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THE ROLE OF VAGAL AFFERENT INFORMATION AND HYPERCAPNIA IN CONTROL OF THE BREATHING PATTERN IN CHELONIA

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SUMMARY

The normal breathing pattern of the turtle, *Chrysemys picta* (Schneider), consists of periods of continuous breathing interspersed with periods of breath holding. During each ventilatory period respiratory frequency and tidal volume are controlled independently. There is a large variability in inspiratory and expiratory gas-flow rates yet tidal volumes are maintained within narrow limits by adjustments of the lengths of the active inspiratory and expiratory intervals. Lung volume information carried within the vagus nerve is responsible for the careful regulation of tidal volume as well as for modulation of the air flow rates and lowering of the threshold of the mechanism initiating expiration following breath holding.

Increases in pulmonary minute ventilation during hypercapnia are caused by increases in respiratory frequency due solely to a shortening of the periods of breath holding. There is some increase in tidal volume but the breath length remains constant and thus the frequency of breathing within each ventilatory period also remains constant.

After vagotomy, changes in minute ventilation due to hypercapnia stem primarily from changes in tidal volume while changes in respiratory frequency are greatly reduced.

INTRODUCTION

A pattern of arrhythmic breathing consisting of a series of one to several breaths separated by a highly variable, respiratory pause (from a few seconds to several hours) commencing at end-inspiration has been described for most species of reptiles (turtles, McCutcheon, 1943; Gans & Hughes, 1967; crocodiles, Naifeh *et al.* 1970; Gans & Clark, 1976; snakes, Glass & Johansen, 1976). Despite the similarities between this breathing pattern and various patterns of abnormal breathing in mammals with defective central respiratory control, evidence indicates that reptiles accurately adjust ventilation to maintain blood pH at a specific, temperature-dependent value (cf. Howell & Rahn, 1976, for review). Although many studies have analysed the effects of respiratory stimuli such as hypoxia, hypercapnia and temperature in reptiles, these studies primarily assess the effects upon metabolism, total pulmonary ventilation or dive length in aquatic species (cf. Wood & Lenfant, 1976, for review).

Very little is known about the factors and mechanisms controlling the arrhythmic breathing pattern.

Hypoxia appears to be the major factor controlling respiration in reptiles (Nielsen, 1961, 1962; Templeton & Dawson, 1963; Glass & Johansen, 1976; Wood & Lenfant, 1976) including turtles (Lenfant *et al.* 1970), yet many of these animals exhibit an incredible tolerance to anoxia (Belkin, 1963*a*, *b*, 1968). Reports on the effects of hypercapnia on ventilation in turtles range from a slight or moderate increase (Millen, Murdaugh & Robin, 1963; Wood & Lenfant, 1976) to a powerful stimulation (Jackson, Palmer & Meadow, 1974). Many of these discrepancies arise from species differences, levels of anaesthesia and the presence or absence of avenues of cutaneous gas exchange. Furthermore, in many studies only ventilation rates and not volumes were measured, which makes comparison of total ventilatory effort in different studies very difficult.

The present study was undertaken to describe and analyse the breathing pattern in the turtle *Chrysemys picta*. The role of pulmonary afferent information carried in the vagus nerve, with and without concomitant hypercapnia (at normoxia and constant temperature), have been studied as a first step in assessing the mechanisms involved in the control of this breathing pattern.

METHODS

Surgical procedures

Experiments were performed on unanaesthetized, lightly restrained specimens of the freshwater turtle, *Chrysemys picta* (600–1200 g), at room temperature (22–23 °C). The optimum temperature range for this temperate species is 20–25 °C (Cagle, 1954). Using a combination of cold (1–4 h at −20 °C) and local anaesthesia (2% lidocaine hydrochloride), a tracheal cannula was inserted and the vagi bilaterally exposed. A pneumotachograph with a side arm for tracheal pressure measurement and gas sampling was attached to the tracheal cannula and, in the majority of cases, the open end of the pneumotachograph was attached to a plastic T-piece. One arm of the T was open to atmosphere and the other was attached to a gas supply. Using a system of air flow meters, the composition of the gas flowing past the end of the tracheal cannula could be altered thus controlling the composition of the inspired air when the turtle breathed. In the intact animal, respiratory pauses occur at end-inspiration and lung volume during the pause is not only variable but influences the respiratory frequency (Milsom & Johansen, 1975). With our procedure, the lung remained open to atmosphere throughout the respiratory pause and thus lung volume returned to a constant functional residual volume during this period eliminating this variable. To establish whether the maintenance of a constant functional residual capacity influenced the normal, resting breathing pattern or time course of the various respiratory phases, the distal end of the tracheal cannula was re-attached to the cut central end of the trachea on several occasions. These animals breathed through an intact glottis and showed no differences in the measured variables from the experimental animals. However, as a consequence of holding the end-respiratory (breath hold) volume constant, any effects of CO_2, vagotomy, tidal volume or breath hold length on this volume will not have been observed.

Recording techniques

Tracheal pressure was measured with a Statham P23V pressure transducer and the pressure drop across the pneumotachograph screen during tracheal air flow with a Hewlett-Packard 268 BC differential pressure transducer. The air flow signal was fed through a Hewlett-Packard 350-3700A integrating preamplifier to give tidal volume; pressure, flow and volume, were continuously recorded on a Sanborn 4 channel chart recorder writing on rectilinear co-ordinates. The O_2 and CO_2 composition of inspired and expired gases was determined either on samples taken through the side arm of the tracheal cannula and measured on a Fisher–Hamilton gas-partitioner or by continuous sampling with a Centronic 200 MGA clinical mass spectrometer (sample rate < 10 ml./min).

Experimental protocol

Animals were allowed to recover from anaesthesia for 4–6 h before experimentation began. The animals were surrounded by opaque screens to shield them from all activities of the experimenters and when resting quietly were presented with mixtures of 0, 5 or 10% CO_2 in air to breathe for periods of 1 h or more in random order. All measurements were recorded continuously but data were selected for analysis only after the responses to each gas mixture had stabilized. Each of these periods represents one trial (*n*). It must be stressed that due to the incompletely divided ventricle of the turtle heart there can be right to left shunting of blood caused by pulmonary arterial vasoconstriction (White, 1976; Milsom, Langille & Jones, 1977; Burggren, 1977), and thus turtles may maintain a considerable partial pressure difference between alveolar and arterial CO_2 for long periods of time following changes in the composition of inspired gases (Glass, Burggren & Johansen, 1979). Consequently, care was taken to ensure that ample time was allowed for stable responses to develop (no changes in mean values of measured variables over 30 min) before any measurements were made.

On the second day of experimentation, the vagi were sectioned under local anaesthesia and the above protocol repeated.

Measurements and analysis

The intervals of the various respiratory phases were all measured from the air flow recordings. The slopes and correlation coefficients of all graphs were computed by simple linear regression analysis of the data on a Digital PDP-12 computer. Unless otherwise stated all values are means ± S.E.

RESULTS

The breathing pattern during normocapnia and hypercapnia, before and after vagotomy

After a variable period of breath holding in the inspiratory position (non-ventilatory period, NVP) a series of one to several breaths commenced with an active expiration and terminated in the end-inspiratory phase (Fig. 1*a*, *c*). This constituted the ventilatory period (VP) (Fig. 1*c*). Each ventilatory period commenced with active expiration regardless of whether the animal had an intact or bypassed glottis (i.e. regardless of lung volume). The time interval of each breath (T_{tot})

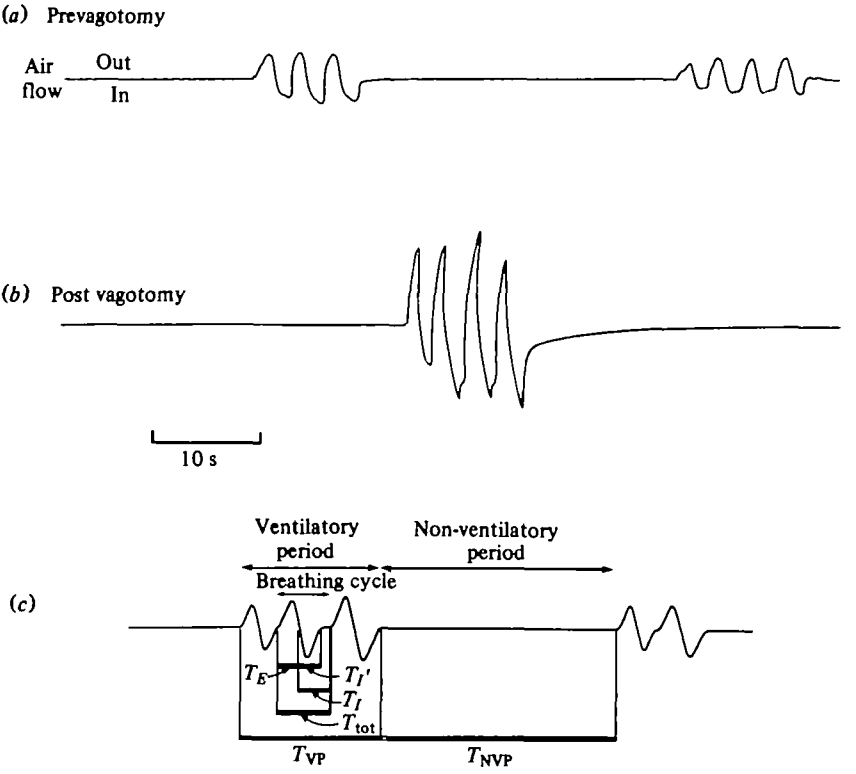

Fig. 1. Representative records of the normal breathing pattern of a turtle (*a*) and the effects of vagotomy (*b*) on this pattern. (Expiration results in an upward deflection of the flow traces; inspiration in a downward deflexion.) (*c*) Schematic diagram of the breathing pattern (taken from the flow trace) in a turtle illustrating the various respiratory intervals. See text for the explanation of all abbreviations.

consisted of the expiratory interval (T_E) and the subsequent inspiratory interval (T_I) which usually consisted of an active inspiratory phase (T_I') (this phase would be similar to the T_I in mammals), and a short interval during which the breath was held at end-inspiration ($T_I - T_I'$) (Fig. 1*c*). The distinction between $T_I - T_I'$, and T_{NVP} is an arbitrary one. Any period of apnoea longer than the mean breath length ($\simeq$ 5·5 s) was designated T_{NVP}. It should be noted, however, that ($T_I - T_I'$) was generally less than 2 s whereas T_{NVP} was on average 12–50 times larger.

Table 1 lists the mean values ($\pm$ S.E.) of several of the variables measured for each trial (n) in 16 animals. Increasing the percentage of CO_2 in the inspired gas (F_{I,CO_2}) resulted in an increased frequency of breathing f (calculated on all breaths taken per unit time), tidal volume (V_T) and total pulmonary ventilation ($\dot{V}_E$). The incidence of ventilatory periods (VP.min^{-1}) increased as a result of the large reduction in the duration of the nonventilatory period (T_{NVP}), while the number of breaths within each ventilatory period (breaths.VP^{-1}) also increased. Since the mean duration of each individual breath (T_{tot}) did not change noticeably, the frequency of breaths within each ventilatory period (f_{VP}) remained unchanged, while the duration of each ventilatory period (T_{VP}) increased as a result of the larger number of breaths in eac

Table 1. *Ventilatory variables of turtles breathing air and CO_2 gases*

	Air				5% CO_2+air				10% CO_2+air			
	n	pre vago-tomy	n	post vago-tomy	n	pre vago-tomy	n	post vago-tomy	n	pre vago-tomy	n	post vago-tomy
f (min^{-1})	31	1·8 ±0·2	10	0·9 ±0·1	16	3·9 ±0·6	4	1·6 ±0·3	19	5·4 ±0·8	5	1·8 ±0·6
f_{VP} (min^{-1})		13·5 ±2·0		9·0 ±1·6		12·2 ±2·7		9·0 ±1·4		13·5 ±2·0		8·4 ±2·0
V_T (ml BTPS.kg^{-1})		13·5 ±0·9		50·6 ±8·4		17·5 ±2·0		82·4 ±24·2		32·6 ±4·8		112·7 ±20·8
$\dot{V}_E$ (ml BTPS.min^{-1}.kg^{-1})		24·8 ±3·4		44·9 ±6·1		65·5 ±11·4		125·1 ±46·8		167·6 ±35·8		184·9 ±47·4
VP.min^{-1}		0·9 ±0·1		0·4 ±0·1		1·4 ±0·3		0·5 ±0·1		1·8 ±0·3		0·5 ±0·1
Breaths.VP^{-1}		2·4 ±0·2		2·6 ±0·4		3·1 ±0·4		3·2 ±0·5		3·2 ±0·3		3·4 ±0·5
T_{tot} (sec)		5·6 ±0·5		6·5 ±0·5		6·0 ±0·7		6·5 ±0·5		5·6 ±0·5		7·1 ±0·9
T_E (sec)		2·6 ±0·1		2·1 ±0·1		2·5 ±0·1		2·3 ±0·2		2·3 ±0·1		2·1 ±0·1
T_I' (sec)		2·6 ±0·1		2·5 ±0·1		2·8 ±0·1		2·6 ±0·2		2·4 ±0·1		2·8 ±0·1
T_I (sec)		3·2 ±0·1		4·3 ±0·3		3·7 ±0·2		3·6 ±0·4		3·0 ±0·1		5·1 ±0·6
T_{VP} (sec)		14·0 ±2·0		16·6 ±2·1		18·9 ±3·2		21·1 ±4·3		18·1 ±2·2		24·3 ±4·2
T_{NVP} (sec)		93·9 ±18·0		169·6 ±39·6		41·2 ±10·8		124·3 ±36·3		24·5 ±7·8		127·8 ±45·1
T_{VP+NVP} (sec)		110·1 ±18·0		185·6 ±40·5		63·4 ±12·5		145·5 ±33·2		48·2 ±9·0		152·8 ±43·1
$T_{VP}/_{(VP+NVP)}.100$		16·2 ±2·4		9·5 ±1·4		36·1 ±4·1		17·4 ±5·0		49·6 ±5·9		25·5 ±10·6

As a consequence of this increase in T_{VP} and the decrease in the subsequent T_{NVP}, the percentage of time spent actively breathing $T_{VP/(VP+NVP)}.100$ increased.

The observed increases in tidal volume when animals were breathing the CO_2 gas mixtures were due to an increase in both force and rate of expiration and inspiration. Despite the increased respiratory drive the breathing pattern remained arrhythmic. At the maximum ventilatory frequencies we measured, under severe hypercapnic stress ($F_{I,CO_2} = 15\%$), breathing became virtually continuous and rhythmic as T_{NVP} was eliminated and f approached f_{VP}, the duration of each breathing cycle being unchanged.

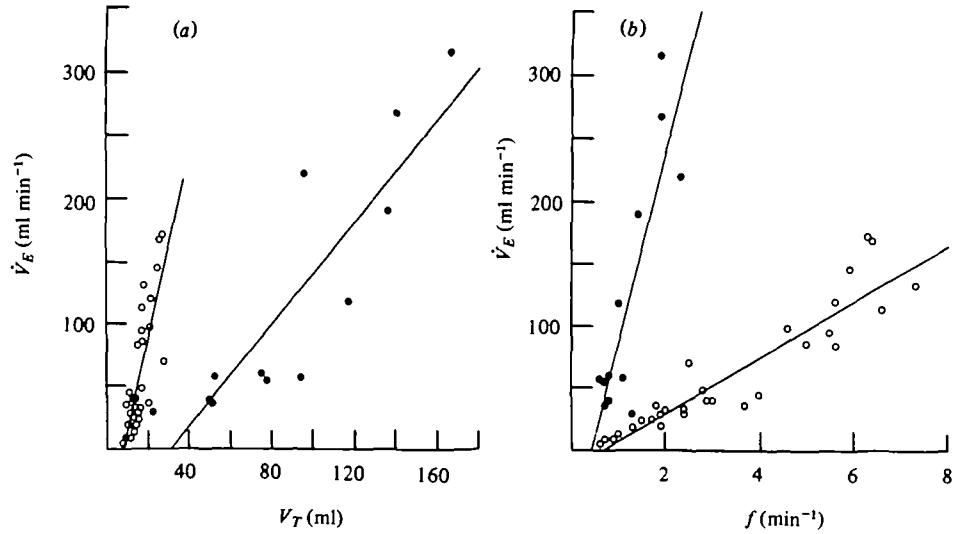

Fig. 2. The relationships between (*a*) minute ventilation ($\dot{V}_E$) and tidal volume (V_T) and (*b*) $\dot{V}_E$ and respiratory frequency (f) for turtles breathing air and CO_2 gas mixtures. Values recorded in intact turtles are represented by open symbols, those recorded post-vagotomy by closed symbols (each symbol represents the mean value for ten successive breaths during one trial in one animal).

The effect of vagotomy on the breathing pattern, studied in five animals (Fig. 1*b*, Table 1), was to decrease the respiratory frequency and increase tidal volume and total pulmonary ventilation. The number of breaths per ventilatory period increased slightly but the frequency of ventilatory periods decreased. The average breath length was prolonged and thus the breathing frequency within each ventilatory period decreased. The net effect of these changes was a slight lengthening of T_{VP} but since T_{NVP} was greatly lengthened the proportion of time spent actively breathing decreased. Increasing the CO_2 concentration in the inspired air of vagotomized animals produced similar trends to those observed in intact animals. Although the amount of time spent actively breathing was less after vagotomy, the presence of 5 and 10% CO_2 in the inspired air produced proportionately the same effect in normal and vagotomized animals, a doubling and tripling respectively of the amount of time spent actively breathing compared to ventilation with room air.

The tidal volume in intact animals breathing room air was approximately 10% of the lung volume (FRC+V_T) (Milsom, 1975). Following vagotomy, tidal volume increased to 39% of the lung volume. Addition of 10% CO_2 to the inspired gas mixture raised these values to 25% and 97% for intact and vagotomized animals respectively.

Regulation of minute ventilation during normocapnia and hypercapnia, before and after vagotomy

In the intact turtle, increases in $\dot{V}_E$ stemmed primarily from increases in f with a relatively small contribution from changes in V_T (Fig 2, open circles). These relationships were not altered by hypercapnia. Following vagotomy both relations remained linear (Fig. 2, closed circles) but now increases in $\dot{V}_E$ were primarily th

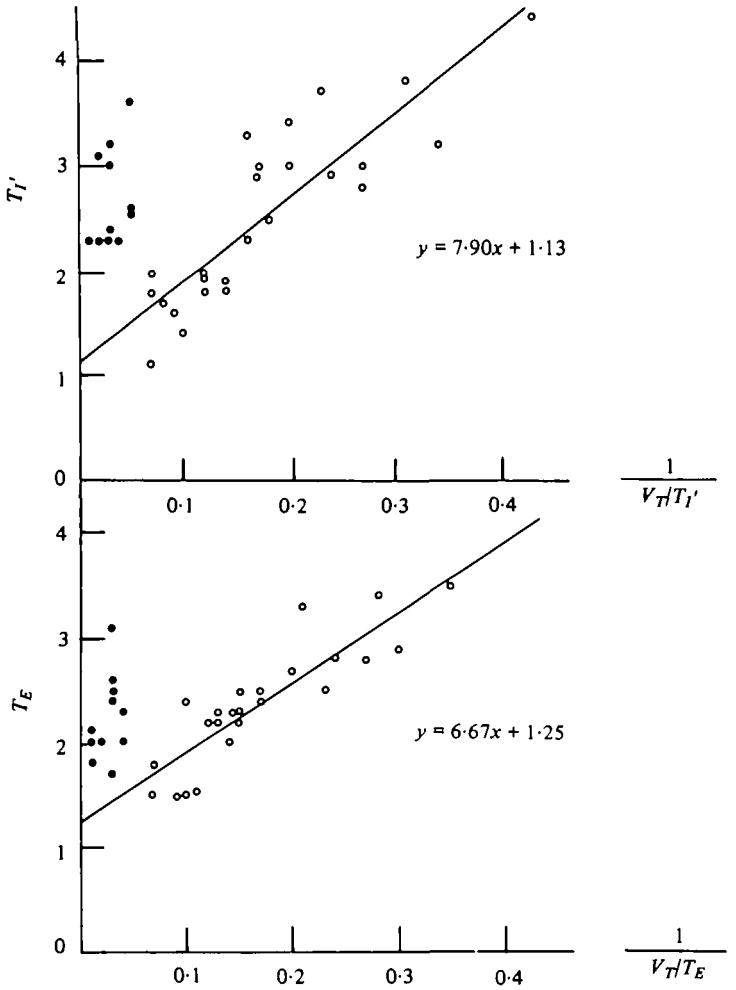

Fig. 3. The relationship between the inspiratory interval (T_I') and the reciprocal of the inspiratory flow rate ($V_T . T_I'^{-1}$) (*a*) and between the expiratory interval (T_E) and the reciprocal of the expiratory flow rate ($V_T . T_E^{-1}$) and (*b*) in intact (open circles) and vagotomized (closed circles) turtles breathing air (symbols as in Fig. 2).

result of increases in V_T. Some acceleration of respiratory frequency in response to increased respiratory drive did remain after the vagi were cut. These relationships were also unaffected by hypercapnia.

Fig. 3 (open circles) shows a strong linear correlation between the inspiratory interval and the inspiratory flow rate (r = 0·863) as well as between the expiratory interval and the expiratory flow rate (r = 0·874). The slopes of these relationships indicated that T_I' and T_E were actively adjusted so that only a small change occurred in V_T despite wide-ranging inspiratory and expiratory air flow rates in animals breathing air. Hypercapnia did not alter these relations.

When all volume-feedback information was removed by vagotomy, the intervals of active inspiration and expiration were restricted in range and independent of tidal

volume. They no longer strongly correlated with the inspiratory and expiratory flow rates (Fig. 3, closed circles) and thus tidal volumes increased, presumably in direct proportion to the central respiratory drive. Unlike mammals, T_I' and T_E values were not equal to maximum prevagotomy values (Bradley *et al.* 1974*a*, *b*).

There was a good correlation between T_I' and T_E ($r = 0{\cdot}80$) which was unaffected by levels of CO_2 and remained following vagotomy. This correlation must therefore be established by some central mechanism which was not influenced by volume feedback information from the lungs.

It should be noted that the respiratory frequency computed during the ventilatory period (f_{VP}) remained constant despite CO_2-induced ventilatory responses (Table 1) and that, although vagotomy resulted in a decreased f_{VP}, this frequency also remained constant as ventilation was increased by hypercapnia.

T_{NVP} was not correlated to either the preceding or following V_T. Such a correlation is not shown by the data of Glass & Johansen (1976) for the snake, although it is argued to be present in the lizard by Jammes & Grimaud (1976). T_{NVP} was, however, strongly correlated to f ($\log f = 1{\cdot}533 - 0{\cdot}688 \log T_{NVP}$; $r = 0{\cdot}899$) and to $\dot{V}_E$ ($\log \dot{V}_E = 2{\cdot}995 - 0{\cdot}857 \log T_{NVP}$; $r = 0{\cdot}875$) so that T_{NVP} decreased as f and $\dot{V}_E$ increased. The similarity between these two relations depicts the major role of changes in frequency in determining changes in $\dot{V}_E$ in the normal intact animal. Since T_{tot} and hence f_{VP} remained relatively constant as $\dot{V}_E$ increased during CO_2 breathing, T_{NVP} was the major determinant of f. CO_2 did not alter the shape of these relations.

Vagotomy did not alter the T_{NVP}, f relationship but did alter the T_{NVP}, $\dot{V}_E$ relationship ($\log \dot{V}_E = 4{\cdot}549 - 1{\cdot}194 \log T_{NVP}$; $r = 0{\cdot}765$). In the absence of lung volume feedback, tidal volume increased dramatically and $\dot{V}_E$ was met at lower respiratory frequencies. Consequently, T_{NVP} rarely fell below a value of 75 s.

DISCUSSION

In resting, spontaneously breathing animals the respiratory pattern was similar to that recorded by other researchers (see Wood & Lenfant, 1976, for review). The mean values recorded for f, V_T and $\dot{V}_E$ (Table 1) fall within the lower range of values reported in the literature for these variables in turtles (McCutcheon, 1943; Millen *et al.* 1963; Frankel *et al.* 1969; Jackson, 1971, 1973; Jackson *et al.* 1974). It is more difficult to find reported values for comparison with the other respiratory variables but published values of T_{tot}, Breaths.VP^{-1}, and $T_{VP/(VP+NVP)} \cdot 100$ (McCutcheon, 1943; Belkin, 1968; Frankel *et al.* 1969; Burggren, 1975; Lucey & House, 1977) encompass those values reported here. CO_2 has consistently been reported as a respiratory stimulant in turtles leading to increases in both f and V_T (Randall, Stulken & Hiestand, 1944; Millen *et al.* 1963; Frankel *et al.* 1969; Jackson *et al.* 1974), although there is some discrepancy concerning the sensitivity of turtles to CO_2 and the magnitude of the ventilatory response it causes. Millen *et al.* (1963) reported that 6% CO_2 introduced into the inspiratory gas of *Pseudemys scripta* (temperature not reported) increased ventilation only slightly ($\dot{V}_E$ increased from 31 ml.min^{-1} to 41 ml.min^{-1}, the body weight of the animals was not reported), whereas Jackson *et al.* (1974), using the same gas mixture and the same animal reported a 10× increase in ventilation ($\dot{V}_E$ increased from 23·8 to 215 ml.min^{-1}.kg^{-1}).

The 3× and 7× increases in pulmonary minute ventilation which we observed when *Chrysemys picta* were exposed to 5% and 10% CO_2 respectively in the inspired air support the contention of Jackson *et al.* (1974) that CO_2 is a powerful respiratory stimulant. Further, these levels of CO_2 are well within physiological levels considering reported values for P_{a,CO_2} of 100–130 mmHg following 2 h of diving in *Pseudemys scripta* (Robin *et al.* 1964; Jackson & Silverblatt, 1974). It should be noted that *Pseudemys scripta* has now been reclassified as *Chrysemys scripta* and there appear to be few ecological or physiological differences between this species and the *Chrysemys picta* used in the present study.

Several studies indicate that O_2 depletion and CO_2 accumulation play a major role in determining the length of the breath hold between ventilatory periods in turtles (Lumsden, 1923; Lenfant *et al.* 1970). Although each ventilatory period must then suffice to raise O_2 levels, decrease CO_2 levels and enable breath holding to be resumed, it is difficult to assess how individual breaths and ventilatory periods are regulated. In spontaneously breathing, eucapnic animals, tidal volume is held within very narrow limits by adjusting T_E and T_I' to the highly variable rates of expiration and inspiration. This requires lung volume feedback presumably from pulmonary receptors running in the vagus nerve.

Although there is much variability in expiratory and inspiratory flow rates, there is also modulation of the gas flow rates by vagal volume-related information as shown by the increase in gas flow rates following vagotomy. In mammals, after vagotomy, the T_I values are prolonged to the maximum recorded before vagotomy, which suggests that there is a maximum interval set by central mechanisms which is normally overridden by peripheral inputs. The increase in V_T following vagotomy is due solely to this prolongation of T_I; the levels of inspiratory activity remain constant (Euler & Trippenbach, 1976*a*, *b*). In turtles, however, the values of T_I' and T_E after vagotomy are frequently in the mid-range of those recorded before vagotomy. The large overall increase in V_T and its variability are not due to prolongation of T_I' at a constant level of inspiratory activity as in mammals but solely to increased levels of inspiratory activity. The fact that T_I' and T_E values are not equivalent to the maximum values measured before vagotomy is not surprising considering that lung volume information prolongs as well as shortens the active inspiratory and expiratory intervals to regulate tidal volume.

When F_{I,CO_2} is increased from 0 to 10%, tidal volume increases roughly 2·5 times yet T_{tot}, T_I, T_I' and T_E do not change appreciably. There is an increase in the expiratory and inspiratory flow rates thus the increase in tidal volume must result from the central excitation of the motor output to inspiratory and expiratory muscles. This central excitation occurs in conjunction with both a proportionate rise in the central tidal volume threshold so that an increased tidal volume is ventilated within the same breath length (T_{tot}) maintaining f_{VP} constant and direct inhibition of pulmonary stretch receptor discharge by CO_2 (Milsom & Jones, 1976; Jones & Milsom, 1979). Pulmonary stretch receptor inhibition will lead to increases in tidal volume necessary to restore volume feedback information to previous levels. Changes in the inspiratory threshold curve and depression of pulmonary stretch receptor discharge are also implicated in hypercapnic induced increases in V_T in mammals (Bradley *et al.* 1975).

The contribution of changes in tidal volume to the increase in minute ventilation

during hypercapnia, however, is small; the major contribution comes from an increase in respiratory frequency. These predominant changes in respiratory frequency occur despite the constancy of individual breath lengths. Thus the frequency of breaths within each ventilatory period (f_{VP}) remains constant while the number of breaths per ventilatory period increases 50% and the length of the nonventilatory period decreases; the number of ventilatory periods per minute doubling when F_{I,CO_2} is increased from 0 to 10%. Under conditions of severe hypercapnic stress ($F_{I,CO_2} \geqslant 15\%$) f approaches f_{VP} and T_{NVP} approaches T_I. Changes in the length of the nonventilatory period appear to be favoured over changes in the rate or depth of active ventilation.

After vagotomy, changes in $\dot{V}_E$ due to hypercapnia are due primarily to changes in V_T. As in mammals (Widdicombe & Winning, 1974; Bradley *et al.* 1974*a*, *b*, 1975), changes in respiratory rate are greatly reduced. After vagotomy, the breath hold interval (T_{NVP}) is lengthened. Since there is a poor correlation between T_{NVP} and V_T ($r = 0{\cdot}495$), which is unchanged by vagotomy, this cannot be due to removal of phasic vagal input but must stem either from removal of excitatory tonic vagal influence from the mechanism initiating respiration following breath holding, or from a decreased sensitivity of this mechanism to O_2 depletion and CO_2 accumulation. Hypercapnia still acts to shorten T_{NVP} but its effects on respiratory frequency are somewhat offset by the increased breath length.

The evidence indicates that although central integration of volume information carried within the vagus in turtles is quite different from that described in mammals, it is essential for normal ventilatory control. The net result of this integration is a breathing pattern where the major controlled variable is the breath hold length. Thus, even during severe hypercapnia there are only relatively small changes in tidal volume and no change in the active ventilation rate (f_{VP}).

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