Functional classification of afferent phrenic nerve fibres and diaphragmatic receptors in cats

A. Bałkowiec, K. Kukuła and P. Szulczyk*

Katedra i Zakład Fizjologii Człowieka, Akademia Medyczna w Warszawie, Krakowskie Przedmieście 26/28, Warszawa 00-325, Poland

1. Single afferent fibres with receptive fields in the diaphragm (272 units) dissected from the right phrenic nerve were classified according to the following properties: reaction to contraction of the diaphragm, resting activity, conduction velocity, location and properties of receptive fields, and reaction to injection of bradykinin and lactic acid into the internal thoracic artery. Nine additional fibres dissected from the phrenic nerve had receptive fields outside the diaphragm. The experiments were performed on chloralose-anaesthetized cats.

2. Ninety-six fibres (36%) had high resting activity when unloaded by contraction of the diaphragm, had low-threshold receptive fields in the muscle and were mostly group II and III fibres. They probably innervated muscle spindles.

3. Eighty-eight fibres (32%) were vigorously activated by contraction of the diaphragm. They had low-threshold receptive fields located in the musculotendinous border and central tendon. Their conduction velocity was in the range for group II and III fibres. We infer that they may innervate tendon organs.

4. Eighty-eight fibres (32%) were slightly affected or not affected by diaphragmatic contraction. They had low- and high-threshold receptive fields located mostly in the muscular part of the diaphragm, and negligible resting activity. Most of them were group III and IV afferent fibres and were activated when bradykinin and lactic acid were applied to their receptive fields. Possibly these low- and high-threshold receptors innervated diaphragmatic ergo- and nociceptors, respectively.

5. Sensory outflow from the diaphragm was found to be somatotopically organized, so that fibres with receptive fields in the sternocostal portion were predominantly located in the upper phrenic nerve root, and those with lumbar receptive fields were in the lower root.

6. It is concluded that the phrenic nerve contains fibres from several distinct classes of sensory receptors: muscle spindles, tendon organs, ergoceptors and nociceptors. The sensory diaphragmatic outflow to the spinal cord is somatotopically organized.

Phrenic nerve afferent fibres that innervate the diaphragm may play an important role in reflex regulation of respiratory and cardiovascular effectors (Road, 1990).

Morphological results indicate that each phrenic nerve in the cat contains approximately 770 myelinated and 1900 unmyelinated fibres. Among them there is a significant proportion of sensory afferent fibres: about 180 myelinated and 300 unmyelinated units (Hinsey, Hare & Phillips, 1939; Duron, 1981; Larnicol, Rose & Duron, 1985).

A few electrophysiological studies have investigated the functional properties of phrenic nerve afferent fibres. The information obtained so far indicates that in cat phrenic nerve the number of fibres that innervate muscle spindles and tendon organs is negligible (Yasargil, 1962; Corda, von Euler & Lennerstrand, 1965). More recently, Graham, Jammes, Delpierre, Grimaud & Roussos (1986) concluded that in the phrenic nerve there are slowly conducting fibres that innervate diaphragmatic receptors sensitive to chemical stimuli. The location of sensory fibres in the phrenic nerve does not guarantee, however, that their receptive fields are in the diaphragm, as it was shown that phrenic nerve afferent fibres also transmit information from thoracic and abdominal visceral receptors (Kostreva & Pontus, 1993*a*, *b*). It is therefore important when classifying afferent fibres, and the diaphragmatic receptors

* To whom correspondence should be addressed.
Authors' names are in alphabetical order.

that they innervate, that the receptive field location for each sensory fibre, isolated from the phrenic nerve, is determined.

Our electrophysiological study was designed to describe the properties of single afferent fibres isolated from the phrenic nerve, to classify these sensory fibres and the receptors they innervate, and to determine receptive fields. Additionally, we wanted to obtain information as to whether afferent fibres with receptive fields located in different portions of the diaphragm project separately to the upper and lower phrenic nerve roots, i.e. whether afferent information from the diaphragm is somatotopically organized.

METHODS

Experiments were carried out on twenty-three cats weighing 2·0–4·0 kg. After initial induction of anaesthesia with ether, 80 mg kg^{-1} α-glucochloralose was injected intraperitoneally. Intubation was performed, and the left femoral vein and artery, and the bladder were cannulated. Blood pressure was monitored continuously from the artery and heart rate from the arterial pressure pulse. A heating pad maintained the animal's body temperature at a constant 37·5 ± 0·2 °C. The animals were not paralysed, were artificially ventilated and a positive end-expiratory pressure of approximately 2–3 cmH_2O was applied. Additional injections of chloralose (10–20 mg kg^{-1}) were given as required to maintain a very deep level of anaesthesia. The depth of anaesthesia was controlled by monitoring the blood pressure, heart rate, pupil size and reactions to noxious stimulation. Mean blood pressure exceeded 90 mmHg throughout the experiment. Arterial P_{O_2}, P_{CO_2} and pH were measured and were maintained within the range reported by Herbert & Mitchell (1971) by means of ventilation adjustment and administration of sodium bicarbonate. Some oxygen was added to the inspiratory air. End-tidal CO_2 was monitored continuously. At the end of the experiments the animals were killed, under very deep anaesthesia, by intravenous injection of saturated KCl solution.

The left phrenic nerve was cut just below the junction of its roots, and the peripheral end was attached to two platinum wire electrodes. Both the phrenic nerve and the stimulating electrodes were covered with Vaseline to insulate them from the surrounding tissue; the wound was then closed. Phrenic nerve roots on the right side were then dissected from the surrounding tissue and put on a black Perspex plate. Strands from the right upper (from C5 segment) phrenic nerve root and, in turn, the lower (from C6 segment) phrenic nerve root were dissected centrally from the rest of the nerve and placed on a monopolar recording electrode. The signal recorded from these strands was amplified using a standard AC amplifier. Phrenic nerve roots on the right were crushed above the recording point at the beginning of the recording session. The diaphragm was therefore isolated on both sides from all motor input coming from the phrenic nerves. A catheter connected to a latex balloon was introduced into the abdominal cavity. The outlet of the catheter was connected to a pressure transducer to monitor the intra-abdominal pressure. The cat's abdomen was dressed with a plaster bandage in order to fix the volume of the abdominal cavity, thus making the intra-abdominal pressure changes more prominent. On the right side the vertebral ends of ribs 9–12 were removed to access the right side of the diaphragm. One centimetre above the diaphragm the right phrenic nerve was isolated from the surrounding tissues and placed on bipolar stimulating electrodes.

The activity of single afferent units isolated from the right phrenic nerve, blood pressure, heart rate, intra-abdominal pressure and trigger pulses were recorded for 'off-line' analysis onto videotapes using a video cassette recorder (Blaupunkt RTV-925), after digitalization at 9 kHz with a VR-100A 8 Exp Interface (Instrutech, Corp., Elmont, NY, USA). The original sensory activity together with the discrimination level were continuously displayed on the screen of an oscilloscope, and histograms of activity were created by computer from standard pulses triggered by the amplified afferent spikes.

The following tests were performed to classify single sensory fibres dissected from the right phrenic nerve.

(1) The latency from an electrical stimulus (0·2 ms, 20 V, 1 Hz), delivered by the electrode positioned on the right phrenic nerve just above the diaphragm, to the evoked spike recorded at the cervical level was determined. The stimulus amplitude was approximately 80 times the threshold amplitude for the lowest threshold units. From this latency and the distance between the recording and stimulating electrodes the conduction velocity of the fibres was calculated. Fibres with conduction velocities below 2·5 m s^{-1}, between 2·5 and 30 m s^{-1} and above 30 m s^{-1} were arbitrarily considered as belonging to groups IV, III and I–II, respectively (Lloyd & Chang, 1948).

(2) We have accepted the following convention in determining the mechanoreceptive thresholds of receptive fields of the tested fibres. The receptive field was denoted as low threshold if the fibre was activated by just-noticeable distortion of diaphragmatic tissue with a blunt wooden probe (1·5 mm in diameter) and a von Frey hair (10 mN). In a few cases the afferent fibres were activated only after squeezing the diaphragmatic tissue using blunt forceps; such squeezing was considered painful when applied to the skin of the experimenter and the receptors were, therefore, considered high threshold. Receptive field locations were entered on a diagram based on Crouch (1969).

(3) Reactions of the sensory fibres to contraction of the diaphragm were determined. Contraction was evoked by a train of electrical stimuli delivered bilaterally to the phrenic nerves (3 pulses, 0·2 ms, 1·0–3·0 V, 300 Hz, once every 30 s). The stimulus amplitude was approximately 8 times threshold for the lowest threshold units included in the phrenic nerve. The time course of the contraction was monitored as an increase in intra-abdominal pressure. Five to ten consecutive responses of the sensory fibres and the intra-abdominal pressure were recorded, summed and averaged, respectively.

(4) A polyethylene catheter (0·6 mm o.d., 0·3 mm i.d.) was introduced through one of the branches of the subclavian artery (costocervical trunk or vertebral artery) into the internal thoracic artery. The blood flow through this artery was preserved. The tip of the catheter in the internal thoracic artery was positioned about 2 cm above the diaphragm, as verified at the end of every experiment. The area of diaphragm supplied by

the internal thoracic artery was determined by injecting Methylene Blue into the artery and observing the colour of the muscle surface. To verify whether solutions injected through the catheter reached the receptive fields of fibres tested, an isotonic KCl injection (0·2 ml) was performed prior to other tests. Only fibres activated by KCl solution were accepted for further investigation. Reactions to bolus injections of bradykinin (acetate salt; Sigma) in doses of 0·01, 0·1, 1·0 and 10·0 μg in 0·2 ml saline (0·9% NaCl) and/or 0·2 ml 0·1 M lactic acid were then tested. Injections of bradykinin were performed at 30 min intervals. For the methodological reasons indicated above, we tested only the fibres with receptive fields located in the area of diaphragm supplied by the right internal thoracic artery.

Statistics

Values given are means ± S.E.M. Analysis of variance (ANOVA) of appropriate design was used. Following ANOVA, Duncan's test for multiple comparisons was applied; the level of significance was set at $P < 0.05$.

RESULTS

The properties of 272 sensory fibres isolated from the phrenic nerve and with receptive fields in the diaphragm are described. Units were classified, with respect to their response to contraction of the diaphragm, into three major groups: 1, unloaded; 2, activated; and 3, not affected or only slightly activated by the contraction. An additional nine fibres had receptive fields located outside the diaphragm.

Receptors unloaded by contraction of the diaphragm

There were ninety-six (36%) fibres of 272 that had a high resting activity suppressed during contraction of the diaphragm (Fig. 1*A*). The mean resting activity of these fibres was 25·0 ± 1·6 spikes s^{-1}. Their conduction velocity was in the range 14–129 m s^{-1} (Fig. 1*B*). All these fibres

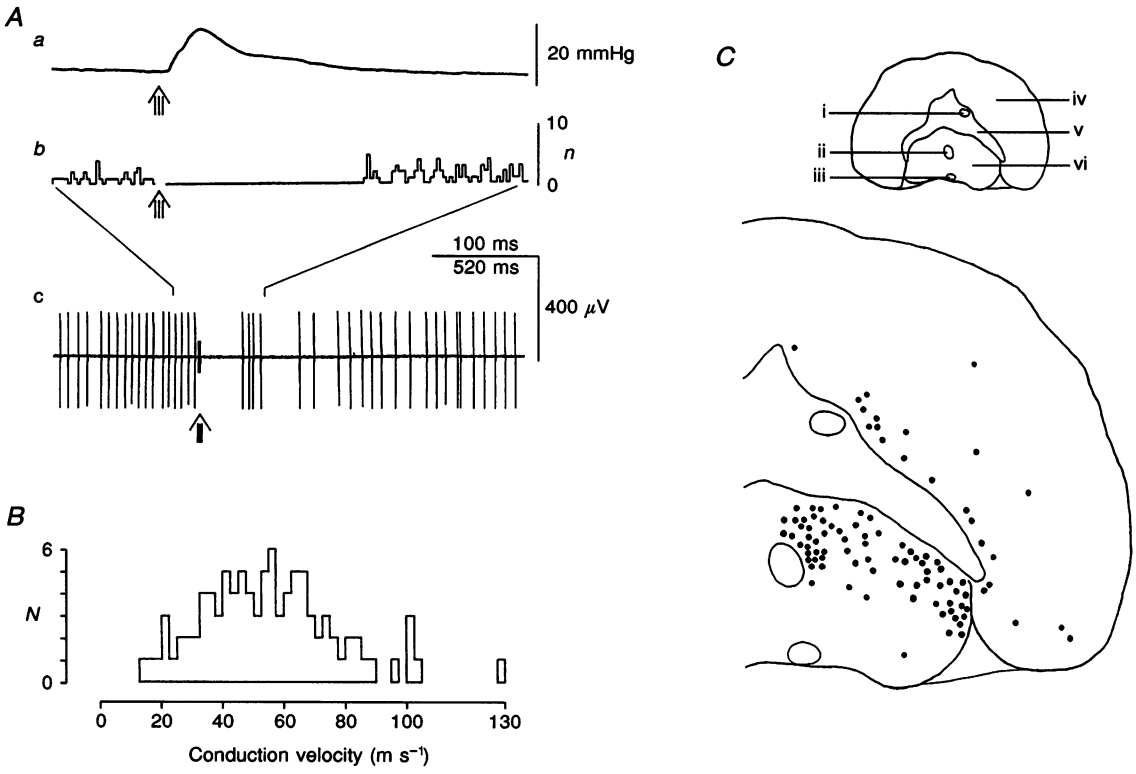

Figure 1. Receptors unloaded by diaphragmatic contraction

A, reaction of a fibre (*b* and *c*) to contraction of the diaphragm evoked by bilateral stimulation (arrows) of phrenic nerves; *a*, contraction of the diaphragm monitored as an increase of the intra-abdominal pressure. In *a* ten responses were averaged and in *b* they were summed. Bin width in the histogram (*b*) is 3 ms. One original response is demonstrated in *c*. Horizontal calibration for *a* and *b* is 100 ms, and for *c* 520 ms; *n*, number of spikes. Here and in Fig. 2, stimulus artifact in *b* was erased and in *c* truncated. The resting activity of the demonstrated fibre was 30·0 spikes s^{-1}, conduction velocity 57·5 m s^{-1} and its receptive field was located in the lumbar portion. *B*, histogram of conduction velocities, bins 2·5 m s^{-1}; *N*, number of fibres. *C*, location of the receptive fields. Inset, anatomy of the diaphragm: i, postcaval foramen; ii, oesophageal hiatus; iii, aortic hiatus; iv, right sternocostal portion; v, central tendon; vi, right lumbar portion.

innervated low-threshold mechanoreceptors. Their receptive fields were located in the sternocostal (23 fibres) and lumbar (73 fibres) portion of the diaphragm. The sternocostal fields were mostly within the narrow strip of muscle along and next to the musculotendinous border (17 fibres). The remaining six receptive fields were dispersed in the sternocostal portion of the muscle. All receptive fields in the lumbar portion were located in a wide band along the musculotendinous border and oesophageal hiatus of the lumbar portion of the diaphragm (Fig. 1*C*). In the most effective experiment we were able to identify thirteen single afferent fibres featuring the above properties; several additional units had to be rejected because there was more than one fibre in the bundle containing them.

Receptors activated by contraction of the diaphragm

Contraction of the diaphragm evoked a high-frequency train of action potentials in eighty-eight of 272 fibres (32%, Fig. 2*A*). The mean increase in the activity evoked by the contraction was $7{\cdot}7 \pm 1{\cdot}0$ spikes ($n = 88$) above control. Of eighty-eight fibres, sixty-four were silent and twenty-four had spontaneous activity ($9{\cdot}0 \pm 1{\cdot}8$ spikes s^{-1}). The conduction velocity of this group of fibres ranged from 4 to 98 m s^{-1} ($n = 88$, Fig. 2*B*). All these fibres innervated low-threshold mechanoreceptors. Their receptive fields were located either in the peripheral area of the muscle (48 fibres) or were distributed in the central tendon (40 fibres). Peripheral receptive fields were found at the sternocostal (23 fields) and lumbar (20 fields) musculotendinous junction or at the points of attachment to the tenth (2 fields) and the eleventh rib (3 fields, Fig. 2*C*). Fibres with conduction velocity below 14 m s^{-1} had receptive fields located exclusively inside the central tendon. In the most effective experiment we were able to test eleven fibres with these properties, but more were found in multiunit preparations that we rejected.

Receptors not or only slightly affected by contraction of the diaphragm

The remaining fibres with receptive fields in the diaphragm (88 of 272, 32%) were unresponsive or only weakly activated by diaphragmatic contraction. These units innervated either low- (75 fibres), or high- (13 fibres) threshold mechanoreceptors.

Fibres innervating low-threshold mechanoreceptors did not react (48 units) or were only weakly activated ($n = 27$); the latter units responded with a mean of $1{\cdot}4 \pm 0{\cdot}2$ spikes per diaphragmatic contraction. Fifty-eight fibres from that group had no resting activity, and

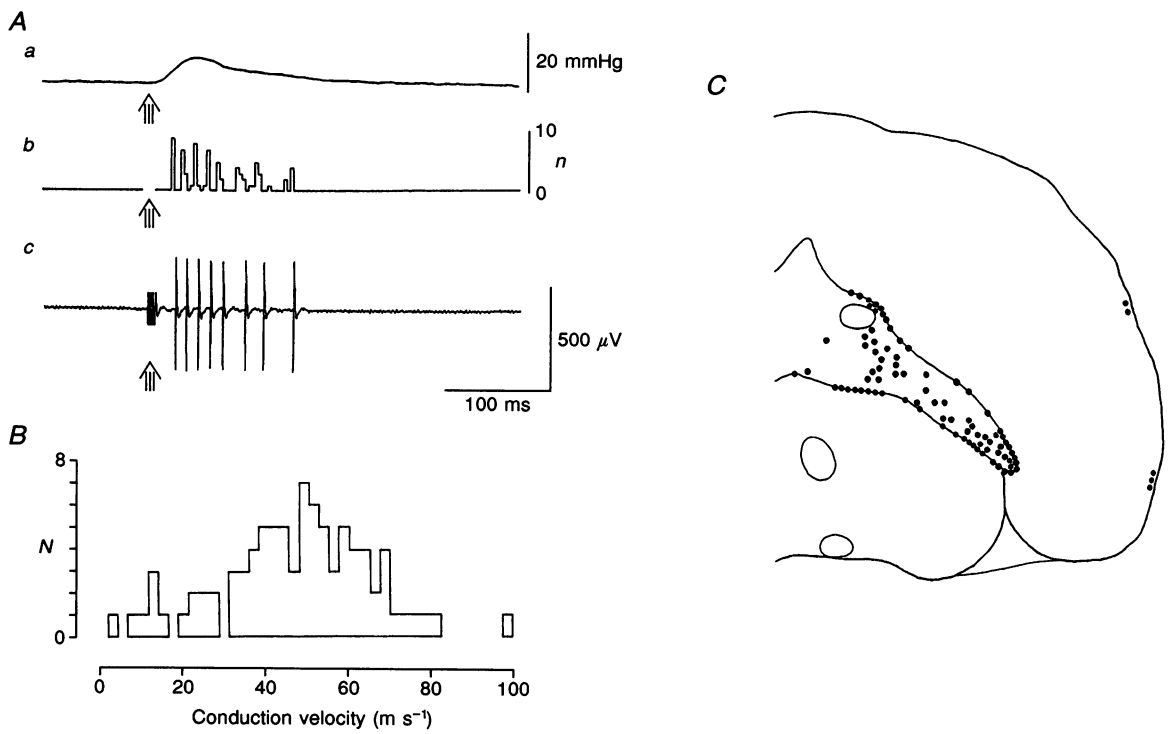

Figure 2. Receptors activated by diaphragmatic contraction

A, reaction of a fibre (*b* and *c*) to contraction of the diaphragm evoked by electrical stimulation of both phrenic nerves (arrows). Bin width for *b* is 3 ms; *n*, number of spikes. This fibre had no resting activity, conduction velocity was 80·0 m s^{-1} and its receptive field was located in the lumbar portion within the musculotendinous border. *B*, histogram of conduction velocities; bins, 2·5 m s^{-1}; *N*, number of fibres. *C*, location of the receptive fields.

seventeen had low irregular resting activity below 1 spike s^{-1}. They had conduction velocities in the range of groups IV (16 fibres), III (45 fibres), and II (14 fibres) (Fig. 3*A*). Low-threshold receptive fields were located in the sternocostal portion (63 fields), lumbar portion (8 fields) and central tendon (4 fields; Fig. 3*B a*). The fibres weakly activated by the contraction had receptive fields in the muscular part of the diaphragm.

Fibres innervating high-threshold receptors did not react to contraction of the diaphragm. Twelve fibres from that group were silent and the spontaneous activity of the remaining one was below 1 spike s^{-1}. Their conduction velocities were in the range of group III (6 units) or group IV (7 units) fibres (Fig. 3*A*, filled columns). The receptive fields were located in the sternocostal portion (10 fields) and central tendon (3 fields; Fig. 3*B b*).

Altogether there were twenty-three group IV fibres in this class (9% of total 272).

Reaction of sensory fibres to chemical stimuli

The influence of lactic acid and/or bradykinin was tested on the receptive fields of nineteen fibres described as 'not affected or slightly affected by contraction'. Figure 4*A* shows data for those units from Fig. 3*B* to which lactic acid and/or bradykinin was applied. We were able to apply the chemicals to the receptive fields located within the range of the internal thoracic artery supply only, which was verified by testing their reaction to prior intra-arterial injection of KCl. All fibres were activated following KCl injection, as is demonstrated in Fig. 4*B*. As expected, these receptive fields were located in the sternocostal portion. All group III and IV fibres tested with these chemicals were activated by them.

Fourteen fibres (7 in both groups III and IV) were activated following the injection of lactic acid into the internal thoracic artery (Fig. 4*C*). The mean number of spikes following the injection of lactic acid was 118·0 ± 25·0 ($n = 14$). All group III fibres tested for lactic acid innervated low-threshold mechanoreceptors, and group IV fibres innervated both low- (4 units) and high- (3 units) threshold mechanoreceptors.

The responses of twelve group III and IV fibres to injections of bradykinin were assessed. The reaction consisted of a short latency prominent activation followed by a secondary long latency low-amplitude response. This late phase was present only after larger doses of bradykinin, as demonstrated in Fig. 4*D* (after 1·0 and 10·0 μg), and gradually disappeared during the interval between consecutive injections. A bradykinin stimulus–response curve was constructed using the first responses of six fibres (5 group III and 1 group IV). The fibres were activated in a dose-dependent manner (doses 0·01–10·0 μg; Fig. 4*D* and *E*). The reactions of six additional fibres (2 group III and 4 group IV) to single doses of 10 μg bradykinin were tested. All together, the mean number of spikes after the injection of 10 μg bradykinin, for these twelve fibres, was 179 ± 32. No reaction was found to the bolus injection of 0·2 ml 0·9% NaCl solution alone into the internal thoracic

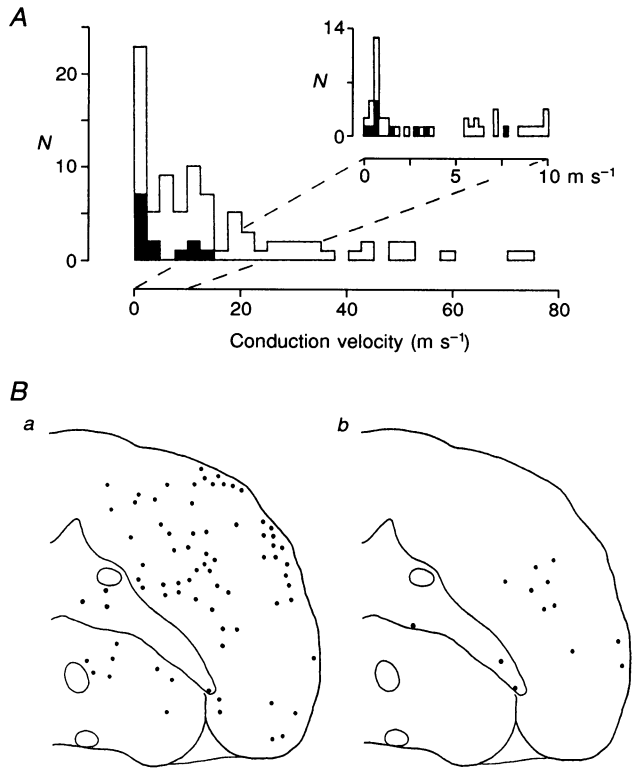

Figure 3. Receptors not affected or slightly affected by contraction

A, histogram of conduction velocities, 2·5 m s^{-1} per bin in main histogram and 0·25 m s^{-1} per bin in the inset. The inset presents data for group IV afferent fibres more clearly. Open columns indicate the conduction velocity of low- and high-threshold units. Filled columns indicate conduction velocity of the high-threshold units only. *B*, location of the receptive fields of low- (*a*) and high- (*b*) threshold mechanoreceptors.

artery (Fig. 4*D*). All fibres tested with bradykinin injections innervated low-threshold mechanoreceptors.

Of fibres with a conduction velocity above 30 m s^{-1}, two from the 'activated' group and one from the 'unloaded' group were tested with the chemical stimuli; others were not tested because the receptive fields of these two groups of fibres were mostly located outside the area supplied by the internal thoracic artery. The three fibres were activated by KCl injection but did not react to lactic acid and bradykinin (10 μg).

Somatotopic organization of sensory innervation of the diaphragm

All together, eighty-eight sensory fibres with receptive fields in the diaphragm were located in the upper and 184 in the lower phrenic nerve root. The ratio of numbers of all sensory fibres in the upper to the lower root was, therefore, approximately 1 : 2. We checked whether the same proportion holds for fibres with lumbar and sternocostal receptive fields. Lumbar fields included all fields in the lumbar muscular part and the lumbar musculotendinous border. For these fibres the upper to lower root ratio was 1 : 6, which means that there are approximately 6 times more fibres in the lower root. Sternocostal fields included those in the sternocostal portion and sternocostal musculotendinous border. The upper to lower root ratio was 1 : 1·4 (instead of the expected value of 1 : 2), indicating that the 'relative density' of sternocostal fibres was greater in the upper than in the lower root (Table 1).

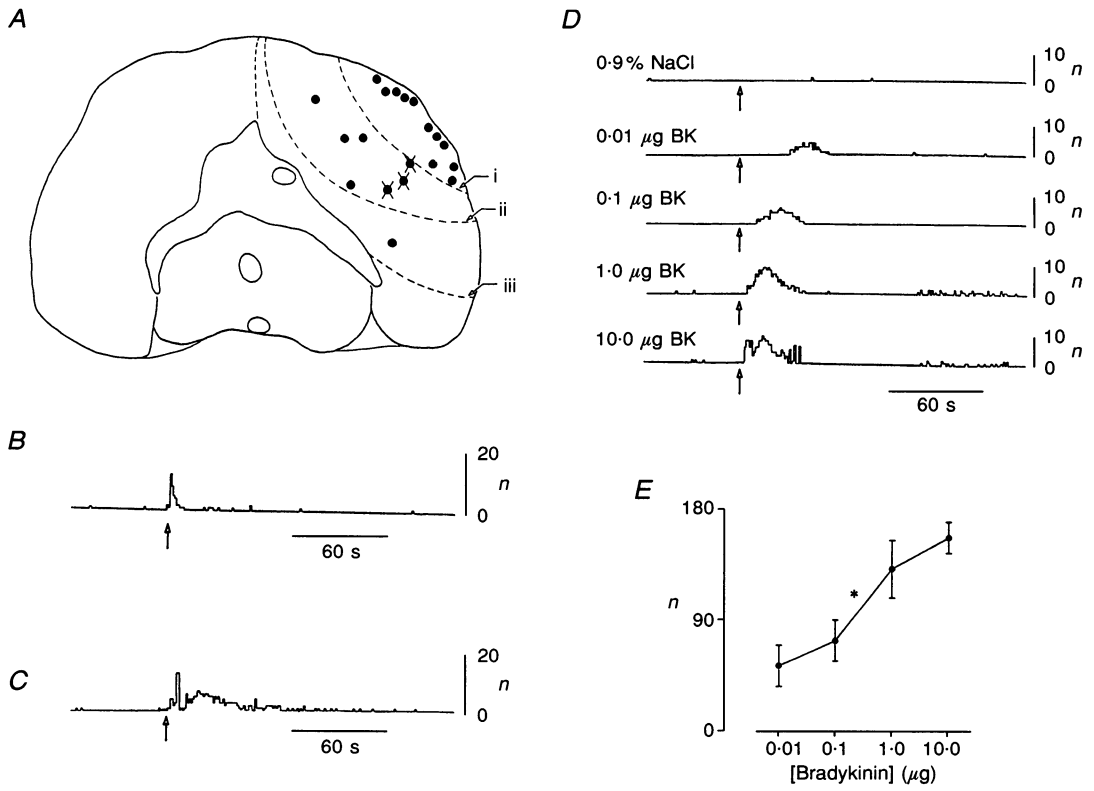

Figure 4. The reaction of afferent fibres to chemical stimuli

A, location of the receptive fields to which lactic acid and bradykinin were applied by injection into the internal thoracic artery. Crossed points indicate high-threshold mechanoreceptors. In 8 experiments 1% Evans Blue solution in 0·9% NaCl was injected into the internal thoracic artery. In all 8 experiments the area delimited by line i was coloured. In 4 experiments the area up to line ii was coloured and in 2 the area to line iii. *B*, *C* and *D* are examples of one fibre with a low-threshold receptive field located in the sternocostal portion of the diaphragm, conduction velocity 2·6 m s^{-1}. Reaction of the fibre to 0·2 ml isotonic KCl injection (*B*), 0·2 ml 0·1 M lactic acid (*C*), and 0·2 ml bradykinin in doses ranging from 0·01 to 10·0 μg. The fibre did not react to a control injection of 0·2 ml 0·9% NaCl (*D*). In *B*, *C* and *D* bin width is 1 s. *E*, the number of impulses (n) above control evoked by the injection of bradykinin in doses from 0·01 to 10·0 μg into the internal thoracic artery, assessed for six fibres. The increase in activity was significantly larger following injection of bradykinin in doses 1·0 and 10·0 μg than 0·01 and 0·1 μg (ANOVA: $F(5; 3) = 12{\cdot}5$, $P < 0{\cdot}001$, $^{*}P < 0{\cdot}05$ Duncan's test). Data are expressed as means ± S.E.M.

Table 1. Numbers and receptive field locations of diaphragmatic sensory fibres passing through upper and lower phrenic nerve roots (central tendon fields excluded)

Receptive field location	Upper root	Lower root	Ratio upper : lower
Lumbar fields	14	87	1 : 6·2
Sternocostal fields	52	72	1 : 1·4

Receptors with receptive fields outside the diaphragm

Nine fibres classified as low-threshold mechanoreceptors had receptive fields outside the diaphragm. The fields were located in the oesophagus (2 fibres), right ventricle (2 fibres), inferior vena cava (3 fibres), pericardium (1 fibre), and ascending aorta (1 fibre). Three fibres were silent and the other six had spontaneous activity (0·1–4·0 spikes s^{-1}). They did not react to diaphragmatic contraction. Two fibres had conduction velocities below 2·5 m s^{-1} and the conduction velocities of the seven remaining fibres ranged from 10 to 21 m s^{-1}.

DISCUSSION

Although recordings from sensory fibres isolated from the phrenic nerve have been performed by other authors (Glebovskii, 1962; Yasargil, 1962; Corda *et al.* 1965), this is, to our knowledge, the first report aiming to classify functional categories of diaphragmatic receptors and their afferent fibres with respect to the location of their receptive fields.

Receptors unloaded by contraction

Electrophysiological studies (Glebovskii, 1962; Yasargil, 1962; Corda *et al.* 1965; Holt, Dalziel & Davenport, 1991) suggest that muscle spindles and tendon organs are quite rare in the diaphragm. It has been reported that in cats the presence of muscle spindles (about 5 per diaphragm) is limited mostly to the lumbar portion (Duron, Jung-Caillol & Marlot, 1978). So far, diaphragmatic muscle spindles have been identified as those unloaded in unison with spontaneous diaphragmatic contractions (Glebovskii, 1962; Yasargil, 1962; Corda *et al.* 1965; Graham *et al.* 1986). We imply that the fibres found in our study with high resting activity, silenced during contraction of the diaphragm and activated by low-threshold mechanical stimuli, most probably innervate diaphragmatic spindles, since their properties were similar to those innervating skeletal muscle spindles (Matthews, 1972). In a single experiment we found a maximum of thirteen fibres that presumably innervated muscle spindles, on one side only. On both sides the total would be at least twenty-six. To our knowledge there is no information about the function of diaphragmatic muscle spindles.

Receptors activated by contraction

These fibres had low-threshold receptive fields and were activated vigorously by diaphragmatic contraction. The receptive fields of half of them were located in the musculotendinous border, which is quite distinctive for skeletal muscle tendon organ location (Jami, 1992). The other half were located in the central tendon. It is unclear, at present, whether all fibres vigorously activated by diaphragmatic contraction, or only the subgroup with receptive fields at the musculotendinous border, innervate diaphragmatic tendon organs. There is unfortunately very little information about the function of diaphragmatic tendon organs. The available evidence suggests that they may be responsible for relaxation of the diaphragm during an increase in abdominal pressure (Revelette, Reynolds, Brown & Taylor, 1992). The unloading of diaphragmatic tendon organs may, in turn, lead to an increase in phrenic nerve discharges (Jammes, Buchler, Delpierre, Rasidakis, Grimaud & Roussos, 1986; Teitelbaum, Borel, Magder, Traystman & Hussain, 1993).

Forty fibres from the group activated by contraction innervated the central tendon, and a further forty-eight fibres had receptive fields in the musculotendinous attachment. We found ninety-six fibres presumably innervating muscle spindles. If we assume that only receptors located at the musculotendinous border are true tendon organs, then the ratio of tendon organs to muscle spindles would be 1 : 2, just as in skeletal muscles (Jami, 1992). If we assume that all receptors vigorously activated by diaphragmatic contraction are tendon organs, then the ratio would be 1 : 1.

The fibres supposedly innervating diaphragmatic spindles and tendon organs had a visibly slower conduction velocity than their skeletal muscle counterparts, which are group Ia and Ib fibres (Jami, 1992). Similarly, it has been demonstrated that α-motoneurones with axons in the phrenic nerve had a significantly slower conduction velocity (Berger, 1979) than those innervating skeletal muscles (Burke, 1967).

Receptors not affected or only slightly affected by contraction of the diaphragm

There was a significant proportion of the fibres (28%) with receptive fields located mainly in the muscle, and a few in

the central tendon, which innervated low-threshold mechanoreceptors, had negligible resting activity and were innervated predominantly by slow conducting group III or IV fibres. We found that fibres from that group were activated by bradykinin and by lactic acid applied to their receptive fields. On the contrary, fibres that were presumed to innervate muscle spindles and tendon organs were not activated by these chemicals. The applied range of bradykinin doses and responsiveness of the fibres were the same as in other studies performed *in vivo* (Mense & Meyer, 1985; Sengupta, Saha & Goyal, 1992; Hong, Han, Yoon & Chung, 1993) and *in vitro* (Mizumura, Minagawa, Tsujii & Kumazawa, 1990). Bradykinin applied locally activates low- and high-threshold skeletal muscle mechanoreceptors innervated by group III and IV afferent fibres, among them muscle nociceptors (Mense & Meyer, 1985). Lactic acid is released from contracting fatiguing muscles (Petrovsky, Phillips, Sawka, Hanpeter & Stafford, 1981). It is known that, when injected into the diaphragmatic circulation, 0·1 M lactic acid activates receptors innervated by slowly conducting fibres (Graham *et al.* 1986; Jammes *et al.* 1986). Our results indicate that slowly conducting fibres with low-threshold receptive fields in the diaphragmatic muscle are activated by algesic substances or substances released from fatiguing muscles. The above-described low-threshold mechanoreceptors are good candidates for 'metaboreceptors' or 'ergoceptors' responsible for mobilization of respiratory and sympathetic systems during muscle work (McCloskey & Mitchell, 1972; Szulczyk, Szulczyk & Żywuszko, 1988). In relation to the respiratory system, the receptors described were located in the effector organ, being under their reflex control.

Diaphragmatic nociceptors

Hinsey & Phillips (1940) suggested that there are nociceptors located in the diaphragm. In our study the possible nociceptors are a few high-threshold mechanoreceptors found in the muscle and central tendon.

Receptors located outside the diaphragm

Just like others (Kostreva & Pontus, 1993*a*, *b*) we found some fibres in the phrenic nerve innervating low-threshold mechanoreceptors and transmitting information from endings located outside the diaphragm.

Comparison with histological findings

Hinsey *et al.* (1939) estimated the number of myelinated and unmyelinated fibres in cat phrenic nerves to be 100 and 300, respectively. More recent results give a similar range and suggest that the total number of sensory fibres is 300–700 (Larnicol *et al.* 1985), of which 180 are myelinated fibres (Duron, 1981). Langford & Schmidt (1983) found 135 myelinated and 185 unmyelinated sensory fibres in one phrenic nerve in rats. Therefore, morphological results indicate that there are more unmyelinated than myelinated sensory fibres in the phrenic nerve. In our study the overwhelming majority of fibres had conduction velocities in the range of myelinated fibres (Lloyd & Chang, 1948). There could be two explanations for this apparent discrepancy. Firstly, we may have missed unmyelinated fibres during dissection. Secondly, we accepted for analysis only those fibres that were activated by the stimulating electrode positioned on the phrenic nerve about 1 cm above the diaphragm. This criterion excluded from analysis those fibres with receptive fields in the thoracic viscera (Kostreva & Pontus, 1993*a*) that join the phrenic nerve more than 1 cm above the diaphragm. It would be expected that fibres innervating thoracic viscera are mainly slowly conducting (Szulczyk & Wilk, 1985). We found nine such fibres; they probably joined the phrenic nerve just above the diaphragm.

Somatotopic organization of the sensory innervation of the diaphragm

Phrenic motoneurones are somatotopically organized, so that motoneurones innervating the sternocostal portion of the diaphragm are located in the C5 spinal cord segment, and those innervating the lumbar portion in C6 (Sant'Ambrogio, Frazier, Wilson & Agostoni, 1963; Duron, Marlot & Macron, 1979). Gordon & Richmond (1990) found somewhat less support for the somatotopic organization of phrenic motoneurones. Such organization was assumed to be an anatomical basis for the independent control of sternocostal and lumbar portions of the diaphragm. Indeed, there is evidence that during vomiting, swallowing and eructation certain anatomical parts of the diaphragm may not contract in unison (Monges, Salducci & Naudy, 1978; Altschuler, Boyle, Nixon, Pack & Cohen, 1985; Oyer, Knuth, Ward & Bartlett, 1989).

The ratio of all sensory fibres passing through the upper and lower roots in our study was found to be 1 : 2, which is in agreement with morphological results (Larnicol *et al.* 1985). Fibres with receptive fields in the sternocostal portion and central tendon preferentially passed through the upper root. On the other hand, of the fibres with receptive fields in the lumbar diaphragm, 6 times more passed through the lower than the upper root. Bearing in mind that the majority of phrenic α-motoneurones innervating the sternocostal and lumbar portion are located in C5 and C6 spinal cord segments, respectively, it may be inferred that lumbar motoneurones are mainly controlled by lumbar receptors and sternocostal motoneurones by sternocostal receptors.

In summary, the present study shows that the phrenic nerve contains numerous fibres from several distinct classes of sensory receptors: muscle spindles, tendon organs, ergoceptors and nociceptors. The sensory diaphragmatic outflow to the spinal cord is somatotopically organized.

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