Physiology and motor innervation of the supralateral radular retractor muscles of the pulmonate snail, *Planorbarius corneus*

J.E.H. Tattersall* and R.C. Brace
Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD, England
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Summary. 1. Some electrophysiological properties of a multifunctional molluscan muscle, the supralateral radular retractor (SLR), were examined. The resting membrane potential was 62.3 ± 10.5 mV and was dependent upon permeabilities to K⁺, Na⁺ and Cl⁻. Current-voltage relationships for SLR muscle fibres were linear, and the fibres had input resistances of 22.5 ± 5.6 MΩ. Indirect evidence suggested that they were not electrically coupled.

2. Miniature EJPs occurred spontaneously in the muscle. Action potentials in the identified motoneurones elicited monosynaptic, chemical EJPs in muscle fibres. The majority of fibres were polyneuronally innervated by up to four motor axons. Action potentials did not normally occur in the muscle fibres, and contraction was proportional to the degree of depolarization produced by EJPs.

3. Contractions elicited in the SLRs by the stimulation of motoneurones were bilaterally symmetrical, and were temporally and spatially characteristic of the type of neurone excited. Temporal differences in contraction between motor units were determined mainly by differences in initial EJP amplitude. The relatively small degree of facilitation shown by all motor units had little effect on the speed of contraction. A linear summation model adequately described the growth of facilitation at terminals of one type of motoneurone, but not at those of the other two types of motoneurones examined.

Introduction

The muscles of most gastropod molluscs, in contrast to the central nervous system, have proved difficult to study with intracellular microelectrodes. The fibres are frequently less than 10 μm in diameter (Heyer et al. 1973), and the stabilization of muscle preparations is hindered by the absence of rigid skeletal elements. Consequently, relatively few studies have been made of neuromuscular transmission, although some of these have demonstrated a considerable capacity for integration and interaction between motor units at the peripheral level (Jacklet and Rine 1977; Banks 1978; Cohen et al. 1978; Peters 1979).

Previous work on the control of the paired supralateral radular retractor (SLR) muscles of *Planorbarius corneus* (L.) has concentrated on the patterns of activity in the motoneurones and on their interactions with premotor cells (Brace and Quicke 1980, 1981; Quicke 1981). The SLRs are multinodular muscles, and are divided into distinct units which can act independently of one another. In particular, the medial parts of the muscle exhibit stronger, more phasic contractions than the lateral parts. This division is reflected in the different patterns of activity in the motoneurones during the feeding cycle (Brace and Quicke 1980, 1981).

The present paper examines some electrophysiological properties of SLR muscle fibres and compares neuromuscular transmission in the different motor units. Only three of the four pairs of identified motoneurones were investigated, due to the
difficulty of maintaining stable penetrations of the remaining pair.

Materials and methods

Animals were collected from localities near Nottingham, and were maintained in 10-l aerated tanks. Those with shell diameters of 2–3 cm were selected for experiments. The saline used was based on that described by Sorokina (1965) and had the following composition (mM): NaCl 36.5; KCl 1.75; CaCl₂ 3.75; MgCl₂ 1.5; Na₂SO₄ 0.15; NaHCO₃ 6.5; NaH₂PO₄ 0.03; Tris 5.0; pH 7.5.

The dissection procedures were as described by Brace and Quicke (1980). In order to simplify the dissection, only one SLR muscle was used from each animal. It was freed from the dissected buccal mass preparation by cutting through the contralateral muscle and the dorsal wall of the buccal mass. The muscle was then pinned onto the Sylgard base of the recording dish, ventral surface uppermost, by pins passing through the odontophoral cartilage and the radula.

In experiments involving changes in the ionic composition of the saline, K⁺ was substituted by Na⁺, Na⁺ by Tris, and Cl⁻ by a number of anions, the principal one being methyl sulphate.

Muscle fibres were penetrated with glass microelectrodes filled with potassium acetate, which had resistances of 60–100 MΩ. Electrodes used to impale motoneurones had resistances of 10–30 MΩ. High-impedance amplifiers equipped with bridge circuits allowed current to be passed through the recording electrodes.

Muscle tension was monitored using a Grass isometric strain gauge, attached to the radular sac by a ligature of dental floss. Simultaneous recordings of intracellular EJPs and muscle tension were obtained by using a staple-shaped pin to fasten the proximal end of the muscle to the bottom of the dish, allowing stable penetrations to be maintained, the distal end being tied to the strain gauge. Permanent records of tension and electrical signals were made using a Grass Polygraph pen recorder or a Medelec recording oscilloscope.

Facilitation at neuromuscular junctions was measured in two ways, either by giving pairs of stimuli to a motoneurone, or by repetitive stimulation of the motoneurone at a range of frequencies. A third method was used to compare facilitation between large numbers of synapses. In the case of paired stimuli, the degree of facilitation, f, was defined according to Mallart and Martin (1967) as f = (V₁ - V₀)/V₀, where V₀ is the amplitude of the first (conditioning) EJP and V₁ the amplitude of the second (test) response. The value of f was plotted against the interval, t, between the two EJPs. The points were fitted by the exponential equation, f = f₁ e⁻ᵇᵗ, where f₁ is the value of f at t = 0 and b is the reciprocal of the time constant of decay of f₁.

In the case of repetitive stimulation, trains of spikes were evoked in motoneurones at frequencies ranging from 0.5–5.0 Hz, and the quantity f = (V₁ - V₀)/V₀ was measured from the resulting EJP trains, where V₀ is the amplitude of the first EJP in the train and V₁ that of the nth EJP after the first. Assuming that the amount of facilitation contributed by each EJP sums linearly with that remaining from preceding responses, plots of f against time (T) after the first EJP in the train can be described by the equation f = k₁(1 - e⁻ᵇᵗ) (Mallart and Martin 1967). The constant k₁ is related to f₁ by the equation k₁ = f₁(eᵇᵗ - 1)⁻¹, where t is the interval between consecutive EJPs in the train. Thus, plots of the growth of f during trains of EJPs may be used to derive values of f₁ and b.

For each train of responses, k₁ was estimated as the mean value of f when the latter had reached a plateau. A regression was then performed on ln(k₁ - f) versus t to find f₁ and b.

The effect of K⁺ upon the membrane potential was examined by varying the external K⁺ concentration ([K⁺]₀) in Cl⁻-free saline. Removal of Cl⁻ from the saline caused a sustained depolarization of approximately 15 mV. Five muscle fibres were impaled in each [K⁺]₀, and their membrane potentials measured. A linear relationship with a slope of 33.5 mV per ten-fold change in [K⁺]₀ was obtained for [K⁺]₀ greater than 1 mM (Fig. 1). At values of [K⁺]₀ lower than this, the slope decreased. Removal of Na⁺ from the saline increased the gradient to 38.8 mV per decade, but the decrease in slope below 1 mM [K⁺]₀, still remained.

Measurements of input resistance (Rᵢᵣ) were made with a single electrode, since it was not possible to impale a single muscle fibre with two electrodes simultaneously. The muscle membrane showed little rectification, the current-voltage relationship being linear between −25 and +25 mV (Fig. 2). Rᵢᵣ, measured from the slopes of these curves, was 22.5 ± 5.6 MΩ (n = 10).

Since attempts to impale two adjacent muscle fibres simultaneously were unsuccessful, an indirect method was used to test for electrical coupling between cells. The Ca²⁺-chelating agent, ethylene-