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Temperature dependent modulation of lobster neuromuscular properties by serotonin

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Summary

In cold-blooded species the efficacy of neuromuscular function depends both on the thermal environmental of the animal's habitat and on the concentrations of modulatory hormones circulating within the animal's body. The goal of this study is to examine how temperature variation within an ecologically relevant range affects neuromuscular function and its modulation by the neurohormone serotonin (5-HT) in *Homarus americanus*, a lobster species that inhabits a broad thermal range in the wild. The synaptic strength of the excitatory and inhibitory motoneurons innervating the lobster dactyl opener muscle depends on temperature, with the strongest neurally evoked muscle movements being elicited at cold (<5°C) temperatures. However, whereas neurally evoked contractions can be elicited over the entire temperature range from 2 to >20°C, neurally evoked relaxations of resting muscle tension are effective only at colder temperatures at which the inhibitory junction potentials are hyperpolarizing in polarity. 5-HT has two effects on inhibitory synaptic signals: it potentiates their amplitude and also shifts the temperature at which they reverse polarity by approximately +7°C. Thus 5-HT both potentiates neurally evoked relaxations of the muscle and increases the temperature range over which neurally evoked muscle relaxations can be elicited. Neurally evoked contractions are maximally potentiated by 5-HT at warm (18°C) temperatures; however, 5-HT enhances excitatory junction potentials in a temperature-independent manner. Finally, 5-HT strongly increases resting muscle tension at the coldest extent of the temperature range tested (2°C) but is ineffective at 22°C. These data demonstrate that 5-HT elicits several temperature-dependent physiological changes in the passive and active responses of muscle to neural input. The overall effect of 5-HT is to increase the temperature range over which neurally evoked motor movements can be elicited in this neuromuscular system.

Key words: neuromuscular, serotonin, lobster, crustacean, muscle tension, temperature.

Introduction

Circulating neurohoromones such as serotonin (5-HT) are important endogenous modulators of neuromuscular function in invertebrates (Worden, 1998). In the lobster *Homarus americanus* serotonin acts both as a neurotransmitter and a circulating hormone to modulate skeletal and cardiac neuromuscular systems (Glusman and Kravitz, 1982; Goy and Kravitz, 1989; Goy et al., 1984; Heinrich et al., 1999; Huber et al., 1997; Kravitz, 1988; Kravitz et al., 1980; Livingstone et al., 1980; Worden et al., 2006; Worden et al., 1995). However, the environmental temperature surrounding the lobster is another major factor that influences lobster physiology and behavior. Like other invertebrates lobsters are cold blooded, and their internal body temperature closely follows that of the surrounding water [see fig. 2 of Worden et al. (Worden et al., 2006)]. Estuary and coastal water temperatures within the habitat of *H. americanus* vary over a 25°C range according to the winds, the tides and the seasons (Cowan et al., 2007; Lawton and Lavalli, 1995; Manning, 2005). Temperature influences multiple parameters of lobster biology, including growth and reproduction (Ennis, 1995; Waddy et al., 1995), cardiac and respiratory performance and its thermal limits (Camacho et al., 2006; Worden et al., 2006), and locomotion into lobster traps (Drinkwater et al., 2006). Lobsters can detect thermal change with exquisite resolution, exhibiting alterations in heart rate in response to thermal changes as small as 0.15°C (Jury and Watson, 2000).

The goal of this study was to examine how changes in environmental temperature might alter the effectiveness of neurohormonal modulation of neuromuscular transmission and muscle physiology. A number of previous studies have characterized the temperature dependence of synaptic output and muscle movements in crustacean neuromuscular systems (Fischer and Florey, 1981; Harri and Florey, 1977; Harri and Florey, 1979; Stephens, 1990; Stephens and Atwood, 1982). However, surprisingly little is known about how temperature might alter the sensitivity of neuromuscular systems to endogenous neuromodulatory hormones. In the crayfish

skeletal phasic abdominal neuromuscular system the neurohormone DF_2 potentiates synaptic transmission much more strongly at cold temperatures compared to warm (Friedrich et al., 1994), suggesting that hormonal modulation might help compensate for low synaptic output in part of the temperature range inhabited by the animal *in vivo*.

Compensating for temperature effects on synaptic transmission may be particularly important in species that spend considerable periods of time living at temperature extremes. *H. americanus* overwintering in the Gulf of Maine waters, for example, spend 6 months of each year at frigid temperatures <5°C (Cowan et al., 2007). *H. americanus* are currently in high abundance in the Gulf of Maine [Atlantic States Marine Fisheries Commission (ASMFC, 2005)], suggesting that colder temperatures are favorable for this species in the wild. In contrast, the same trawl and landings data show that fishing mortality is high and lobster abundance is low in warmer waters south of New England, particularly in Long Island Sound, where bottom water temperatures can average 20°C in August and September (Howell et al., 2005) and the commercial fishery has collapsed. Understanding the thermal limits of lobster physiological function will be important for predicting the environmental consequences of global warming for the health and survival of this commercially valuable species.

In this study we examine how the environmental temperature and thermal change affects neurotransmission and contraction in the lobster dactyl opener skeletal muscle. We demonstrate that neuromuscular function is strongly temperature dependent and that 5-HT modulates the system to extend the effective temperature range for neuromuscular function.

Materials and methods

Dissection and temperature control

Adult lobsters (*Homarus americanus* Milne Edwards) were obtained from commercial sources and maintained in artificial seawater. Prior to experimentation the lobsters were held at 6°C for several weeks in order to ensure they became physiologically acclimated to temperature (Camacho et al., 2006). The first or second walking legs were dissected in chilled saline (462 mmol l^{-1} NaCl, 16 mmol l^{-1} KCl, 26 mmol l^{-1} $CaCl_2$, 8 mmol l^{-1} $MgCl_2$, 11 mmol l^{-1} glucose and 5 mmol l^{-1} Hepes buffer adjusted to pH 7.4). Part of the exoskeleton and the entire dactyl closer muscle were removed from the propopodite segment of the leg to expose the dorsal surface of the dactyl opener muscle. The specific opener inhibitory (OI) axon and the opener excitatory (OE) axon innervating the dactyl opener were exposed in the meropodite. The axon of the common inhibitory motoneuron (CI) also provides innervation to all the fibers of the lobster dactyl opener (Worden and Camacho, 2006), but we did not examine its physiological effects in this study.

After dissection, the preparation was pinned to Sylgard™ in the bottom of a 1.5 ml saline-filled chamber made of resin, through which flowed refrigerated coolant. Saline was superfused over the preparation at a rate of 1–2 ml min^{-1}; the tubing through which this saline flowed was also chilled by refrigerated coolant. Using this system, the temperature of the bath could be regulated over the range 1–23°C. In experiments in which temperature was varied, the rate of change of bath temperature was approximately 0.67°C min^{-1}. All experiments began at 2°C and the temperature was warmed over the course of the experiment. In experiments examining the temperature dependence of 5-HT effects, the following protocol was used. The bath was warmed from 2°C to 20°C while physiological measurements were made in control saline. The bath was then chilled to 2°C. Serotonin (5-HT) (Sigma Chemical, St Louis, MO, USA) was added to the saline superfusing the preparation at 2°C and the effects monitored for at least 15 min to ensure that intracellular responses and neurally evoked contractions had stabilized. Finally, the bath was warmed again from 2°C to 20°C while physiological measurements were repeated. The concentrations of 5-HT used in these experiments were in the range 50–100 nmol l^{-1}, approximately one order of magnitude higher than the concentration found in lobster hemolymph under resting conditions (Livingstone et al., 1980).

Electrophysiological and tension recording

Using suction electrodes, excitatory junction potentials (EJPs) and inhibitory junction potentials (IJPs) were elicited in the muscle by selective stimulation of the axons of the dactyl opener excitatory motoneuron (OE) and specific opener inhibitory motoneuron (OI), which run separate courses in the meropodite. Synaptic responses were recorded intracellularly in areas of the muscle in which IJPs appeared largest; these included fibers in the most proximal region of the muscle as well as the most proximal fibers of the central region. Intracellular recordings were performed using 3–5 MΩ pipettes filled with 3 mol l^{-1} potassium acetate. Changes in muscle membrane potential were recorded differentially between the microelectrode and the bath, grounded through a silver/silver chloride electrode, and amplified by an Axopatch-1D amplifier (Axon Instruments, Union City, CA, USA). Input resistance was measured by determining the change in muscle membrane potential elicited by a hyperpolarizing current injection.

Tension was measured almost isometrically by attaching a surgical thread from the apodeme of the dactyl opener muscle to a tension transducer (model FT03, Grass Instruments, West Warwick, RI, USA) connected to an amplifier (Cyberamp 320, Axon Instruments). At the start of each experiment muscles were stretched to a degree that resulted in 2 g tension at 2°C. Outputs from the microelectrode and the tension transducer were digitized using a Digidata 1200 interface (Axon Instruments) at sampling rates of 2.9–13.3 kHz, recorded on VCR tape *via* a digital data recorder (Model 10B, Instrutech Corp., Port Washington, NY, USA), and analyzed on- and off-line using pClamp software (versions 6.0-7.0, Axon Instruments). Tension recordings were filtered at 300 Hz and calibrated in grams (g). In this study we examined muscle contractions stimulated by the excitatory motoneuron (OE) as well as muscle relaxations stimulated by the specific inhibitory

motoneuron (OI). Both inhibitory motoneurons that innervate the dactyl opener (OI and CI) can inhibit muscle contractions stimulated by the excitatory motoneuron OE (Worden and Camacho, 2006), but we did not explore these physiological effects in this study.

Data analysis

In some experiments, stimulation of the excitatory and/or the specific inhibitory motoneuron innervating the dactyl opener muscle was applied at a constant low frequency (0.5–1 Hz), and the amplitude of junction potentials was determined by averaging recordings from ten consecutive trials. The effect of 5-HT on junction potential amplitude was determined by comparing the average of ten trials in the presence of 5-HT to control responses at the same temperature. EJP amplitudes were corrected for non-linear summation according to the equation $Ejp'=Ejp/(1–Ejp/Er)$ (Martin, 1955) where the corrected value for the size of the EJP (Ejp') is determined from the measured value of the EJP (Ejp) and the reversal potential (Er) of the synaptic current for the opener excitor motoneuron, assumed to be +11.5 mV as demonstrated in crayfish (Onodera and Takeuchi, 1975). In experiments in which IJPs were elicited by stimulus trains, the amplitude of the last IJP in the inhibitory postsynaptic response was measured with respect to baseline. In some experiments, muscle movements were elicited by applying brief (200–600 ms) stimulus trains (10–30 Hz) to the axons of the motoneurons. In each experiment, identical stimulus protocols were used under control conditions and in the presence of 5-HT. Neurally evoked muscle contractions and relaxations were measured by averaging ten consecutive tension responses to stimulus trains and determining the peak of the contraction, or relaxation, with respect to the baseline tension. Resting muscle tension was determined as the level of muscle tension during periods when motoneurons were not being stimulated and any muscle movements due to prior motoneuron stimulation had decayed. Graphing and statistical analysis were performed using Origin Software (Microcal Software, Inc., Northampton, MA, USA). All quantitative data are reported as means ± s.e.m., if not indicated otherwise.

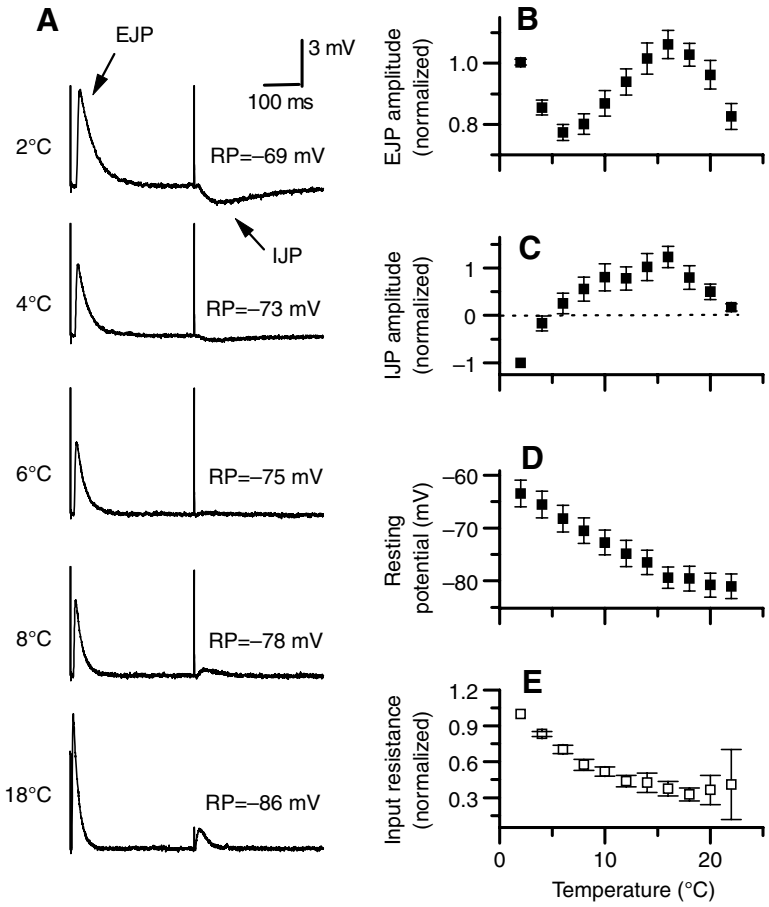

Fig. 1. The amplitudes of EJPs and IJPs vary as a function of temperature. (A) Intracellular recordings from a muscle fiber in which a single EJP and a single IJP are elicited in each stimulus trial over the temperature range 2–18°C. Traces are averaged from ten trials at each temperature; the resting potential (RP) recorded at each temperature is indicated above each trace. In this experiment, the IJP reversed polarity at 6.0°C. (B) Amplitude of EJPs as a function of temperature in nine muscle fibers. Data are normalized to the value measured at 2°C; at this temperature EJP amplitude in these fibers ranged from 1.4 to 12.9 mV. EJPs are depolarizing in polarity over the entire temperature range. (C) Amplitude of IJPs as a function of temperature in six muscle fibers. Data are normalized to values measured at 2°C; at this temperature IJP amplitude in these fibers ranged from –0.1 mV to –5.2 mV. The broken line indicates the reversal of the IJP polarity; data below the broken line represent IJPs of hyperpolarizing polarity. (D) Muscle resting potential (mean ± s.e.m.) measured as a function of temperature in 18 muscle fibers, including those for which data is presented in B and C. (E) Input resistance of muscle fibers (mean ± s.e.m.; N=11) measured in response to hyperpolarizing current pulses. Values are normalized to those measured at 2°C.

Results

Neuromuscular transmission and neurally evoked muscle contraction and relaxation are temperature dependent

Changes in temperature strongly affect the postsynaptic response of the dactyl opener to stimulation of the excitatory (OE) and specific inhibitory (OI) motoneurons (Fig. 1A). Excitatory junction potentials (EJPs) are depolarizing over the entire voltage range from 2°C to 22°C. However, in a majority of muscle fibers (9 out of 12) EJP amplitude exhibited a biphasic pattern of temperature dependence with one maximum at (or below) 2°C and another maximum in the range 14–16°C (Fig. 1A,B). In contrast, both the size and the polarity of the inhibitory junction potentials (IJPs) depend on temperature. At 2°C, IJPs are hyperpolarizing, but as the temperature warms they decrease in amplitude and ultimately become depolarizing (Fig. 1A,C). As the temperature warms the kinetics of both the

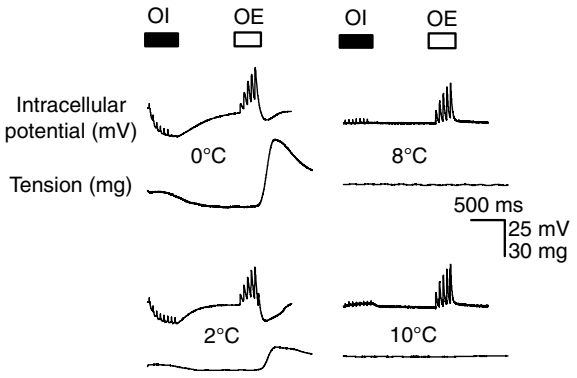

Fig. 2. Electrical and contractile responses of muscle are temperature dependent. Intracellular and tension recordings from dactyl opener muscle in response to trains of stimuli delivered alternately to the inhibitory (OI) and excitatory (OE) motoneurons. The timing of the stimulus trains (12 Hz for 700 ms for the inhibitory motoneuron OI; 10 Hz for 500 ms for the excitatory motoneuron OE) is indicated above the traces. Intracellular recordings of muscle membrane potential are shown above the corresponding tension recordings, traces are averaged from ten stimulus trials at the indicated temperatures. Muscle resting potential was –56 mV at 2°C.

excitatory and the inhibitory junction potentials become faster (Fig. 1A) and the muscle resting potential becomes progressively more hyperpolarized (Fig. 1D). The input resistance of the dactyl opener muscle fibers decreases by approximately 50% relative to its initial value at 2°C as the temperature warms to 12°C and remains relatively low at warmer temperatures (Fig. 1E).

To examine whether motor movements might also depend on temperature, muscle tension was monitored as short stimulus trains were delivered alternately to the axons of OI and OE. Fig. 2 shows that brief stimulus trains delivered to the axon of the inhibitory (OI) motoneuron produced relaxation of the dactyl opener. Brief stimulus trains delivered to the excitatory (OE) motoneuron produced partial tetanic contractions, consistent with the physiological role of the dactyl opener as a relatively slowly contracting tonic muscle. Both types of muscle movement are strongest at cold temperatures. Neurally evoked muscle relaxations become smaller as the temperature warms and the amplitude of IJPs decreases; these relaxations disappear at temperatures where the IJPs reverse polarity and become depolarizing. When the polarity of IJPs is depolarizing, neurally evoked relaxations are not observed even when stimulus train parameters are increased to 50–60 Hz for 10 s (not shown). Neurally evoked contractions also decrease in strength as a function of increasing temperature (Fig. 2), but can be rescued at warm temperatures by increasing the frequency of the stimulus train to 30 Hz or higher (not shown). Thus, whereas neurally evoked contractions can be evoked over the entire temperature range, neurally evoked relaxations can only be evoked at cold temperatures where the IJPs are hyperpolarizing.

Serotonin enhances EJPs and twitch contractions

Serotonin (5-HT) potentiates both EJP amplitude and neurally evoked contractions of lobster dactyl opener muscle (Fig. 3A). Its potentiating effects on EJPs have a threshold of approximately 1 nmol l^{-1}, and saturate at approximately 1 μmol l^{-1} (Fig. 3B). Serotonin does not affect the muscle resting potential significantly; 5 nmol l^{-1} and 50 nmol l^{-1} serotonin change muscle resting potential by 2.0±2.1 mV (mean ± s.d.; N=6) and –0.8±3.0 mV (N=27), respectively. The potency of serotonin's effect on EJP amplitude strongly depends on the size of the EJP before serotonin is added; small (<1 mV) EJPs are potentiated more strongly than larger (>2.5 mV) EJPs (Fig. 3C). Analysis of data from 15 experiments in which 5-HT potentiated EJP amplitude over a temperature range of 2–20°C showed no evidence for a temperature-dependent effect of 5-HT on EJP (data not shown), in agreement with data in Fig. 3C showing that serotonin's effect on excitatory neurotransmission is similar at 2°C and 16°C (Fig. 3C).

Interestingly, in 6 of 12 experiments in which the excitatory motor axon was stimulated in short stimulus trains, muscle fiber action potentials appeared when serotonin was present in the bath. The appearance of the action potential varied in different experiments; Fig. 3D illustrates recordings from an experiment in which the fourth EJP in a series gave rise to an action potential in the presence of 50 nmol l^{-1} serotonin. In general, the action potential appeared later in the series of EJPs as the bath temperature warmed, and no action potentials were observed at temperatures above 5°C. Thus, whereas the potentiating effect of serotonin on neuromuscular transmission appears to be independent of temperature (Fig. 3C), serotonin frequently induced hyperexcitability of the muscle at low temperatures (Fig. 3D).

Serotonin increases both muscle relaxation and the temperature range over which IJPs are effective in relaxing muscle

To examine the temperature dependence of inhibitory neuromuscular function, trains of IJPs were elicited and the corresponding motor movements measured as a function of temperature. 5-HT increases IJP amplitude as well as the magnitude of the IJP-evoked muscle relaxations (Fig. 4A,B). In addition, 5-HT shifts the temperature at which IJPs reverse polarity to warmer temperatures (Fig. 4B), enabling relaxation in a temperature range where IJPs would otherwise be depolarizing and ineffective in triggering relaxation. For example, in the preparation illustrated in Fig. 4B, muscle relaxations could not be evoked in the absence of 5-HT at temperatures >4°C where IJPs were depolarizing. However, in the presence of 5-HT, the IJP reversed polarity at 9°C and relaxations could be observed up to temperatures <8°C. The traces in Fig. 4C demonstrate that IJPs are depolarizing under control conditions but hyperpolarizing in the presence of 5-HT when recorded in the same muscle fiber at 6°C. Data averaged from four experiments examining muscle relaxation are shown in Fig. 4D. 5-HT most effectively potentiates muscle

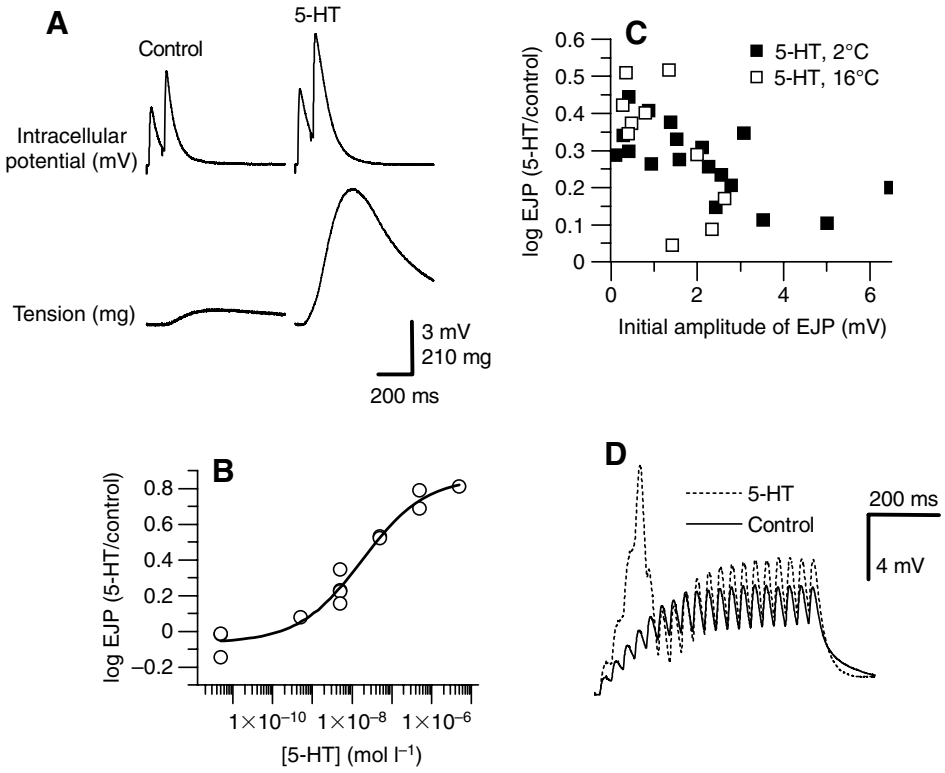

Fig. 3. Serotonin (5-HT) increases the amplitude of EJPs and neurally evoked contractions. (A) Representative recordings of EJPs (upper traces) and the corresponding twitch contractions (lower traces) at 2°C are shown under control conditions and 22 min after the addition of 50 nmol l^{-1} serotonin. Each trace is the average of ten consecutive recordings in response to a 200 ms train of 10 Hz stimulation. Serotonin increased EJP size in this muscle fiber by approximately 30% and increased twitch tension recorded from the entire muscle by 800%. (B) Serotonin elicits a dose-dependent increase in EJP amplitude, with a threshold of approximately 1 nmol l^{-1} and maximal effects at 1 μmol l^{-1}. Results are expressed as the logarithm of the ratio of EJP amplitude after application of serotonin with respect to its control value. Symbols represent 13 measurements from 10 different preparations at a temperature of 2°C. (C) Serotonin is most effective at potentiating small EJPs. Symbols illustrate the magnitude of potentiation of EJPs by 50 nmol l^{-1} serotonin recorded at bath temperatures of 16°C (squares) and 2°C (triangles) expressed as the logarithm of the ratio of EJP amplitude after application of serotonin with respect to its control value. Each symbol represents a measurement from a different preparation. Slopes of linear fits to data (not shown) are negative and significantly different from zero (at 2°C slope=–0.03, P=0.002; at 16°C slope=–0.13, P=0.03). (D) Serotonin triggers muscle action potentials. At 3°C in the presence of 50 nmol l^{-1} serotonin (broken trace) an action potential appeared superimposed on the fourth EJP evoked by a 30 Hz, 600 ms stimulus train. The control recording at the same temperature is shown as a solid trace.

relaxations at the coldest temperature tested (by an average of 17.6-fold at 2°C) and enables neurally evoked relaxations of muscle at temperatures between 4°C and 10°C.

To test whether 5-HT might change the reversal potential for GABA-gated chloride flux through the postsynaptic muscle membrane the temperature and membrane potential at which IJPs reversed polarity were measured in the presence and absence of 5-HT. Compared to that measured under control conditions, 5-HT (100 nmol l^{-1}) shifts the temperature at which IJPs reverse from 6.6±2.6°C (mean ± s.d.) to 13.2±6.1°C (N=8; P<0.05 in a paired t-test) without changing the membrane potential at which the IJP reverse (–64.8±8.7 mV compared to –66.5±7.6 mV; P>0.05). To control for the possibility that the temperature of IJP reversal might shift as a result of warming the preparation twice (see Materials and methods), another set of experiments was performed in the absence of 5-HT. In the control experiments, warming the preparation twice did not significantly change the temperature at which IJPs reversed (7.0±3.5°C *versus* 5.3±2.7°C (N=6; P>0.05) (see below).

The increase in IJP amplitude elicited by 5-HT has been reported to be due to an increase in GABA release (Vyshedskiy et al., 1998); however, it is also possible that 5-HT increases the driving force for chloride flux through the GABA-gated channel. To test this possibility, the effects of 5-HT on resting membrane potential were measured. At 2°C, 100 nmol l^{-1} 5-HT depolarized muscle fibers by a mean (± s.d.) value of 4.8±4.0 mV (N=8) (Fig. 5).

The plot in Fig. 5 illustrates how the effect of 5-HT on the temperature at which IJPs reverse can be understood in terms of the temperature dependence of the membrane potential. Muscle resting potential hyperpolarizes as the temperature warms under both control (open symbols) and experimental (closed symbols) conditions. Under control conditions the muscle resting potential hyperpolarizes by approximately 7.5 mV when the temperature warms to the temperature at which IJPs reverse (T_{rev}=6.6°C). 5-HT depolarizes the same muscle fibers at 2°C by nearly 5 mV relative to their control values, resetting the membrane potential at value that is further depolarized from the chloride (IJP) reversal potential. To reach the membrane potential at which IJPs reverse the muscle resting potential must hyperpolarize by 5 mV more than is necessary under control conditions. Therefore, a relatively small depolarization produced by 5-HT produces a substantial shift (to approximately 13.2°C) in the temperature at which IJPs reverse.

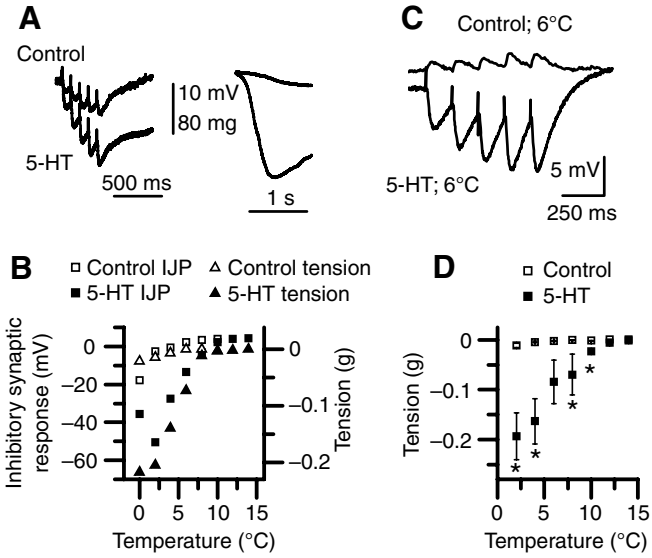

Fig. 4. Serotonin (5-HT) potentiates both IJP amplitude and the size of neurally evoked relaxations. (A) Intracellular recordings of IJPs (left) evoked at 2°C by a stimulus train (7 Hz, 700 ms) delivered to the inhibitory (OI) motoneuron in the absence (control) and in the presence of 100 nmol l^{-1} 5-HT. 5-HT depolarized the resting potential of this muscle fiber at 2°C from –66 mV to –60 mV. Corresponding relaxations of muscle tension are shown at right. Both intracellular and tension traces are averaged from five stimulus trials. (B) Plot of IJP amplitude (squares) and neurally evoked relaxations (triangles) as a function of temperature in the absence (open symbols) and presence (solid symbols) of 5-HT. Data in B are from the same experiment as in A. The reversal potential of the IJPs in this experiment was 4.3°C under control conditions and 9°C in the presence of 5-HT. (C) Intracellular recording of IJPs from the same muscle fiber at 6°C under control conditions (upper trace) and in the presence of 5-HT; data are from the same experiment as in A and B. (D) Data pooled from four experiments; symbols represent the amplitude of relaxations evoked by stimulus trains delivered to the inhibitory (OI) motoneuron in the absence (open symbols) and the presence (solid symbols) of 5-HT. At each temperature N=4 except as follows: in control data N=3 at 10°C and N=1 at 12°C; in 5-HT data N=2 for 8°C, 10°C and 12°C. Asterisks indicate values that are significantly different from controls (paired t-test, P<0.05).

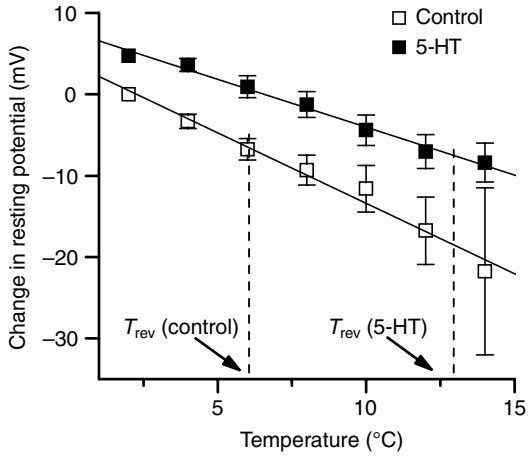

Fig. 5. Temperature dependence of the muscle fiber resting potential in the presence and absence of serotonin (5-HT). Symbols represent the change in resting potential with respect to the value measured at 2°C measured in the same eight muscle fibers as a function of temperature in the absence (solid symbols) and presence (open symbols) of 5-HT. Data are normalized in both cases to the values of the resting membrane potential recorded at 2°C. Addition of 5-HT (100 nmol l^{-1}) at 2°C produced a mean change in resting membrane potential of +4.75 mV; the data measured in the presence of 5-HT is offset accordingly. Arrows indicate the temperatures at which IJPs reverse polarity (T_{rev}) under control conditions (6.6°C) and in the presence of 5-HT (13.2°C). At each temperature N=8 except as follows: for 10°C N=6, for 12°C N=4, for 14°C N=2.

The potentiation of resting muscle tension and neurally evoked contractions by serotonin depends differentially on temperature

The temperature dependence of 5-HT modulation of neurally evoked contractions was examined by stimulating trains of EJPs and monitoring the corresponding contractions of the muscle. Results from a typical experiment are illustrated in Fig. 6A, and data pooled from multiple experiments are shown in Fig. 6B,C. Under control conditions, neurally evoked contractions evoked with brief stimulus trains are maximal at 2°C and are virtually abolished as the temperature approaches 18°C (lower traces of Fig. 6A, open symbols in Fig. 6B). In the presence of 5-HT, neurally evoked contractions are potentiated over the entire temperature range (upper traces of Fig. 6A, solid symbols in Fig. 6B). Quantitatively, the effect of 5-HT in potentiating neurally evoked contractions was significantly stronger at 18°C than it was at other temperatures (Fig. 6C). This result is consistent with the raw data traces shown in Fig. 6A, which show that at 18°C 5-HT enabled large neurally evoked contractions that were nearly undetectable under control conditions.

To examine whether 5-HT might directly modulate muscle independently of its modulatory effects on neurotransmission, resting muscle tension was monitored in the absence of neural input both in the presence and absence of 5-HT. Results from a typical experiment are shown in Fig. 7. During the first 30 min in control conditions, resting muscle tension decreases by half from its original value of 2 g (Fig. 7B) as temperature increases from 2°C to 22°C (Fig. 7A). This effect reverses as the temperature cools back to 2°C (over 30–100 min). 5-HT (hatched bar above Fig. 7A) added to the bath at a constant temperature of 2°C increases muscle tension significantly to 2.8 g (over 100–117 min). As temperature warms to 22°C in the presence of 5-HT, muscle tension decreases to reach a value at 22°C comparable to that observed at 22°C under control conditions. Finally, as the temperature chills back to 2°C (160–210 min), muscle tension recovers nearly to its potentiated value of 2.8 g. Thus, in this experiment, 5-HT maximally increased resting muscle tension at the coldest temperature of 2°C and had almost no effect at 22°C.

The temperature dependent modulation of resting muscle tension by 5-HT was highly reproducible. Fig. 7C shows results from seven preparations in which the experimental protocol shown in Fig. 7A was repeated. Under control conditions muscle tension decreased significantly (by an average of 26.9%) as temperature warmed from 2°C to 22°C (Fig. 7B) and recovered when the muscles were chilled to 2°C. Adding 50 nmol l^{-1} 5-HT (hatched bar in Fig. 7C) at 2°C significantly increased muscle tension (by an average of 46.9%). As the temperature warmed from 2°C to 22°C in the presence of 5-HT muscle tension relaxed, becoming significantly lower (by an average of 28.2%) than it had been at 2°C before 5-HT was added and approximately the same as it was at 22°C in the absence of 5-HT. Thus, 5-HT strongly potentiates resting muscle tension at the coldest temperatures, and has no effect on muscle tension at the warmest temperature.

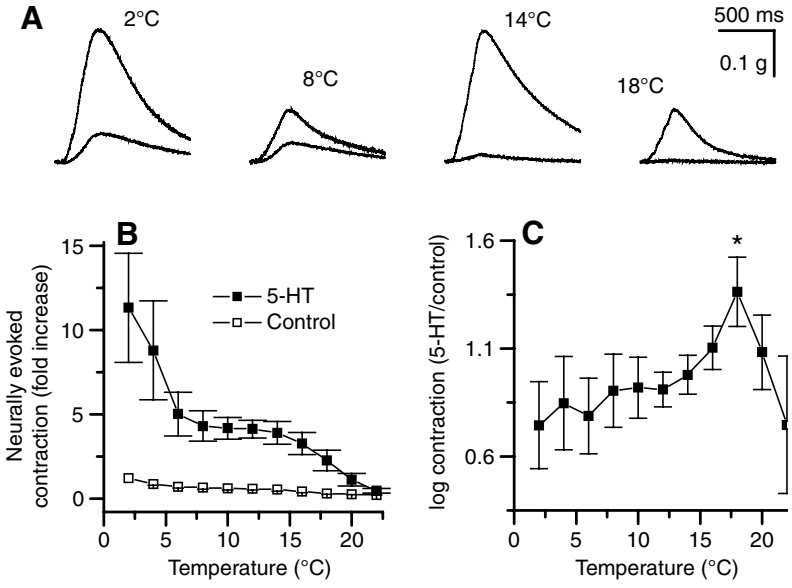

Fig. 6. Modulation of neurally evoked contractions by 5-HT is temperature dependent. (A) Neurally evoked contractions recorded under control conditions (lower traces) and in the presence of 50 nmol l^{-1} 5-HT (upper traces) at the indicated temperatures. Each trace is the average of ten consecutive recordings. (B) Temperature dependence of neurally evoked contraction strength in muscle fibers (N=6) in the absence (open symbols) and presence (closed symbols) of 5-HT (50 nmol l^{-1}). Control data are normalized relative to the average contraction amplitude recorded at 2°C (error bars are not larger than the symbols); 5-HT data are normalized relative to the potentiating effect of 5-HT on contraction amplitude (an increase of 1112%) at 2°C in the same preparations. (C) Potentiation of neurally evoked contractions by 50 nmol l^{-1} 5-HT recorded at bath temperatures between 2°C and 22°C (N=5 muscle fibers at 20°C and 22°C, N=6 at all other temperatures). Symbols illustrate the logarithm of the ratio of neurally evoked contraction strength after application of 5-HT with respect to the control value at the same temperature. Asterisks indicate values that are significantly different from data recorded at 2°C (independent t-test, P<0.05).

Discussion

For invertebrate ectotherms, which comprise >95% of animal species, the temperature of the surrounding environment is a major factor determining physiology and behavior (Johnston and Bennett, 1996). Here we describe the temperature-dependent physiology of the lobster dactyl opener muscle and its modulation by the circulating neurohormone serotonin (5-HT). The lobster dactyl opener is a popular model system for studies of synaptic plasticity (Bykhovskaia et al., 1999; Bykhovskaia et al., 2001; Bykhovskaia et al., 2004; Glusman and Kravitz, 1982; Goy and Kravitz, 1989; Hamilton et al., 2006; Kravitz et al., 1980; Vorob'eva et al., 1999; Worden et al., 1997), as is the homologous crayfish dactyl opener muscle (Beaumont and Zucker, 2000; Delaney et al., 1991; Dixon and Atwood, 1989a; Dixon and Atwood, 1989b; Qian and Delaney, 1997; Vyshedskiy et al., 1998; Vyshedskiy and Lin, 1997; Wang and Zucker, 1998). We demonstrate that (1) thermal change within a biologically relevant range of temperatures profoundly affects the neurophysiological properties of the lobster dactyl opener neuromuscular system, and (2) the modulatory effects of serotonin on neuromuscular function have distinctly different temperature dependencies and extend the thermal range over which motor movements are effective. Fig. 8 summarizes the temperature dependence of lobster neuromuscular function and behavior over the biologically relevant temperature range for *H. americanus* in the wild.

The temperature dependence of the dactyl opener neuromuscular system

Our observations that neurally evoked contractions weaken and resting muscle tension decreases as temperature warms are similar to those reported in studies of the closer muscle of the crayfish walking leg (Harri and Florey, 1977) and the crayfish claw opener and stretcher muscles (Jacobs and Atwood, 1981a; Jacobs and Atwood, 1981b). Further, the temperature dependence of both the resting membrane potential and the muscle input resistance in the dactyl opener (Fig. 1D,E) is similar to that reported in previous studies of neuromuscular systems in crayfish (Harri and Florey, 1977; Harri and Florey, 1979; Fischer and Florey, 1981), lobster (Colton and Freeman, 1975b) and crab (Stephens, 1990; Stephens and Atwood, 1982).

Our results differ from those reported in previous studies, however, in that the pattern of temperature dependence of EJP amplitude is biphasic with two temperature optima: EJP amplitudes are maximal both in the temperature range from 14–18°C and at very cold temperatures (around 2°C) (Fig. 1A,B). Other studies of crayfish muscles report different patterns of temperature dependence of EJPs. EJP amplitudes are maximal at 2°C but progressively smaller as the temperature warms in dactyl opener muscle (Fischer and

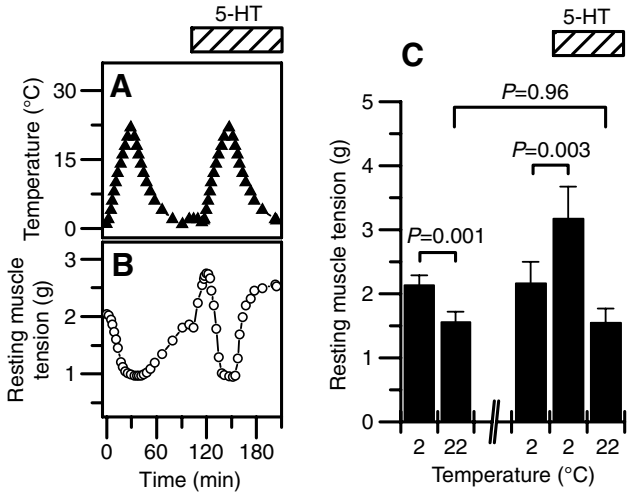

Fig. 7. Serotonin increases resting muscle tension most strongly at the coldest temperatures. (A) Bath temperature recorded as a function of time illustrating the change from 2°C to 22°C and back to 2°C before and during application of 50 nmol l^{-1} 5-HT (hatched bar). (B) Resting muscle tension in the same preparation recorded at temperatures indicated in A. (C) Muscle tension (mean ± s.e.m.) recorded at 2°C and 22°C in the absence and presence (hatched bar) of 5-HT (50 nmol l^{-1}) in the same seven preparations. Values for *P* (paired *t*-test) are indicated by horizontal brackets. The break in the *x*-axis indicates the distinction between the first temperature warming protocol and the second.

Florey, 1981; White, 1983), whereas they increase in size over the temperature range 10–20°C in deep abdominal extensor muscle (Dunn and Mercier, 2003).

The cellular mechanism underlying the temperature dependence of EJP amplitude in lobster dactyl opener muscle cannot be explained by the temperature dependence of the muscle input resistance (compare Fig. 1B,E). However, there are several other possibilities. For example, the synaptic conductance underlying the EJP may change as a function of temperature, as suggested by the observation that the ionic response of lobster muscle to bath-applied glutamate is relatively more selective for sodium at 3°C compared with 18°C (Colton and Freeman, 1975a) and by reports that sodium influx is critical for hormone-induced membrane potential oscillations at 15°C but not at 21°C in a lobster stomatogastric neuron (Johnson et al., 1992). Another factor is that the time course for neurally evoked synaptic vesicle release depends on temperature. Quantal neurotransmission at dactyl opener muscle synapses is asynchronous at cold (<5°C) temperatures (Bykhovskaia et al., 1999; Worden et al., 1997) and becomes more synchronous at warmer temperatures. Finally, it is also possible that temperature regulates the relative amount of neurotransmitter released from synaptic terminals, as demonstrated in crayfish (Dunn and Mercier, 2003).

Since IJPs in the lobster dactyl opener result from the release of GABA from the inhibitory motoneurons (Otsuka et al., 1966), the temperature dependence of the IJP amplitude and polarity can be understood in terms of the driving force for chloride flux through the GABA-gated chloride channel. At the coldest temperatures where the resting potentials are relatively depolarized (see Fig. 1D), we suspect that IJP amplitudes are large and hyperpolarizing because the driving force for chloride influx is large. Cold (2°C) temperatures have been used experimentally in studies of inhibitory neurotransmission in the dactyl opener because these temperatures enhance the signal-to-noise ratio for inhibitory synaptic signals and favor large inhibitor-evoked muscle relaxations (Worden and Camacho, 2006). As temperature warms to approximately 6°C and the muscle fibers hyperpolarize, the resting membrane potential approaches the reversal potential for chloride flux when the IJP becomes indistinguishable from the baseline (see data at 6°C in Fig. 1A). At warmer temperatures, IJPs are depolarizing, presumably because the muscle resting potential become more negative than the reversal potential for chloride flux. Notably, depolarizing IJPs do not generate motor movements, contradicting the idea that the classical inhibitory signals generated by OI in the dactyl opener will act as 'excitatory inputs' under experimental conditions where IJPs are depolarizing (Segev and Parnas, 1983). The decline in amplitude of IJPs and EJPs at temperatures warmer than 15°C may reflect several factors, including a temperature-dependent reduction of transmitter output, a decrease in muscle fiber input resistance and the metabolic stress of warm temperatures for this species (Chang et al., 1998).

Finally, it is important to note that many aspects of physiology are temperature dependent in crustacea. For example, thermal change alters the firing rate of specific lobster neurons (Konishi and Kravitz, 1978), the release of hormones from lobster neurosecretory organs (Kuramoto and Tani, 1994), the strength of long-term facilitation at crayfish neuromuscular synapses (Jacobs and Atwood, 1981a; Jacobs and Atwood, 1981b), the physiological properties of crayfish gap junctional synapses (Heitler and Edwards, 1998), and the conduction velocity of action potentials in crabs (Young et al., 2006).

Temperature dependent modulation of neuromuscular movements by serotonin

In agreement with previous studies of the lobster dactyl opener muscle (Kravitz et al., 1980; Glusman and Kravitz, 1982; Goy and Kravitz, 1989; Worden et al., 1995) 5-HT has multiple modulatory effects on the dactyl opener neuromuscular preparation. Interestingly, although 5-HT modulated neurally evoked contractions and relaxations as well as resting muscle tension in temperature-dependent ways, it potentiated excitatory neurotransmission in a temperature-independent manner. In contrast, the neuromodulatory effect of peptide DF_2 on crayfish phasic abdominal extensor muscles is three times as strong at 7–9°C as it is at 15–17°C (Friedrich et al., 1994), an effect that has been attributed to the greater ability of the peptide to increase calcium influx into presynaptic

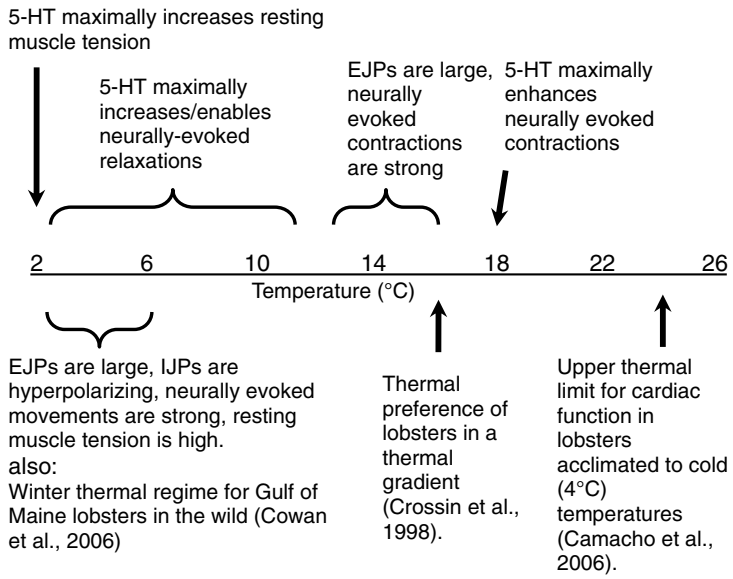

Fig. 8. Schematic diagram illustrating the temperature dependence of physiology and behavior in the lobster *H. americanus*. With the exception of the data reported by Crossin et al. (Crossin et al., 1998), the indicated temperatures are those reported for lobsters acclimated to cold (<6°C) temperatures.

terminals of the motoneurons at low temperatures (Dunn and Mercier, 2003).

However, it is important to note that the electrophysiological results reported in this study were collected mainly in the most proximal fibers of the dactyl opener muscle. Different regions of the dactyl opener have fibers with different properties (Mykles et al., 2002) and we cannot rule out the possibility that the central and distal fibers of the lobster dactyl opener may exhibit different synaptic responses to serotonin and/or temperature. On the other hand, our tension recordings reflect the physiological output of all the fibers of the entire muscle. Therefore, the thermal and hormonal modulatory effects we observe on contractility are characteristic of the output of the dactyl opener as a whole.

In the presence of 5-HT, neurally evoked contractions are particularly large not only at 2°C but in the temperature range 12–16°C, which includes the thermal preferendum of lobsters allowed to freely move in thermal gradients (16°C) (Crossin et al., 1998). Quantitatively, the maximal potentiation of contractions by 5-HT occurs at 18°C, a temperature that nearly abolished neurally evoked contractions triggered by the brief stimulation protocols employed in this study. Thus 5-HT extends the upper thermal limit for neurally evoked contractions of the dactyl opener muscle. In contrast, the potentiating effect of serotonin on the strength and frequency of the lobster heartbeat does not depend on temperature (Worden et al., 2006).

In dactyl opener skeletal muscle 5-HT also acts by shifting the temperature at which IJPs elicited by the specific inhibitory motoneuron reverse polarity to warmer temperatures, thereby extending the upper limit of the temperature range over which the specific inhibitor acts to actively relax resting muscle tension. In agreement with an earlier report (Kravitz et al., 1980), we find that 5-HT does not modulate the reversal potential for chloride flux through the GABA channel. Transient neurally evoked muscle relaxations [which can be triggered by both of the inhibitory motoneurons innervating the dactyl opener (Worden and Camacho, 2006)] have also been observed in crayfish muscle [for example, see fig. 12 of Harri and Florey (Harri and Florey, 1977)], and may serve *in vivo* to facilitate contractions of the antagonist dactyl closer muscle and movement of the dactyl. These relaxations may be particularly important under the frigid (<5°C) environmental conditions endured by some lobster populations for over 6 months each year in the wild (Cowan et al., 2007), because in this thermal range resting muscle tension is high and the effects of 5-HT in potentiating resting muscle tension are strong (see Fig. 7). It is interesting to speculate that a relatively high level of resting muscle tension at cold temperatures may be physiologically adaptive for tonic muscles in that it permits sustained contractions under thermal conditions where metabolism is significantly slowed and the energetic costs of neural electrical activity are therefore high.

Modulation of muscle tone by temperature and by serotonin

Contraction of crustacean striated muscle is proportional to the level of muscle fiber depolarization (Harri and Florey, 1977; Orkand, 1962), but the mechanism by which resting muscle tension is generated is poorly understood. An early suggestion that the relatively depolarized muscle membrane potentials recorded at cold temperatures might be sufficient to exceed the threshold for excitation–contraction (e–c) coupling (Harri and Florey, 1977) was contradicted by a subsequent report that the threshold for e–c coupling in crayfish dactyl opener muscle is both temperature independent and at least 15 mV more depolarized than the resting membrane potential over the entire temperature range [see fig. 3 of Fischer and Florey (Fischer and Florey, 1981)]. It is unlikely that resting muscle tension in the lobster dactyl opener is due to calcium influx through voltage-dependent ion channels because the threshold for the voltage-dependent calcium current is –35 to –45 mV, which is approximately 20 mV more depolarized than the resting potentials of the muscle fibers (Kravitz et al., 1980) (and see Fig. 1D).

Our observation that 5-HT strongly modulates resting muscle tension at cold temperatures (see Fig. 7B,C) is also difficult to understand in terms of an effect on ion channels. Although 5-HT-mediated increases in the resting muscle tension of dactyl opener muscle depend on extracellular calcium levels (Kravitz et al., 1980), 5-HT changes neither the threshold for e–c coupling in crayfish dactyl opener (Fischer and Florey, 1983) nor the threshold for the voltage-dependent calcium current in lobster dactyl opener (Kravitz et al., 1980). Resting muscle tension in crayfish superficial extensor muscle

also increases at colder temperatures (Quigley and Mercier, 1997), although it has not been reported whether neuromodulators are more effective at modulating tension at colder temperatures. It is likely that one or more muscle membrane conductances in the dactyl opener is temperature dependent, given that muscle input resistance is highest at the coldest temperatures. Thermosensing ion channels have been described previously both in invertebrate and in vertebrate species (Viswanath et al., 2003).

Given the muscle fiber heterogeneity in the dactyl opener muscle (Mykles et al., 2002), one confounding issue is whether muscle tone and changes in tone might arise from the action of a small number of fibers of a special type. In order to elucidate the physiological pathway linking membrane excitation and tension it will be important to measure electrophysiological and contractile properties at the level of single muscle fibers and to determine the mechanism by which temperature alters the muscle resting membrane potential. Previous studies of crustacean muscles have suggested that temperature might alter the activity of sodium pump or the relative permeability of the muscle to potassium and sodium ions (Florey and Hoyle, 1976; White, 1983). In addition, temperature may alter the activity of the Na^+/Ca^{2+} exchanger, which has previously been implicated as a mediator of tonic muscle contractions (Goblet and Mounier, 1982; Mounier and Goblet, 1987). Elucidating the cellular mechanism(s) mediating resting muscle tension will be an important first step in understanding how muscle contractility can be modulated by temperature and by 5-HT.

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