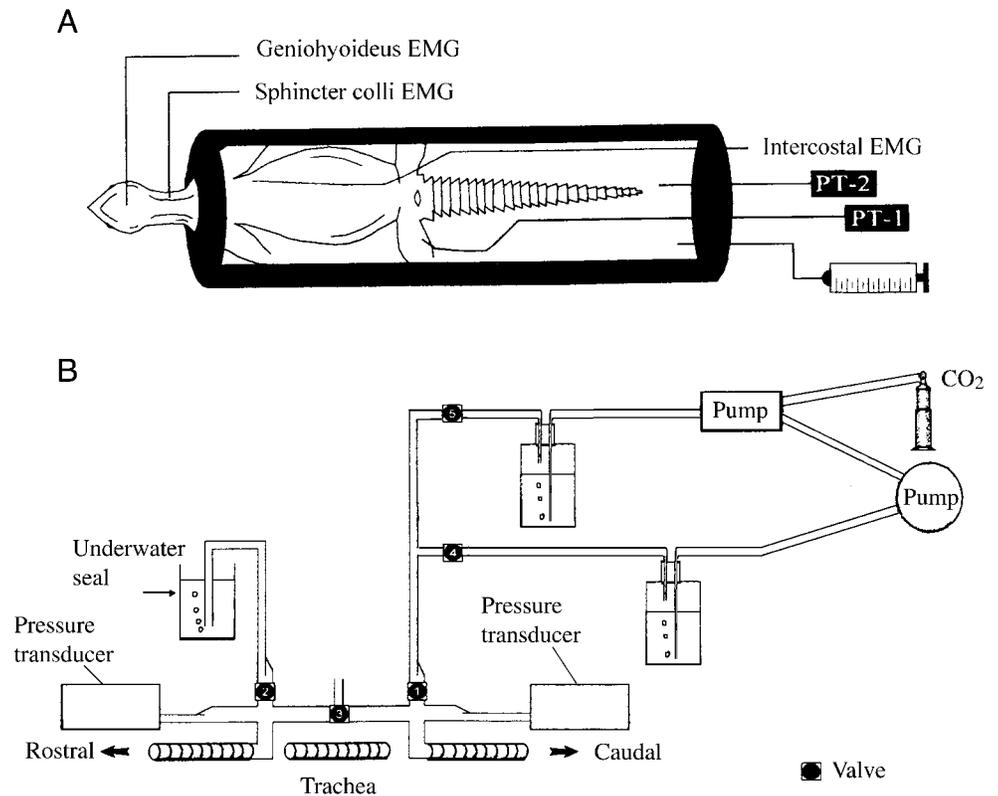
wall, between the last two ribs (Fig. 1A). This configuration invariably also recorded the electrocardiogram (ECG) (see Fig. 2). The signals were fed into a preamplifier (Isleworth Electronics type AIOI) and then further amplified and filtered (5–5000 Hz) (Digitimer; Neurolog NL 105, 106, 115, 120, 125).

Plethysmography

The tidal volumes generated by the buccal and thoracic respiratory pumps were explored across a range of temperatures (25–35 °C) using whole-body plethysmography. Whole-body plethysmography was performed by placing the lizard in a closed cylindrical tube (50 cm in length, 9 cm diameter). The animal's head and neck protruded from one end through a sealed rubber membrane so that the buccal pump could operate freely (Fig. 1A). A port for measuring pressure within the plethysmograph was connected to a pressure transducer as described above. EMG signals and blood pressure (P_b) were recorded while the animal was in the cylinder. A second port served for calibration purposes. A 10 ml syringe (BD Plastipak) was used to inject a known volume of air, and the corresponding pressure was noted.

Experimental protocol

To assess the maximal inflationary pressure generated by the buccal pump, the rostral tracheal cannula was connected to a tube (2 mm diameter) that opened under a column of water

(Fig. 1B, valves 2 and 3 turned). The isolated buccal pump contracted against this linear auxotonic load and, after several episodes of buccal breathing, the maximal inflationary pressure during these auxotonic buccal contractions was noted as the maximal depth of water against which airflow was sustained. Following this, the buccal pump tube was connected to a pressure transducer (Fig. 1B, valves 2 and 3 affected). The other tracheal cannula (i.e. the one facing the lungs) could be patent, obstructed or inflated under positive pressure. It was connected to two alternative pressure sources *via* an exchange valve to administer short square-wave pulses of pressure. One source consisted of an air pump used to inflate the lungs with room air, delivered *via* a Wolff bottle containing water of measured depth. The other unit consisted of a gas-mixing pump (H. Wösthoff OHG; Bochum, Germany; type L9 725) delivering a range of CO_2 mixtures (Fig. 1B). A range of square-wave pressure pulses ($2.5–7.5\,cmH_2O$; $1\,cmH_2O= 98.1\,Pa$) of humidified air or a range of CO_2 concentrations (3, 5, 7 and 10 % in humidified air) were applied. These lung-inflation procedures were repeated on animals (*N*=3) following bilateral thoracic vagotomy.

All signals were fed into an intelligent computer interface (Cambridge Electronic Design; CED 1401) and displayed on a computer using data-capture software (Spike2 CED). The data were sampled at 100–1000 Hz and stored on hard disk for subsequent analysis. Values for measured variables are given as means ± S.D.

Results

Aspiration pump

Two respiratory patterns were identified in *Uromastyx*. One consisted of triphasic lung inflation and deflation [active expiration (stage I)–active inspiration–passive expiration (stage II)], in which the intercostal muscles were involved (Fig. 2). This thoracic ventilatory cycle was the prevailing mode of respiration throughout most recording periods. At the initiation of each cycle, the glottis opened (this was noted by opening the mouth and looking directly at the glottis) and an active expiration-I occurred, followed immediately by an active inspiration with no intervening pause. Following this inspiration, a short passive expiration-II occurred (Figs 2, 3). The rib cage movement terminated with a pause of variable duration (Fig. 3). The EMG trace from intercostal muscles for this pattern confirmed that both expiration-I and inspiration involved active contractions of these muscles, while expiration-II was passive (Figs 2, 3). Each pause was interrupted by an active expiration-I, then a new cycle began.

Buccal pump

The alternative breathing pattern involved a buccal pump, which was shown to inflate the lungs. Buccal movements usually commenced as body temperature rose from 30 to 35 °C during progressive body warming (see below). Some conscious lizards were easily provoked to perform buccal ventilation by touching their skin or lightly squeezing their tail or feet. The buccal pump always commenced operation during the respiratory pause that occured immediately after a short passive expiration-II in a triphasic thoracic breath (Fig. 4A).

Buccal pumping was achieved by muscles inserted in the floor of the buccal chamber. EMG recordings from the sphincter colli and geniohyoid muscles revealed that both were activated simultaneously. Contraction of these muscles was associated with a transient rise in pressure within the plethysmograph, indicating that the buccal pump was filling the lung sacs. Often a bout of these buccal inflations resulted in a progressive increase in baseline pressure indicative of lung inflation (Fig. 4A). Calibration of the whole-body plethysmograph enabled the tidal volumes associated with buccal and aspiration pumps to be measured. In three lizards, the tidal volume (measured from base to peak) of the buccal pump was 2.76 ± 0.7 ml ($N=487$ breaths), whereas the tidal volume of the thoracic pump (from end stage I expiration to peak inspiration) was 4.30 ± 1.6 ml ($N=253$ breaths). The maximum pressures generated by a bout of auxotonic activity in the buccal pump were measured in six lizards as having a mean of 9.33 ± 1.71 cmH$_2$O (range 6–17 cmH$_2$O).

Breathing patterns

Thoracic and buccal respiratory patterns never occurred simultaneously. The former often occurred independently, and occasionally continuously for more than 30 min, at temperatures between 20 and 30 °C, but the latter always took place during thoracic breathing, following expiration-II. Continuous recordings of respiratory activity as animals were gradually warmed over their normal diurnal range of body temperatures showed irregular bouts of thoracic breathing at 20 °C leading to more regular thoracic breathing at approximately 30 °C. These thoracic breaths often showed regular patterning with short pauses, especially when body
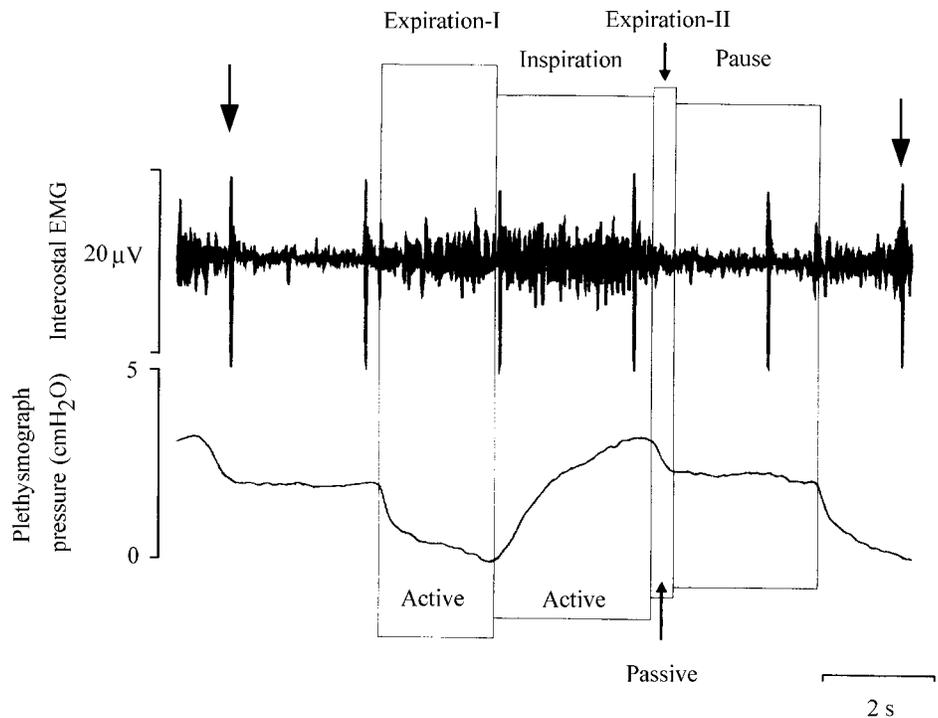

Fig. 2. Recording of electromyographic (EMG) activity from intercostal muscles, together with the pressure generated within the plethysmograph by an aspiratory lung-inflation cycle. The inflation cycle is triphasic, with active expiration-I leading to active inspiration then a brief, passive, partial expiration-II, followed by a ventilatory pause with the lungs inflated, terminated by the next cycle. The sharp deflections in the intercostal recording are electro-cardiographic complexes (large arrows). 1 cmH$_2$O=98.1 Pa.
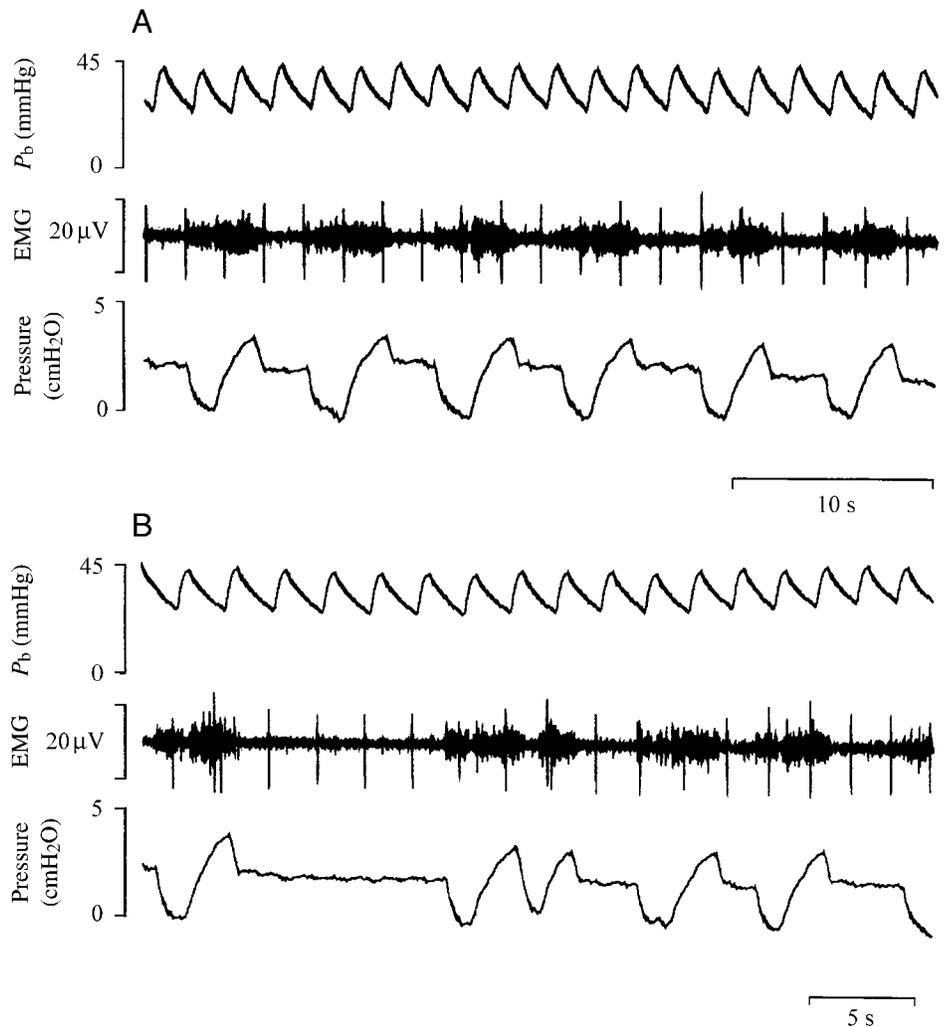
Fig. 3. A series of thoracic, aspiratory cycles of lung ventilation recorded from an anaesthetised *Uromastyx*. (A) An animal breathing regularly at 30 °C; (B) an animal breathing irregularly at 25 °C. P_b is the femoral arterial blood pressure (1 mmHg=0.133 kPa). Electrocardiographic activity is apparent in the recording of intercostal electromyographic (EMG) activity. 1 cmH$_2$O=98.1 Pa.

temperatures were relatively high (30–37 °C) (Fig. 3A), but at other times, generally at lower body temperatures (20–30 °C), a variety of breath-holds occurred (Fig. 3B). Above this temperature, buccal breathing movements were progressively recruited until, at approximately 35 °C, they became a component of each breathing cycle (Fig. 4B). The number of buccal pumping movements varied from two to 40 per respiratory cycle. These movements typically resulted in a progressive increase in lung volume to a point where the next pause in the breathing cycle began (Fig. 4A).

The effects of lung inflation

Inflating the lungs with a square-wave pressure of room air, immediately after the first buccal contraction in a bout, caused the buccal pump to stop immediately (Fig. 5). Application of a range of lung inflation pressures (2.5–7.5 cmH$_2$O) revealed that, while inflation at 2.5 cmH$_2$O pressure allowed the buccal pump to undergo a single contraction after the inflation, the higher inflation pressures all stopped buccal breathing immediately (Fig. 6). Subsequent EMG activity in the buccal musculature and the associated rise in pressure in the plethysmograph were abolished without affecting the standing

pressure in the buccal cavity (Fig. 6). Repeated cessation of the buccal pump as a result of successive lung inflations during one buccal breathing cycle is shown in Fig. 7A. A transient inflation of the lungs caused immediate cessation of activity in the buccal musculature. This resumed 4–5 s later (Fig. 7A). Inflating the lungs with gas mixtures enriched with CO$_2$ (3–10 %) also caused immediate cessation of the buccal pump (Fig. 8).

Bilateral thoracic vagotomy

After bilateral thoracic vagotomy, the effect on the buccal pump of inflating the lung with air was abolished, so that buccal movements continued unabated when the lungs were inflated (Fig. 7B). Bilateral vagotomy not only removed the inhibitory effect of lung inflation on buccal breathing but also affected the amplitude and the duration of the EMG of the intercostal muscles. After vagotomy, the variance of both these latter parameters increased. The mean amplitude of intercostal muscle EMG before vagotomy was 15.1±11.2 μV (87 breaths in three lizards); after vagotomy, it was 28.6±19.2 μV (102 breaths in three lizards). The mean duration of the EMG recorded from intercostal muscles also changed from
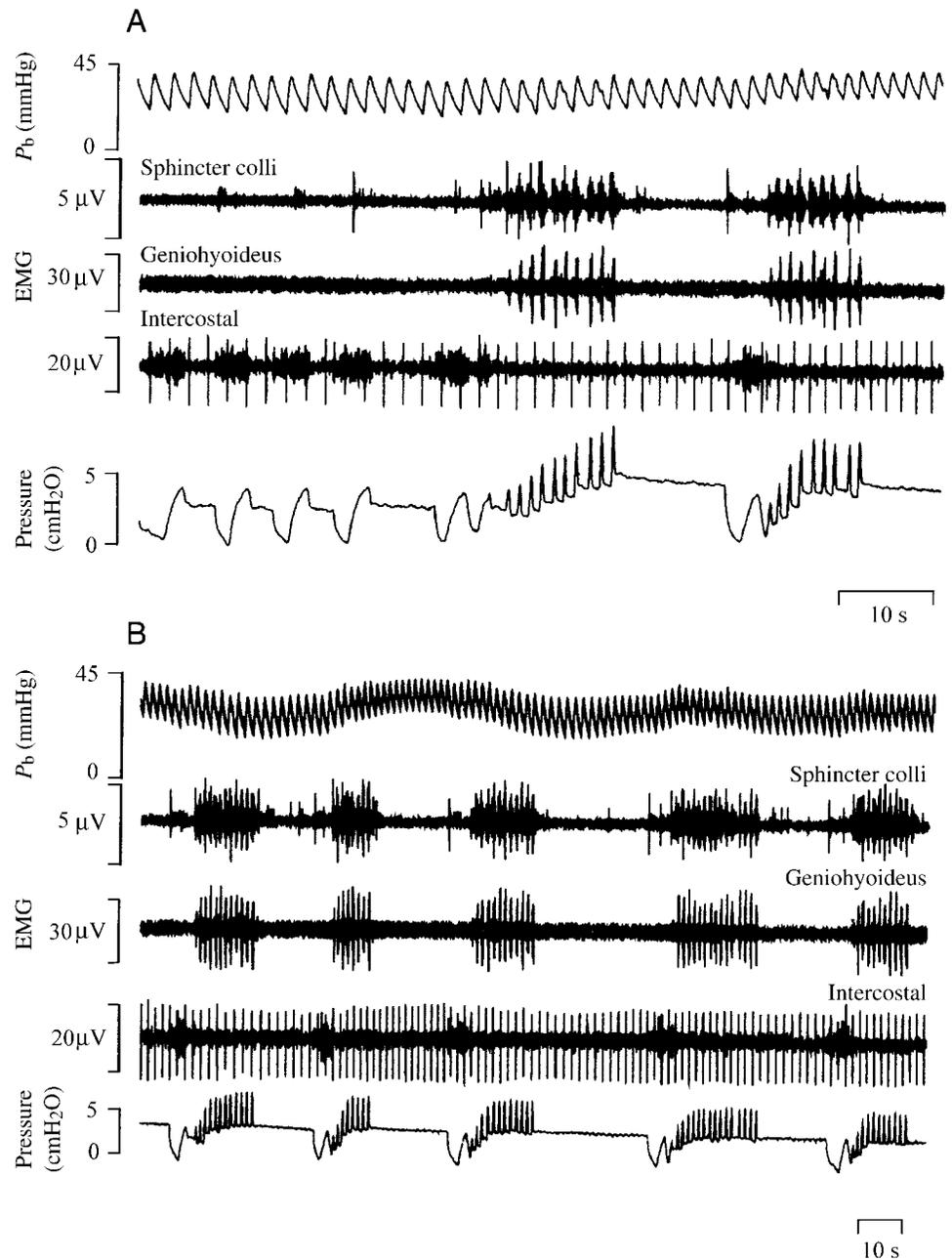
Fig. 4. A series of thoracic plus buccal ventilatory cycles recorded from an anaesthetised *Uromastyx* using whole-body plethysmography. (A) Animal at 30 °C, a cycle of thoracic ventilatory cycles terminates in the addition of buccal pumping. This starts in the respiratory pause immediately after passive expiration and results in additional inflation of the lung signified by an increase in baseline plethysmograph pressure, which is held until the subsequent active expiration. (B) A regular series of thoracic plus buccal ventilatory cycles recorded from an animal at 35 °C. Note the short interval between one respiratory pumping cycle and the next. P_b is the femoral arterial blood pressure (1 mmHg=0.133 kPa). EMG, electro-myographic activity. 1 cmH$_2$O=98.1 Pa.

3.84±1.8 s (87 breaths in three lizards) to 7.72±4.2 s (102 breaths in three lizards) after vagotomy.

Discussion

The events in the thoracic respiratory cycle of *Uromastyx* are similar to those previously reported in *Uromastyx aegyptius* (von Saalfeld, 1934) and a variety of lizards (Boelaaert, 1941; Templeton, 1960; Templeton, 1964; Templeton and Dawson, 1963; Porcell and Gonzalez, 1986; Carrier, 1989). The cycle consisted of three elements, active expiration-I, active inspiration and passive expiration-II followed by a pause. Extensive research has reported that reptiles ventilate their lungs triphasically (Couvereur, 1898; Willem and Bertrand,

1936; Randall et al., 1944; Templeton and Dawson, 1963; Templeton, 1964; Bennett, 1973; Rosenberg, 1973; Gatz et al., 1975; Jammes and Grimaud, 1976; Porcell and Gonzalez, 1986). Gans (Gans, 1970) states that all reptiles show this triphasic pattern in their ventilatory movements but that the resulting tidal volume was always diphasic. Since the present study did not measure airflow at the mouth, it is not known whether passive stage II expiration results in airflow or whether the glottis is closed during this stage.

In addition to triphasic thoracic ventilation, the present study described diphasic buccal ventilation in *Uromastyx*, similar to that described in amphibians. In amphibians, the buccal oscillations pump air into and out of the buccal cavity, and more powerful movements of the buccal floor are associated

Fig. 5. The effects of a forced inflation of the lungs with air on buccal ventilation in an anaesthetised *Uromastyx* following tracheostomy to separate the thoracic and buccal pumps (see Fig. 1B for details). The arrow indicates the moment of inflation, immediately after the first buccal contraction in the second ventilatory cycle. Lung inflation terminated the bout of buccal contractions. Each bout of buccal contractions followed a thoracic contraction, as indicated by the recordings of specific pressures and electromyographic (EMG) activity. $1\,cmH_2O=98.1\,Pa$.
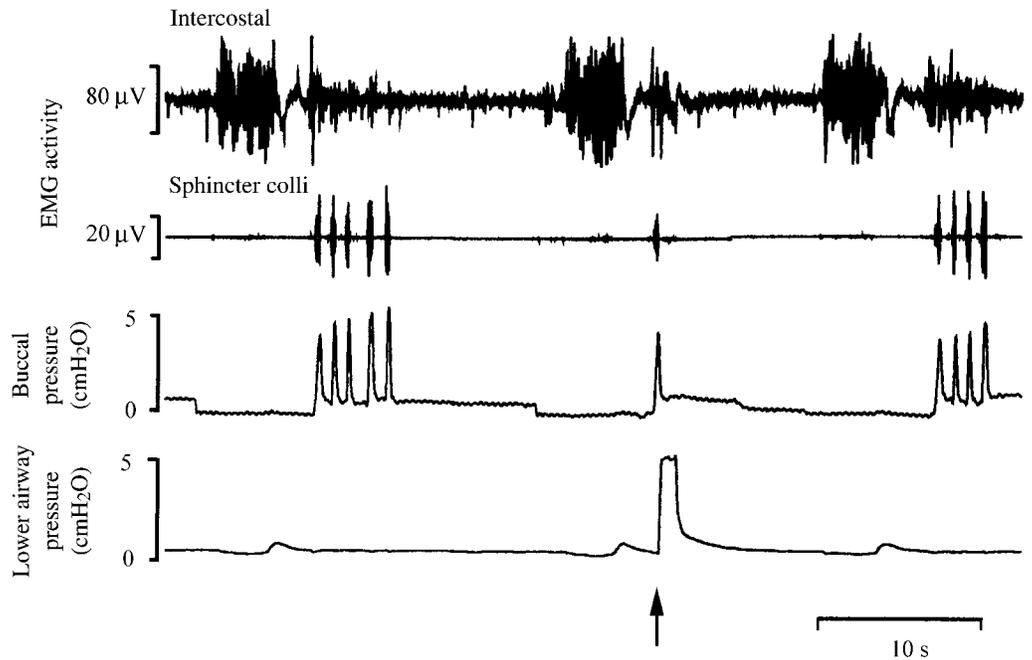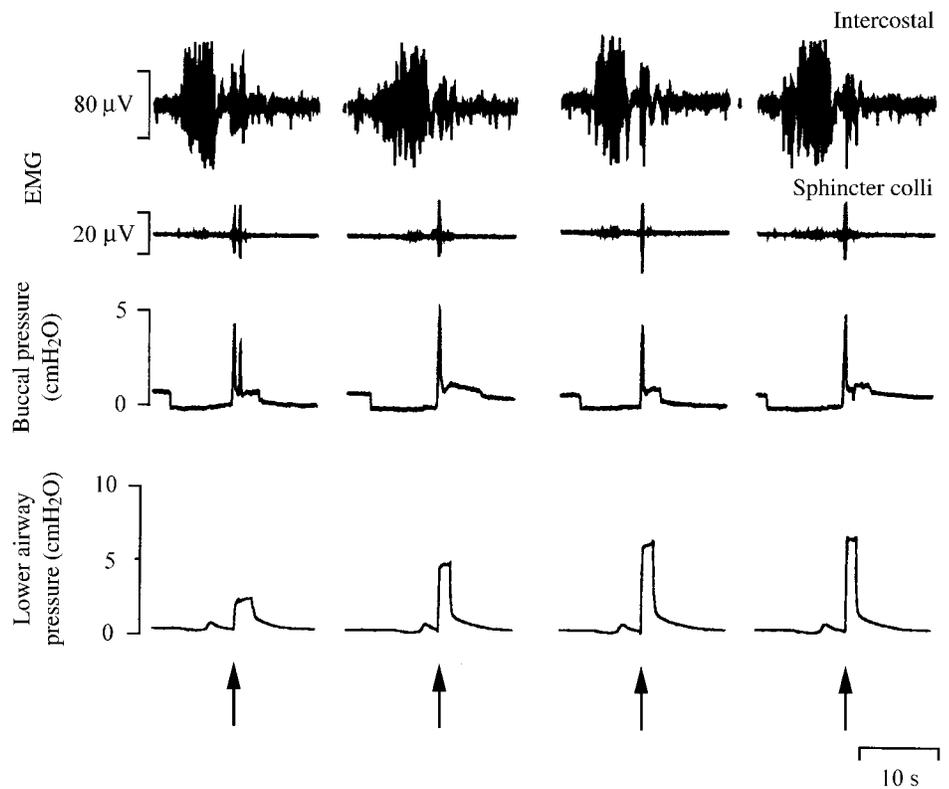
with lung ventilation (Jongh and Gans, 1969; Macintyre and Toews, 1976; Boutilier and Toews, 1977; West and Jones, 1975).

Previous studies have reported buccal pumping in reptiles. Agassiz (Agassiz, 1857) stated that rhythmic gular motion fulfilled an important inspiratory function in turtles. Later, McCutcheon (McCutcheon, 1943) determined that the glottis was closed during gular motion in the terrapin and suggested

an olfactory role for these movements. Boelaert (Boelaert, 1941) suggested a non-respiratory role for gular movements in lizards. In *Uromastyx*, buccal respiration was reported to serve as a defence mechanism to wedge the animal so tightly into a hole or between rocks that it could not be removed by a predator (Smith, 1946), although Templeton (Templeton, 1964) ascribed a respiratory role to these buccal movements. In the lizard *Sauromalus obesus*, the mechanism of buccal

Fig. 6. The effects of a forced inflation of the lungs with air at a range of inflation pressures on buccal ventilation in an anaesthetised *Uromastyx* following tracheostomy to separate the thoracic and buccal pumps. The arrows indicate the moments of inflation, immediately after the first buccal contraction. The individual traces show the effects of inflation at increasing pressures. These are, from the left: 2.5, 5.2, 6.6 and $7.4\,cmH_2O$ ($1\,cmH_2O=98.1\,Pa$). Inflation caused immediate cessation of buccal ventilation at all pressures except $2.5\,cmH_2O$, when a single contraction followed inflation. EMG, electromyographic activity.

Pre-vagotomy
Sphincter colli

EMG   100 μV

Lower airway pressure (cmH_2O)   6   0

5 s

Post-vagotomy
Sphincter colli

EMG   50 μV

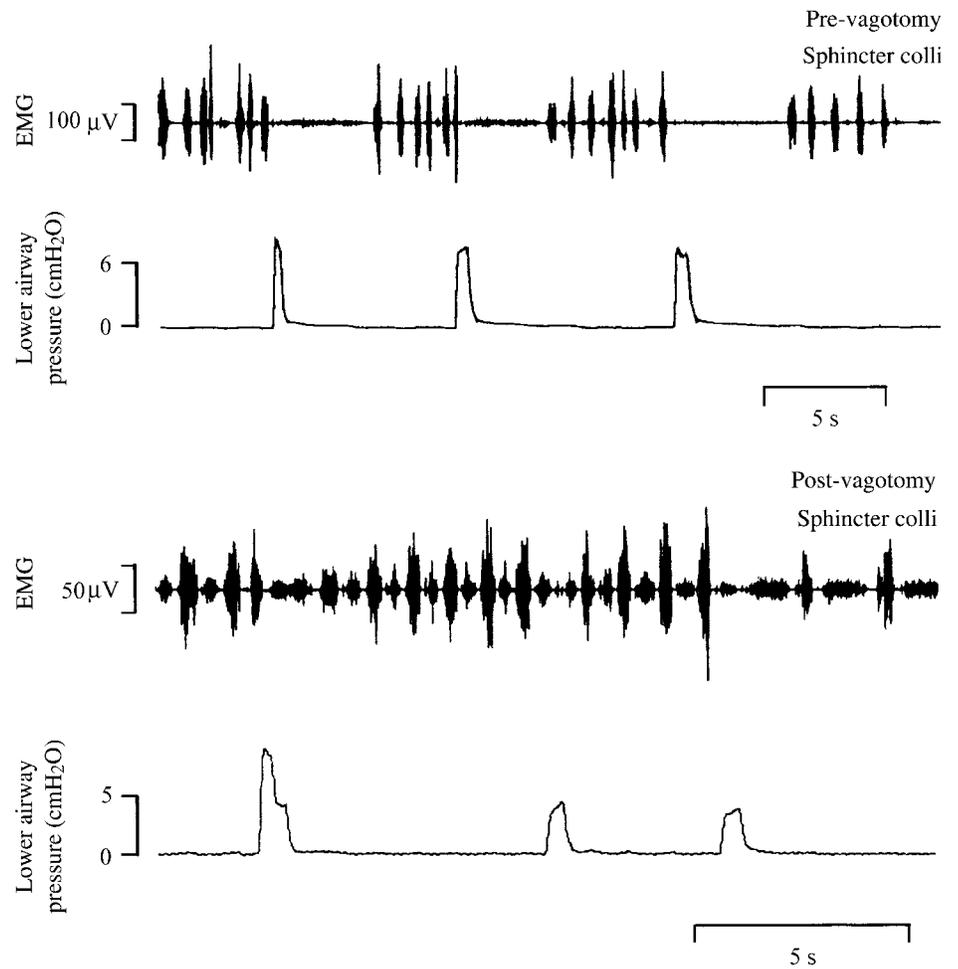Lower airway pressure (cmH_2O)   5   0

5 s

Fig. 7. The effects of a forced inflation of the lungs with air on prolonged bouts of buccal ventilation in an anaesthetised *Uromastyx* (A) before bilateral thoracic vagotomy and (B) following bilateral thoracic vagotomy. Transient inflation of the lungs in the intact animal caused immediate cessation of buccal contractions, with recovery after 4–5 s. Following bilateral vagotomy, lung inflation was no longer effective in arresting the buccal pump. EMG, electromyographic activity. $1\,cmH_2O=98.1\,Pa$.

3% CO_2 10% CO_2

EMG (intercostal)   100 μV

EMG (sphincter colli)   40 μV

Lower airway pressure (cmH_2O)   5   0

Buccal pressure (cmH_2O)   5   0
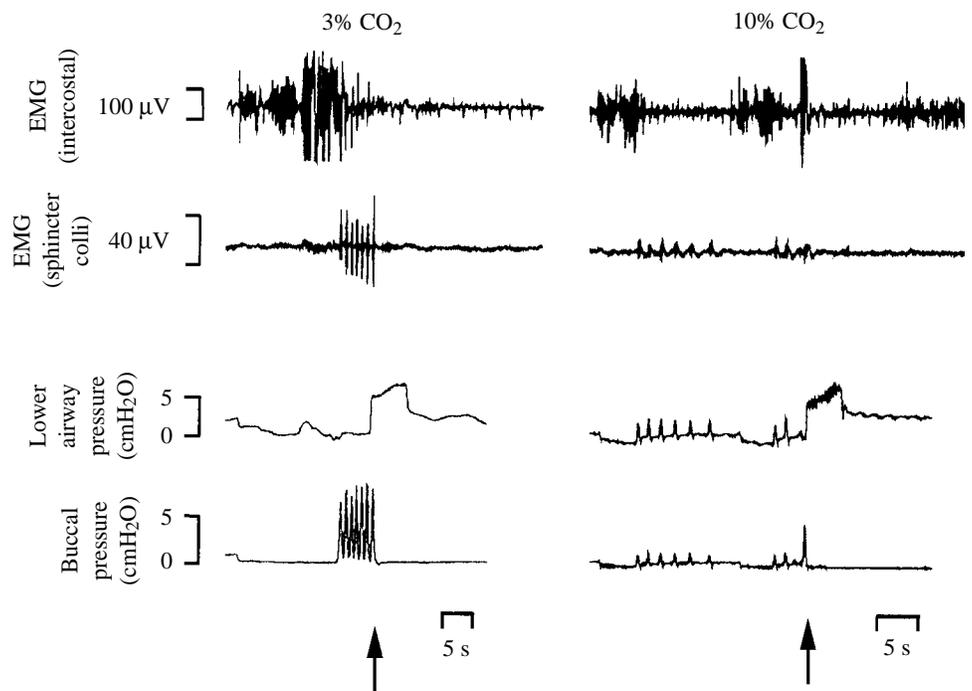
5 s   5 s

Fig. 8. The effects of a forced inflation of the lungs with CO_2-rich air on buccal ventilation in an anaesthetised *Uromastyx*. Transient inflation of the lungs caused immediate cessation of buccal contractions with both 3 % and 10 % CO_2. EMG, electromyographic activity. $1\,cmH_2O=98.1\,Pa$.

pumping (the swallowing mechanism) begins as the buccal pouch expands and air is drawn in through the nares; the tongue is then raised and placed against the roof of the closed mouth, effectively sealing off the internal nares. The glottis then opens, and the buccal pouch contracts, forcing air into the lungs (Templeton, 1964). Cragg (Cragg, 1978) classified the throat movement in reptiles into three types. First, a bucco-pharyngeal pump, which is similar to an amphibian-type pump (Gans, 1970). This type of pump has been reported only in lizards and does not involve respiratory action. The other two types were termed gular pulsations; one occurs randomly and the other in sequence with all thoracic movements.

In this study on *Uromastyx aegyptius microlepis*, the buccal pump was always activated after a sequence of thoracic movements and made a clear contribution to lung inflation. Pressures of 6–17 cmH$_2$O were recorded from the isolated buccal pump, and pumping the air by buccal movements clearly resulted in inflation of the lungs. The tidal volume of thoracic breathing was almost double the buccal tidal volume. So, the present study has unequivocally demonstrated that *Uromastyx* possesses both the buccal pump of amphibians and the aspiration pump characteristic of mammals. These two mechanisms are temporally separated and never occur simultaneously because buccal breaths are always initiated immediately after expiration-II of a thoracic breath.

Evidence of neural control of the buccal pumping mechanism was sought in the present study by investigating lung stretch-receptor reflexes. By mechanically separating the inflationary pressures associated with the two breathing patterns, it was possible to show that the buccal pump is under the influence of a powerful Hering–Breuer reflex. A range of air pressures were used to inflate the lungs, and all but the lowest were equally effective in stopping the buccal pump. This indicates that *Uromastyx* has both intrapulmonary stretch receptors with a low threshold and a strong inspiratory-terminating reflex, mediated through the vagus nerve and similar to that described for mammals.

It should be emphasised that these results do not establish that the buccal pump plays a respiratory role. Although the consistent recruitment of the buccal pump after each thoracic breath, at high temperatures, implies that it has a respiratory role, buccal pumping may also contribute to thermoregulation or to inflation of the animal for defence or courtship displays, as mentioned above. At high temperatures, the rate of buccal pumping was equal to, and transiently synchronous with, that of the heart beat (Al-Ghamdi, 1995). Again, this may either affect gas exchange or possibly the rate of cooling by a countercurrent mechanism in the airways. Clearly, more work is needed to test these speculative hypotheses.

Hering–Breuer-type reflexes have been widely reported in the vertebrates. Slowly adapting pulmonary stretch receptors are important in providing information, *via* vagal afferents, about the rate and depth of lung inflation. Lung stretch receptors have been identified in lungfish, amphibians, reptiles and mammals (Milsom and Jones, 1985; Coleridge and Coleridge, 1986; Shelton et al., 1986). In mammals, the slowly

adapting pulmonary stretch receptors are inhibited by airway CO$_2$ (Scheid and Piiper, 1986; Shelton et al., 1986), whereas in birds the intrapulmonary chemoreceptors are extremely sensitive to intrapulmonary CO$_2$ but relatively insensitive to lung stretch (Scheid and Piiper, 1986).

The presence and functional characteristics of pulmonary stretch receptors in reptiles have been reviewed recently (Wang et al., 1998). Pulmonary stretch receptors, sensitive to changes in lung volume, have been described in turtles, lizards, snakes and alligators. Snakes and alligators have both rapidly and slowly adapting lung stretch receptors (Furilla and Bartlett, 1988; Powell et al., 1988). Consistent with results in mammals, the sensitivity of these receptors is depressed or even silenced by CO$_2$. In the tegu lizard *Tupinambis nigropunctatus*, the lung contains two receptor systems, one of which is sensitive to intrapulmonary CO$_2$ concentration but not to stretch of the lung, while the other shows a rapidly increasing discharge frequency when the lung is stretched, but is insensitive to intrapulmonary CO$_2$ levels (Fedde et al., 1977). A CO$_2$-sensitive receptor has also been reported in tortoises (Jones and Milsom, 1979; Milsom and Jones, 1976; Milsom and Jones, 1979; Ishii et al., 1986). *Uromastyx* appears to employ only the second receptor type because the lung-inflation reflex was apparently unaffected by inclusion of CO$_2$ (3–10 %) in the gas mixture used to inflate the lungs.

In *Uromastyx*, the amplitude and duration of intercostal muscle EMG activity were approximately doubled after vagotomy. Bilateral vagotomy alters the breathing pattern, producing an elevation in tidal volume, in both the Tokay lizard (*Gekko gecko*) (Milsom, 1984) and alligators (Douse and Mitchell, 1992), emphasising the role of vagally mediated reflex control in the regulation of lung ventilation in reptiles. Bilateral vagotomy also removed the inhibitory effect of lung inflation on buccal ventilation in *Uromastyx*. This supports the presence of a functional Hering–Breuer reflex regulating both the buccal pump and the aspiration pump, with its afferent limb running through the vagus nerve.

The Hering–Breuer reflex has previously been reported in lizards and snakes. In lizards, the discharge frequency of the receptors was rapidly increased when the lung was stretched. These receptors are variably sensitive to CO$_2$ (Fedde et al., 1977). In some snakes, the breathing pattern is influenced by a vagally mediated, volume-related feedback with a weak reflex effect (Bartlett et al., 1986). Both slowly and rapidly adapting receptors exist in amphibian lungs, and they respond to changes in lung volume. This mechanism limits the degree of inflation (Tenney and Leiter, 1995). Stretch receptors, associated with a Hering–Breuer-type reflex, have also been described in some fishes. Work with dogfish indicated that inflation of the pharynx reflexly inhibited ventilation (Satchell, 1959; Satchell and Way, 1962). There are also previous reports of lung-inflation-inhibiting activity in a buccal pump, both in the African lungfish *Protopterus annectens* (Pack et al., 1992) and in the toad *Bufo marinus* (Wang et al., 1999). The demonstration that this is also the case in *Uromastyx* provides strong supporting evidence of a role in lung inflation for the

buccal pumping action observed at high temperatures in this lizard and implies a respiratory role for this inflation. The co-existence of two different respiratory motor mechanisms in an extant amniote may offer novel perspectives on the central integration of these different neural outflows.

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