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THERMAL ACCLIMATION IN A CRUSTACEAN NEUROMUSCULAR SYSTEM

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SUMMARY

1. Effects of temperature on the muscle fibre membrane and synapses of stretcher muscle preparations made from autotomized limbs of the Pacific shore crab (*Pachygrapsus crassipes*) were investigated.

2. Acclimation of the crabs to different temperatures modified properties of both muscle fibre membrane and synapses.

3. Increased temperature produced an increase in membrane potential of the muscle fibres. A semi-log plot of these data revealed two linear phases of the membrane potential–temperature relationship, with a change in slope near the acclimation temperature.

4. Maximum values for excitatory junction potential (EJP) amplitude and time constant of EJP decay, and minimum values for facilitation were obtained at temperatures close to the acclimation temperature. It is suggested that the decline in EJP amplitude and time constant of decay produced by deviations in temperature from the acclimation temperature is compensated for by an increase in the amount of facilitation. In this way, maximum tension can be produced by the stretcher muscle in a range of at least 8 °C around the acclimation temperature.

INTRODUCTION

The body temperature of most cold-blooded animals, or poikilotherms, is dependent upon the temperature of the environment. Certain environments provide near constant thermal conditions and permit cold-blooded animals to maintain stable body temperatures. Physiological and behavioural observations made on poikilotherms from such thermally stable environments have revealed limited tolerance to sudden temperature changes (Hammel, Stromme & Myrhe, 1969; Florey & Hoyle, 1961, 1976). By contrast, other poikilotherms live in environments with dramatic thermal fluctuations, both short term (on a daily basis) and long term (on a seasonal basis). These animals must tolerate and adapt to the temperature changes in order to maintain functional integrity.

In the crayfish, a poikilotherm that lives in a thermally fluctuating environment, neuromuscular systems function over a wide temperature range. Rapid alterations in

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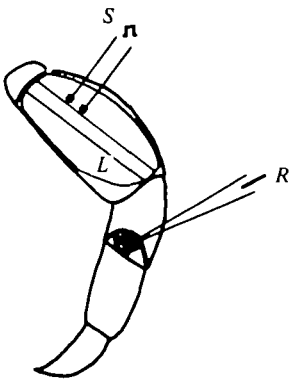

Fig. 1. Diagram of a preparation made from an autotomized walking limb. Stimulus shocks (*S*) were applied to bundles of the exposed limb nerve (*L*) in the meropodite, and evoked junctional potentials were recorded (*R*) via microelectrodes in single stretcher muscle fibres. Scale: 1 cm.

temperature provoke changes in membrane potential, and changes in amplitude, time course, and facilitation of the junction potential (Harri & Florey, 1977). These responses can be modified by prior acclimation of the crayfish to different temperatures (Harri & Florey, 1979). In fish, thermal acclimation has a profound effect upon the saturation and fluidity of synaptosomal membrane lipids (Roots & Johnston, 1968; Driedzic, Selivonchick & Roots, 1776; Cossins, Friedlander & Prosser, 1977; Matheson, Oei & Roots, 1980). It has been suggested that, in crayfish, thermal acclimation modifies membrane and synaptic properties by influencing membrane lipid composition (Harri & Florey, 1979).

In the present paper, we report observations on the single excitatory motor axon that innervates the stretcher muscle in walking legs of the shore crab, *Pachygrapsus crassipes*. Previously, we reported that short term changes in temperature produce changes in muscle fibre membrane and synaptic properties (Atwood & Stephens, 1980). Here, we report that these responses are modified by acclimation of the crabs to different temperatures.

MATERIALS AND METHODS

Shore crabs (*Pachygrapsus crassipes*) were obtained from the Pacific Biomarine Laboratories in Venice, California, and were retained individually at 12 °C (cold acclimation) or 21 °C (warm acclimation). Feeding (Purina fish chow) and changes of artificial seawater were performed twice a week. Under these conditions the crabs lived for at least 6 months.

Preparations were made from walking legs removed from crabs which had been acclimated for at least 6 weeks. Observations were carried out on the stretcher muscle in the carpopodite, which is innervated by a single excitor (*E*) axon (Wiersma & Ripley, 1952). Autotomized limbs were bathed in a crab saline, composed of 470 mM-NaCl, 8 mM-KCl, 20 mM-$CaCl_2$, 10 mM-$MgCl_2$ and 5 mM-Hepes buffered to pH 7·2. The limb nerve was exposed in the meropodite and platinum hook electrodes were

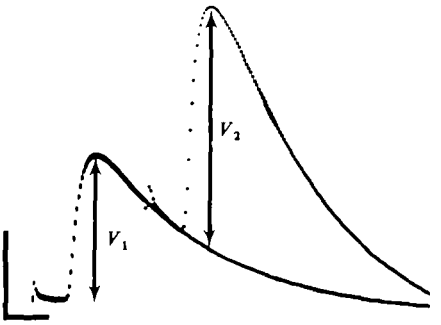

Fig. 2. Calculation of facilitation (F). A photograph taken directly from the signal averager of two superimposed traces showing a single EJP and a pair of EJP's (separated by 25 ms) recorded from a stretcher muscle fibre. Each trace represents the mean response of 32 successive sweeps. EJP amplitudes (V_1 and V_2) were corrected for summation (see text). Calibration: 2 mV and 10 ms.

used to deliver single stimulating pulses, or pairs or trains of pulses (0·1 ms duration) to a bundle containing the E axon (Fig. 1). A small window was drilled in the distal portion of the carpopodite and the exposed stretcher muscle fibres were penetrated with glass microelectrodes filled with 3 M-KCl (3 to 15 MΩ). Values for the membrane potential of single muscle fibres were obtained from a digital voltmeter. Excitatory junctional potentials (EJP's), evoked by E axon stimulation, were displayed and photographed on an oscilloscope. EJP amplitude, and time constant (defined as the time taken for the EJP to decline from peak amplitude to $1/e$ of peak amplitude) were measured with a signal averager (Fabritek Instruments Inc., Model 1072).

Measurements of facilitation were made by applying pairs of stimuli (at an inter-pulse interval of 25 ms) to the E axon and using the signal averager to analyse the mean of 16 response-pairs. Mean amplitudes of EJP's were measured as shown in Fig. 2 and corrected for non-linear summation (Martin, 1955), using a value for reversal potential of 0 mV (Taraskevich, 1971; Takeuchi & Onodera, 1973). The relatively slow time course of the EJP made it unnecessary to apply further corrections. The degree of facilitation (F) was calculated using the equation:

$$F = \frac{V_2 - V_1}{V_1}.$$

Two EJP's with the same amplitude produce an F value of 0. A positive F value denotes facilitation, whereas a negative F value is obtained when the second EJP is smaller than the first, and reflects de-facilitation or depression.

Tension produced by the whole stretcher muscle was measured with a force-displacement transducer (Grass FT. 03) attached to the propodite. The E axon was stimulated with 1·5 second trains of electrical shocks at frequencies between 5 and 75 Hz. The amplified signals were photographed on an oscilloscope.

A Peltier battery provided base support for the preparation and was used to control the temperature of the saline. A uniform temperature was achieved throughout the saline bath by weak circulation currents created by constant perfusion with fresh crab saline, and by suction used to maintain constant bath depth. Temperature changes

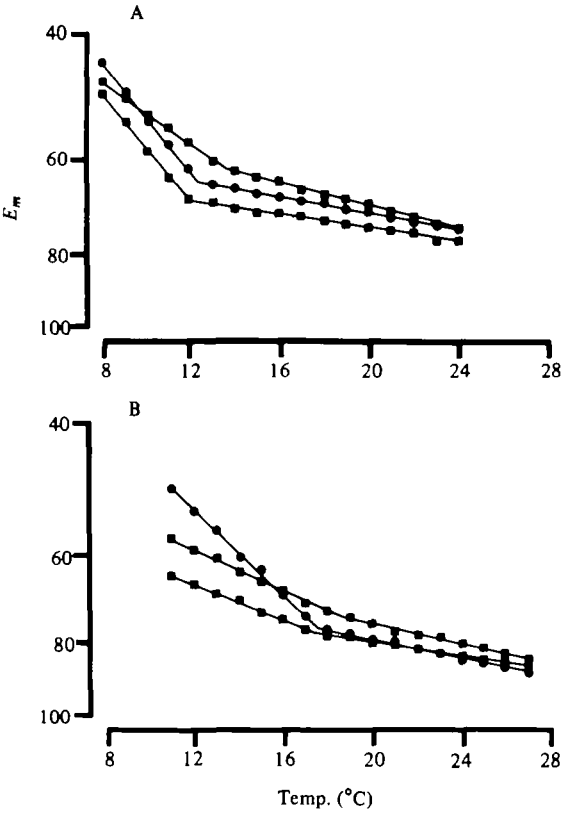

Fig. 3. The effect of temperature on the membrane potential (E_m) of stretcher muscle fibres in 3 cold (A) and 3 warm (B) acclimated preparations. The semi-log plot shows two linear components. All correlation coefficients were > 0·9.

were made at about 0·2 °C/min and observations were made after a period of at least 5 min at the test temperature.

RESULTS

Cold-acclimated preparations (acclimated to 12 °C) were cooled to 8 °C and observations made during subsequent warming to 23 °C. Warm-acclimated (21 °C) preparations were cooled to 13 °C and then warmed to 27 °C. The lower limit of each temperature range was chosen to avoid irreversible change in EJP amplitude, which occurred at 6 °C in cold-acclimated preparations and at 11 °C after warm acclimation. Upper limits were chosen to keep the temperature below the critical threshold at which a single orthodromic action potential in the *E* axon generated additional spikes in the periphery (Stephens & Atwood, 1981).

Membrane potential

As temperature was raised, membrane potential increased exponentially, first with one slope, then another (Fig. 3). A similar biphasic relationship between membrane potential and temperature has been reported for crayfish closer muscle fibres (Harri &

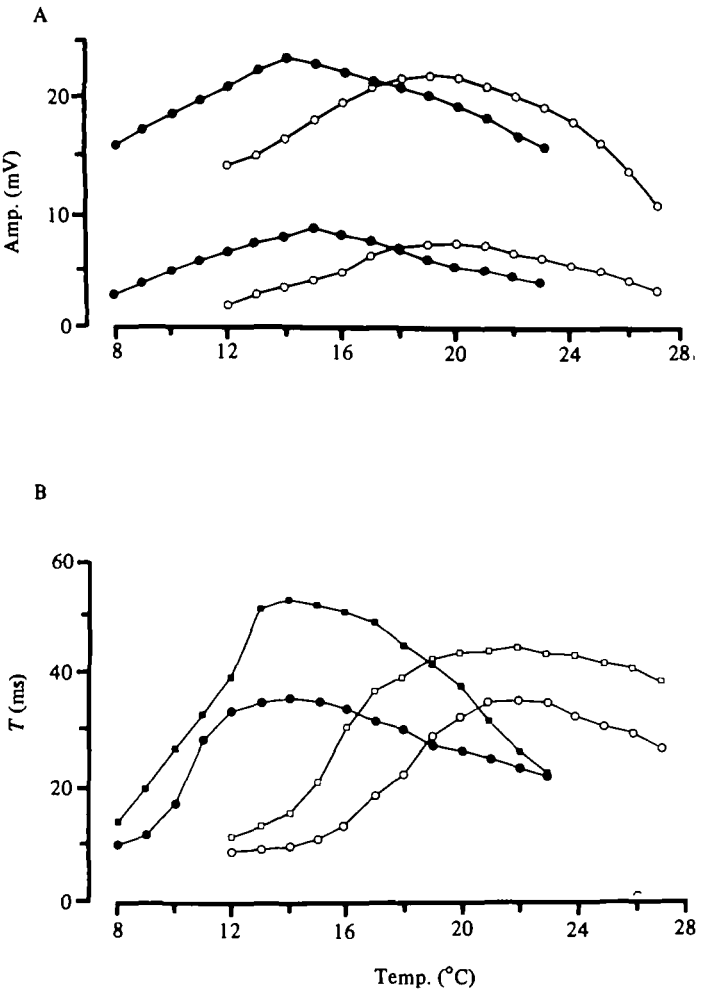

Fig. 4. The effect of temperature on (A) EJP amplitude and (B) time constant (T) in 2 cold acclimated preparations (solid symbols) and 2 warm acclimated ones (open symbols) showing shift of peak values along the temperature axis with acclimation.

Florey, 1979). In the present study, the mean temperature at which the two components intersected was 12·7 °C for cold-acclimated preparations, and 18·7 °C for warm-acclimated preparations (Table 1). These values are significantly different ($P < 0{\cdot}01$, Student's t-test).

Single EJP's

In both cold- and warm-acclimated preparations, increasing the temperature caused the EJP amplitude and time constant to increase initially to a maximum value, and then to decrease (Fig. 4). For 16 cold-acclimated and 16 warm-acclimated preparations, maximum EJP amplitude was observed at mean temperatures of 11·2 °C and 18·6 °C, respectively, and maximum EJP time constant was obtained at 12·9 °C

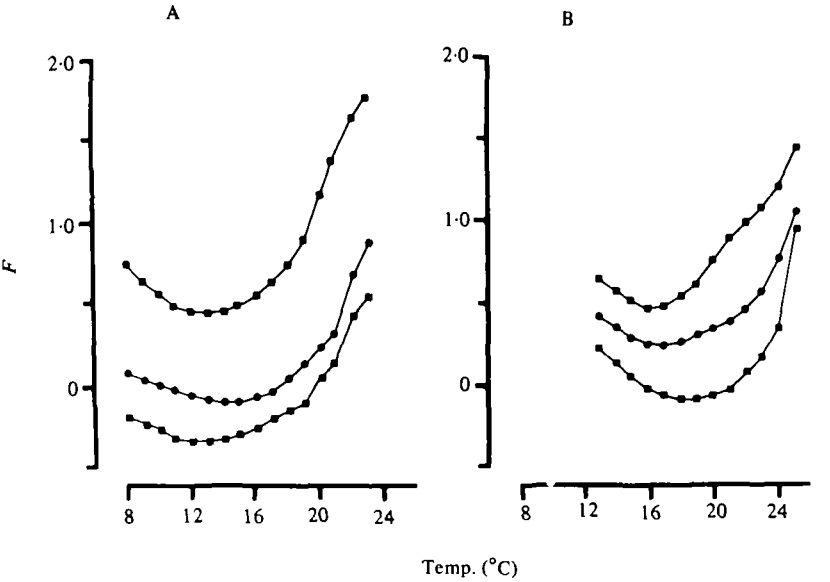

Fig. 5. The effect of temperature on facilitation F (measured using pairs of EJP's separated by 25 ms) in 3 cold (A) and 3 warm (B) acclimated preparations. F attains a minimal value close to the acclimation temperature, in all fibres examined.

Table 1. *A resumé of results showing the dependence of certain synaptic and membrane properties upon temperature in cold- and warm-acclimated preparations. The values represent mean temperatures* (+S.D.) *at which EJP amplitude and time constant were maximal; the temperature at which the minimum amount of EJP facilitation was recorded; and the temperature at which the slope of the membrane potential–temperature curve changed* (*Fig.* 3).

	Acclimation temperature	
	Cold (12 °C)	Warm (21 °C)
Membrane potential	12·7 ± 0·7 °C (23 preparations)	18·7 ± 0·9 °C (20 preparations)
Maximum EJP amplitude	11·2 ± 0·8 °C (16 preparations)	18·6 ± 2·4 °C (16 preparations)
Maximum EJP time constant	12·9 ± 1·9 °C (16 preparations)	19·5 ± 2·3 °C (16 preparations)
Minimum facilitation	12·6 ± 1·5 °C (23 preparations)	16·7 ± 1·2 °C (12 preparations)

and 19·5 °C (Table 1). The values for cold- and warm-acclimated preparations are significantly different (Student's t-test, $P < 0{\cdot}01$).

Pairs of EJP's

Microelectrode recordings from different fibres in the stretcher muscle revealed EJP's with different amplitudes and facilitation properties (Atwood & Bittner, 1971). In the present study, the extent of EJP amplitude variation encountered in the small exposed region of the stretcher muscle is indicated in the examples of Fig. 4. For studies of short-term facilitation using pairs of pulses, measurements were taken from fibres with similar EJP amplitudes. In all fibres, the curve relating facilitation to

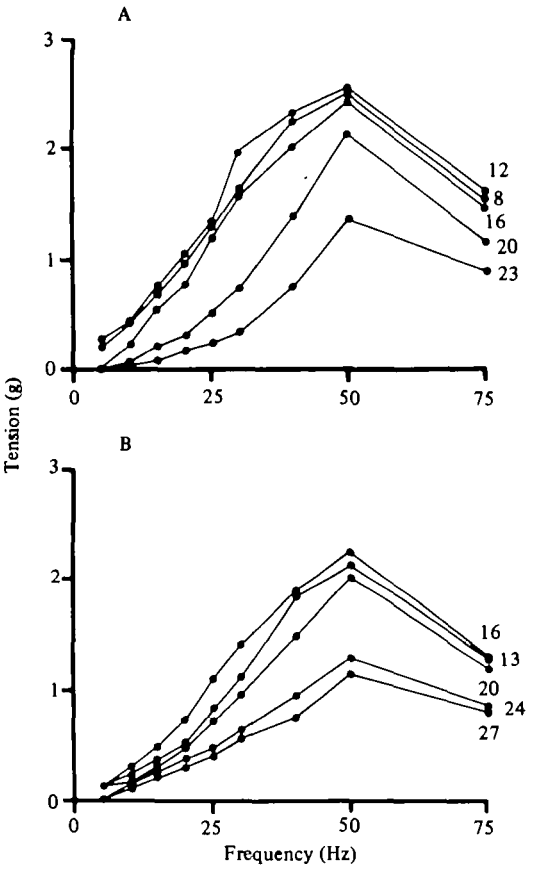

Fig. 6. The effect of stimulation frequency on the maximum tension produced by the stretcher muscle. The data were taken at five temperatures (inset figures in C) from 1 cold (A) and 1 warm (B) acclimated preparation.

temperature had the same form, although absolute values for facilitation differed from one fibre to another (Fig. 5). In cold- and warm-acclimated preparations, computer analysis of pairs of EJP's evoked at an interval of 25 ms revealed that increasing the temperature produced an initial decrease in facilitation (F) followed by a gradual increase (Fig. 5). The mean temperatures at which minimum F values were recorded were 12·6 °C for cold-acclimated preparations, and 16·7 °C for warm-acclimated preparations (Table 1). These values are significantly different (Student's t-test, $P < 0{\cdot}01$).

Tension response

EJP's were evoked by trains of stimuli (lasting 1·5 s) delivered to the motor nerve, at frequencies from 5 to 75 Hz. Tension in the stretcher muscle increased with frequency between 5 and 50 Hz. Between 50 and 75 Hz, tension declined.

In cold-acclimated preparations, the frequency-response curves were similar at 8, 12 and 16 °C (Fig. 6A). Further increases in temperature to 20 °C and then to 23 °C

resulted in an overall decline in the amount of tension produced. By contrast, in warm-acclimated preparations, the frequency-response curves were similar at 13, 16 and 20 °C. (Fig. 6B). A decline in muscle tension was observed at higher temperatures. Therefore, it is apparent that acclimation temperature influences the temperature range over which maximum tension can be produced by the stretcher muscle.

DISCUSSION

Our present data show that membrane potential of stretcher muscle fibres in the shore crab *Pachygrapsus crassipes* is closely related to temperature (Fig. 3) and that certain muscle membrane properties can be modified by thermal acclimation (Table 1). Similar changes in membrane properties with temperature have been described in other crustacean muscles (Fatt & Katz, 1953; Colton & Freeman, 1975; Harri & Florey, 1977) and modification of these properties with acclimation has been reported (Harri & Florey, 1979). Axonal membranes of crustaceans are also affected by temperature (Dalton & Hendrix, 1962; Stephens & Atwood, 1981).

In the brain of goldfish, lipid fluidity and degree of saturation of lipids in synaptosomal membranes are dependent upon acclimation temperature (Roots & Johnston, 1968; Driedzic *et al.* 1976; Cossins *et al.* 1977; Matheson *et al.* 1980). Similar observations have been made for crustacean membranes (Chapelle, 1977, 1978; Chapelle *et al.* 1979). If such changes take place in *Pachygrapsus*, as seems likely, it is possible that thermal acclimation modifies the properties of the axons and muscles by producing fundamental changes in the membrane lipid composition. The operation of ionic channels could thereby be affected, and resultant changes in total membrane conductance and in conductance of specific channels could be altered. Both pre- and post-synaptic mechanisms could be affected. Further detailed work on specific membrane channels is required.

The amount of tension produced by a train of *E* axon spikes is dependent upon the frequency of firing, the acclimation temperature of the crab, and the temperature of the preparation (Fig. 6). Maximum values for cold-acclimated preparations were recorded between 8 and 16 °C, whereas for warm-acclimated preparations maximal values were observed between 13 and 20 °C. Therefore, the temperature range at which maximum muscle tension is produced is dependent upon the acclimation temperature.

The shift in maximal tension with acclimation temperature may be explained in terms of the changes that take place in EJP properties, and in the amount of facilitation. Maximum values for EJP amplitude and time constant were recorded near the acclimation temperature (Table 1). Moreover, it was at this temperature that the minimum amount of EJP facilitation was recorded. Any changes in the temperature of the preparation produced a decline in both amplitude and time constant of the EJP (Fig. 4). However, increases in the amount of facilitation also occur (Fig. 5). It seems possible that the decreases in EJP amplitude and time constant are compensated for by an increase in facilitation. In this way, the nerve–muscle system is capable of retaining its functional integrity over a wide thermal range about the acclimation temperature.

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