Website publication 22 July 1998

NeuroReport 9, 2589–2593 (1998)

Heat shock protects synaptic transmission in flight motor circuitry of locusts

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We investigated the effects of heat shock on the temperature sensitivity of synaptic transmission in the motor circuit for flight in *Locusta migratoria*. In heat shocked animals synaptic transmission failed at 5–6°C higher than in control animals and recovery of transmission was more than three times faster upon return to room temperature. We also found that synaptic delay was rendered insensitive to increases in temperature by heat shock. Thus we have shown in the locust that heat shock has important protective effects on synaptic transmission, thereby extending the upper temperature limit for the motor patterns that generate flight. This is the first description of an effect of heat shock that preserves neuronal communication under subsequent stressful conditions. *NeuroReport* 9: 2589–2593 © 1998 Rapid Science Ltd.

Key words: Flight; Heat shock; Insect; Motor circuit; Postsynaptic potential; Temperature

Introduction

Organisms exhibit a heat shock response in which increased expression of stress proteins (or heat shock proteins, HSPs) is coincident with the induction of thermotolerance (the ability to survive normally lethal temperatures). There is considerable interest in the role of HSPs in nervous tissue because heat shock (HS) can protect neurons against ischaemic damage[1] and this protection may be mediated by HSPs.[2] Much is known about the molecular biology of HSPs and the HS response[3] and about the distribution of HSPs in nervous tissue,[4] yet nothing is known of how HS or HSPs alter neuronal function during and after stress. In poikilotherms the nature of the HS response correlates with the ecological niche of the organism[5] and HSPs allow certain organisms to behave in extreme environments.[6] Preserved behaviour implies preserved neuronal function and suggests that the operation of motor circuits is protected by HS. Previously we have described protective effects of HS on the ability to generate flight rhythms of the motor circuitry in the migratory locust, *Locusta migratoria*.[7] To determine whether these effects may be mediated by the protection of synaptic transmission we investigated the effect of HS on parameters of excitatory postsynaptic potentials (EPSPs) in this system.

Locusts in the wild are often exposed to environmental temperatures in excess of 40°C and the heat produced by active muscles during flight can increase thoracic temperatures by as much as 10°C above ambient.[8] HS treatment of 45°C for 3 h induces the expression of HSPs in locusts,[9] confirming that they show a normal HS response. Recently we have shown that HS has profound effects on the operation of the flight system, reducing the thermosensitivity of flight rhythm generation and allowing the circuit to operate at 5–7°C higher temperatures.[7] Much is known of the operation of the flight circuitry in locusts[10] and of the effects of temperature on neuronal[11] and synaptic properties[12] in this system. The frequency of rhythm generation is not affected by the amplitude of postsynaptic potentials but is affected by changing conduction delays between elements in the circuit.[12,13] A preliminary investigation showed that HS reduces the conduction velocity of action potentials and reduces the thermosensitivity of the amplitude of an extracellularly recorded action potential.[14] In this study we investigated the parameters of PSPs generated in flight interneurons by action potentials of the forewing hinge stretch receptor (fSR). The fSR is a peripherally accessible single-celled proprioceptor at the base of each wing[15] and its connections[16] and role[17] in the generation of flight rhythms are now well known. We describe effects of HS on synaptic transmission in the flight system and show that HS had protective effects that would permit motor circuit operation at higher temperatures than normal.

Materials and Methods

Heat shock: Mature locusts were collected from a crowded colony maintained at Queen's University. Experimental locusts were heat shocked in a humid incubator at 45°C for 3 h, which induces thermotolerance in our colony.[7] Control locusts were

kept for the same period of time in a similar container at room temperature. Experiments were performed 1–12 h after the HS treatment.

Preparation: The thoracic ganglia were exposed in a semi-intact preparation[18] and intracellular recordings were made from neuropil segments of flight interneurons using glass microelectrodes (2 M KAc, –40 MΩ). The axon of the forewing stretch receptor, which travels from the periphery in nerve 1d2 to the mesothoracic ganglion, was stimulated with just suprathreshold square voltage pulses (0.01 ms duration) at 5 Hz using a suction electrode at the junction of nerves 1d1 and 1d2. Intracellularly recorded EPSPs were judged to be monosynaptic using standard criteria.[12] For the second dataset (see below), fSR action potentials were recorded with a second suction electrode located proximal to the stimulating electrode. This enabled calculation of axonal conduction velocity after measuring the distance between the electrodes with fine calipers. The preparation was superfused with saline (147 mM NaCl, 10 mM KCl, 4 mM $CaCl_2$, 3 mM NaOH, 10 mM HEPES buffer) the temperature of which was controlled with a Nichrome heating coil around the inlet pipette that led from a reservoir maintained at room temperature. A flow rate of 0.1 ml/s ensured that the preparation was flushed with fresh saline within 10 s. Saline temperature was monitored with a copper/constant-an thermocouple (0.2 mm diameter, BAT-12, Sensortek, Clifton, NJ) located adjacent to the mesothoracic ganglion.

Procedure: To avoid frequency-dependent changes in synaptic strength, the axon of the fSR was stimulated at 5 Hz. It took ~3 min for the heater to increase the temperature of the superfusing saline to 50°C and this dropped to room temperature within 1 min after the heater was turned off. Electrophysiological and temperature traces were digitized and recorded continuously on VHS videotape for subsequent off-line analysis using Brainwave analysis software (Datawave Technologies, Longmont, CO). EPSP parameters were measured from averages of 25 consecutive EPSPs over 5 s. EPSP amplitudes could not always be measured to failure because of the tendency of the interneuron to generate bursts of action potentials close to failure that obscured the underlying EPSP. Amplitude and latency values are presented relative to the values at room temperature due to variability in the initial values, however, in the second dataset, absolute conduction velocities and synaptic delays could be calculated for each preparation at each temperature tested.

Calculation of synaptic delay: Conduction delay was subtracted from the measured latency to derive synaptic delay for individual EPSPs at different temperatures. Synaptic delay includes the time taken for transmission after arrival of the action potential at the terminals and includes presynaptic calcium entry, vesicle release, transmitter diffusion and postsynaptic receptor activation. Our values for conduction delays (time for the action potential to travel from the stimulus site to the presynaptic terminals) were under-estimated by assuming a constant axonal diameter of the fSR. Hence our values for synaptic delay were over-estimated. Calculations show that the likely error in our reported values are minimal and do not affect our conclusions. Assuming (a) that the diameter of the fSR axon narrows on average to half the diameter of the axon in nerve 1 over the 400 μm from the point of entry into the mesothoracic ganglion to the presynaptic terminals;[19] and (b) that conduction velocity is proportional to the square root of axonal diameter; then synaptic delay is over-estimated in control preparations from 0.05 ms at 25°C above room temperature to 0.08 ms at room temperature and in HS preparations from 0.06 ms at 25°C above room temperature to 0.01 ms at room temperature. These over-estimates would mostly be hidden in the s.e. bars of Fig. 3D. Moreover the difference in the over-estimate of synaptic delay between control and HS is only 0.02 ms at room temperature and 0.01 ms at 25°C above room temperature.

Dataset: The first dataset contained eight control and 10 HS animals. For these experiments we did not measure conduction velocity. To confirm our results and to obtain values for conduction velocity in each preparation a second dataset of 12 controls and 12 HS preparations was collected using a second suction electrode. Equivalent data from the two datasets were not significantly different and were pooled. A preliminary dataset of 10 penetrations in six control preparations and 20 penetrations in 11 HS preparations, collected the year previously, showed the same effects but is not presented here. Data for statistical comparison were tested for normality and equal variance and appropriate parametric or non-parametric tests were applied using commercial software (Sigmastat, Jandel Scientific, Corte Madera, CA). Significance was assessed at $p < 0.05$.

Results

PSPs were recorded intracellularly from the neuropil processes of different flight interneurons in the mesothoracic ganglion (Fig. 1A). Raising the temperature of the superfusing saline had characteristic effects on the amplitude and latency of excitatory EPSPs[12] (Fig. 1B). Temperature was increased in

a ramp-like fashion until failure of transmission (Fig. 1C). We considered the synapse to have failed when no depolarization following the stimulus artifact was detected. When possible we also noted the time taken for transmission to recover (i.e. to the first detectable sign of an EPSP following the stimulus artifact). Subsequently we measured five parameters of EPSPs (Fig. 1D) but report only parameters that changed significantly. The data presented here were obtained from two datasets collected separately 6 months apart (see Materials and Methods).

HS significantly increased the temperature at which synaptic transmission fails by 5–6°C (Fig. 2A). EPSPs in control preparations failed at 38.8 ± 0.4°C (n = 19 preparations) whereas EPSPs in HS preparations failed at 44.4 ± 0.8°C (n = 16). The difference (5.6°C) was significant (t-test: t = 6.58, DF = 33, $p < 0.0001$). Moreover the time period taken for synaptic transmission to recover after the heater had been turned off was significantly shorter by ~90 s in HS preparations (Fig. 2B). EPSPs took 129.1 ± 15.0 s (n = 11) to recover in control preparations but only 38.0 ± 15.7 s (n = 7) in HS preparations. The difference (91.1 s) was significant (t-test: t = 4.03, DF = 16, $p < 0.001$).

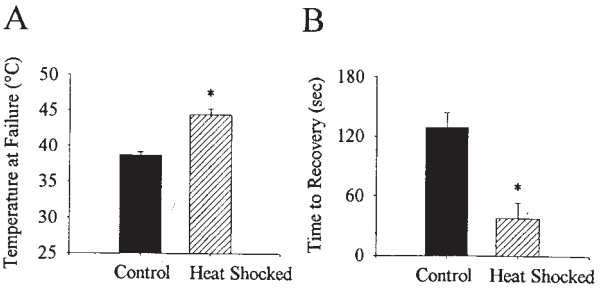

FIG. 2. Heat shock increased the temperature at which EPSPs from the fSR fail, and reduced the time to recover from failure. Asterisks indicate significant differences between control and HS values. Bars indicate mean ± 1 s.e. (**A**) EPSPs in HS preparations failed at higher temperatures than those in control preparations. (**B**) EPSPs in HS preparations recovered from failure sooner after a return to room temperature than those in control preparations. For eight control and nine HS preparations it was not possible to record the time to recover due to loss of the penetration.

There were also significant effects of HS on the thermosensitivity of synaptic parameters. The relative amplitude of EPSPs in HS preparations was

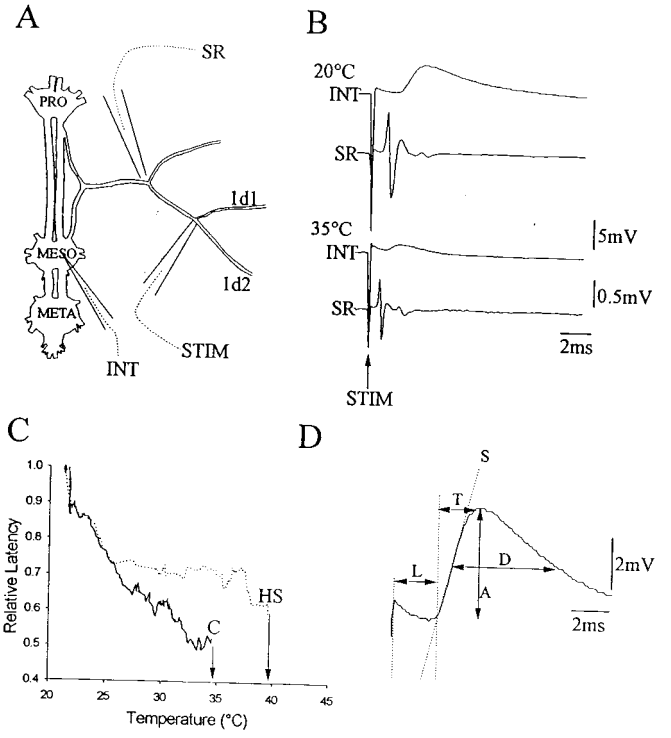

FIG. 1. Temperature sensitivity of excitatory synaptic potentials (EPSPs) in the locust flight system .(**A**) Experimental arrangement. PRO, MESO, META, prothoracic, mesothoracic and metathoracic ganglia; INT, intracellular electrode; STIM, extracellular stimulating electrode; SR, extracellular recording electrode. (**B**) Sample recordings (25 sweeps averaged) of the triphasic extracellular fSR action potential (SR) and an EPSP recorded in a flight interneuron (INT) at 20°C (upper panel) and at 35°C (lower panel). (**C**) Sample data of the temperature sensitivity of synaptic latency measured from sequential individual EPSPs (i.e. not averaged) from one control (C) and one heat-shocked (HS) preparation. Latency values are displayed relative to the latency at room temperature (absolute values varied due to differing location of the stimulating electrode). Arrows indicate the temperatures when synaptic transmission failed for these individual synapses. (**D**) Parameters of the EPSP (25 sweeps averaged) that were measured (L, latency from the stimulus; T, rise time; S, slope; A, amplitude; D, duration at half amplitude) and analyzed off-line.

more resistant to increased temperature compared with controls (Fig. 3A). Assessed using a two-way repeated measures ANOVA there was a significant effect of HS treatment (F = 6.94, DF = 1, $p = 0.016$) and a significant effect of temperature (F = 43.07, DF = 4, $p < 0.001$). HS EPSP amplitudes were reduced by 10–20% less than control EPSPs for all temperature increases. In addition, HS significantly affected the temperature sensitivity of relative synaptic latency (Fig. 3B). Whereas the latency of control EPSPs reduced in an exponential fashion with increasing temperature, as described previously,[12] the latency of HS EPSPs started to decline in parallel with control EPSPs but levelled off after 5–10°C above room temperature. There was a significant effect of HS treatment (two-way repeated measured ANOVA: F = 14.18, DF = 1, $p < 0.001$), a significant effect of temperature (F> 1e20, DF = 4, $p < 0.001$), and a significant interaction between HS treatment and temperature (F > le20, DF = 4, $p < 0.001$). Synaptic latencies in control preparations were reduced to 40% by a 20–25°C increase above room temperature whereas in HS preparations they were reduced only to 60% by the same temperature increase. With the second dataset we were able to calculate conduction velocity of the fSR action potential and thus obtain a measure of conduction delay from the point of stimulation to the intracellular recording site. The effect of HS on conduction velocity[14] was confirmed (Fig. 3C; Kruskal Wallis one-way ANOVA on ranks: H = 7.27, DF = 0.007). We used specific values of conduction delay to calculate synaptic delay (Fig. 3D). For synaptic delay there was a significant effect of temperature (two-way repeated measures ANOVA: F = 10.67, DF = 5, $p < 0.001$) and a significant interaction between HS treatment and temperature (F = 5.48, DF = 5, $p < 0.001$). The lack of significance in the effect of HS treatment alone is not surprising given that the relationships cross over. In control locusts synaptic delay decreased exponentially from 1.2 ± 0.03 ms at room temperature to 0.56 ± 0.08 ms in the 15–20°C range above room temperature. Interestingly, in HS locusts synaptic delay at room temperature (1.01 ± 0.04 ms) was shorter than in controls. Also, after an initial decrease to 0.73 ± 0.07 ms in the 0–5°C range (paralleling the decrease in control synaptic delay) HS synaptic delay abruptly increased before levelling off such that it was essentially insensitive to temperature in a 25°C range from 0 to 5°C above room temperature to failure.

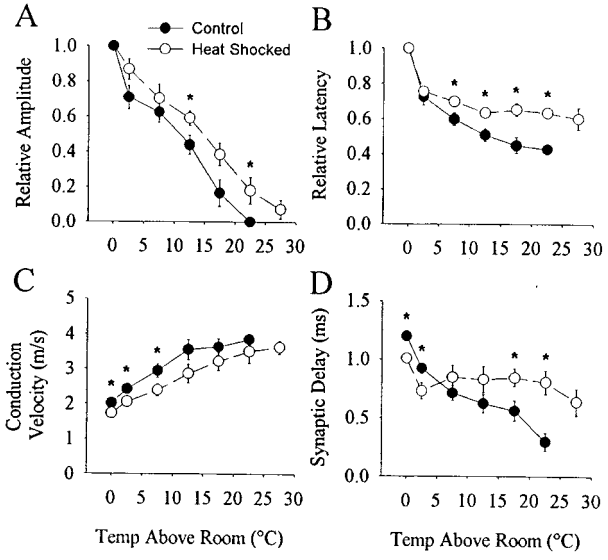

FIG. 3. Heat shock reduces temperature sensitivity of EPSP parameters. In each graph the results have been collected into bins of 5°C relative to room temperature (21.1 ± 0.2°C, $n = 42$) and are presented as means ± s.e. (error bars may be hidden in the symbol). Each animal contributes only one value to any one bin. Note that HS preparations have an extra bin compared to control because they failed at higher temperatures. Asterisks indicate significant differences between control and HS values. (**A**) Temperature sensitivity of relative EPSP amplitude in control and HS preparations. There are significant differences between individual bins in the 10–15°C range (t-test: t = 2.29, DF = 17, $p = 0.04$) and in the 20–25°C range (t-test: t = 2.54, DF = 13, $p = 0.024$). (**B**) Temperature sensitivity of relative synaptic latency in control and HS preparations. There are significant differences between individual bins in the 5–10°C range (t-test: t = 2.36, DF = 39, $p = 0.023$), the 10–15°C range (t-test: t = 2.94, DF = 32, $p = 0.006$), the 15–20°C range (t-test: t = 3.66, DF = 26, $p = 0.0015$) and the 20–25°C range (t-test: t = 7.08, DF = 6, $p = 0.0004$). (**C**) Temperature sensitivity of conduction velocity in control and HS preparations. There are significant differences between individual bins at room temperature (Mann-Whitney rank sum test: U = 200.5, DF = 22, $p = 0.004$), in the 0–5°C range (Mann-Whitney rank sum test: U = 197, DF = 22, $p = 0.007$) and in the 5–10°C range (Mann-Whitney rank sum test: U = 99, DF = 21, $p = 0.045$). (**D**) Temperature sensitivity of synaptic delay in control and HS preparations. There are significant differences between individual bins at room temperature (t-test: t = 3.63, DF = 22, $p = 0.001$), in the 0–5°C range (t-test: t = 2.34, DF = 22, $p = 0.03$), in the 15–20°C range (t-test: t = 2.47, DF = 16, $p = 0.025$) and in the 20–25°C range (t-test: t = 2.72, DF = 6, $p = 0.034$).

Discussion

HS had profound effects on synaptic transmission in the locust flight circuitry without any detectable deleterious effects. HS locusts are thermotolerant, behaving normally and flying when tethered in a wind tunnel.[7] Thus we believe that the altered neuronal properties we describe above were protective by enabling synaptic transmission at temperatures 5–6°C higher than in control animals. The synapses we studied were all from a single peripheral afferent (the fSR) but other synapses in the circuit should behave similarly. The effect of temperature on interneuronal synapses is similar to the effect on fSR synapses.[12] In addition, synaptic delay at a central synapse in a locust is highly sensitive to increases in temperature with values very similar to those from our control locusts.[20] There is a strong correlation between the temperature sensitivity of synaptic delay and that of motor patterns and the results we

describe here can explain the effects of HS on flight behaviour in intact animals and on rhythm generation in deafferented preparations.[7] Wingbeat frequency in intact tethered animals previously exposed to HS is insensitive to increases in temperature which we propose is due to the temperature insensitivity of synaptic delay after HS. In deafferented preparations from HS locusts, the centrally generated flight rhythm initially is of higher frequency than in controls but this levels off to become insensitive for temperatures > 32°C.[7] Similar effects were seen on synaptic delay in this investigation. The temperature sensitivity profile of synaptic delay is intriguing and suggests that a mechanism was activated by a 5–10°C temperature increase to protect flight capability in HS locusts. Strikingly, HS increases the upper temperature limit for rhythm generation in the flight system by 6–7°C,[7] which is similar to the increase in the upper limit for synaptic transmission reported here. The amplitude of PSPs in the flight system can be reduced to 40% of normal values by removing calcium ions from the superfusing saline without preventing the generation of flight rhythms or significantly affecting their frequency.[13] The present data on thermosensitivity of relative EPSP amplitude show that there is about a 5°C difference between the temperatures at which EPSP amplitude falls below 40% in control and HS preparations. This temperature differential increases for greater percent reductions of amplitude. We have not investigated the effect of HS on plateau potentials that are known to be generated in the flight system,[21] but these are normally triggered by synaptic input and would require EPSPs above a certain threshold amplitude to be activated. Flight rhythms will fail when synaptic transmission in the system fails, or when PSPs fall below a threshold amplitude, and thus HS protects the operation of the motor circuitry allowing locusts to behave, not to mention survive, in higher environmental temperatures.

The sub-cellular mechanisms underlying these phenomena remain to be determined but it is an exciting possibility that synaptic transmission is protected by the action of HSPs in the nervous system. HSPs are produced in response to HS in *Locusta migratoria*.[9] A preliminary report[22] demonstrates that locust thoracic ganglia contain proteins that cross-react with antibodies raised against *Drosophila* HSP70 and HSP83 and that HS has no effect on the temperature sensitivity of lipid order (microfluidity) of a membrane fraction obtained from thoracic ganglia. Cysteine string proteins (CSP) that have a 'J' domain suitable for molecular interaction with HSP70 are integral components of the synaptic release machinery linking synaptic vesicles to calcium secretion coupling, and CSP null mutants cause a temperature-sensitive block of evoked neurotransmission.[23] Furthermore, an affinity purification procedure to isolate SNAP (soluble NSF attachment protein, NSF–n-ethylmaleimide sensitive factor) receptors implicated in vesicle targeting and fusion revealed two peptides that were from HSP7O.[24] A testable hypothesis is that HSPs stabilize the protein machinery underlying synaptic transmission, allowing continued neural function during and after stress.

Conclusion

Prior heat shock stabilizes the properties of postsynaptic potentials during subsequent stressful conditions. The effects described can explain how heat shock reduces the thermosensitivity of the flight circuitry and thus enables it to generate motor rhythms and tethered flight at extreme high temperatures. To our knowledge, this work provides the first evidence in any organism that heat shock protects neural function through actions at the level of synaptic transmission. We anticipate that future research motivated by the increasing interest in the protective effects of HS and HSPs in stroke will reveal similar effects upon mammalian neural circuits.

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ACKNOWLEDGEMENTS: This research was supported by the Natural Sciences and Engineering Research Council of Canada and by the Faculty of Graduate Studies and Research at Queen's University. Experiments were performed in compliance with the Canadian Council for Animal Care. We thank R.D. Andrew, H.L. Atwood and P.K. Rose for their constructive criticism of a previous version of this paper.

Received 26 May 1998; accepted 29 May 1998