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Comparison of the motor discharge to the crural and costal diaphragm in the rat

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Abstract

We compared the efferent innervation of the crural and costal regions of the mammalian diaphragm with regard to axonal motor discharge patterns and conduction speeds. Recordings were obtained from single crural (2 3 3) and costal (1 3 3) phrenic motoneurones. Median conduction speeds, calculated by spike triggered averaging (13.7 m s^{-1} crural and 11.8 m s^{-1} costal), and frequency histograms of conduction speed were not statistically significantly different between the two populations ($p = 0.27$: Mann–Whitney test and $p = 0.9$: Kolmogorov–Smirnov test, respectively). There was no difference in the proportions of inspiratory, post-inspiratory or non-respiratory units encountered in the crural and costal phrenic branches. Units that lacked respiratory rhythm did not express cardiac rhythm and were insensitive to ganglion blockade. In conclusion, there were few differences noted between the two motor pools and this may be related to the fact that the rat does not differentially regulate its diaphragm during swallowing and is not an emetic species.

Keywords: Phrenic; Crural; Costal; Diaphragm

1. Introduction

The diaphragm should be considered as two separate muscles, the crural and costal (De Troyer et al., 1981). One question that follows is whether the differences between the two muscles are reflected in their motor innervation. In the rat, the phrenic nerve consistently divides into three branches, two innervate the costal diaphragm, and one innervates the crural diaphragm (Laskowski et al., 1991). In the present study we analyse the properties of single crural and costal phrenic efferent neurons, primarily in terms of activity pattern and conduction speed and classify the neuronal pool into functional groups, using axonal recordings of the phrenic branches. The neurone types (based on respiratory discharge pattern), or ratio of types, found in the crural and costal branches are of particular interest. Analysis of differences between the crural and costal diaphragm at the level of the single axons innervating each muscle has not been carried out before in the rat, or any other species. Previous attempts to measure conduction speed in the rat phrenic nerve failed because of the difficulty in discriminating between overlapping stimulus artefact and evoked potential. Spike triggered averaging obviates this problem, as there is no stimulus artefact.

With regard to the onset times of inspiratory firing, Kong and Berger (1986) described two discrete populations of early and late units in the rat phrenic nerve. The relative proportions of these two types in costal and crural populations is unknown. In the dog, the onset of contraction with inspiration is earlier in the crural diaphragm than in the costal diaphragm (Easton et al., 1987, 1995; Darian et al., 1989).

Since the crural diaphragm, but not the costal diaphragm, has been reported to be a site of concentration of muscle spindles in cat and rat (Balkowiec et al., 1995; Hill, 2001), we were motivated to search for a gamma fusimotor population in the crural branch. We wished to test the following specific hypotheses: first, the frequency distribution of conduction velocities of the crural motoneurones is bimodal and significantly different to a unimodal costal distribution. Second, the median conduction speed of the crural population is statistically significantly slower than the costal population. Third the inspiratory onset times differ between crural and costal populations. Fourth, there are more tonically firing units in the crural population (i.e. units without respiratory rhythm which may contribute to basal lower oesophageal tone).

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2. Methods

2.1. Surgical preparation

Twenty-two adult male Wistar rats (approximately 8 weeks of age) were used in these experiments (220–365 g, mean = 281 ± 41 g S.D.). All experiments were performed in accordance with the Cruelty to Animals Act, 1876 and the European Communities (Amendment of Cruelty to Animals Act, 1876) Regulations, 1994, and with the approval of the local ethics committee.

Anaesthesia was induced with pentobarbitone sodium (Sagatal, 60 mg kg^{-1} i.p.) and maintained with α-chloralose administered in a bolus loading dose (25 mg kg^{-1} i.v.) followed by a lower maintenance dose as necessary (12 mg kg^{-1} h^{-1} i.v.). The level of anaesthesia was assessed by the absence of a withdrawal reflex and cardiovascular response to paw pinch. Temperature was measured with a rectal thermometer and maintained at 37 °C using a Harvard homeothermic blanket system. The femoral vein was cannulated for administering supplemental anaesthesia and fluids, and the femoral artery was cannulated for the recording of arterial pressure. Arterial pressure was measured with a Statham P23Db transducer (Statham Ltd., Hato Rey, Puerto Rico). The trachea was cannulated below the level of the thyroid, and the animal was artificially ventilated throughout the experiment with humidified, O_2-enriched air at a rate of between 50 and 60 ventilations (FI_{O_2}: 0.8; V_T: 1.8 ml) per minute using a Harvard rodent ventilator (model 683). The rate of ventilation was adjusted to a level just above the apnoeic threshold where a spontaneous breath occurred with each stroke of the ventilator (P_{CO_2} approximately 40 mmHg, pHa: 7.4). A midline sternotomy was performed and an end expiratory pressure of 1–3 cm H_2O was applied to prevent complete collapse of the lungs.

A sample of femoral arterial blood was taken from each animal once surgery was complete for measurement of dissolved gases and pH, using a Rapidlab 860 analyser (Chiron Diagnostics, East Walpole, MA, USA), and haematocrit. Sodium bicarbonate solution (1 M) was administered as required for pH correction. A 1 mM solution of polyvinylpyrrolidone (Sigma) in 0.9% (w/v) saline, a polysaccharide with a molecular weight of 40,000 that acts as an oncotic agent, was administered to counteract some effects of blood loss. Administration of polyvinylpyrrolidone solution was based on a subjective assessment of the level of blood loss, and administered in doses of 0–2 ml per animal.

The left or right phrenic nerve was dissected away from the connective tissue all along its path through the thorax from the point where it passes under the superior vena cava to the point where it reaches the diaphragm. The three branches of the nerve were separated at this latter point, and their projections traced as far as possible to identify the crural branch. When the crural branch was identified, the other two branches were cut, and the main trunk of the phrenic was placed on the heart. Contraction of only the crural diaphragm at a cardiac rhythm verified the identification of the crural branch. Then the crural branch was cut, but in such a way that it was longer than the costal branches to allow identification later. The cut central end was placed in a Perspex recording chamber filled with room temperature Hepes-buffered Tyrode's solution (pH 7.4) (NaCl (136.89 mM), KCl (2.68 mM), $CaCl_2$ (1.85 mM), $MgCl_2$ (1.11 mM), NaH_2PO_4 (0.4 mM), Glucose (5.6 mM), Hepes (10 mM)). This solution also contained collagenase (1 mg ml^{-1}; Sigma, 12,800–1330 units of collagenase activity mg^{-1}). After 5 min, the collagenase containing solution was replaced with a collagenase-free Hepes-buffered Tyrode solution. The epineurium and any residual connective tissue, loosened by the activity of the collagenase, were then stripped away from the nerve all along the length in the tissue bath. The tissue bath was composed of two chambers. In the first (closest to the central end of the nerve) the phrenic nerve was placed over a bipolar electrode constructed from silver wire (250 μm in diameter), and the chamber was emptied of solution, dried, and filled, covering the nerve, with a mixture of 50% paraffin oil and 50% petroleum jelly. This mixture gave electrical insulation and prevented desiccation of the nerve. The cut branches of the nerve lay in the second chamber, which remained filled with room temperature Hepes-buffered Tyrode's solution. Electrical activity of single axons in these branches was recorded using glass suction electrodes made from borosilicate glass (World Precision Instruments), pulled to a fine tip using a vertical two-stage puller (Narishige), broken back with a fine dissecting forceps to give a tip with an internal diameter of 10–30 μm, and backfilled with Hepes-buffered Tyrode's solution. Fire polishing of the electrode tip with a microforge was performed in early experiments, but not doing so did not appear to affect the recordings, so this step was subsequently omitted. The suction and bipolar electrode potentials were amplified, filtered and integrated (NL104, NL125, NL704 Neurolog system, Digitimer Ltd., Welwyn Garden City, UK). The signals were then passed through an A/D converter, (1401, Cambridge Electronic Design (CED), Cambridge, UK) and recorded using Spike2 Software (CED). The distance between the suction electrode and bipolar electrode (7–12 mm) was measured in each experiment to calculate conduction speed. The bipolar phrenic recording was rectified and a moving time average over a width of 10 ms created offline to provide an index of central inspiratory activity.

In all experiments, spike channels were recorded at a sample rate of 28–62 kHz, depending on the sampling requirements of other channels. The whole phrenic neurogram was sampled at 10 kHz, and all other channels were sampled at 100 Hz.

2.2. Spike capture and sorting

Activity recorded with the glass suction electrode was not stored as a complete continuous waveform. Instead, portions of the channel, 2–4.5 ms in duration and centred on the spike peak were recorded as spikes occurred. This was necessary to compare spike shapes to each other, and also allowed high sampling rate (thus high fidelity capturing of short events, i.e. spikes) with acceptable computational load. This process of sampling only those "slices" where a spike occurred (spike capture) was carried out by the CED 1401 firmware controlled via Spike2.