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SOME REFLEX CARDIOINHIBITORY RESPONSES IN THE CAT AND THEIR MODULATION BY CENTRAL INSPIRATORY NEURONAL ACTIVITY

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SUMMARY

1. Cats were anaesthetized with a mixture of chloralose and urethane, and were artificially ventilated.

2. An open pneumothorax was provided by two large-bore tubes which were sealed in the sixth intercostal space on each side. They were connected to a Fleisch pneumotachograph. Phasic changes in central inspiratory neuronal activity were measured quantitatively as changes in the volume of the pneumothorax during temporary interruption of artificial respiration, the volume of the lungs being held constant at their end-expiratory level. In this way the activity of slowly adapting pulmonary stretch receptors was maintained constant.

3. Reflex cardioinhibitory responses were elicited by stimulation of (a) the carotid body chemoreceptors by intracarotid injections of cyanide; (b) the arterial baroreflex by controlled elevations of the blood pressure; (c) cardiac receptors by left atrial injections of veratridine; and (d) pulmonary C fibres (including J receptors) by right atrial injections of phenylbiguanide.

4. The effects of central inspiratory neuronal activity on pulse interval were assessed by comparing the values observed during the inspiratory and expiratory phases of the respiratory cycle in the control state and during stimulation of each cardiovascular receptor group.

5. The carotid chemoreceptor-induced bradycardia measured during the expiratory phase of respiration was reduced during inspiration to a value of about 15% of control. The central inspiratory drive was less effective in altering the reflex responses from the arterial baroreceptors and cardiac receptors, the corresponding values being 42 and 51% respectively.

6. In contrast, the bradycardia evoked by pulmonary C fibre stimulation was not significantly affected by the central inspiratory drive.

7. The differential nature of the modulation by the central inspiratory drive occurred independently of the integrity of the sympathetic nerve supply to the heart indicating that the cardiac efferents involved were largely fibres in the vagus nerves.

8. The possible explanation of these results in terms of central mechanisms is discussed.

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INTRODUCTION

It has been shown previously that the effectiveness of excitatory inputs from baroreceptors and chemoreceptors to cardiac vagal motoneurones is dependent on the phase of the respiratory cycle. The effects of such stimuli are maximal during the expiratory phase of respiration; during the inspiratory phase, the cardiac vagal motoneurones are partly or wholly refractory to such excitatory stimuli. This inspiratory modulation of the cardiac vagal motoneurones is due to two components: an increase in central inspiratory neuronal activity and an increased activity of slowly adapting pulmonary stretch receptors (Anrep, Pascual & Rössler, 1936*a*, *b*; Davidson, Goldner & McCloskey, 1976; Gandevia, McCloskey & Potter, 1978; McAllen & Spyer, 1978*a*, *b*; Potter, 1981; Daly & Kirkman, 1989). It appears, however, that not all excitatory inputs to cardiac vagal motoneurones are affected in a similar way. Whereas the cardioinhibitory responses resulting from excitation of carotid chemoreceptors and of cardiac receptors were potentiated under conditions of a reflexly induced apnoea (Daly, Kirkman & Wood, 1988), the response resulting from stimulation of pulmonary C fibres was little affected (Daly & Kirkman, 1988). This finding implied that the excitatory input from pulmonary C fibres to cardiac vagal motoneurones, in contrast to that from each of the other three receptor groups, was unaffected by changes in either the central inspiratory neuronal activity or the activity of pulmonary stretch afferents.

A study by Daly & Kirkman (1989) on the second of the two components went some way towards substantiating this view. They showed that in the absence of central inspiratory neuronal activity, inflation of the lungs wholly or partly inhibited the bradycardia resulting from stimulation of the carotid chemoreceptors and baroreceptors and of cardiac receptors, but was without effect on that due to excitation of pulmonary C fibres.

The possibility has now been examined that there is also a differential modulation by the activity of the central inspiratory neurones on the excitatory input from various cardiovascular receptors. The results of the study are described in this paper and have been reported briefly elsewhere (Daly, 1990).

METHODS

Cats of either sex and varying in weight from 2·5 to 5·03 kg (mean $3{\cdot}21 \pm 0{\cdot}8$ (S.D.) kg) were anaesthetized with an intraperitoneal injection of a mixture of 2% α-chloralose (Kuhlmann, Paris; or Vickers Laboratories Ltd, UK; 52 mg kg^{-1}) and 20% urethane (British Drug Houses Ltd; 520 mg kg^{-1}) dissolved in 85 parts of sodium chloride solution (154 mM) and 15 parts of polyethylene glycol (mol. wt 200; 'Carbowax', Union Carbide Ltd).

Respiration

The arrangement for artificial ventilation of the lungs is shown in Fig. 1. A tracheostomy tube was inserted, and a bilateral pneumothorax was created by insertion of a 10 mm bore brass tube in the left and right sixth intercostal spaces near the sternum. Positive pressure ventilation was applied by means of a Starling 'Ideal' pump via a four-way tap and the lungs collapsed passively against a resistance of 1·5–3 cmH_2O pressure provided by the water trap *a*. The inspired O_2 concentration was maintained at about 35%.

Central respiratory neuronal activity was measured in the absence of lung movements in the following way: the four-way tap (Fig. 1) was turned clockwise by 90 deg. This procedure

temporarily disconnected the lungs from the pump which now vented through port *X*. The lungs collapsed passively against a similar resistance provided by water trap *b* and were thus held at constant volume. The two tubes that had been passed through the chest wall were connected to a pneumotachograph (Godart). In the absence of movements of the lungs, changes in volume of the

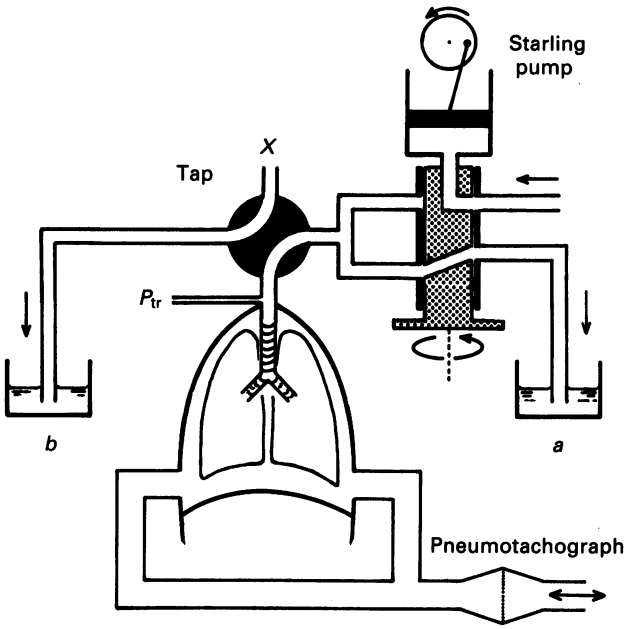

Fig. 1. Diagram of the arrangement of (1) artificial ventilation of the animal by means of a Starling 'Ideal' pump and (2) temporarily disconnecting the pump from the animal and measuring quantitatively changes in the volume of the pneumothorax in the absence of lung movements, as an index of the output of the respiratory centres. The pump is shown in its 'expiratory' position during normal artificial ventilation, with the lungs collapsed passively against a pressure provided by water trap *a*. The four-way tap allows selection of the mode of artificial inflation of the lungs (as shown), or, by turning it 90 deg clockwise, of cessation of lung movements for measurement of changes in the volume of the pneumothorax by means of a pneumotachograph. During this latter mode, lung volume is held constant at an expiratory pressure provided by a second water trap (*b*), the pressure being the same as provided by *a*. Port *X* is used for venting the pump. Turning the tap a further 90 deg clockwise restored normal artificial ventilation. The stippled area represents the rotary valves on the Starling pump controlling the lung inflation and passive deflation phases of the respiratory cycle, which are linked to the movement of the piston. P_{tr}, tracheal pressure. For clarity, the remainder of the animal is not shown. For further details, see text.

pneumothorax (V_{pn}) provided a quantitative measure of the phasic output of the respiratory centres through movements of the diaphragm and rib-cage. At the end of the period of observation, usually 10–15 s, the tap was turned through a further 90 deg so that normal artificial respiration was resumed. It follows that the changes in volume the pneumothorax measured *during* artificial respiration are due to a combination of phasic changes in lung volume controlled by the pump, and in movements of the diaphragm and rib-cage through activity of the respiratory centres.

In some experiments the background central inspiratory drive was enhanced by adding CO_2 to the inspiratory side of the respiratory pump to raise the arterial CO_2 partial pressure (P_{a,CO_2}). End-tidal CO_2 was monitored continuously by an infra-red analyser (P. K. Morgan).

In some experiments the central inspiratory drive was interrupted by simultaneous stimulation of the central ends of both superior laryngeal nerves. The nerves were dissected, cut close to the

larynx, and their central ends mounted on platinum wire electrodes. They were stimulated with electrical pulses of 2–4 V, 2 ms duration at a frequency of 30 Hz (Grass S88 stimulator) through an isolation unit.

Elicitation of cardioinhibitory responses

The carotid body chemoreceptors were stimulated by small doses of sodium cyanide (0·01–0·1 % w/v) injected into a common carotid artery in volumes of less than 0·2 ml via a catheter inserted into the external carotid or lingual artery.

Pulmonary C fibres were stimulated by injections of phenylbiguanide (200 μg ml^{-1}) into the right atrium via a catheter inserted into the right external jugular vein.

Cardiac receptors were excited by veratridine (50 μg ml^{-1}) injected into the left atrium via a catheter inserted through the atrial appendage.

The arterial baroreceptors were stimulated by a sustained rise in blood pressure in the aortic arch and carotid circulation, thereby eliciting an arterial 'baroreflex'. For this purpose, the pressure was raised by controlled partial inflation of a balloon (Swan-Ganz monitoring double-lumen catheter, size 4F; American Edwards Laboratories, Irvine, USA) inserted into the descending thoracic aorta via the left femoral artery.

Measurement of pressures

Pressures were measured in the aorta and trachea via catheters attached to Statham strain-gauge transducers (model P23Gb). Mean aortic pressure was obtained electrically by passing the output of the amplifier through a simple *R–C* network with a time constant of 1 s and was recorded separately. Zero pressures were established post-mortem by exposing to air the tips of the catheters *in situ*.

In some experiments, both stellate ganglia, together with the thoracic sympathetic chains to the level of T3, were surgically excised. They were approached through a trans-sternal thoracotomy in the third intercostal space. The chest was closed around the two brass tubes through which changes in the volume of the pneumothorax were measured.

All variables were recorded on a direct writing ultraviolet light recorder (S.E. Laboratories Ltd, Feltham, Middlesex, UK).

The urinary bladder was catheterized suprapubically and continually drained to prevent reflexes arising from this organ (Daly, Ward & Wood, 1986).

Blood gas analysis

Samples of arterial blood were drawn anaerobically into oiled glass syringes and analysed immediately. The P_{O_2}, P_{CO_2} and pH of the blood were determined using a calibrated electrode system (Model 158, Corning Medical and Scientific). Determinations were made at a temperature of 37·5 °C. Metabolic acidosis was corrected by an infusion of a mixture of 1 M-sodium bicarbonate solution (one part) and distilled water (three parts). Packed cell volume was determined by centrifuging samples of blood in capillary haematocrit tubes for 5 min at 10000 *g*.

Drugs

The following drugs were used. Veratridine free base (Sigma Chemical Co.), 1-phenylbiguanide (Aldrich Chemical Co. Inc.), sodium cyanide (British Drug Houses Ltd), atropine sulphate (BDH Chemicals Ltd), hexamethonium bromide (May and Baker Ltd), propranolol hydrochloride (Inderal, Imperial Chemical Industries Ltd) and atenolol (ICI Pharmaceuticals).

Statistical analysis

Data for controls and experimental values are expressed as means ± S.E.M. unless otherwise stated. Where appropriate, Student's paired *t* test was used to evaluate the significance of the differences between sets of paired data. Values were taken as being significant if $P < 0.05$.

Experimental procedure

All tests were carried out under conditions in which artificial ventilation of the lungs was temporarily interrupted in the expiratory position. The animal's spontaneous respiratory movements continued, the changes in volume of the pneumothorax being monitored. The cardiac

chronotropic effects were measured and taken as the peak pulse interval during the centrally induced inspiratory and expiratory phases of three or four respiratory cycles. Artificial respiration was then restarted. The test was then repeated, but this time the stimulus to a group of cardiovascular receptors was carried out one or two respiratory cycles after stopping artificial respiration. Comparison of the cardiac responses to stimulation of the receptors during the inspiratory and expiratory phases of the respiratory cycle was made, with the corresponding values measured during control tests using time-matched points. In the case of the right atrial injections of phenylbiguanide, the cardiac responses were measured within 5 s of injection, that is within the right atrium to left atrium circulation time, to exclude effects on receptors in the systemic circulation.

RESULTS

The initial control values for respiratory, cardiovascular and blood gas variables are shown in Table 1. In all animals the P_{a,O_2} was greater than 100 mmHg.

Effects of central respiratory activity on cardioinhibitory reflexes

Control observations

Control tests were made under conditions in which artificial respiration was temporarily interrupted with the lungs held at constant volume in the end-expiratory position and without stimulation of any of the cardiovascular receptor groups. The typical response is illustrated by Fig. 2*A*, and the averaged control responses for the observations relating to tests of individual inputs from the four cardiovascular receptor groups are shown in Fig. 3 (hatched bars).

In seventeen animals this resulted in a mean V_{pn} of $21{\cdot}4 \pm 1{\cdot}5$ ml kg^{-1}. Beat-by-beat analysis of the pulse interval indicated that in ten of the animals the phasic increase in V_{pn} was accompanied by a small acceleration of the heart, there being no change in the remaining seven. For all observations, the mean pulse interval during expiration of $301{\cdot}1 \pm 10{\cdot}5$ ms fell to $290{\cdot}3 \pm 8{\cdot}2$ ms during inspiration; the difference was not statistically significant.

Stimulation of the carotid body chemoreceptors

In all twenty tests (eleven animals), intracarotid injections of sodium cyanide ($4{\cdot}9 \pm 0{\cdot}3$ μg kg^{-1}) during temporary cessation of artificial respiration caused bradycardia, the pulse intervals, measured during the expiratory phase of the respiratory cycle, increasing by $380{\cdot}5 \pm 52{\cdot}5$ ms from a control value of $308{\cdot}3 \pm 10{\cdot}5$ ms. During the inspiratory phase of the cycle there was an acceleration of the heart corresponding to a reduction in pulse interval of $338{\cdot}8 \pm 47{\cdot}4$ ms and this response was associated with a V_{pn} of $25{\cdot}8 \pm 1{\cdot}3$ ml kg^{-1}. Thus, the pulse interval during inspiration was reduced to a value of only $57{\cdot}5 \pm 13{\cdot}6$ ms greater than the control value without stimulation of the carotid bodies (Fig. 2*B*). Considering the level of bradycardia evoked by chemoreceptor stimulation during expiration, the pulse interval was reduced, by the central inspiratory neuronal activity, to 15·1 % of its original size.

Besides causing an increase in V_{pn} and acceleration of the heart, activity of the central inspiratory neurones evoked an increase in mean blood pressure of $7{\cdot}8 \pm 2{\cdot}3$ mmHg from an expiratory value of $124{\cdot}1 \pm 4{\cdot}7$ mmHg (Fig. 3). Analysis of

individual tests indicated that the inspiratory acceleration of the heart occurred independently of the blood pressure which either increased, decreased or remained unchanged.

Elicitation of the arterial baroreflex

Step increases in arterial pressure of 39·5 ± 2·9 mmHg from an initial control value of 120·7 ± 3·5 mmHg resulted in an increase in pulse interval of 515·7 ± 45·4 ms,

TABLE 1. Initial control values for respiratory, cardiovascular and blood gas variables

No. of animals	21
Body weight (kg)	3·21 ± 0·8
P_{tr} (mmHg)	
Lung inflation	6·7 ± 1·2
Lung deflation	1·6 ± 0·5
Heart rate (beats min^{-1})*	196 ± 55·0
Pulse interval (ms)*	297·4 ± 45·8
Mean BP (mmHg)	121·0 ± 12·6
Arterial blood	
P_{O_2} (mmHg)	180 ± 50
P_{CO_2} (mmHg)	41·2 ± 6·5
pH	7·392 ± 0·049
Haematocrit (%)	47·3 ± 4·9
Rectal temperature (°C)	37·7 ± 0·9

Values are means ± S.D. Animals artificially ventilated. BP, arterial blood pressure; P_{tr}, tracheal pressure. * Averaged over 15 s.

measured during the expiratory phase of respiration, the initial value being 318·3 ± 12·3 ms. At the height of inspiration the size of the bradycardia was reduced to within 42·3 % of its original size (Figs 2*C* and 3). The blood pressure increased by an average value of 3·5 ± 0·8 mmHg.

Stimulation of cardiac receptors

Left atrial injections of veratridine (4·0 ± 0·2 $\mu g\ kg^{-1}$) caused bradycardia, the value for pulse interval measured during the expiratory phases of the respiratory cycle increasing by 502·3 ± 89·2 ms from the corresponding pre-injection value of 306·1 ± 13·3 ms. During the inspiratory phase of respiration acceleration of the heart occurred, the value for pulse interval being decreased to within 51·5 % of the control level (Figs 2*D* and 3). The inspiratory and expiratory levels of the blood pressure were not significantly different.

Stimulation of pulmonary C fibres

Right atrial injections of phenylbiguanide (18·6 ± 1·1 $\mu g\ kg^{-1}$) increased the expiratory value of pulse interval by 406·6 ± 32·2 ms from an initial value of 293·9 ± 6·2 ms. In contrast to the effects on the cardioinhibitory responses elicited by the inputs from the carotid chemoreceptors, arterial baroreceptors and cardiac receptors, activity of the central inspiratory neurones had no significant effect on the bradycardia evoked by stimulation pulmonary C fibres ($P > 0·1$; $n = 42$; Figs 2*E* and 3). It will be noted from Fig. 3 that the value for V_{pn} was 14·1 ± 0·9 ml kg^{-1}, a

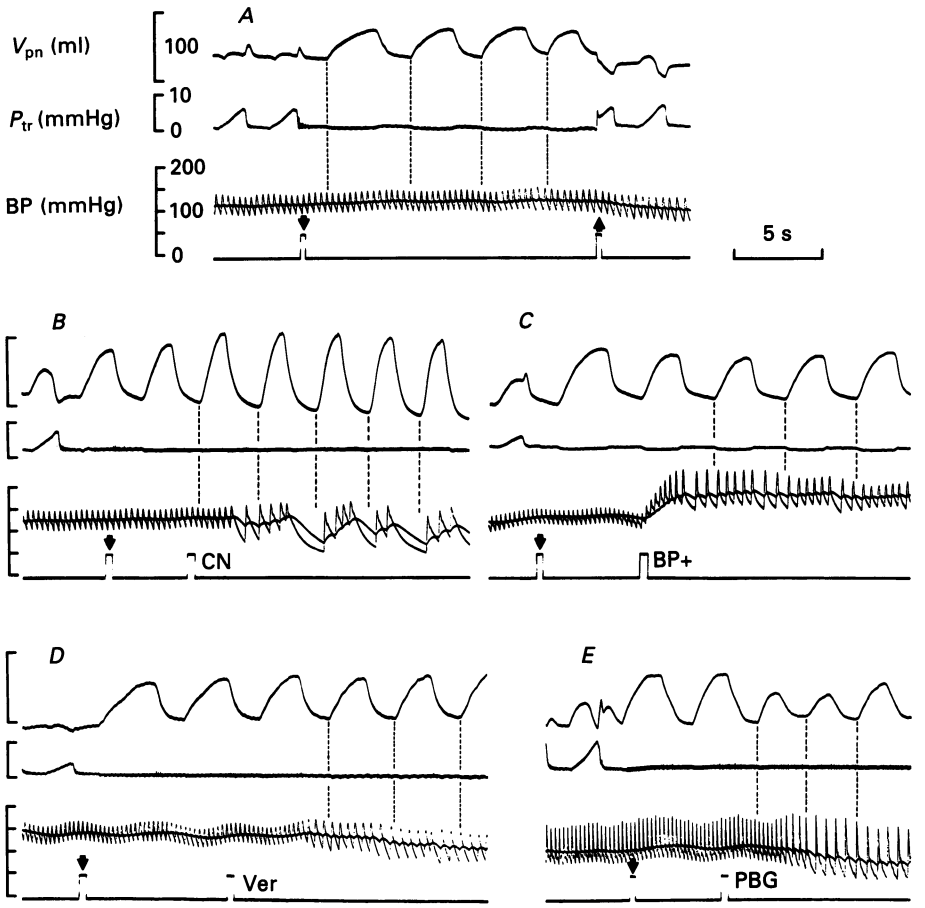

Fig. 2. The effects of phasic central inspiratory neuronal activity on the cardioinhibitory responses elicited by stimulation of various cardiovascular receptor groups. Bilateral upper thoracic sympathectomy. Positive pressure ventilation. Open pneumothorax. Each reflex cardioinhibitory response was studied under conditions in which artificial respiration was temporarily interrupted, with the lungs held at their expiratory volume, expiratory pressure 1·5 mmHg, as indicated by the downward arrow in each panel. *A*, cessation of artificial respiration alone between the two arrows. *B*, stimulation of the carotid body chemoreceptors by intracarotid cyanide (CN; 4·8 μg kg^{-1}); *C*, elicitation of the arterial baroreflex alone by raising the arterial pressure by 40 mmHg; *D*, stimulation of the cardiac receptors by left atrial injection of veratridine (Ver; 4·1 μg kg^{-1}); *E*, stimulation of pulmonary C fibres by right atrial injection of phenylbiguanide (PBG; 16·2 μg kg^{-1}). Vertical interrupted lines indicate beginning of each inspiratory phase of the respiratory cycle. Records from above downwards: V_{pn}, changes in volume of the pneumothorax (inspiration upwards); P_{tr}, tracheal pressure; BP, phasic and mean arterial blood pressure. Time calibration, 5 s.

response that was smaller than those elicited by the other three cardiovascular inputs. This can be accounted for by the known initial depressant effect of phenylbiguanide on respiration (Dawes & Fastier, 1950; Dawes & Mott, 1950). The arterial blood pressure fell, on average, by 4·5 mmHg during inspiration.

So as to extend the range of V_{pn} over which respiratory variations in pulse interval could be observed, tests were carried out on five occasions under conditions of raised P_{a,CO_2} by adding CO_2 to the inspired gas to give end-tidal CO_2 concentrations of about 7·5%. The results of these tests are included in the averaged responses shown in Fig. 3.

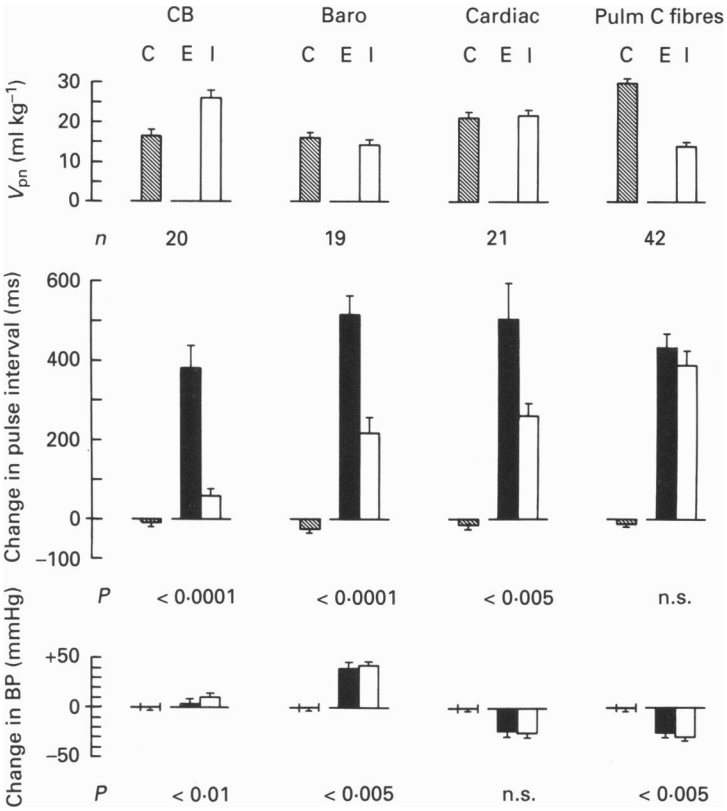

Fig. 3. Summary of the effects of changes in central respiratory neuronal activity (E, expiration, filled blocks; I, inspiration, open blocks) on the cardioinhibitory responses to stimulation of the carotid body chemoreceptors (CB; intracarotid sodium cyanide, 4·9 ± 0·3 μg kg^{-1}), the arterial baroreflex (Baro; increase in blood pressure, 39·5 ± 2·9 mmHg), cardiac receptors (Cardiac; left atrial veratridine, 4·0 ± 0·2 μg kg^{-1}), and pulmonary C fibre endings (Pulm C fibres; right atrial phenylbiguanide, 18·6 ± 1·1 μg kg^{-1}). C (hatched blocks), control changes in physiological variables without stimulation of cardiovascular receptors. All tests carried out during temporary interruption of artificial respiration with the lungs held at their expiratory volume. From above downwards: measurements of V_{pn} (volume of pneumothorax); changes in pulse interval; and changes in BP (mean arterial blood pressure). Statistical values refer to comparisons of paired data in filled (E) and open (I) blocks. *n*, number of tests.

Considering individual cardiac responses to central inspiratory neuronal activity, pulse interval was reduced in twenty-four of forty-two tests (57%), increased in fifteen (36%) and was unchanged in three (7%). These variations in the response were not related to the concomitant changes in blood pressure.

Relationship of cardioaccelerator response to central inspiratory activity

To see whether the degree of modulation of an excitatory input from each cardiovascular receptor group was affected by the size of the central inspiratory drive, the data were replotted so as to relate the inspiratory and expiratory values

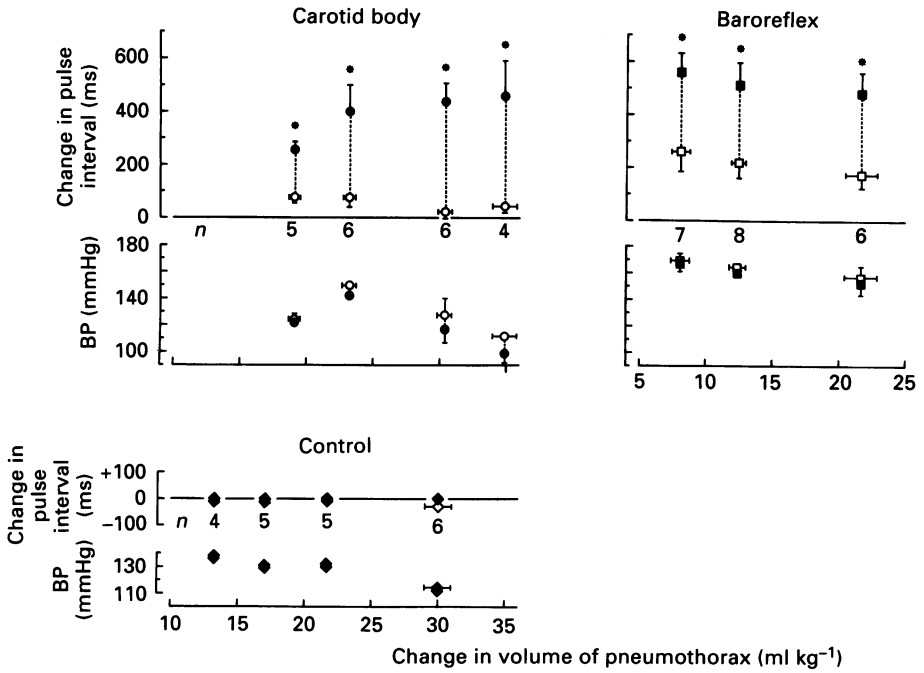

Fig. 4. Data from Fig. 3 relating to respiratory changes in pulse interval during stimulation of the carotid body chemoreceptors and arterial baroreceptors, and the change in volume of the pneumothorax. The lower panel shows control responses occurring during interruption of artificial ventilation without stimulation of cardiovascular receptors. Values for pulse interval and change in volume of the pneumothorax are means ± S.E.M. Where no error bars are given, the S.E.M. is less than the size of the symbol. BP, mean arterial blood pressure. $*P < 0{\cdot}05$.

of pulse interval to the level of V_{pn}. The results are shown in Figs 4 and 5. Control observations carried out during temporary interruption of artificial respiration alone indicate there were no changes in pulse interval or blood pressure at any level of V_{pn} (Fig. 4). The expiratory bradycardia, however, evoked by stimulation of the carotid bodies (Fig. 4), the arterial baroreflex (Fig. 4) and the cardiac receptors (Fig. 5), was partly suppressed by central inspiratory neuronal activity over the observed limits of the range of spontaneous changes in V_{pn} ($P < 0{\cdot}05$). The degree of suppression was greater in the case of the carotid chemoreceptor input than with either the arterial baroreceptor or cardiac receptor inputs, and was not significantly related to V_{pn}. Central inspiratory activity had no effect on arterial blood pressure at any level of V_{pn} (Figs 4 and 5).

On the other hand, the cardiac response to pulmonary C fibre stimulation was not significantly affected by the central inspiratory activity at any level of V_{pn} up to 24 ml kg^{-1} (Fig. 5), nor did central inspiratory activity have any effect on blood pressure (Fig. 5).

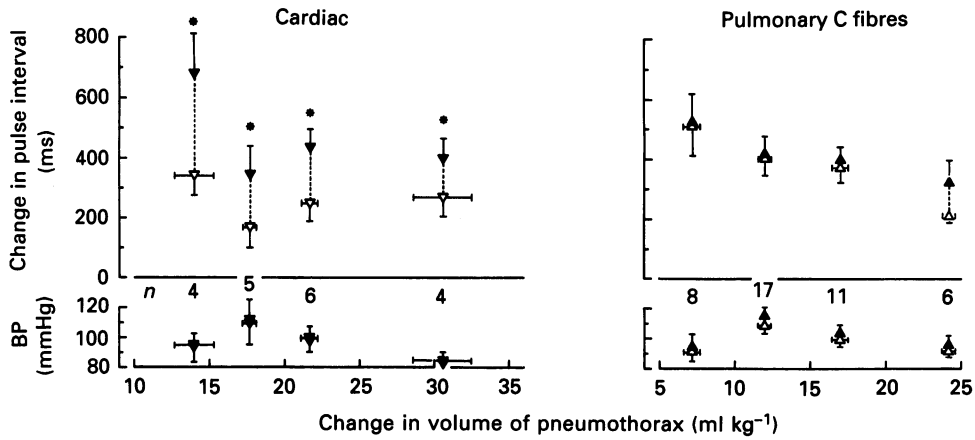

Fig. 5. Data from Fig. 3 relating to the respiratory changes in pulse interval during stimulation of the cardiac receptors and pulmonary C fibre endings, and the change in volume of the pneumothorax. Values for pulse interval and change in volume of the pneumothorax are means ± S.E.M. Where no error bars are given, the S.E.M. is less than the size of the symbol. BP, mean arterial blood pressure. $*P < 0.05$.

Effects of interruption of the cardiac sympathetic pathway

The patterns of the cardiac chronotropic responses with respect to the inputs from carotid chemoreceptors, arterial baroreceptors, cardiac receptors and pulmonary C fibre receptors, and their differential modulation by central inspiratory neuronal activity, were similar after interruption of the cardiac sympathetic supply (see Methods, three experiments, Fig. 2) or following the administration of the β-adrenergic blocking agents propranolol (0·5 mg kg^{-1} I.V.) and atenolol (3 mg kg^{-1} i.v.) (two experiments each). These results indicate that the cardiac effects are mediated largely by way of the vagus nerves.

Effects of inhibition of central inspiratory neuronal activity

Evidence that the central inspiratory drive is responsible for the inspiratory acceleration of the heart rests on information from two types of experiments. First, when the phasic changes in central inspiratory activity were inhibited by electrical stimulation of the superior laryngeal nerves during a test of excitation of the carotid chemoreceptors, the arterial baroreflex or cardiac receptors, there was an immediate cessation of the bursts of cardioacceleration, with the result that a steady pulse interval ensued. On cessation of the electrical stimulus there was a resumption of phasic central inspiratory activity, accompanied by bursts of cardioacceleration (Fig. 6*A* and *B*). In other experiments, illustrated by Fig. 7, it was found that the central inspiratory cardioaccelerator response occurring during excitation of the

arterial baroreflex (*A*) was absent when the test was repeated during the reflexly induced inhibition of respiration (*B*). On the other hand, the bradycardia evoked by pulmonary C fibre stimulation was little affected by phasic changes in central inspiratory drive and, accordingly, the pattern of response when the test was

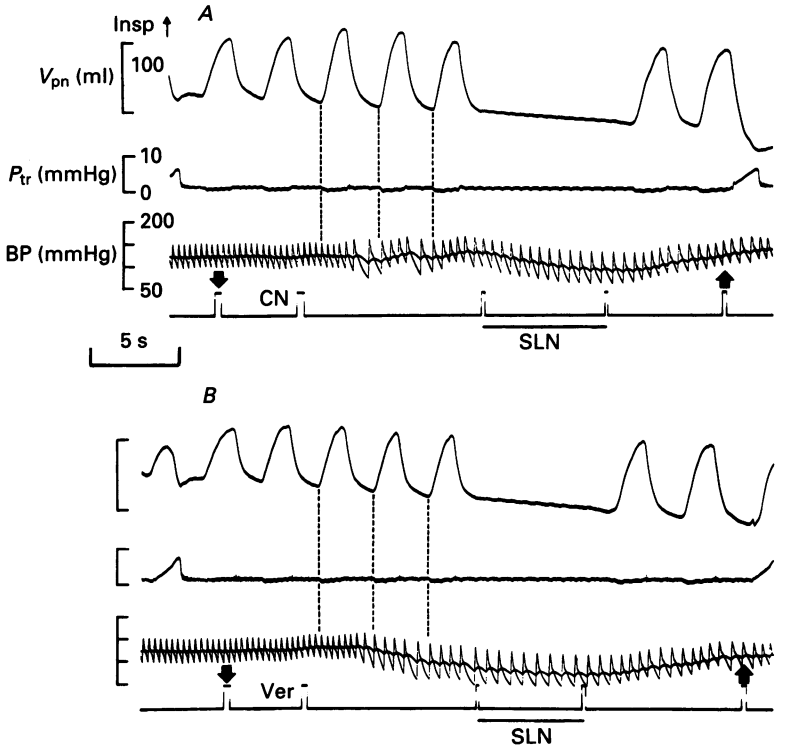

Fig. 6. The effects of electrical stimulation of the central ends of the superior laryngeal nerves on the phasic inspiratory neuronal activity and pulse interval. Positive pressure ventilation, open pneumothorax. Artificial respiration temporarily interrupted between arrows, the lungs being held at constant volume at their expiratory level. Spontaneous changes in volume of the open pneumothorax measured with a Fleisch pneumotachograph. *A*, stimulation of the carotid body chemoreceptors by intracarotid sodium cyanide (CN; 4·7 μg kg^{-1}) followed by electrical stimulation of the superior laryngeal nerves (SLN; 3 V, 2 ms, 30 Hz). *B*, stimulation of the cardiac receptors by left atrial injection of veratridine (Ver; 4·3 μg kg^{-1}) followed by electrical stimulation of the superior laryngeal nerves (SLN; 3 V, 2 ms, 30 Hz). Vertical interrupted lines indicate beginning of each inspiratory phase of the respiratory cycle. Records from above downwards: V_{pn}, changes in volume of the pneumothorax (inspiration upwards); P_{tr}, tracheal pressure; BP, phasic and mean arterial blood pressure. Time calibration, 5 s.

repeated during a reflexly induced inhibition of respiration was similar (Fig. 7*C* and *D*).

Second, the animals were over-ventilated to reduce the end-tidal CO_2 to a level (3·5–4·0 %) which abolished spontaneous central inspiratory activity. When the tests of stimulation of the carotid chemoreceptors, the arterial baroreflex and cardiac

receptors were repeated during temporary interruption of artificial ventilation, bradycardia occurred but without the changes in V_{pn} or periods of cardioacceleration that were seen in control tests carried out at normal levels of end-tidal CO_2 (5·0–6·2 %).

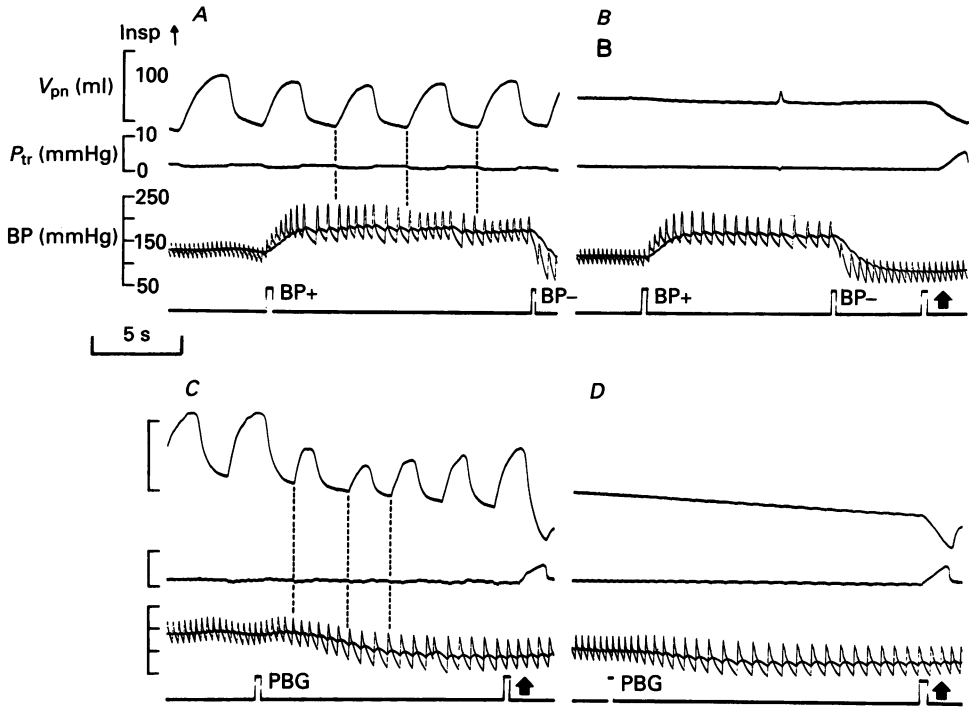

Fig. 7. The effects of electrical stimulation of the superior laryngeal nerves on the phasic inspiratory neuronal activity and pulse interval. Positive pressure ventilation. Open pneumothorax. Artificial respiration temporarily interrupted 2 s before recording began, and restarted at the arrow, the lungs being held at constant volume at their expiratory level. Spontaneous changes in volume of the open pneumothorax measured with a Fleisch pneumotachograph. Upper panels, arterial baroreceptors stimulated by raising the blood pressure by 50 mmHg: *A*, alone; and *B*, during cessation of inspiratory neuronal activity produced by electrical stimulation of the superior laryngeal nerves (3 V, 2 ms, 30 Hz). Lower panels, stimulation of pulmonary C fibre endings by right atrial injections of phenylbiguanide (PBG, 17·1 $\mu g\ kg^{-1}$) before (*C*) and during (*D*) electrical stimulation of the superior laryngeal nerves (3 V, 2 ms, 30 Hz). Vertical interrupted lines indicate beginning of each inspiratory phase of the respiratory cycle. Records from above downwards: V_{pn}, changes in volume of pneumothorax (inspiration upwards); P_{tr}, tracheal pressure; BP, phasic and mean arterial blood pressure. Time calibration, 5 s.

In these two types of experiments, therefore, there was always a close association between the presence of phasic changes in V_{pn} and bursts of cardioacceleration.

Effects of atropine and hexamethonium

The cardioinhibitory responses to stimulation of each of the four groups of cardiovascular receptors were abolished by intravenous atropine (1 mg kg^{-1}) and so also were the inspiratory-related cardioaccelerator effects (two experiments). Intravenous injections of hexamethonium (10 mg kg^{-1}) also abolished the brady-

cardia evoked by stimulation of the carotid chemoreceptors, arterial baroreceptors and pulmonary C fibre endings (five experiments), and the response to excitation of cardiac receptors (two experiments). Again, all observed inspiratory-related cardiac responses were abolished in the same experiments.

DISCUSSION

The present experiments show that whereas the central inspiratory drive can modulate the vagally induced cardioinhibitory response elicited by stimulation of cardiac receptors by veratridine, in addition to the responses from carotid chemoreceptors and arterial baroreceptors as found previously (Koepchen, Lux & Wagner, 1961; Koepchen, Wagner & Lux, 1961; Davidson *et al.* 1976; McAllen & Spyer, 1978*a*, *b*; Gilbey, Jordan, Richter & Spyer, 1984), it does not significantly affect the bradycardia resulting from stimulation of pulmonary C fibre endings. On the basis that the two components of respiratory modulation, central inspiratory neuronal activity and activity of lung stretch afferents, act in concert (Anrep *et al.* 1936*b*), then the present results are consistent with previous findings that a similar differential modulation of the inputs from the four groups of cardiovascular receptors occurs as a result of activity of pulmonary stretch afferents (Daly & Kirkman, 1989; Fig. 8 of this paper) and of a reflexly induced apnoea (Daly *et al.* 1988).

An important consideration in the choice of method for measuring the phasic changes in the central inspiratory neuronal activity was the need to minimize the input from slowly adapting pulmonary stretch receptors which are known to play an important role in modulating the effectiveness of at least some excitatory inputs to cardiac vagal motoneurones (Daly & Scott, 1958; Angell-James & Daly, 1969, 1978; Gandevia *et al.* 1978; Daly & Kirkman, 1989; see also Spyer, 1982; Jordan & Spyer, 1986) and at the same time maintain the integrity of the pathway for pulmonary C fibres. The measurement of V_{pn} included the contribution of the intercostal muscles and accessory muscles of respiration in addition to that of the diaphragm to the phasic changes in thoracic volume and therefore closely resembled the relative contributions of the various groups of respiratory muscles in normal breathing. In this connection the contribution of the diaphragm over the range of tidal volume is, in man at least, only about 64%, and that for the rib-cage about 36% (Wade, 1954; Agostoni, Mognoni, Torri & Saracino, 1965). The values vary, however, with the size of the tidal volume, position of the body, anaesthesia and between species (see Campbell, Agostoni & Newsom Davis, 1970). Thus, the method of recording phrenic nerve discharge alone, while giving valuable information about the characteristics of inspiratory neuronal activity, would have excluded an unknown contribution to which the rib-cage was making under the conditions of the present experiments.

Furthermore, the measurement of changes in V_{pn} enabled a quantitative comparison to be made of the results obtained in previous experiments in which the differential modulation by pulmonary afferents driven by varying degrees of lung inflation was studied (Daly & Kirkman, 1989; Fig. 8 of this paper).

The maintenance of a constant lung volume at a slightly less than normal functional reserve capacity ensured that the low steady level of activity of pulmonary stretch afferents was achieved. This period of interruption of artificial

respiration meant that for the period of time equivalent to three or four normal breaths, the P_{a,O_2} progressively fell, while the P_{a,CO_2} increased. Analysis of the arterial blood showed, however, that at the time the observations were made, the P_{a,O_2} remained above 100 mmHg, whereas the P_{a,CO_2} increased, on average, by 9 mmHg. It is unlikely, however, that these changes modified the cardiac responses to stimulation of arterial chemoreceptors, baroreceptors and cardiac responses. Quantitatively similar results were obtained in experiments in which the responses obtained under the present conditions were compared with those in which the lungs were subsequently denervated (Daly & Scott, 1958) and the P_{a,CO_2} maintained constant by continuous artificial respiration (M. de B. Daly, unpublished observations).

The observed values for V_{pn} can all be considered to have occurred within the normal physiological range, taking the normal tidal volume of the cat to be about 15 ml kg^{-1}. The largest value of 35 ml kg^{-1} was therefore only slightly more than twice the normal tidal volume. It will be noted from Figs 3 and 4, however, that in the case of the carotid chemoreceptor input, no effects of values for V_{pn} less than 18·5 ml kg^{-1} were elicited. The reason for this is that in this type of experiment there is no control of the degree of bradycardia *and* the level of V_{pn} on stimulation of the carotid bodies or, for that matter, of any of the other three groups of cardiovascular receptors. Stimuli that cause bradycardia affect spontaneously V_{pn} through the normal reflex effect on respiration. This is in contrast to the study of the effects of lung inflation on cardioinhibitory reflex responses in which the volumes used for inflating the lungs could be independently controlled (Daly & Kirkman, 1989; Fig. 8 of this paper).

The inspiratory modulation of the bradycardia evoked by stimulation of the carotid chemoreceptors, arterial baroreceptors and cardiac receptors frequently showed three phases typified by the responses shown in Fig. 2: an acceleration of the heart during the inspiratory phase of the respiratory cycle, bradycardia, which was particularly marked during carotid chemoreceptor stimulation during the early expiratory phase, followed by escape of the bradycardia during the late expiratory phase. This pattern of response is consistent with the observations of Gilbey *et al.* (1984). In their studies involving intracellular and extracellular recordings from the cardiac vagal motoneurones, they showed that the neurones did not fire during the ramp phase of phrenic nerve activity corresponding to inspiration, but were active during stage I expiration (post-inspiration) and during stage II expiration when the phrenic nerve was silent (Richter, 1982; Remmers, Richter, Ballantyne, Bainton & Klein, 1986). Maximum values of the membrane potential (−50 to −60 mV) during inspiration were followed by depolarization at the onset of stage I expiration when they discharged some action potentials. The frequency of this discharge then decreased or even ceased when the membrane slowly repolarized during stage I expiration. The cardiac vagal motoneurones discharge during post-inspiration as a result of inspiratory inhibition; their increased excitability may also be reinforced by direct post-inspiratory excitation (Richter & Spyer, 1990). It is still not clear, however, whether the fall in their expiratory discharge is the result of adaptation resulting from intrinsic membrane properties of the neurones or to stage II expiratory inhibition (Richter & Spyer, 1990.) The same doubt exists concerning the observation that during stimulation of the superior laryngeal nerve, the frequency of

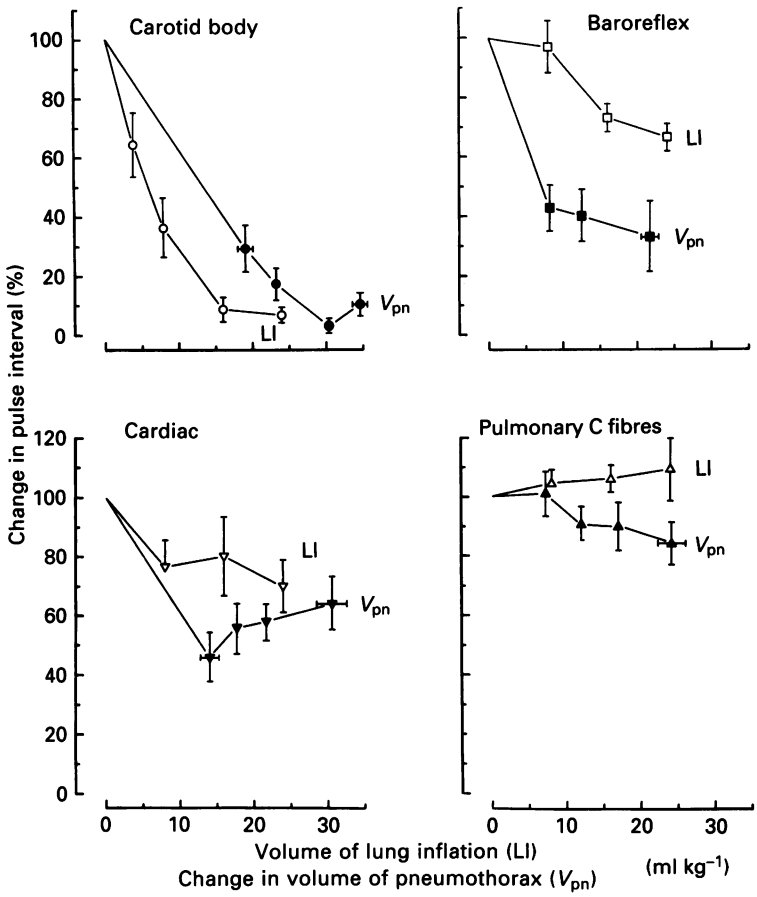

Fig. 8. Normalized data taken from Figs 4 and 5 showing the effects of phasic central inspiratory neuronal activity, measured as changes in volume of the pneumothorax (V_{pn}), on the cardioinhibitory responses to stimulation of the carotid body chemoreceptors, the arterial baroreflex, cardiac receptors and pulmonary C fibre endings. The increase in pulse interval evoked by stimulation of each of the four inputs is taken as 100% measured during the expiratory phase of the respiratory cycle, the expiratory volume of the pneumothorax being taken as zero. The inspiratory values observed at increasing levels of V_{pn} are expressed as a percentage of that occurring at zero volume. For comparison, the figure shows the normalized data for the effects of inflation of the lungs with different volumes of room air on the cardioinhibitory responses elicited by stimulation of the same four groups of cardiovascular receptors in the absence of activity of the central inspiratory neurones. The data are taken from Fig. 5 of Daly & Kirkman (1989). The increase in pulse interval evoked by stimulation of each of the four inputs is taken as 100%, measured during cessation of central inspiratory neuronal activity evoked reflexly by electrical stimulation of the superior laryngeal nerves. Filled symbols, changes in volume of the pneumothorax (V_{pn}), representing the changes in central inspiratory neuronal activity; open symbols, lung inflation volume.

the steady heart rate seen under these conditions in the present experiments is faster than the nadir associated with the post-inspiratory phase of the spontaneous respiratory cycle. As shown by Remmers *et al.* (1986), stimulation of the superior

laryngeal nerve caused respiratory arrest by prolonging the period of depolarization of post-inspiratory neurones in stage I of expiration ('central post-inspiratory apnoea').

Possible mechanisms of respiratory modulation

The cardiac vagal motoneurones in the cat are situated largely in the nucleus ambiguus (McAllen & Spyer, 1976, 1978*a*, *b*), but also in the dorsal vagal motor nucleus (Kalia & Mesulam, 1980; Bennett, Kidd, Latif & McWilliam, 1981). The former group can be activated synaptically by excitation of baroreceptors and chemoreceptors (see Spyer, 1982; Jordan & Spyer, 1987), by intravenous injections of veratridine (Lipski, McAllen & Spyer, 1976), and by right atrial injections of phenylbiguanide (D. Jordan & K. M. Spyer, personal communication). They are the main site for respiratory 'gating' of excitatory inputs from chemoreceptors and baroreceptors (McAllen & Spyer, 1978*a*, *b*; Gilbey *et al.* 1984; Mifflin, Spyer & Withington-Wray, 1988).

On the premise that the cardiac vagal motoneurones are a homogenous group (Gilbey *et al.* 1984), the differential modulation of reflex cardioinhibitory responses by inflation of the lungs (Daly & Kirkman, 1989) could be explained, as proposed by Potter (1981), by the fact that pulmonary stretch afferents have an independent effect earlier in each vagal excitatory pathway, although not directly on the afferent terminals (Jordan & Spyer, 1979). Such a hypothesis could explain the quantitative differences in the effects of central inspiratory activity on the four excitatory inputs to cardiac vagal motoneurones.

The absence of any effect of lung inflation on the cardioinhibitory response to pulmonary C fibre stimulation led Daly & Kirkman (1989) to postulate an alternative view that there are two populations of cardiac vagal motoneurones, one of which can be inhibited by pulmonary stretch afferents, the other cannot. The present results are not inconsistent with such a hypothesis. However, it was suggested that the pulmonary C fibre-evoked bradycardia could involve vagal non-myelinated preganglionic fibres with their cells in the dorsal vagal motor nucleus of the cat (Bennett *et al.* 1981; Donoghue, Fox, Kidd & Koley, 1981; Bennett, Ford, Kidd & McWilliam, 1984; Jordan, Spyer, Withington-Wray & Wood, 1986). These neurones are cardioinhibitory in function (Woolley, McWilliam, Ford & Clarke, 1987) and can be activated synaptically by electrical stimulation of non-myelinated pulmonary vagal afferents (Bennett, Goodchild, Kidd & McWilliam, 1985) but not by lung inflation (Bennett *et al.* 1984). Woolley *et al.* (1987) showed in the rabbit that the cardioinhibitory response to stimulation of the vagal non-myelinated fibres was abolished by atropine but not by hexamethonium. On this evidence it would be expected that, in the present experiments, the cardioinhibitory response to excitation of pulmonary C fibres would have persisted after hexamethonium, but this was not so. There are, however, considerable species differences in the nature and function of cardiac vagal non-myelinated fibres (Woolley *et al.* 1987). An alternative site for this population of cardiac vagal motoneurones, that is in the nucleus ambiguus, is not ruled out.

Finally, in this connection, consideration must be given to the possibility that the differential modulation of various excitatory inputs to cardiac vagal motoneurones is determined, at least to some extent, by the differing patterns of evoked activity

in various functional groups of inspiratory and expiratory neurones. Variable reciprocal and non-reciprocal effects on inspiratory and expiratory medullary neurones and in activity of inspiratory and expiratory nerves occur with respect to respiration (Koepchen, Klüssendorf, Borchert, Lessmann, Dinter, Frank & Summer, 1977; Mifflin, Spyer & Withington-Wray, 1987), to the input from pulmonary stretch afferents (Koepchen *et al.* 1977; St. John & Zhou, 1990), and as a result of excitation of the carotid chemoreceptors, arterial baroreceptors and pulmonary C fibres (Koepchen, Kalia, Sommer & Klüssendorf, 1977; D. Jordan, K. M. Spyer & M. de B. Daly, unpublished observations). These latter experiments by us were designed to test this hypothesis and indicate that it is unlikely to explain the present results or the mechanism of the differential modulation of excitatory inputs to cardiac vagal motoneurones by pulmonary stretch afferents (Daly & Kirkman, 1989).

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