Serotonin modulates muscle function in the medicinal leech Hirudo verbana

Shannon P. Gerry* and David J. Ellerby

Department of Biological Sciences, Wellesley College, 106 Central Street, Wellesley, MA 02481, USA
*Author for correspondence (sgerry@wellesley.edu).

The body wall muscles of sanguivorous leeches power mechanically diverse behaviours: suction feeding, crawling and swimming. These require longitudinal muscle to exert force over an extremely large length range, from 145 to 46 per cent of the mean segmental swimming length. Previous data, however, suggest that leech body wall muscle has limited capacity for force production when elongated. Serotonin (5-HT) alters the passive properties of the body wall and stimulates feeding. We hypothesized that 5-HT may also have a role in allowing force production in elongated muscle by changing the shape of the length–tension relationship (LTR). LTRs were measured from longitudinal muscle strips in vitro in physiological saline with and without the presence of 10 μM 5-HT. The LTR was much broader than previously measured for leech muscle. Rather than shifting the LTR, 5-HT reduced passive muscle tonus and increased active stress at all lengths. In addition to modulating leech behaviour and passive mechanical properties, 5-HT probably enhances muscle force and work production during locomotion and feeding.

Keywords: serotonin; length–tension; obliquely striated muscle

1. INTRODUCTION

Sanguivorous leech body wall muscles have an unusually diverse functional repertoire, powering crawling, swimming and suction feeding behaviours [1]. These have distinct kinematic and motor control patterns: dorsoventral undulation of the body axis during swimming; cycles of body elongation, anterior anchoring, then posterior retraction during crawling; and antero-posterior waves of contraction to create suction pressure during feeding [2]. Feeding contractions are maintained, and locomotor movements rapidly resumed [3] despite the extreme body volume changes that stretch the body wall during feeding. This kinematic variability and maintenance of function during body distension suggests that leech body wall muscle is capable of exerting force and performing mechanical work across an unusually wide range of muscle lengths.

Leech muscle is obliquely striated: adjacent sarcomeres are displaced along the long axis of the muscle, creating a diagonal sarcomere pattern in longitudinal sections [4]. This muscle is present in a number of invertebrate groups, and may have a broader length–tension relationship (LTR) than that found in vertebrate cross-striated muscle [4]. The relationship for leech muscle is asymmetrical, however, allowing maintenance of force production at shorter relative lengths than in cross-striated muscle, yet having similarly impaired force production when elongated [4]. Consequently, how can leech body wall muscle remain functional during, and immediately after feeding?

Hormonal modulation of body wall mechanical properties may provide an explanation. Serotonin (5-HT) is important in the initiation and control of feeding behaviour in sanguivorous leeches [2] and may increase the compliance of body wall tissue [1,5,6] to accommodate body distension. No data are available, however, concerning the effects of 5-HT on the LTR, or that can place the LTR in context with the in vivo strain of the body wall muscle during locomotion and feeding.

We hypothesized that the elevated 5-HT levels associated with feeding were essential for preserving effective mechanical function of the longitudinal body wall muscles during the distension created by a large blood meal, by shifting the muscle LTR to support force production at increased muscle strains. We tested this using sonomicrometry to quantify the longitudinal strain of body segments during swimming, crawling and feeding in the medicinal leech. These in vivo operating strains have been compared with the LTR of leech longitudinal body wall muscle determined in vitro both with and without elevated 5-HT levels.

2. MATERIAL AND METHODS

(a) Experimental animals

Medicinal leeches (Hirudo verbana) were purchased from a commercial supplier (Leeches USA Ltd., Westbury, NY, USA) and maintained on a 12 L:12 D cycle at 21°C. Commercially supplied white defibrinated sheep blood (Product no.DSB500, Hemostat Laboratories) was added to the experimental tank. The leeches attached via their anterior sucker to an access port covered with Parafilm and fed until sated.

(b) In vivo experiments

Longitudinal body segmental strain of five fasted leeches was measured using strain-gauge transducers (Sonometrics Corporation, London, Ontario, Canada) secured dorsally at the anterior and posterior margins of a body segment by cyanoacrylate. Sonomicrometry uses the transit time of an ultrasonic pulse to detect the relative spacing of transducer pairs. Segmental length change data were collected using a Sonometrics TRX Series Sonomicrometer (Sonometrics Corporation). Segmental strains were calculated relative to average swimming strain, Ls.

Segmental strains were recorded during swimming and crawling in a 20 l tank of 21°C water, and while the leeches fed from an apparatus described in Cladin et al. [3]. Briefly, a tissue bath containing defibrinated sheep blood (Product no.DSB500, Hemostat Laboratories, Dixon, CA, USA) at 38°C was introduced to the experimental tank. The leeches attached via their anterior sucker to an access port covered with Parafilm and fed until sated.

(c) In vitro experiments

Six leeches were anesthetized by placing them on ice. The dorsal portion of three adjoining body segments was excised and placed in saline containing (in millimolar): NaCl, 115.0; KCl, 4.0; CaCl2, 1.8; MgCl2, 2.0; and HEPES, 10.0; pH 7.4 at 21°C, then divided into narrow longitudinal strips, of which four per leech were typically used for mechanical measurements. The ends of these were placed in aluminium foil clamps leaving only the middle segment exposed, and
secured with cyanoacrylate. One-half of the preparations were placed in saline containing 10 μM 5-HT (Sigma) solution.

LTRs for the non-5-HT and 5-HT preparations were quantified using a muscle ergometer (300B-LR, Aurora Scientific, Aurora, Ontario, Canada). Passive tonus and peak force in response to a 1 ms pulse, 80 Hz, 200 ms total duration electrical stimulus delivered via platinum electrodes parallel to the muscle strips were recorded in 20% increments from 40 to 160% of control muscle length with a 3 min relaxation time at each increment. Stimulus current was adjusted for each muscle strip to elicit maximal active force production. The force and position data were captured to a PC via a 604A A to D interface (Aurora Scientific) and a PCI A to D card (PCI-6503, National Instruments, Austin, TX, USA).

(d) Statistical analysis
A two-way ANOVA (SPSS, v. 17.0; SPSS Inc., Chicago, IL, USA) was used to test for differences in peak strain, minimum strain and shortening rate during stimulation (figure 2). Contrary to our initial hypothesis, force production increased at all muscle lengths, not only at the higher segmental

3. RESULTS
Swimming produced cyclical strain changes (figure 1) with a peak-to-peak amplitude of 16.8 ± 4.2% L<sub>m</sub> at a frequency of 2.58 ± 0.32 Hz (mean ± 1 s.d.). Crawling and feeding strain patterns were qualitatively similar but shifted to a significantly higher strain range during feeding (figure 1, table 1 and the electronic supplementary material, table S1). Exposure to 5-HT was associated with increased stress during stimulation (p < 0.001; figure 2a; the electronic supplementary material, table S2). Individual leeches differed in the magnitude of their response to 5-HT (p = 0.007), yet all individuals followed the same trend. Residual tonus increased with muscle length and was significantly lower overall in the muscle strips exposed to 5-HT (p = 0.001; figure 2b and the electronic supplementary material, table S3). Exposure to 5-HT did not alter the shape of the LTRs for active or passive stress (p > 0.05), but shifted them relative to the y-axis.

4. DISCUSSION
Leech longitudinal muscles exert force across a remarkably wide length range in comparison to the muscle of other organisms (figure 1 and table 1). The in vivo operating range is approximately 75–240% of L<sub>m</sub>, the length at which maximal force was exerted (figure 2). Exposure to 5-HT decreases residual tonus and increases force production in response to stimulation (figure 2). Contrary to our initial hypothesis, force production increased at all muscle lengths, not only at the higher segmental

<table>
<thead>
<tr>
<th>behaviour</th>
<th>maximum length (% L&lt;sub&gt;m&lt;/sub&gt;)</th>
<th>minimum length (% L&lt;sub&gt;m&lt;/sub&gt;)</th>
<th>shortening rate (L&lt;sub&gt;m&lt;/sub&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</th>
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<tr>
<td>crawling</td>
<td>107.8 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.0 ± 5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.72 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>early feed</td>
<td>99.8 ± 9.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.2 ± 10.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>mid feed</td>
<td>145.2 ± 16.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>96.6 ± 23.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.50 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>late feed</td>
<td>142.5 ± 25.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>101.4 ± 25.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.50 ± 0.34&lt;sup&gt;b&lt;/sup&gt;</td>
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Figure 1. Representative longitudinal muscle strains during swimming (solid line), crawling (dotted line) and suction feeding (dashed line) behaviours. The traces correspond to the shortening phases of crawling and feeding because these are directly relevant to the LTR. Strain is expressed as a percentage of mean swimming length (L<sub>s</sub>).
with which cross-bridge formation is possible [4,8]. The mechanisms that reduce residual tonus and increase active force production in the presence of 5-HT are less clear in comparison.

The persistent tonus may be generated by a ‘catch’ mechanism similar to that described in Mytilus anterior byssus retractor muscle (ABRM). In the ABRM, an accessory protein—twitchin—acts as a tether between the thick and thin filaments, maintaining force production at low metabolic cost with minimal cross-bridge cycling [12]. Twitchin phosphorylation in the presence of 5-HT relaxes the catch state [13–15]. Twitchin-like proteins have been isolated from the obliquely striated muscle of other invertebrates, including Hirudo [16]. Also, several invertebrates show reduced tonus in the presence of 5-HT [7,17,18]. Together, this provides circumstantial evidence that a 5-HT controlled catch mechanism may be operating in Hirudo, although confirmation would require detailed investigation of the differential response of active contraction and tonus to treatments that eliminate cross-bridge formation, and the binding properties of Hirudo twitchin in relation to its phosphorylation state.

No comparable data are available for Hirudo obliquely striated muscle stimulated to elicit maximal force production in the presence of 5-HT. However, 5-HT does enhance force production in the body wall muscle of other annelids during spontaneous [19,20], acetylcholine-induced [17] and electrically stimulated contractions [21]. There is a similar potentiating effect of 5-HT on Aplysia muscle [22], associated with a 5-HT-mediated increase in inward calcium currents across muscle cell membranes [23,24]. Confirmation of a similar 5-HT-mediated mechanism in Hirudo muscle requires further investigation.

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