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Review

Effects of heat stress on axonal conduction in the locust flight system

John R. Gray, R. Meldrum Robertson *

Department of Biology, Queen's University, Kingston Ont. K7L 3N6, Canada

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Abstract

Pretreatment of tissues or whole organisms with high, sublethal temperatures (heat shock) induces thermotolerance to normally lethal temperatures. It is of interest whether heat shock induces protection of neuronal function at normally lethal temperatures by investigating effects of heat shock on the temperature sensitivity of neuronal parameters in the locust flight system. The rhythm frequency of the deafferented flight motor was measured as well as the conduction velocity and amplitude of extracellularly recorded action potentials conveyed along the forewing stretch receptor axon. Measurements were made at temperatures ranging from 10 to 50°C in heat shocked and control animals. The deafferented rhythm was less sensitive to temperature changes above 35°C in heat shocked animals. The conduction velocity and relative amplitude of action potentials conveyed along the stretch receptor axon were less sensitive to temperature increases above 20°C in heat shocked animals. These data suggest that heat shock conserves the operation of the flight system at high temperatures. This may be accomplished by a decrease in the thermosensitivity of the conduction velocity and amplitude of action potentials within the central flight circuitry. The latter effect may serve to protect synaptic interactions and thus allow the circuitry to operate within optimal parameters.

Keywords: Heat shock; Thermotolerance; Motor pattern; Action potential; Conduction velocity; Locust; Flight

1. Introduction

The nervous system of poikilothermic animals is more strongly influenced by physical variations in the environment than is that of homeothermic animals that posses internal regulatory mechanisms. Variations in ambient temperature, therefore, can have a more profound effect on neuronal function in poikilotherms. Nevertheless, poikilotherms can survive and thrive in conditions of extreme temperatures and large temperature ranges. They may be able to avoid temperature extremes by behavioural means or they may posses compensatory mechanisms that allow them to function within a wide range of temperatures.

Heat shock occurs when an organism is exposed to brief periods of relatively high, sublethal temperatures and it may involve the acquisition of thermotolerance to normally lethal temperatures and/or the production of heat shock proteins (HSPs). Although thermotolerance has been correlated with HSP production [4,8,23], the physiological mechanisms by which it is acquired are not yet well understood. Within nervous tissue, the production of HSPs may be related to attenuation of increased excitatory neurotransmission that accompanies thermal stress (see [7] for a review), however, the precise role is by no means clear. The question addressed in this investigation is, therefore, does heat shock protect neuronal function at high temperatures?

Insects are useful models for exploring this question because their activity levels are dependent upon the ambient temperature and they can produce HSPs in

* Corresponding author. Tel.: +1 613 5456533; fax: +1 613 5456617; e-mail: robertrm@biology.queensu.ca

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response to thermal stress [4,23,26]. Exposing a locust to 45°C for 3 h results in the production of HSPs [23] and the development of thermotolerance to normally lethal temperatures [17,23]. Heat shock has also been shown to induce thermotolerance of the flight system of the locust, which is manifested as a decrease in the thermosensitivity of the wingbeat frequency [17]. Since locusts are native to semi-arid regions of equatorial Africa where the ambient temperature can be 32°C [19] and the thoracic temperature of a flying locust can be 6–10°C above the ambient temperature [21,22], it is conceivable that locusts may routinely be exposed to heat shock conditions in the wild. Moreover, the permissive upper limit for flight of a locust under laboratory conditions is 42°C [9,21], which is potentially lower than the ambient temperature to which a wild locust may be exposed.

The locust flight system is an invaluable model for studying principles of neuronal function as there is a wealth of literature that describes the interactions between the neurons that form the flight circuitry (for reviews, see [11,12,14]). In particular, the forewing stretch receptor (fSR) is ideal for examining the function of a single neuron which has a strong influence on the operation of the flight system as a whole. The fSR is a single-celled proprioceptor that fires spontaneously at about 10 Hz when the wings are at rest and makes synaptic connections with flight motoneurons [1] and interneurons [10]. This activity facilitates measurements of conduction velocity of the fSR action potentials that are conveyed along the axon which makes the fSR an attractive model for examining the effects of heat shock on neuronal function.

It is known that increases in temperature can increase the rhythm frequency of the flight system [5,24] and can also modify parameters of synaptic transmission [13]. It is also known that heat shock can reduce the thermosensitivity of the wingbeat frequency and the deafferented rhythm frequency between 32 and 50°C [17]. It is not known if heat shock affects thermosensitivity of the flight system at temperatures below 20°C. It was, therefore, the specific aim of the experiments described in this paper to determine if: (1) heat shock affects thermosensitivity below 20°C; and (2) heat shock protects neuronal function at high temperatures. As a first step neuronal parameters of the fSR were examined in control and heat shocked animals to determine if preconditioning influenced the response of these parameters to increasing temperature. The data presented here suggest that heat shock-induced thermotolerance of the flight system is mediated partly by decreasing the thermosensitivity of action potential generation and conduction at temperatures above 20°C.

2. Methods

2.1. Animals

Adult *Locusta migratoria* were obtained from a colony (28°C, 18:6 light:dark) maintained at the Department of Biology at Queen's University. Animals that were at least 3 weeks past the imaginal moult were selected.

2.2. Heat Shock

Locusts were heat shocked by placing ten individuals in a covered, well ventilated, plastic pail that was placed into an incubator (45°C) for 3 h. A dish of water was also placed in the incubator to maintain high humidity and prevent evaporative cooling during heat shock. This procedure was not lethal to the animals yet has been demonstrated to induce thermotolerance and the production of HSPs [23]. The locusts were allowed to recover for 3–24 h after heat shock before the experiments were started. The control animals were placed into a separate identical pail and maintained at room temperature (about 23–25°C). A total of five groups of animals were used. Acquisition of thermotolerance was assessed in a sample group by placing ten marked, heat shocked and ten unmarked, control locusts into an incubator set at 55°C for 3 h. The locusts were then left at room temperature and the percent survival was determined 24 h later.

2.3. Experimental setup

A standard preparation [15] was used to expose the thoracic ganglia and appropriate nerves for each series of experiments. The temperature of the preparations was maintained by heating saline (NaCl, 147 mM; KCl, 10 mM, $CaCl_2$, 4 mM; NaOH, 3 mM: HEPES buffer, 10 mM) that dripped into the thoracic cavity from a gravity perfusion system. The saline was heated as it entered the preparation by varying the current flow through a Nichrome wire (24 gauge) that was wrapped around the shaft of a pasteur pipette. Controlling the current flow through the wire and the flow rate of the saline allowed for reliable control of the temperature of the preparation (usually ± 0.5°C). The DC output of a copper/constantan thermocouple (BAT-12, Sensortek, Clifton, NJ) that was placed in the saline next to the appropriate nerve was used to monitor the temperature. Temperatures ranged from 10 to 50°C in 5°C increments for each animal and was increased over about 5 min to allow the temperature to equilibrate. After the animals were exposed to the highest temperature of the experiment they were returned to room temperature and base measurements were repeated. Measurements were made about 3–5 min after the target temperature

was attained. A total of ten control and ten heat shocked locusts were used for each series of experiments.

2.4. Deafferented flight rhythm

All nerve roots of the meso and metathoracic ganglia except for Meso N1 (nerves named according to [3]) were cut to prevent conflicting sensory information from reaching the flight motor. Meso N1 was cut more distally so as to leave the cut end of $N1D_1$ (which contains the axon of the forewing dorsal longitudinal motoneuron, fDLMn) exposed. Blowing on the head reliably generated flight rhythms lasting 5–10 s. An extracellular suction electrode was placed on the cut end of $N1D_1$ to record the deafferented, centrally generated flight rhythm (Fig. 1A). The instantaneous rhythm frequency for each trial was determined by taking the reciprocal of the interburst period.

2.5. Axonal conduction

One suction electrode was placed on Meso N1 near its branch with prothoracic nerve 6 (Pro N6) and the other suction electrode was placed on Meso $N1D_2$. The spontaneous action potentials were recorded at each electrode conveyed along the fSR nerve as it propagated medially from the periphery (Fig. 1B). At the end of each experiment the distance between the two suction electrodes was measured using fine calipers.

The parameters of the recorded fSR action potential measured were: (1) conduction delay (from the negative peak of the triphasic extracellular recording of each electrode); (2) the conduction velocity of the propagated signal; and (3) the amplitude and duration of the action potential recorded from the distal electrode (Fig. 1B; for each temperature this was normalized to measurements recorded at 25°C because of the variability of the amplitude and positioning of the electrode between preparations).

2.6. Analysis

All data were digitized and recorded onto VCR tape for later analysis using Datawave Technologies (Longmont, CO) acquisition and off-line analysis software. The significance was assessed using a paired t-test. The samples were considered significantly different at $P < 0.05$.

3. Results

The heat shock treatment used killed one locust out of 50, otherwise the treatment was not lethal and did not appear to affect adversely the behaviour of the locusts. A test of the sample group showed that 90% of the heat shocked animals survived exposure to 55°C for 3 h whereas only 40% of the control animals survived. The greatest degree of thermotolerance was found in animals that were tested within 3 h after heat shock. These data agree with previous findings [17,23] and confirm that the heat shock treatment can induce thermotolerance.

3.1. Deafferented rhythm

The period of rhythmic fDLMn activity from control and heat shocked locusts decreased with increasing temperature (Fig. 2, upper graphs). Both graphs were well fit by a double exponential decay (r^2: control =

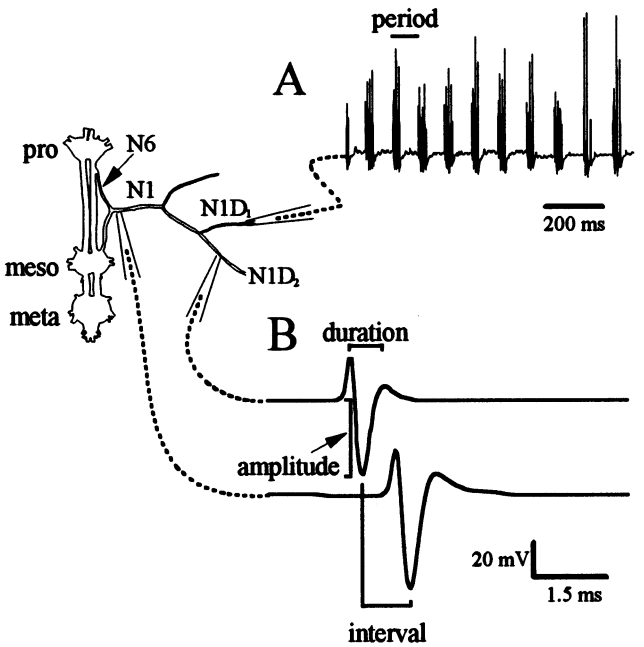

Fig. 1. Diagram of experimental setup. A preparation similar to that described by Robertson and Pearson [15] was used to expose the thoracic ganglia. To record the deafferented flight rhythm (A) a suction electrode was placed on the cut end of Meso $N1D_1$ (nerves named after [3]). The instantaneous frequency was calculated as the reciprocal of the period between bursts of the dorsal longitudinal motoneuron. Measurements of fSR conduction velocity were made by placing one suction electrode on Meso $N1D_2$ and a second suction electrode on the main trunk of Meso N1 (B). The conduction velocity was determined as the distance between the electrodes divided by the interval between the negative peaks of the triphasic waveforms. The amplitude of the extracellularly recorded action potential was measured as the voltage from the baseline to the negative peak. The duration was measured as the interval between the two positive peaks of the triphasic waveform. Because of the variability of the extracellular signal between preparations, the amplitude and duration at each temperature was normalized to that recorded at 25°C. Both amplitude and duration were measured from the more distal electrode since the signal from the fSR propagates medially from the periphery. Meso, mesothoracic ganglion; meta, metathoracic ganglion; N1, mesothoracic nerve 1; $N1D_1$, D_1 branch of mesothoracic nerve 1; $N1D_2$, D_2 branch of mesothoracic nerve 1; pro, prothoracic ganglion.

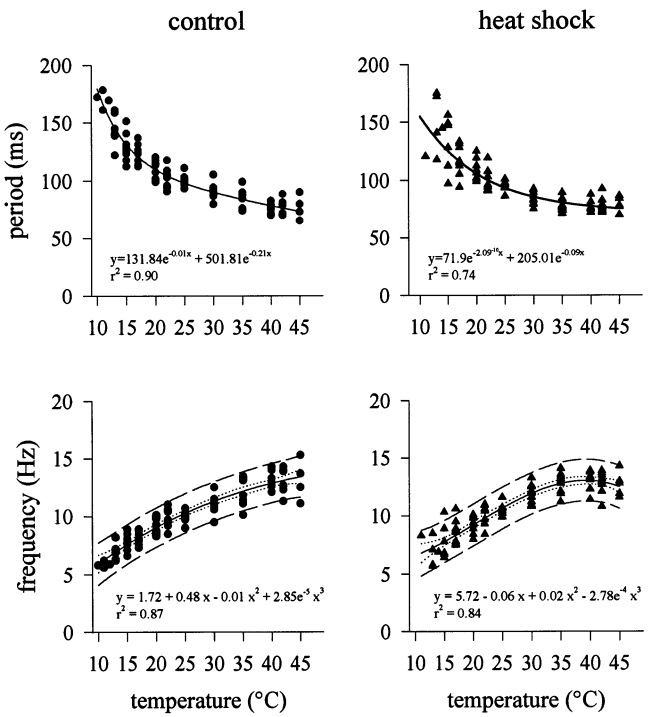

Fig. 2. The deafferented flight rhythm of heat shocked locusts is less sensitive to temperature changes above 35°C than is that of control locusts. The periods between bursts of the dorsal longitudinal motoneuron of control ($n = 10$) and heat shocked ($n = 10$) locusts are plotted in the upper two graphs and fit with lines that describe a double exponential decay. The deafferented rhythm frequencies (the reciprocal of the periods) of control and heat shocked locusts (bottom graphs) are fit with third order regressions (solid lines) as well as 95% confidence limits (dashed lines) and 95% predictor intervals (dotted lines). Above 35°C the regression of the heat shock frequencies decreases. All data are scatter plots of the means from 50–100 cycles at each temperature from each animal. The equation of the line and r^2 value are indicated in each graph.

0.90, heat shock = 0.74). Although no attempt was made to describe the mechanisms of the period decrease using this fit, unpublished observations suggest that synaptic parameters within the flight system are similarly temperature dependent.

The deafferented rhythm frequency of control and heat shocked locusts increased with increasing temperatures (Fig. 2, bottom graphs) and both groups were well fit by a third order regression (r^2: control = 0.87, heat shock = 0.84). At higher temperatures (35–45°C) the frequency of control animals continued to increase (Fig. 2, bottom left) whereas that of the heat shock animals leveled off (Fig. 2, bottom right). These data agree with previous findings [17] and demonstrate that the deafferented rhythm of heat shocked locusts was less sensitive to temperature changes above 35°C. It is shown that below 35°C there was no apparent difference between control and heat shocked groups.

3.2. Axonal conduction

The conduction velocity of fSR signals increased with temperature for control and heat shocked animals (Fig. 3A). Plotting the means of the conduction velocities at different temperatures for all animals (data for a given temperature from each animal is from a mean of 50–100 events) shows that heat shocked animals were less sensitive than control animals. The data were fit with second regressions (r^2: control = 0.98, heat shock = 0.99) and the means were significantly different using a paired t-test ($P = 0.001$).

For control and heat shocked animals the relative amplitude of the fSR action potentials increased with increasing temperature between 15 and 25°C and decreased with increasing temperatures above 25°C (Fig. 3B). However, in heat shocked animals the amplitude was less sensitive to temperature changes above 35°C. The data were fit with a third order regression (r^2: control = 0.99, heat shock = 0.96) and the means were significantly different using a paired t-test ($P = 0.04$).

There was no significant difference in the duration of the action potentials between heat shocked and control animals at equal temperatures (data not shown).

4. Discussion

Preconditioning of locusts at high, sublethal temperatures (i.e. heat shocking) imparts thermotolerance upon the flight system [17]. The experiments described here were designed to test the hypothesis that the decreased thermosensitivity of the heat shocked flight system is

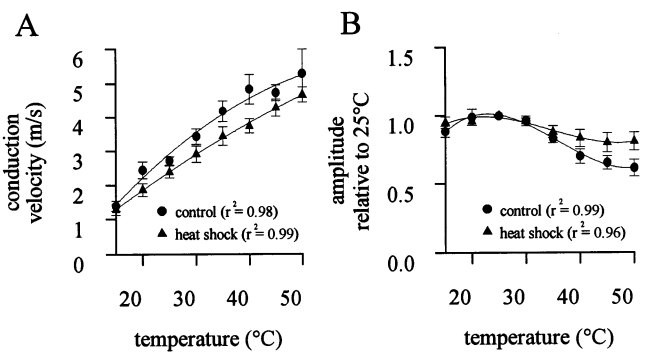

Fig. 3. The conduction velocity and amplitude of signals propagated along the fSR are less sensitive to temperature changes above 35°C in heat shocked locusts than in control locusts. At a given temperature the conduction velocity (A) measured from control locusts ($n = 10$) was significantly higher than that measured from heat shocked locusts ($n = 10$; $P = 0.001$) and the amplitude (B) measured from control locusts was significantly lower than that measured from heat shocked locusts ($P = 0.04$). Data are the mean ± S.E.M. of the means calculated from 50–100 events at each temperature from each animal. The data for conduction velocity were fit with a second order regression and the data for amplitude were fit with a third order regression. r^2-Values are given for each regression line.

manifested via conservation of neuronal function of the flight circuitry. To this end, previous findings have been confirmed [17] that the deafferented flight rhythm of heat shocked locusts is less sensitive to temperature increases between 35 and 45°C and it is shown here that there is no difference at temperatures below 20°C. Moreover, it was found that the conduction velocity and amplitude of action potentials conveyed along the axon of the fSR are less sensitive to temperature increases above 20°C.

It has been proposed that the rhythm frequency of the flight motor pattern generator is determined, in part, by the temporal properties of the constituent neurons [16]. Within limits, increases and decreases of conduction velocity could serve to increase or decrease the frequency, respectively. Outside of these limits, the rhythm could be sufficiently disrupted so as to prevent proper coordination between flight neurons and consequently should prevent rhythm generation. Thus there may be an optimal range of conduction velocities that allows the flight system to function properly. It has also been shown that the circuitry of the motor pattern generator is sensitive to temperature changes [5,13,24,25]. The wingbeat frequency of intact locusts and the rhythm frequency of deafferented locusts increase with temperature between 20 and 40°C [5,24]. Moreover the conduction velocity of a flight motoneuron increases within a similar temperature range [24]. This study shows that the temperature-dependent increase of conduction velocity of heat shocked preparations is less pronounced that of control animals, suggesting that effects of heat shock can compensate for temperature increases between 20 and 45°C. Indeed, if conduction velocity does play a part in determining the flight motor pattern frequency, then heat shock could serve to reduce the thermosensitivity of the system partly via compensatory effects on conduction velocity. That is, heat shock could help to maintain the system closer to optimal operating conditions.

The effect of heat shock on the amplitude of the extracellularly recorded fSR action potential may also be involved in the increased thermotolerance of the flight system. As the temperature of the presynaptic neuron (e.g. the fSR) increases, the action potential amplitude and duration decrease [2,6,18,20], which could attenuate the response of the postsynaptic neuron (e.g. flight interneurons or motoneurons; [13]). If the temperature sensitivity of fSR-evoked PSPs is indicative of those evoked from central flight neurons, which is a reasonable assumption, then the amplitude of central PSPs should also decrease with increasing temperature. Attenuation below some critical level could serve to disrupt the timing of the flight motor and ultimately prevent normal rhythm generation. Experiments to study the effects of heat shock on PSP parameters could provide insight into whether thermotolerance of the flight system may be manifested at the synaptic level.

It has been confirmed that heat shock reduces thermosensitivity of the flight system and is shown here that it also reduces thermosensitivity of certain neuronal functions. The next step in determining the specific effect of heat shock on the flight system is to record postsynaptic events from elements of the central flight circuitry. It may even be possible to determine if HSPs are involved in effects that were observed since it is clear that the heat shock conditions described here can induce HSP production in locusts [23]. Moreover, preliminary, unpublished, observations suggest that HSPs are expressed in the thoracic ganglia in response to heat shock. This is an exciting proposition as it could provide insight into a role for HSPs at the cellular or even behavioural level.

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