J. Physiol. (1973), **233**, *pp.* 45–62
With 9 text-figures
Printed in Great Britain

EXTRA-SEGMENTAL REFLEXES DERIVED FROM INTERCOSTAL AFFERENTS: PHRENIC AND LARYNGEAL RESPONSES

BY JOHN E. REMMERS

From the Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire, U.S.A.

(*Received 1 January 1973*)

SUMMARY

1. Phrenic and recurrent laryngeal efferent responses were evoked by brief tetani or single shocks to the cut external intercostal nerves of anaesthetized cats. The reflexes derived from middle thoracic segments (T5 and 6) were compared with those emanating from caudal thoracic segments (T9 and 10).

2. During inspiration, middle intercostal nerve stimulation transiently inhibited the spontaneous discharge in both efferent neurograms, whereas stimulation of caudal intercostal nerves facilitated phrenic discharge and usually inhibited recurrent laryngeal activity.

3. During expiration, stimulation at either thoracic level enhanced recurrent laryngeal discharge while provoking little or no phrenic response.

4. Superficial lesions of the lateral cervical cord, ipsilateral to the stimulus sites, above or below the phrenic outflow, eliminated all reflex responses except the phrenic response to caudal thoracic stimuli. Similarly, in the spinal animal, middle intercostal afferents could not be shown to decrease phrenic excitability. Caudal intercostal afferents cause phrenic excitation by a spinal reflex.

5. Group I afferents of the mid-thoracic segments and group II afferents of the caudal thoracic segments initiate these extra-segmental reflexes.

6. The recurrent laryngeal responses manifest, for the most part, changes in the discharge of fibres innervating the posterior cricoarytenoid muscle. The responses fit the overall pattern of response to middle intercostal nerve stimulation, namely, inhibition of inspiratory muscles and excitation of expiratory muscles. Intercostal afferent stimulation also activated the laryngeal adductor muscles.

7. The results support the view that intercostal mechanoreceptors initiate an array of extra-segmental respiratory reflexes, including spinal and supraspinal arcs. The simplest way to account for the various responses

to stimulation of middle intercostal afferents is to postulate a reflex involving supraspinal respiratory neurones.

8. The observed reflexogenic differences correlate with anatomical differences between the middle and caudal ribs. Possible functional implications of this relationship are discussed.

INTRODUCTION

Afferent impulses from intercostal muscle receptors can influence reflexly the motor activity of non-intercostal respiratory muscles. Downman (1955) first demonstrated this clearly by showing that intercostal afferent stimulation caused phrenic inhibition in the decerebrate cat and phrenic excitation in the spinal animal. Garcia Ramos (1959) reported similar observations and, in addition, noted activation of abdominal expiratory muscles. Two recent investigations, one in the decerebrate cat (Decima & Euler, 1969*b*) and one in the anaesthetized animal (Remmers, 1970), explored the subject further, but left two questions unanswered:

(1) What is the exact pattern of phrenic response? Decima & Euler (1969*b*) demonstrated a bi-phasic response to caudal (T9–T12) intercostal stimuli. Remmers (1970) stimulated more cranial interspaces and elicited only phrenic inhibition. Whether this difference is attributable to the difference in receptive field or to differences in experimental methods is uncertain.

(2) What neural structures mediate phrenic inhibition by intercostal afferents? This question has not been answered directly. Decima & Euler (1969*b*), like previous investigators (Downman, 1955; Garcia Ramos, 1959), speculated that the inhibitory intercostal-to-phrenic (I-P) reflex may be a spinal reflex, pointing out that descending influences seemed to determine the expression of excitatory and inhibitory I-P reflexes as is the case for intercostal-to-intercostal and splanchnic-to-intercostal reflexes (Alderson & Downman, 1966). Remmers (1970) noted that the phrenic inhibitory response was associated with other reflex effects, namely, a change in respiratory rate and inhibition of inspiratory intercostal discharge, and he attempted to explain both by postulating supraspinal inhibition.

The present investigation is directed at both questions. The first was approached by comparing middle and caudal intercostal afferents with regard to the pattern of phrenic response and the type of afferent fibre responsible for the reflex. Evidence relating to the second question was obtained from the response of supraspinal respiratory motoneurones and from the phrenic response after interruption of the probable ascending tract for this supraspinal reflex. The latter, in effect, separated the I-P

reflex into spinal and supraspinal components. Similar separation was inferred from the changes in phrenic motoneurone excitability caused by intercostal afferent stimulation in the spinal animal.

A preliminary account of these investigations has already appeared (Remmers & Jurancich, 1972).

METHODS

Successful experiments were carried out on twenty-eight cats, weighing 2·5–4·0 kg, anaesthetized by an I.P. injection of Dial with urethane (allobarbital, 60 mg/kg; urethane, 240 mg/kg). In most experiments the trachea was intubated orally, and the lungs were ventilated by a positive pressure respirator. Most animals were paralysed with gallamine administered intravenously (0·5 mg/kg). Rectal temperature was maintained within 1 degree of 38° C by external heating. Arterial blood pressure was recorded continuously, and an experiment was terminated if the diastolic value fell below 60 mm Hg. Arterial blood P_{O_2}, P_{CO_2} and pH were determined by electrodes. Metabolic acidosis was often present and was corrected by I.V. infusion of a sodium bicarbonate solution.

The animal was placed prone in a stereotaxic head holder. The phrenic C5 root and the recurrent laryngeal nerve were dissected free, cut distally and placed on bipolar, platinum-iridium electrodes immersed in a mineral oil pool. Action potentials were amplified by a differential a.c. coupled amplifier (Tektronix 122) and displayed on a multi-channel storage oscilloscope. They were also fed into a pulse-frequency analyser (Transidyne 1260), the output of which was proportional to the frequency of spikes in a train. The 90 % response time of the instrument was 0·2 sec so that its over-all behaviour was similar to an R-C 'integrator' with leak. In some experiments electromyograms (EMGs) of the intrinsic laryngeal muscles were recorded with fine wire electrodes (Basmajian & Stecko, 1962) placed in the thyroarytenoid and posterior cricoarytenoid muscles under direct vision. In these cases the trachea was intubated through a tracheostomy performed well below the larynx.

For afferent stimulation of intercostal nerves, selected external intercostal nerves were exposed as described by Sears (1964), cut distally and mounted on bipolar stimulating electrodes. These electrodes were usually held by attachment to a spinous process and submerged in a mineral oil pool maintained at 38° C by a lamp. The stimulus was either a single shock or a 4–6 msec pulse train (500 pulses/sec). The timing of stimuli relative to the respiratory cycle was set by a ramp-pulse generator which was triggered by the initial spikes of the phrenic burst and which, in turn, gated the stimulator after a pre-set delay. In most cases, two pairs of external intercostal nerves, on the same side, were stimulated: T5 and 6 and T9 and 10. Both nerves of a pair received identical pulses.

A wide cervical laminectomy was performed and the cord was exposed throughout the experiment. This allowed reflex responses to be tested immediately before and after partial cord section. Superficial lesions of the lateral cervical cord were made either at the C3 or the C7 level using a narrow piece of razor blade fixed in a forceps with the blade protruding 1 mm. At the termination of the experiment the cervical cord was removed and examined to determine the extent of the lesion. In some cases the cord was bathed in India ink, fixed in 10 % formalin, and cut transversely into 0·5 mm sections which were examined under a dissecting microscope. In other cases the cord was fixed in formalin, sectioned sagittally or horizontally, and stained with haematoxylin and eosin.

In experiments in which dorsal root potentials were recorded, the chest was opened

bilaterally to minimize thoracic motion, and a T5–T10 laminectomy was performed. Stimulating and recording electrodes were mounted on micromanipulators, and a large mineral oil pool was formed covering the cord and intercostal nerves. Using a dissecting microscope, one dorsal root filament at T6 or T9 was cut and its peripheral segment placed on small bipolar silver wire electrodes. To obtain mono-phasic recordings of action potentials, the crushed, cut end was positioned on one of the electrode wires. To eliminate spontaneous afferent traffic, the internal intercostal nerve of T6 and T9 was cut. The test stimulus was a single shock (0·1 msec) delivered to the central stump of the corresponding external intercostal nerve. To enhance the reflex response to this stimulus, the adjacent external intercostal nerve, T5 or T10, was stimulated with a pulse train of sufficiently low intensity as to cause no detectable phrenic or recurrent laryngeal response. This conditioning stimulus was delivered during the 4 msec period immediately preceding the test stimulus.

RESULTS

Phrenic and recurrent laryngeal responses

General. Pulse trains were more effective than single shocks in provoking phrenic and recurrent laryngeal responses. Similarly, the stimulus intensity necessary for a reflex response was less when two adjacent nerves, rather than a single nerve, were stimulated. These results are consistent with the existence of a pool of interneurones on to which there may be considerable convergence. Responses became more reproducible and the thresholds and latencies more constant when brief pulse trains were delivered to two adjacent nerves. As would be expected from previous work (Remmers, 1970), the phrenic and recurrent laryngeal inhibitory responses became more pronounced during the latter part of inspiration. However, when the timing of stimuli relative to the respiratory cycle was held constant, the stimulus thresholds were constant and the responses were virtually superimposable for periods of 30–45 min.

Initial experiments revealed two basic patterns of phrenic response: stimulation between the levels, T4 and T8, produced purely inhibition; stimulation at the T9 level and below augmented and then inhibited phrenic discharge. This distribution of reflexogenic capability led to the operational definition of the segments, T4–T8, 'middle' thoracic segments and of the segments, T9–T13, 'caudal' thoracic segments. The results agree with those of Decima & Euler (1969*b*) in the decerebrate cat, where stimulation of T8, the most cranial segment tested, caused only phrenic inhibition, but more caudal nerves evoked biphasic responses. In the present study only two pairs of nerves were studied extensively, T5 and 6 and T9 and 10. The responses to stimulation of T5 and 6 were typical of other middle intercostal nerves, and responses to stimulation of T9 and 10 were similar to those obtained with T11 or T12 stimuli. Identical responses were derived from ipsilateral and contralateral recordings.

Responses during inspiration. Stimuli delivered to the 5th and 6th external intercostal nerves during inspiration consistently inhibited phrenic and recurrent laryngeal discharges. Typical responses are shown in Fig. 1 (slow sweep speed) and in Fig. 2 (higher sweep speed). The latencies varied inversely with stimulus intensity and were 13–20 msec and 15–25 msec

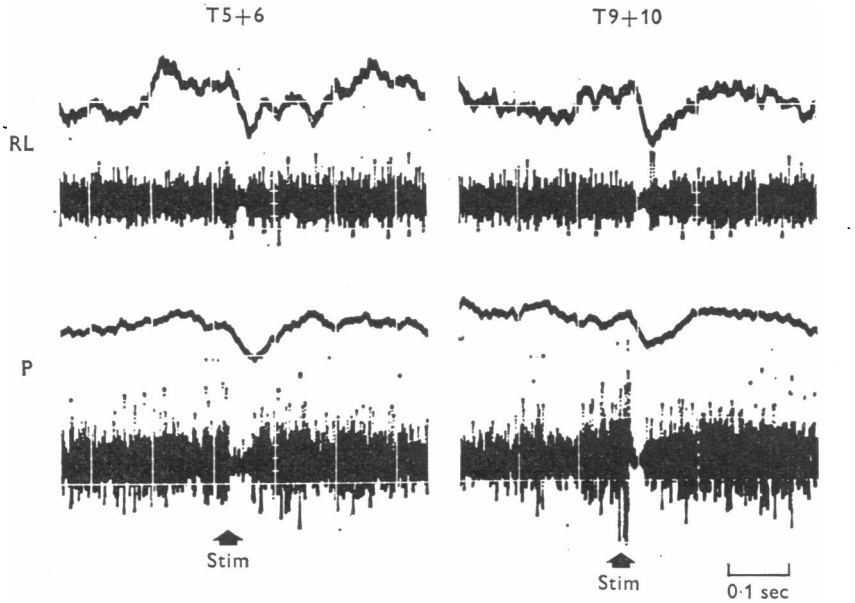

Fig. 1. Cat, anaesthetized with Dial with urethane and breathing spontaneously. Recordings were made midway through two inspirations. During one inspiration, a single shock was applied (at the arrow) to the cut external intercostal nerves T5 and 6 (left panel); during the other inspiration, a single shock was applied (at the arrow) to the cut external intercostal nerves T9 and 10 (right panel). Upper two traces derived from the recurrent laryngeal nerve (RL) and the lower pair from the phrenic nerve (P). In each pair, upper trace indicates impulse frequency and lower trace displays action potentials. The spontaneous discharge in both nerves was inhibited by the stimulus at either thoracic level. Phrenic discharge was augmented just before the inhibition caused by the T9 and 10 stimulus.

for the phrenic and recurrent laryngeal responses, respectively. With low stimulus intensity the latency for the recurrent laryngeal inhibitory response exceeded that for the phrenic inhibitory response by 5–8 msec. A similar difference in latency has been reported for anaesthetized cats in hypocapnic apnoea (Irani, Megirian & Sherry, 1972). With higher stimulus intensities this difference in apparent latencies sometimes increased owing to the appearance of an initial excitatory component in the recurrent

laryngeal response. Much of the difference in latency for the inhibitory response results from the greater peripheral conduction distance of the recurrent laryngeal nerve. The difference in peripheral nerve conduction time for the two nerves was estimated from the conduction time observed when a stimulus was applied to the vagus nerve, 2 cm distal to its exit from the skull, and the response was recorded off the ipsilateral recurrent laryngeal nerve. Values of 3–5 msec were obtained in four cats.

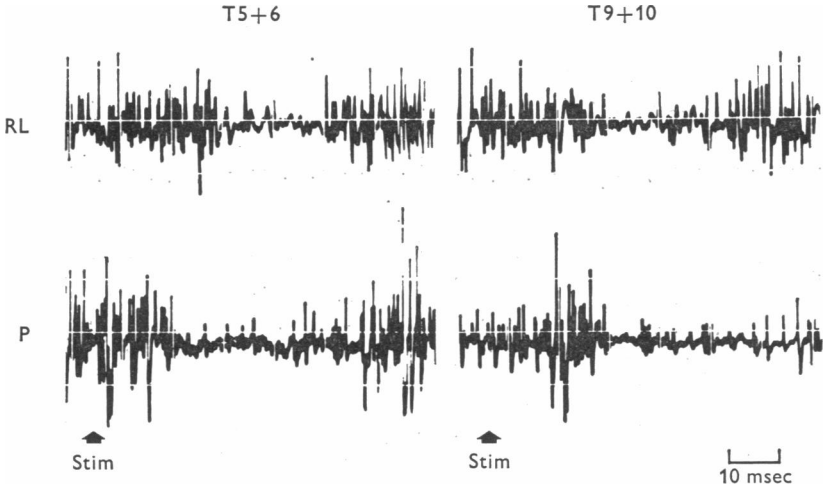

Fig. 2. Cat, anaesthetized with Dial with urethane and breathing spontaneously. Same configuration of responses as in Fig. 1, recorded at higher sweep speed. Top race: recurrent laryngeal discharge (RL); bottom trace: phrenic discharge (P). Single shocks were applied, at the arrows, to the external intercostal nerves. Inhibitory responses were evoked in all instances, and a volley preceded phrenic inhibition caused by the T9 and 10 stimulus.

Stimulus intensities 5–10 db above threshold were sometimes associated with more complex response patterns in the recurrent laryngeal neurogram. Biphasic (excitation–inhibition) or triphasic (inhibition-excitation-inhibition) patterns sometimes appeared, as is shown in Fig. 3, probably as a result of opposing responses in fibres innervating the thyroarytenoid (TA) and the posterior cricoarytenoid (PCA) muscles, as described below. Phrenic response to mid-thoracic stimuli were exclusively inhibitory at all levels of stimulus intensity. Over-all, these responses are reminiscent of those reported by Megirian (1968) using superior laryngeal nerve stimulation.

Intercostal stimulation at the T9 and 10 level during inspiration yielded biphasic phrenic responses which were entirely similar to those found by

Decima & Euler (1969*b*) (Figs. 1, 2 and 3). Recurrent laryngeal discharge, on the other hand, was either uninfluenced (Fig. 3) or inhibited by caudal stimuli. The latencies for the phrenic and recurrent laryngeal inhibitory responses to caudal intercostal stimuli were generally 3–5 msec longer than the corresponding latencies with middle intercostal stimuli.

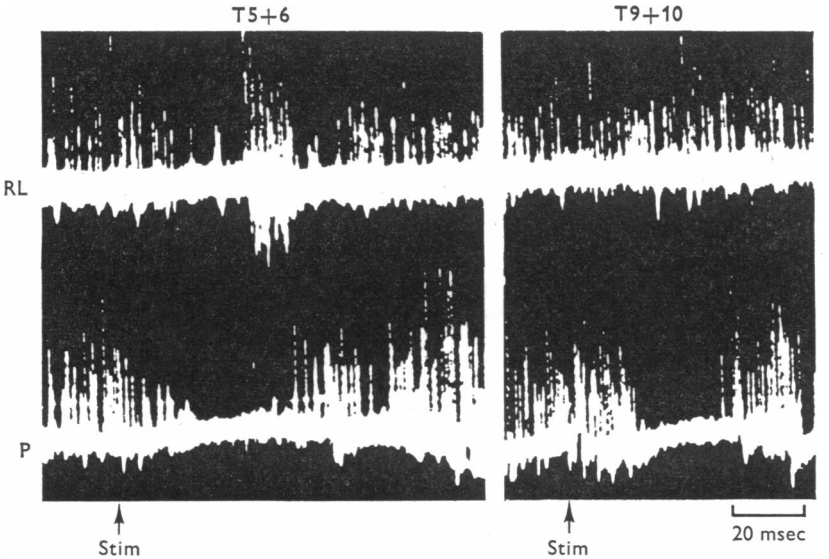

Fig. 3. Cat, anaesthetized with Dial with urethane and breathing spontaneously. Same configuration of responses as in Figs. 1 and 2. Top trace: recurrent laryngeal discharge (RL); bottom trace: phrenic discharge (P). A pulse train (6 msec, 500/sec) was applied during one inspiration (at arrow) to the external intercostal nerve, T5 and 6 (left panel), and T9 and 10 (right panel). Note that the T5 and 6 stimulus evoked a recurrent laryngeal volley which falls within the inhibitory interval, while T9 and 10 stimulus caused no recurrent laryngeal response.

Responses during expiration. Intercostal stimuli delivered at either thoracic level during expiration evoked no response in the phrenic neurogram other than a brief volley after caudal stimuli of high intensity. Recurrent laryngeal responses always reversed during the expiratory half-cycle: stimuli, which during inspiration caused only inhibition, during expiration provoked excitation. Typically, the threshold for this expiratory volley was somewhat lower than that associated with inhibition of inspiratory discharge. Middle and caudal intercostal stimuli elicited similar responses. This reversal of the laryngeal motor response is illustrated in the laryngeal EMGs shown in Figs. 4 and 5. During inspiration (Fig. 4) discharge from the PCA electrodes is inhibited by stimulation at either

thoracic level; during expiration (Fig. 5) the same stimulus causes bursts of discharge in both the PCA and the TA electrodes.

Effects of lateral cord section. The superficial region of the lateral cervical cord was sectioned in twenty experiments, and the position and extent of

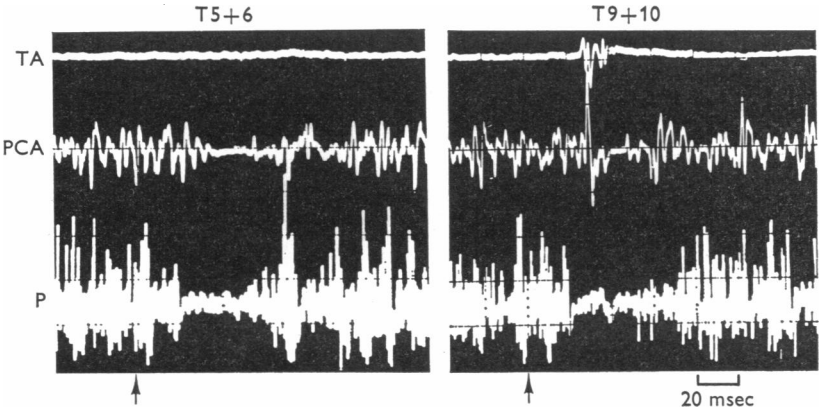

Fig. 4. Cat, anaesthetized with Dial with urethane and breathing spontaneously. The upper two traces are EMGs recorded from electrodes placed in the thyroarytenoid (TA) and the posterior cricoarytenoid (PCA) muscles. Bottom trace: phrenic discharge (P). A 4 msec tetanic stimulus was delivered (at the arrows) during one inspiration to the external intercostal nerves, T5 and 6 (left panel), and during another inspiration to the corresponding nerves of T9 and 10 (right panel). The stimuli evoked little or no TA response but caused transient inhibition of both PCA and phrenic discharge.

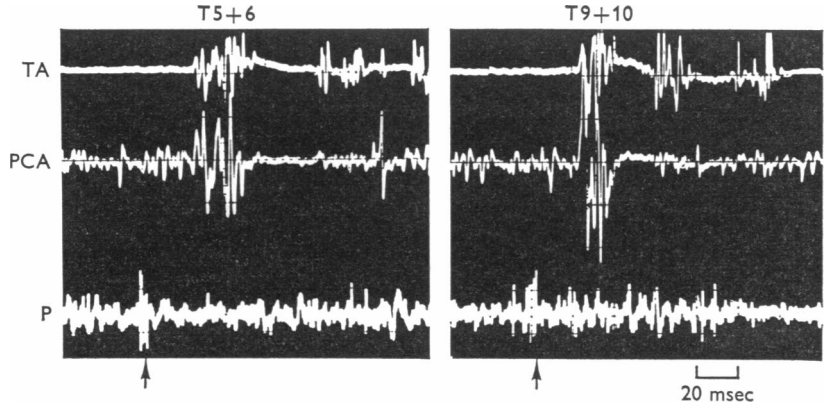

Fig. 5. Expiratory record. Same experiment as in Fig. 4. The same tetanic stimulus, delivered (at the arrow) to the external intercostal nerves, T5 and 6 (left panel), and T9 and 10 (right panel), caused volleys in both laryngeal muscles but not in the phrenic nerve. Note after discharge in the TA recording.

the sections were similar to those reported elsewhere (Remmers & Tsiaras, 1973). The 1 mm deep cut, above or below the level of the phrenic outflow, had little influence on the spontaneous phrenic or recurrent laryngeal discharges in most cases. Similar observations were made by Remmers & Tsiaras (1973), who, in addition, found no significant change in end-tidal P_{CO_2}. Occasionally, a lesion at the C3 level, ipsilateral to the site of phrenic recording, diminished spontaneous phrenic discharge, and in one case all rhythmic discharge ceased.

Lesion of the lateral cord ipsilateral, but not contralateral, to the stimulus site drastically and promptly changed the picture of reflex responses. Ipsilateral lesions at the level of C3 or C7 consistently eliminated all recurrent laryngeal responses and the phrenic inhibitory response to mid-thoracic stimuli, as well. Such a change can be seen by comparing Fig. 6 with Fig. 2. The control responses shown in Fig. 2 had been reproduced over a period of 1 hr. The lateral cord was then sectioned superficially at C3, ipsilateral to the stimulus sites, and 5 min later the responses shown in Fig. 6 were obtained. The inset shows the extent of cord section in this experiment. The only response evident after cordotomy is the phrenic volley and a subsequent period of subnormal discharge in response to stimulation of the 9th and 10th intercostal nerves. Whether this post-excitatory depression of phrenic discharge manifests a separate I-P reflex cannot be stated. The phrenic volley sometimes increased in size and duration after cordotomy, but never to the same extent as after cord transection. This change after cordotomy may be the consequence of loss of descending inhibition or simply the removal of an antagonistic reflex.

Bilateral dorsal column section had no effect on the reflexes, in agreement with the report of Garcia Ramos & Lopez Mendoza (1959).

Laryngeal responses

Responses from electrodes placed in the PCA and TA muscles were recorded in five cats. The PCA leads exhibited activity which was phasic with the respiratory cycle and the TA leads were silent, a combination of findings which has already been reported (Nakamura, Uyeda & Sonoda, 1958; Mårtensson, 1963; Murakami & Kirchner, 1972). As was expected from the recurrent laryngeal responses, middle and caudal intercostal stimuli elicited qualitatively similar responses in the laryngeal EMGs. Stimuli delivered during inspiration inhibited the discharge recorded from the PCA electrodes and usually evoked no response at the TA electrodes (Fig. 4). With higher stimulus intensities a brief volley was produced at the TA electrodes but excitatory responses were never recorded from the PCA electrodes during inspiration. This suggests that the complex recurrent laryngeal responses which occur during inspiration stem from simultaneous

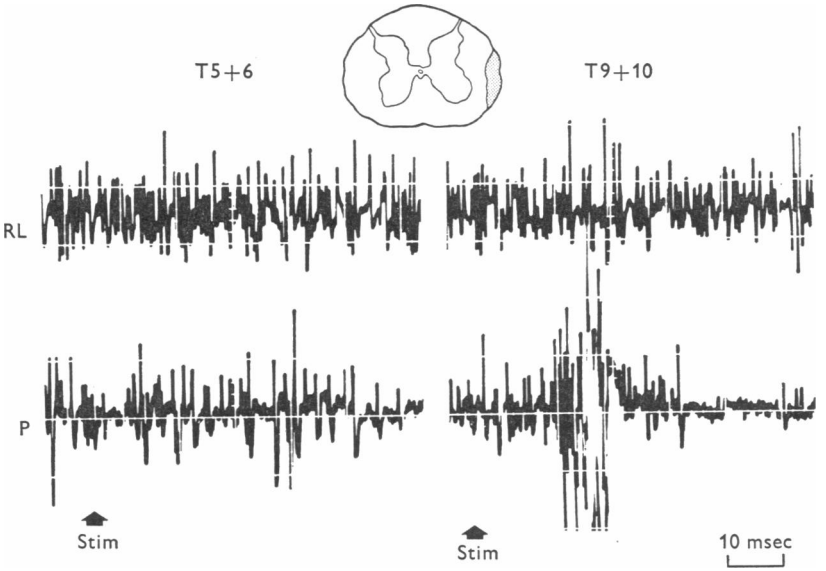

Fig. 6. Same experiment as shown in Fig. 2. Top trace: recurrent laryngeal discharge (RL); bottom trace: phrenic discharge (P). The lateral cord, ipsilateral to the stimulus sites, was sectioned superficially at the C3 level shortly before this record was obtained. The same stimulus was applied during inspiration to the same intercostal nerves as in Fig. 2 (left panel: T5 and 6; right panel: T9 and 10). All the responses have disappeared except the phrenic response to the caudal stimulus. Inset shows the extent of cord section in this case.

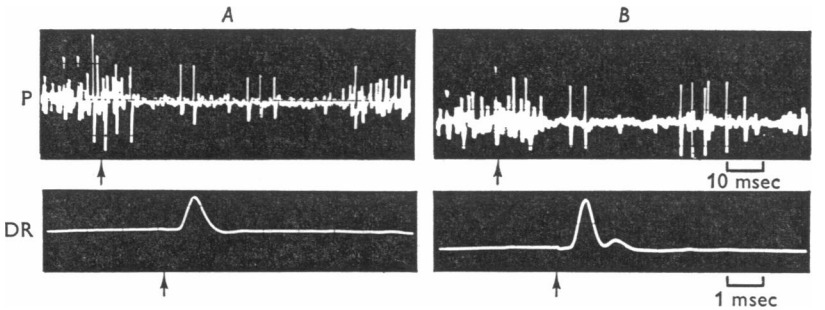

Fig. 7. Cat, anaesthetized with Dial with urethane and paralysed, artificially ventilated. Inspiratory record. Simultaneous recording at different sweep speeds of phrenic discharge (P: top trace) and T6 dorsal root discharge (DR: bottom trace). The top trace (slow sweep) was triggered first and the lower trace (fast sweep) was triggered after a delay. A single shock stimulus was applied at the arrows to the T6 external intercostal nerve, having been immediately preceded by a 4 msec tetanic subthreshold stimulus to the T5 external intercostal nerve. In *B* the intensity of the T6 stimulus was 2 db higher than in *A*, causing recruitment of Group II fibres but no change in the phrenic response.

excitation of TA motor fibres and inhibition of PCA motor fibres. During expiration, afferent stimulation at either thoracic level facilitated discharge at both TA and PCA electrodes, with TA volleys sometimes recurring (Fig. 5). The discharges in the TA and PCA leads differed sufficiently in onset and duration to negate the possibility of a spurious response of one EMG owing to electronic spread of potentials generated in the vicinity of the other pair of electrodes.

Dorsal root recordings

Dorsal root recordings were made in five cats. When the intensity of the stimulus delivered to the 6th external intercostal nerve was just sufficient to produce a compound action potential in the T6 dorsal root neurogram, slight but reproducible efferent responses were usually apparent. The fibres of this lowest threshold population conducted at approximately 110 m/sec and were, presumably, Group Ia afferents. As the size of this mass entry wave grew with increasing stimulus intensity, the associated phrenic and recurrent laryngeal responses were enhanced. A secondary hump in this wave, which might indicate activation of Group Ib fibres, could not be identified. A typical simultaneous recording of the T6 dorsal root discharge and phrenic responses are shown in Fig. 7*A*. Figure 7*B* shows that the phrenic response was unchanged by recruitment of a second population of afferent fibres, presumably Group II, conducting at 40–50 m/sec. These results indicate that Group I afferents are responsible for the phrenic and recurrent laryngeal responses to middle intercostal stimulation.

In addition, Group II afferents of the mid-thoracic segments may contribute to the intercostal-to-recurrent laryngeal reflex during expiration. Fig. 8*A* shows a facilitatory recurrent laryngeal response evoked during expiration by activating Group I fibres. The record in Fig. 8 *B* was made after the stimulus intensity was increased 2 db and shows that Group II afferents were recruited and that the efferent volley was enhanced. This increase in response may have resulted from the activation of Group II fibres but the associated increase in Group I discharge may have been responsible for this effect.

Results with caudal intercostal stimuli were entirely comparable to those reported by Decima, Euler & Thoden (1969). Phrenic excitation was not provoked by Group I afferents of the 9th external intercostal nerve, but appeared coincident with the recruitment of Group II fibres. Recurrent laryngeal responses, when present, behaved similarly.

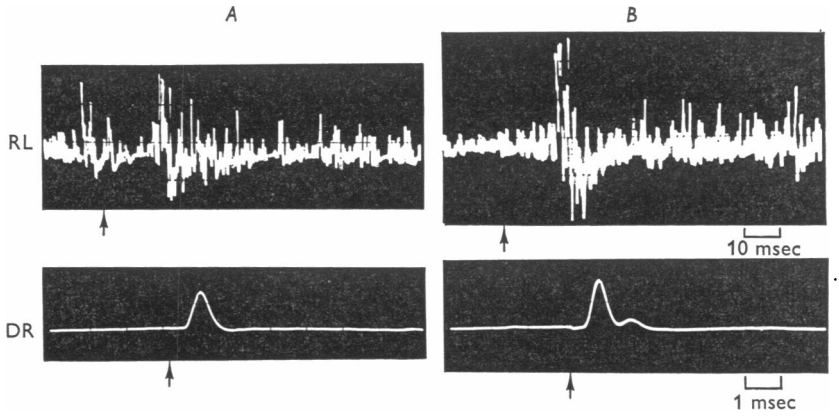

Fig. 8. Cat, anaesthetized with Dial with urethane, paralysed and artificially ventilated. Simultaneous recording at different sweep speeds of recurrent laryngeal discharge (top trace: RL) and T6 dorsal root discharge (bottom trace: DR). The top trace (slow sweep) was triggered first and the bottom trace (fast sweep) was triggered after a delay. A single shock stimulus was applied, at the arrows, to the T6 external intercostal nerve, having been immediately preceded by a 4 msec tetanic stimulus of subthreshold intensity to the T5 external intercostal nerve. In *B* the intensity of the T6 stimulus was 2 db higher than in *A*. Group II afferents were recruited in *B* and the recurrent laryngeal volley was augmented.

Spinal reflexes

As expected from the results of Decima *et al.* (1969), caudal intercostal stimuli evoked a phrenic volley and middle intercostal stimuli caused no overt phrenic response in the spinal animal. The effect of middle intercostal stimulation on the excitability of phrenic motoneurones was examined in five animals after spinal cord transection at the C2 level. The external intercostal nerves, T5 and 6, received a 5 msec pulse train, and a single shock, test stimulus was delivered to the corresponding nerves of the 9th and 10th interspaces. The stimuli were staggered such that the T5 and 6 stimulus preceded by 5–30 msec the phrenic volley caused by the test stimulus, and the number of spikes in the phrenic volley was measured.

The average firing probability never differed more than 20% from control, and neither the number of spikes nor the size of the evoked volley displayed a time-dependency. The results do not evidence a depression in phrenic motoneurone excitability, which could have resulted from a spinal, inhibitory I-P reflex. It is worth noting that this lack of influence of middle intercostal afferents on the evoked phrenic response is, in fact, equivalent to the lack of influence of middle intercostal stimuli on the spontaneous phrenic discharge after superficial section of the lateral cord at the C3 level.

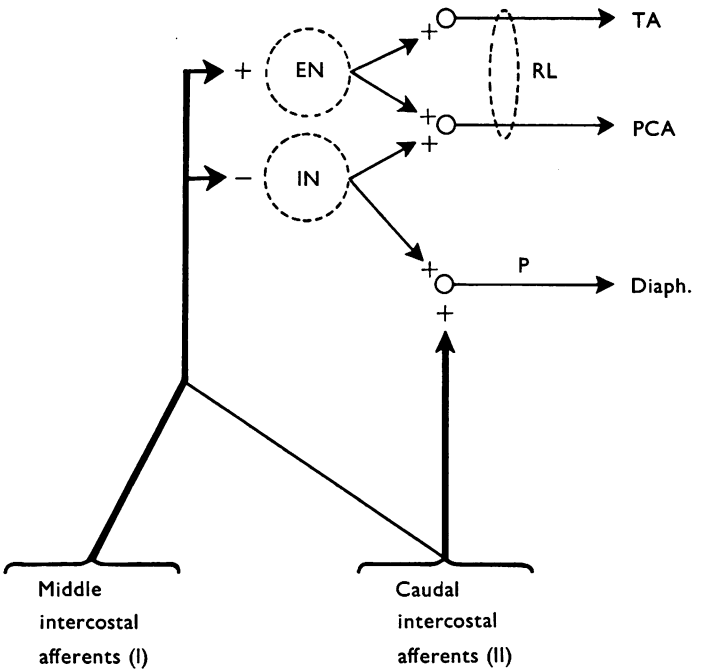

Fig. 9. Schematic summary of reflex effects of middle and caudal intercostal afferents. EN and IN denote 'expiratory' and 'inspiratory' interneurones, respectively, which are postulated to be facilitated during inspiration and expiration. TA: thyroarytenoid muscle; PCA: posterior crycoarytenoid muscle; Diaph.: diaphragm; RL: recurrent laryngeal nerve; P: phrenic nerve.

DISCUSSION

The dependence of the pattern of phrenic response on receptive field reconciles the apparent differences between previous studies (Decima & Euler, 1969*b*; Remmers, 1970). This pattern is similar to that recently reported by Irani *et al.* (1972). The laryngeal responses support the view that the inhibitory I-P reflex is one component of a generalized reflex linking intercostal muscles to other respiratory muscles. In addition, the response of the laryngeal adductors (TA) manifests an intercostal-to-laryngeal reflex which probably serves primarily non-ventilatory actions requiring glottal closure or the development of intrapulmonary pressures in the static or quasi-static respiratory system. This facilitatory reflex may correspond to the excitatory intercostal-to-recurrent laryngeal reflex recently described for cats in hypocapnic apnoea (Irani *et al.* 1972), since the excitability of TA motoneurones is increased in this state (Murakami & Kirchner, 1972).

Spinal cord tracts interrupted by lateral cord section

The observation that lateral cord section at the C3 level left phrenic motor activity substantially intact is not inconsistent with results of a recent investigation of descending spinal pathways to phrenic motoneurones by Newsom Davis & Plum (1972). The lesions which these workers used to eliminate diaphragmatic activity were more extensive, ventrally, than the ones used in the present study. Similarly, Nakayama & Baumgarten (1964) identified respiratory tracts in a large portion of the ventrolateral quadrant of the cervical cord.

Tonic descending tracts, inhibitory to intercostal-intercostal and splanchnic-intercostal reflexes have been shown to arise in the medullary reticular formation (Alderson & Downman, 1966). Similar influences, arising in the cerebellum, have been shown to control the I-P reflex in the decerebrate cat (Decima & Euler, 1969*b*). Cordotomy may have interrupted these tracts, though those identified by Downman & Hussain (1958) lie dorso-medial to the sections used in the present study. However, such tonic descending influences are usually minimal in the anaesthetized animal with the C.N.S. intact (Alderson & Downman, 1966), and their removal could hardly be expected to eliminate all recurrent laryngeal response to intercostal afferent stimulation. Disruption of ascending tracts carrying intercostal proprioceptive traffic seems, therefore, to be a likely explanation, but its proof must await a more direct experimental approach.

Neural structures mediating extra-segmental intercostal reflexes

A fundamental issue is whether or not the reflex actions of middle intercostal afferents reflect alterations of central respiratory activity, i.e. of bulbopontine neuronal activity involved in the generation of descending respiratory traffic. There is no direct evidence against this possibility. One might argue that the shortest latency for the phrenic inhibitory response, 13 msec, is roughly the same as that for the spinal I-P reflex from the caudal intercostal afferents and is too brief for a supraspinal arc. However, this latency is also the same as that reported for the phrenic response to median nerve stimulation in the cat, a reflex which seems to traverse brain stem structures (Calma, 1952). Although no information is available regarding the intercostal-to-reticular formation conduction time, the latency for intercostal projection to the cerebellum is 7 msec (Coffey, Godwin-Austin, MacGillivray & Sears, 1971). The argument advanced by Calma (1952), indicating that the observed latency provides ample time for traversing supraspinal synapses, seems valid in the present context.

The postulate that middle intercostal afferents act on bulbopontine respiratory neurones is supported by two findings in the present study:

(1) the lack of phrenic response after partial or complete cord section, and (2) the parallel response of spinal and supraspinal respiratory motoneurones.

Phrenic response after cord section. Were the phrenic inhibitory action of middle intercostal afferents the result of a spinal arc, some manifestation of it could be anticipated after lateral cordotomy or after complete cord transection at the C2 level. The negative evidence on this point, while not conclusive, strongly favours the possibility that the phrenic inhibitory reflex derived from middle intercostal afferents involves supraspinal connexions.

Behaviour of supraspinal respiratory motoneurones. The behaviour of laryngeal motoneurones also relates, though circumstantially, to the question. That the PCA muscle, indeed, constitutes a 'respiratory' muscle which regulates air flow by determining the degree of glottal abduction and, therefore, the glottal resistance, rests on three pieces of evidence: the PCA phasically abducts the vocal cords synchronously with the respiratory cycle (the TA being inactive normally); hypercapnia increases both inspiratory and expiratory abduction by the PCA; and lung inflation inhibits PCA discharge during inspiration and excites PCA discharge during expiration (Mårtensson, 1963; Murakami & Kirchner, 1972; Bartlett, Remmers & Gautier, 1973). This evidence bespeaks a dual control of PCA activity by both inspiratory and expiratory mechanisms. The present results are in accord with this concept; PCA responses paralleled phrenic responses during inspiration and during expiration were similar to those described for abdominal expiratory muscles (Garcia Ramos, 1959).

The simplest way to explain the opposite responses of PCA motoneurones in inspiration and expiration and to account for the parallel response of PCA and other respiratory muscles (diaphragm, inspiratory intercostals, and abdominals) is to postulate a reflex action of middle intercostal afferents on medullary inspiratory and expiratory neurones. Any other hypothesis requires two sets of intercostal-to-PCA interneurones with appropriate centrifugal connexions to medullary inspiratory and/or expiratory neurones. In addition, separate sets of interneurones, also with centrifugal respiratory connexions, may be postulated to mediate the other extra-segmental intercostal reflexes. Obviously, the postulate of a single reflex involving medullary respiratory neurones is a very convenient one.

The pattern of phrenic and recurrent laryngeal responses to middle intercostal afferent stimulation, including the dependence on respiratory phase, is essentially the same as that reported by Megirian (1968) using vestibular and superior laryngeal stimuli. This may indicate that these three unrelated afferent inputs impinge on the respiratory effector mechanism at a common point. Biscoe & Sampson (1970) have demon-

strated that medullary inspiratory neurones constitute at least part of the neural substrate for superior laryngeal reflexes, and have suggested that the phrenic inhibition involved disfacilitation.

A reflex schema. A general schema summarizing the observed reflexes is presented in Fig. 9. 'Inspiratory' and 'expiratory' neurones simply denote interneurones, most likely supraspinal, which are facilitated during inspiration and expiration, respectively. They may or may not be medullary 'respiratory centre' neurones. Group I intercostal afferents of the mid-thoracic segments inhibit the 'inspiratory' interneurones and facilitate the 'expiratory' interneurones. Caudal intercostal afferents of group II, while contributing to this reflex, facilitate phrenic motoneurones through a separate, spinal loop. Though it is simplest to include the facilitatory reflex to TA motoneurones as a part of the reflex mediated by the 'expiratory' interneurones, a separate set of interneurones may be involved. The present results imply that respiratory mechanisms control this reflex, and medullary expiratory mechanisms appear to facilitate TA motoneurones (Kurozumi, Tashiro & Harada, 1971).

Structural–functional correlates

What functional importance should be attached to extra-segmental intercostal reflexes is uncertain. A notable exception is the excitatory I-P reflex, which has been convincingly shown to constitute a load compensating mechanism dependent on the mechanical coupling between the diaphragm and the caudal ribs (Decima & Euler, 1969*a*). Related to this is the correspondence between rib structure and reflexogenic capability of the various thoracic segments. In the cat, the transition between the complete and incomplete ribs occurs at T9, the most cranial segment initiating phrenic excitation. The caudal ribs (9–13) form the loci of attachment of the diaphragm and the abdominal expiratory muscles. Forces exerted on these ribs are related to the forces in these muscles suggesting that, in addition to providing the load compensation mechanism already mentioned, the reflex may participate in motor acts requiring simultaneous contraction of the diaphragm and abdominal muscles, e.g. expulsive or postural manoeuvres.

It is difficult to predict under what circumstances the extra-segmental reflexes arising from middle intercostal mechanoreceptors may be active. They may be a part of postural control systems, and the documentation of cerebellar projections of intercostal afferents is of considerable interest (Coffey *et al.* 1971). The complete or circumferential ribs, and their corresponding intercostal muscles withstand forces related to transpulmonary pressure, and, in addition, intercostal γ-efferent discharge varies phasically with the respiratory cycle (Critchlow & Euler, 1963; Eklund, Euler &

Rutkowski, 1963; Sears, 1963). If the extra-segmental reflexes derived from the mid-thoracic region are mediated by bulbopontine respiratory mechanisms, they may, by inhibiting inspiration and facilitating expiration, participate in setting the respiratory pattern in some situations. This possibility is discussed in another paper (Remmers & Tsiaras, 1973).

The author gratefully acknowledges the help of Miss J. Jurancich in carrying out some of these experiments. This work was supported by a Public Health Service Research Grant (1-KO1-HL 14330–01) from the National Heart and Lung Institute. The author is a Markle Scholar in Academic Medicine and a recipient of a Public Health Service Research Career Development Award (1-KO4-HL 50282–02) from the National Heart and Lung Institute.

REFERENCES

ALDERSON, A. M. & DOWNMAN, C. B. B. (1966). Supraspinal inhibition of thoracic reflexes of somatic and visceral origin. *Archs ital. Biol.* **104**, 309–327.

BARTLETT, D., REMMERS, J. & GAUTIER, H. (1973). Laryngeal regulation of respiratory airflow. *Resp. Physiol.* (in the Press).

BASMAJIAN, J. V. & STECKO, G. (1962). A new bipolar indwelling electrode for electromyography. *J. appl. Physiol.* **17**, 849.

BISCOE, T. J. & SAMPSON, S. R. (1970). An analysis of the inhibition of phrenic motoneurones which occurs on stimulation of some cranial nerve afferents. *J. Physiol.* **209**, 375–393.

CALMA, I. (1952). The reflex activity of the respiratory centre. *J. Physiol.* **117**, 9–21.

COFFEY, G. L., GODWIN-AUSTIN, R. B., MACGILLIVRAY, B. B. & SEARS, T. A. (1971). The form and distribution of the surface evoked responses in cerebellar cortex from intercostal nerves in the cat. *J. Physiol.* **212**, 129–145.

CRITCHLOW, V. & EULER, C. v. (1963). Intercostal muscle spindle activity and its γ motor control. *J. Physiol.* **168**, 802–847.

DECIMA, E. E. & EULER, C. v. (1969*a*). Excitability of phrenic motoneurones to afferent input from lower intercostal nerves in the spinal cat. *Acta physiol. scand.* **75**, 580–591.

DECIMA, E. E. & EULER, C. v. (1969*b*). Intercostal and cerebellar influences on efferent phrenic activity in the decerebrate cat. *Acta physiol. scand.* **76**, 148–158.

DECIMA, E. E., EULER, C. v. & THODEN, U. (1969). Intercostal-to-phrenic reflexes in the spinal cat. *Acta physiol. scand.* **75**, 568–579.

DOWNMAN, C. B. B. (1955). Skeletal muscle reflexes of splanchnic and intercostal nerve origin in acute spinal and decerebrate cats. *J. Neurophysiol.* **18**, 217–235.

DOWNMAN, C. B. B. & HUSSAIN, A. (1958). Spinal tracts and supraspinal centres influencing visceromotor and allied reflexes in cats. *J. Physiol.* **141**, 489–499.

EKLUND, G., EULER, C. v. & RUTKOWSKI, S. (1963). Intercostal γ-motor activity. *Acta physiol. scand.* **57**, 481–482.

GARCIA RAMOS, J. (1959). On the integration of respiratory movements. *Acta physiol. latinoam.* **9**, 246–256.

GARCIA RAMOS, J. & LOPEZ MENDOZA, E. (1959). On the integration of respiratory movements. II. The integration at spinal level. *Acta physiol. latinoam.* **9**, 257–266.

IRANI, B., MEGIRIAN, D. & SHERRY, J. H. (1972). An analysis of reflex changes in excitability of phrenic, laryngeal and intercostal motoneurones. *Expl Neurol.* **36**, 1–13.

KUROZUMI, S., TASHIRO, T. & HARADA, Y. (1971). Laryngeal responses to electrical stimulation of the medullary respiratory centers in the dog. *Laryngoscope, St Louis* **81**, 1960–1967.

MÅRTENSSON, A. (1963). Reflex responses and recurrent discharges evoked by stimulation of laryngeal nerves. *Acta physiol. scand.* **57**, 248–269.

MEGIRIAN, D. (1968). Vestibular control of laryngeal and phrenic motoneurons of cat. *Archs ital. Biol.* **106**, 333–342.

MURAKAMI, Y. & KIRCHNER, J. A. (1972). Respiratory movements of the vocal cords. An electromyographic study in the cat. *Laryngoscope, St Louis* **82**, 454–467.

NAKAMURA, F., UYEDA, Y. & SONODA, Y. (1958). Electromyographic study on respiratory movements of the intrinsic laryngeal muscles. *Laryngoscope, St Louis* **68**, 109–119.

NAKAYAMA, S. & BAUMGARTEN, R. v. (1964). Lokalisierung absteigender Atmungsbahnen im Rückenmark der Katze mittels antidromer Reizung. *Pflügers Arch. ges. Physiol.* **281**, 231–244.

NEWSOM DAVIS, J. & PLUM, F. (1972). Separation of descending spinal pathways to respiratory motoneurons. *Expl Neurol.* **34**, 78–94.

REMMERS, J. E. (1970). Inhibition of inspiratory activity by intercostal muscle afferents. *Resp. Physiol.* **10**, 358–383.

REMMERS, J. E. & JURANCICH, J. (1972). Phrenic and recurrent laryngeal responses to intercostal stimuli. *Fedn Proc.* **31**, 362.

REMMERS, J. E. & TSIARAS, W. G. (1973). Effect of lateral cervical cord lesions on the respiratory rhythm of anaesthetized, decerebrate cats after vagotomy *J. Physiol.* **233**, 63–74.

SEARS, T. A. (1963). Activity of fusimotor fibres innervating muscle spindles in intercostal muscle of the cat. *Nature, Lond.* **197**, 1013–1014.

SEARS, T. A. (1964). Efferent discharges in alpha and fusimotor fibres of intercostal nerves of the cat. *J. Physiol.* **174**, 295–315.