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Stress-induced thermotolerance of ventilatory motor pattern generation in the locust, *Locusta migratoria*

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Abstract

Ventilation is a crucial motor activity that provides organisms with an adequate circulation of respiratory gases. For animals that exist in harsh environments, an important goal is to protect ventilation under extreme conditions. Heat shock, anoxia, and cold shock are environmental stresses that have previously been shown to trigger protective responses. We used the locust to examine stress-induced thermotolerance by monitoring the ability of the central nervous system to generate ventilatory motor patterns during a subsequent heat exposure. Preparations from pre-stressed animals had an increased incidence of motor pattern recovery following heat-induced failure, however, prior stress did not alter the characteristics of the ventilatory motor pattern. During constant heat exposure at sub-lethal temperatures, we observed a protective effect of heat shock pre-treatment. Serotonin application had similar effects on motor patterns when compared to prior heat shock. These studies are consistent with previous studies that indicate prior exposure to extreme temperatures and hypoxia can protect neural operation against high temperature stress. They further suggest that the protective mechanism is a time-dependent process best revealed during prolonged exposure to extreme temperatures and is mediated by a neuromodulator such as serotonin.

Keywords: Ventilation; Thermotolerance; Locust; Heat shock; Serotonin

1. Introduction

Many organisms are exposed to and challenged by natural environmental changes such as diurnal temperature fluctuations or low oxygen and in order for an organism to survive in extreme environments, the neural processes underlying behaviors must be preserved. Neuronal circuits will fail and imperil the animal long before cells and tissues die at extreme temperatures. It has been well established that prior exposure to stress induces tolerance and cross-tolerance to subsequent stress (Burton et al., 1988; Morimoto and Santoro, 1998; Wu et al., 2002), but mechanisms mediating thermotolerance of neural function remain to be determined.

A good model system for examining the mechanisms of stress tolerance is the African migratory locust, *Locusta migratoria*, an organism that is tolerant of high temperature and low-oxygen stress. The locust's desert ecology and accessible neuronal and muscular physiology allows a detailed examination of the effects of fluctuating temperatures and low oxygen on organismal function. It has been shown that prior heat shock can exert neuroprotective effects in the flight system (Robertson et al., 1996; Dawson-Scully and Robertson, 1998; Wu et al., 2001) and in the neuromuscular control of the hindleg (Barclay and Robertson, 2000). In other model systems, heat shock extends life span (Khazaeli et al., 1997; Shama et al., 1998) as well as protecting against apoptosis, and excitotoxicity (Lowenstein et al., 1991; Mailhos et al., 1993).

A crucial motor activity to the locust is ventilation, and therefore it was of interest to determine whether prior stress could increase thermotolerance of ventilation as has been demonstrated for flight and neuromuscular function. We also explored other means of manipulating thermotolerance such as exposure to cold and hypoxic stresses. Sequential cold shocks have been shown to

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increase the molting incidence in a hemipteran (Garcia et al., 2001), and exposure to hypothermia or hypoxia increases the survival rate of retinal ganglion cells subsequently exposed to a stressor (Caprioli et al., 1996). Exposure to severe hypoxia has also been shown to increase the activity of antioxidant enzyme activity in larval insects thus prolonging survival when subsequently stressed (Hermes-Lima and Zenteno-Savin, 2002). Whereas, there is ample evidence demonstrating the protective effects of heat shock and anoxia on intermittent activity such as flight, little is understood about the effects of prior stress on a continuous motor activity such as ventilation. Ventilatory motor patterns offer advantages, compared to flight motor patterns, in their vital role and continuous nature. It could be argued that if any motor circuit is to be protected at high temperatures, it should be the ventilatory motor circuit.

The mechanisms underlying these protective responses are not yet known, however, thermoprotection may be mediated by neuromodulator such as octopamine, which has been shown to increase in circulating locust hemolymph during heat stress (Davenport and Evans, 1984). Alternatively, serotonin may regulate the development of thermotolerance by modulating potassium currents in a manner similar to heat shock, i.e. the induction of potassium channel phosphorylation, which decreases potassium currents (Parker, 1995; Byrne and Kandel, 1996). The natural circulation of this neuromodulator makes it an appropriate candidate for investigation and if serotonin is indeed involved in thermoprotection, the application of serotonin may mimic the thermoprotective effects of heat shock. In this study, thermotolerance as a result of prior stress in the locust ventilatory system was examined at the neuronal level using an electrophysiological approach. We hypothesized that ventilatory motor activity would demonstrate a robust protective response to prior environmental stress that would be evident in an increase in the upper temperature limit for circuit function, and would be mimicked by treatment with serotonin.

2. Materials and methods

2.1. Animals and experimental treatments

Mature male *L. migratoria*, 1.5–3 weeks past imaginal ecdysis, were collected from a crowded laboratory colony at Queen's University. The colony was maintained at 30 °C, as described previously (Robertson et al., 1996). Locusts were distributed among four experimental groups: control (C), heat shock (HS), cold shock (CS) and anoxia (AN). Control locusts were kept in a 2 L plastic container at room temperature for 4 h. Locusts receiving a heat shock treatment were kept in a similar container and placed in a humid incubator for 3 h at 45 °C and then allowed to recover at room temperature (22 ± 1 °C) for 1 h (Robertson et al., 1996). Cold-shock animals were subjected to a similar protocol except that they were maintained at 3 °C in a refrigerator for 3 h before 1 h recovery. Animals receiving anoxia treatment were exposed to a pure nitrogen atmosphere for 2 h then allowed to recover for 2 h as previously described (Wu et al., 2002). Experiments were performed 1–6 h following treatment.

In a separate study, the application of serotonin was used to determine whether it could mimic the effects of heat shock. The other stressful stimuli were not investigated. Locusts receiving serotonin treatment were perfused with saline containing 10^{-3} M serotonin for 12 min prior to thermal stress, control animals were also used and perfused with standard saline prior to thermal stress.

2.2. Extracellular recording

To expose ventilatory muscles for electrophysiology experiments, the legs, wings and pronotum were removed. A dorsal incision was made to open the thorax and the animals were pinned to a cork surface. Locusts were dissected in standard extracellular saline as previously described (Wu et al., 2002). The gut was removed and muscles on the right side of the thorax and first three abdominal segments were exposed by removing fat bodies and air sacs (Fig. 1). All nerves, including the nerve to muscle 161, were preserved. In experiments involving serotonin application, nerve 1 on both left and right sides of the metathoracic ganglion were cut to ensure circulation of serotonin within the ganglion,

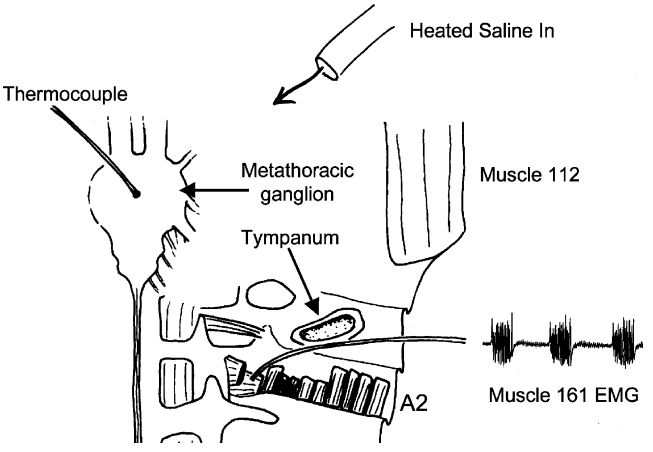

Fig. 1. Diagram of a semi-intact locust preparation displaying the location for electromyographic (EMG) recording from expiratory muscle 161 located in the second abdominal segment (A2). Anterior is towards the top and the metathoracic ganglion, tympanum and right hindwing dorsal longitudinal muscle (muscle 112) are indicated. For clarity, the metathoracic and abdominal nerves have been omitted from the diagram though they were left intact during most experiments. Heated saline was superfused through the preparation. The temperature was monitored with a thermocouple positioned just dorsal to the metathoracic ganglion.

where the pattern generator for ventilation is located. Appropriately, in these experiments, animals not receiving serotonin treatment also had nerve 1 cut.

Extracellular recordings of ventilatory activity were obtained using an electromyographic (EMG) electrode made from 0.1 mm diameter copper wire insulated except at the tip and placed in the expiratory muscle 161 (numbered according to Snodgrass (1935) and Albrecht (1953)). A saline perfusion, either standard or containing serotonin, was maintained at room temperature for 12 min to allow ventilation to stabilize. Saline temperature was controlled using nichrome wire as a heating coil wrapped around an inlet pipette. Temperature was monitored with a thermocouple (Bat-12, Physitemp Instruments Inc.) held just above the metathoracic ganglion, where the ventilatory motor pattern is generated (Miller, 1960). The temperature of the saline perfusion was increased in a ramp-like manner (approximately 6 °C/min) until ventilatory pattern generation failed. Or, during experiments exposing heat shock and control locusts to prolonged high temperatures, temperature was ramped from room temperature to 43 or 47 °C and held for 30 min or until failure. Failure was characterized as loss of motor patterning and by a tonic high frequency firing of motor neurons in excess of 90 Hz. Post failure, the saline was allowed to cool to 25 °C to permit recovery and animals were observed for 20 min. In experiments involving serotonin, the temperature was ramped and then held at 45 °C for 20 min or until failure, and 30 min were given to observe recovery. Forty-five degrees centigrade was an intermediate high temperature and therefore was appropriate for comparing the effects of serotonin with previous experiments. Recovery was monitored and recorded as the time required until movement was detected in the expiratory muscle after heat-induced failure. This coincided with a resumption of the recorded motor pattern but was easier to detect. Signals were amplified, digitized and stored on videotape for later analysis using Axoscope 8.2 (Axon Instruments Inc., Union City, CA, USA). Ventilatory motor patterns were characterized by measuring rhythm frequency and duration of individual bursts.

2.3. Statistical analyses

Experiments were performed over several months and animals receiving different treatments were interspersed over time with each other. Our aim was to obtain 10 animals in each treatment group for statistical comparison but actual numbers differed from this. All successful experiments are included in the analysis. The effects of different stresses on failure during ramp-like temperature increases were assessed using 11 control, 9 heat shock, 12 cold shock and 10 anoxia treated animals. To compare the effect of prior heat shock on the ability to withstand a prolonged high temperature, we used 15 C and 8 HS at 43 °C and 17 C and 9 HS at 47 °C. In serotonin experiments, the effects of heat shock and serotonin on pattern generation on heat-induced failure were assessed using a set of 15 C, 15 HS, 14 control with serotonin (C + 5HT), and 14 heat shock with serotonin (HS + 5HT). Data were plotted using SigmaPlot 6.0 graphing software (Jandel Scientific, San Rafael, CA, USA). Statistical significance was assessed using the appropriate parametric or non-parametric tests, as indicated in the text. Statistical analysis was performed using SigmaStat 2.0 software (Jandel Scientific, San Rafael, CA, USA). Differences were considered significant at $p < 0.05$. The mean and standard error are given as the measures of central tendency and variability, respectively.

3. Results

As the internal temperature was increased, ventilatory rhythm frequency increased and burst duration decreased (Figs. 2 and 3). Rhythm frequency was significantly lower upon recovery after heat-induced failure compared with the frequency at the same temperature prior to the temperature ramp (Fig. 3) (*t*-test, $t = 4.959$, df = 16, $p < 0.05$) while burst duration significantly increased (*t*-test, $t = 4.105$, df = 15, $p < 0.05$). Prior heat shock, cold shock and anoxic treatments did not affect the thermosensitivity of ventilatory pattern generation as measured by rhythm frequency (two-way RM ANOVA, $F = 1.510$, $p > 0.05$, df = 12) or burst duration (two-way RM ANOVA, $F = 1.600$, $p > 0.05$, df = 16). The temperature at which the circuit failed during a ramped temperature increase did not differ across the four conditions (Fig. 4A; one-way ANOVA, $F = 2.633$, $p > 0.05$, df = 3). However, significantly fewer (45%, 5 of 11) control animals recovered, whereas 100% of pre-stressed animals recovered (*z*-tests for pairwise comparisons of proportionate data; summary of comparison, $z \geq 2.158$, $p \leq 0.05$). The time to recovery after such heat-induced fail-

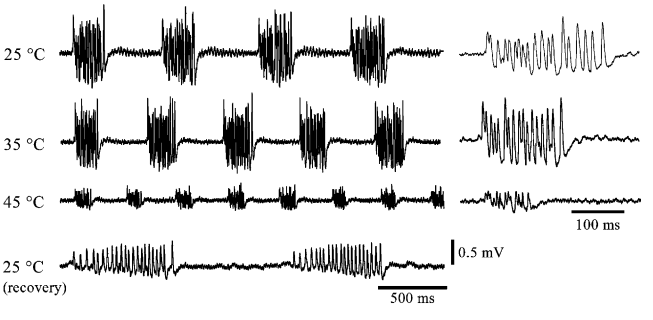

Fig. 2. Expiratory activity of a semi-intact locust preparation during a heat ramp as monitored by EMG recordings from muscle 161. Patterns are shown at 25, 35, and 45 °C, respectively, of a control preparation, with burst expansions displaying intraburst activity. At 25 °C, an extracellular recording from muscle 161, 20 min after recovery from heat-induced failure is also shown.

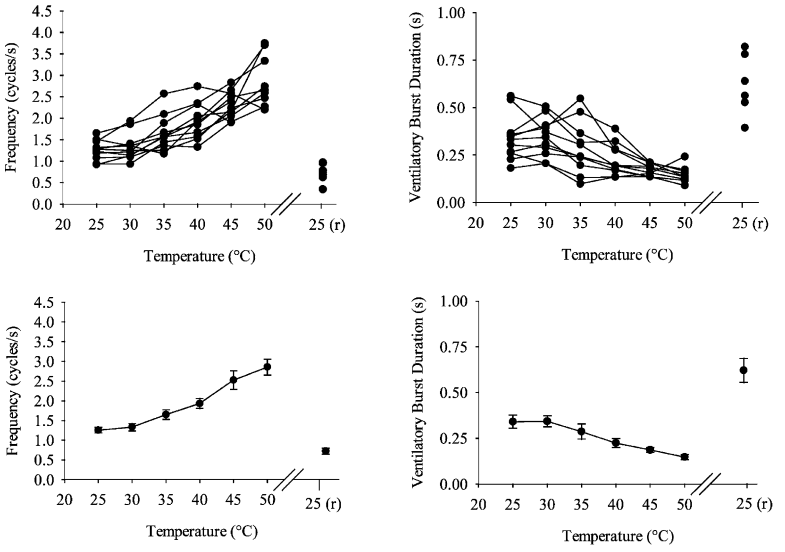

Fig. 3. Effect of increasing temperature on expiratory burst frequency (left graphs) and burst duration (right graphs). The top two graphs show the relationships for individual preparations of control animals and these data are collapsed into means ± S.E. in the bottom two graphs. At the extreme right of each abscissa, the axis is interrupted and the data following were obtained at 25 °C after recovery [25(r)]. See text for details.

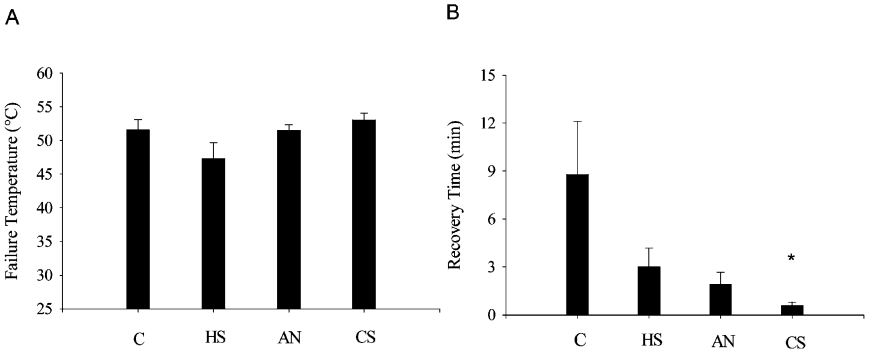

Fig. 4. Failure temperature and time to recovery after heat-induced failure. (A) Failure temperature did not differ significantly between the four different conditions (C, control $n = 11$; HS, heat shock $n = 9$; AN, anoxia $n = 10$; CS, cold shock $n = 12$). (B) Of the animals that recovered after heat-induced failure (C = 5/11; HS = 9/9; AN = 10/10; CS = 12/12), time to recovery of the ventilatory mechanism was shorter in pre-stressed locusts (see text for details). Data are means ± S.E. and an asterisk indicates significant difference from control group.

ure showed an obvious trend displaying a quicker time to recovery for those animals exposed to prior stress but these differences were not significant. This lack of significance is likely due to the low recovery of control animals. To adjust for this, we performed the statistical analysis using a recovery time of 20 min for those animals that did not recover and there was a highly significant difference between the recovery times of C and CS animals (Fig. 4B) (Kruskal-Wallis, $H = 11.545$, $p < 0.05$, df = 3).

The ability of all pre-stressed preparations to recover and the apparent decrease in time to recovery suggested the existence of a time-dependent process of protection in these animals. To determine whether a prior stress protects neural operation in locusts from a subsequent exposure to prolonged sub-lethal internal temperature, the saline in control and heat-shocked locusts was held at 43 and 47 °C. Animals were monitored for a maximum of 30 min. All heat-shocked locusts continued to display ventilatory activity throughout the 30 min period, whereas the time to failure was significantly lower for control animals at both 43 (*t*-test, $t = -2.602$, df = 21, $p < 0.05$) and 47 °C (*t*-test, $t = -4.337$, df = 24, $p < 0.001$), with the greater difference at 47 °C (Fig. 5).

To investigate the possibility of neuromodulator mediation of the thermoprotective response, animals treated with serotonin were compared to control and heat-shocked animals in their ventilatory response to increased internal temperatures. At high temperatures (45 °C), the frequency of burst generation of C animals was significantly lower than that of HS (*t*-test, $t = 8.00$, $p < 0.05$), C + 5HT (*t*-test, $t = 15.13$, $p < 0.05$), and HS + 5HT (*t*-test, $t = 8.03$, $p < 0.05$; Fig. 6). However, no differences in frequency or burst duration were evident during the temperature ramp up to, and including, 40 °C. When exposed to prolonged high internal

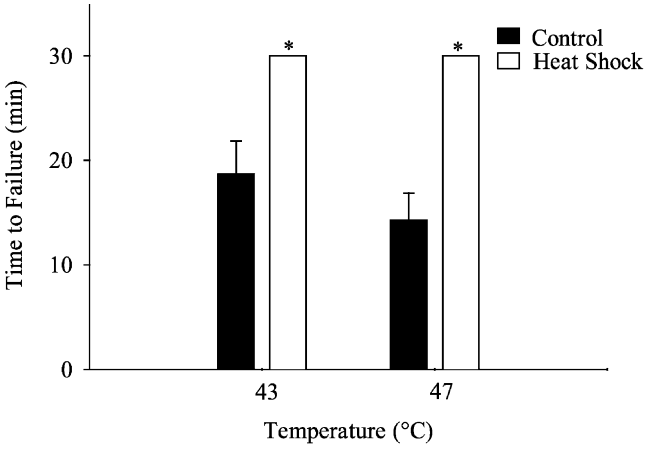

Fig. 5. The effect of prior heat shock treatment on the ventilatory rhythm in the locust exposed to sustained heat application. Internal temperature was maintained until rhythm failure or for a maximum of 30 min, at which point animals still ventilating were deemed not to have failed. Animals exposed to prior heat shock ventilated for significantly longer than controls at 43 °C (C, control $n = 15$; HS, heat shock $n = 8$) and 47 °C (C, control $n = 17$; HS, heat shock $n = 9$), (see text for details). Data are means ± S.E. and asterisks indicate significant difference from the control group at a set temperature.

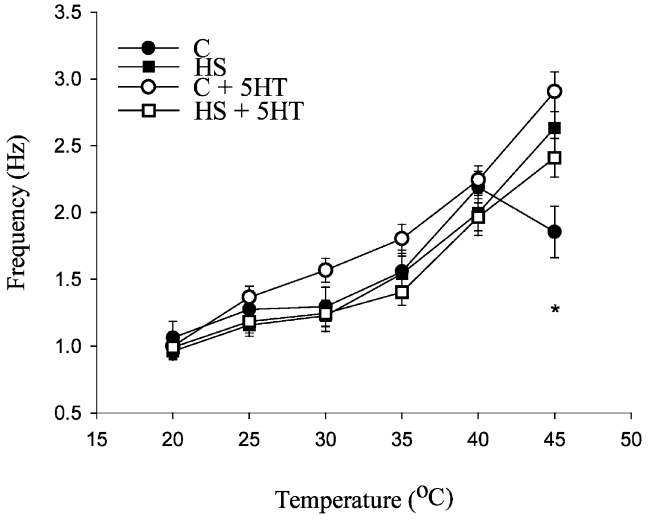

Fig. 6. Thermosensitivity of the ventilatory motor pattern, demonstrated by frequency of bursting of control and heat-shocked animals as well as those treated with serotonin (C, control $n = 15$; HS, heat shock $n = 15$; C + 5HT, control with serotonin $n = 14$; HS + 5HT, heat shock with serotonin $n = 14$). Data are means ± S.E. and asterisk indicates significant difference between controls and other three treatment groups.

temperature (45 °C), ventilatory motor pattern generation failed for 66% of C, 33% of HS, 36% of C + 5HT, and 38% of HS + 5HT (Fig. 7A). Of those animals that failed 60% of C, 100% of both HS and C + 5HT, and 83% of HS + 5HT experienced recovery of the motor pattern (Fig. 7B).

4. Discussion

Research on thermal stress and insect thermotolerance has important applications, not only to provide model systems for determining the neuronal mechanisms underlying protection of motor pattern generation, but also to investigate the feasibility of temperature manipulation for non-chemical pest control (Hallman and Denlinger, 1998). In this study, we tested the hypothesis that ventilatory motor activity would demonstrate a robust protective response to prior environmental stress that would be evident in an increase in the upper temperature limit for circuit function. Although there was no stress-mediated increase in the temperature at which ventilatory pattern generation failed in response to a ramp-like increase in temperature, we found that prior environmental stress decreased the time it took the system to recover from failure. Indeed, more than half of the control animals did not recover after heat-induced failure. We also found that in response to a maintained high temperature, preparations from heat-shocked animals continued to generate a ventilatory motor pattern after control preparations had failed. Moreover, fewer heat shock and control preparations treated with serotonin failed within the time limit of the experiment. Thus our main conclusion is that prior stress results in thermoprotection of ventilatory motor pattern generation in the locust and that serotonin mimics this effect of heat shock.

The central pattern generator for ventilation in locusts is robust and is capable of generating higher-level patterns of ventilatory control even in an isolated CNS (Bustami and Hustert, 2000; Bustami et al., 2002). Our intention was to monitor the output of the ventilatory central pattern generator and we minimized afferent input by immobilizing the abdomen and by cutting many of the nerves originating in the metathoracic and fused abdominal ganglia. We are confident that the motor activity we recorded was a true representation of central pattern generator activity.

4.1. Pattern thermosensitivity

Thermosensitivity of motor patterns was demonstrated in all of our treatment groups by the overall increase in rhythm frequency and decrease in burst duration as temperature increased. This reaction has been well documented in previous studies, most recently in the hyperthermic response of the respiratory network of mice (Tryba and Ramirez, 2003), and including pattern generators for other types of motor rhythms (e.g. Walker, 1975; Foster and Robertson, 1992). One functional explanation for increasing ventilatory frequency is that animals that are stressed by an increase in temperature or alteration in behavioral state, such as in flight, increase expiratory compression of the abdomen to facilitate

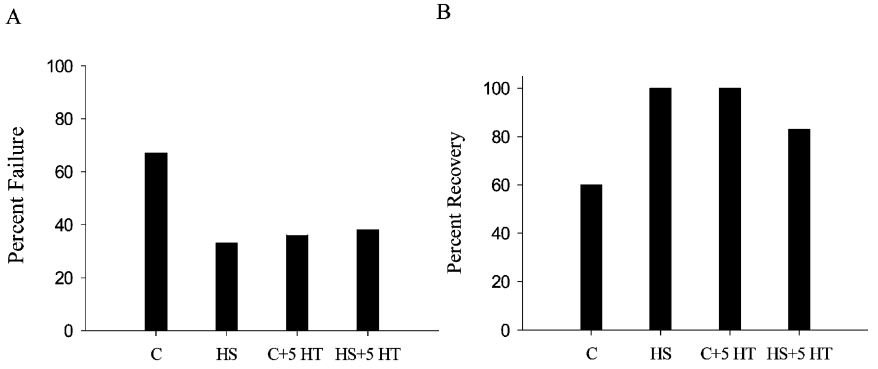

Fig. 7. (A) Percentage failure of the ventilatory motor pattern when internal temperature was held at 45 °C (C, control; HS, heat shock; C + 5HT, control with serotonin; HS + 5HT, heat shock with serotonin). A greater proportion of control animals failed than those from the other three treatment groups. (B) Subsequent to motor pattern failure, control animals were less likely to recover than those from the other three treatment groups.

greater airflow to meet increased metabolic demands (Ramirez, 1998). Any heat-induced reduction in expiratory amplitude is compensated for by more frequent ventilatory bursts (Hustert, 1975). Another explanation is that increased ventilatory rate is an adaptive response to dissipate body heat (Prange, 1990). Whether to meet increased metabolic demands or dissipate heat, adaptive responses could be mediated via internal thermoreceptors or as a direct response to heating the pattern generating circuit. At present, we cannot distinguish between these possibilities but note that, to our knowledge, internal thermoreceptive sense organs have not been described in locusts. Given the direct effects of temperature, specific thermoreceptors may be unnecessary. Similarly, direct effects on pattern generator neurons, equivalent to the direct effects of hypoxia on an insect motorneuron (Le Corronc et al., 1999), could mediate the ventilatory responses of the isolated CNS to oxygen and carbon dioxide levels (Bustami et al., 2002).

4.2. High temperature failure of pattern generation

Although we found an increase in thermoprotection during prolonged heat exposure, an interesting question is why we did not observe an increase in the failure temperature of the ventilatory motor pattern during ramp-like increases of temperature. Similar experiments in locusts have shown that heat shock or anoxia can protect the flight motor system and the neuromuscular control of the jumping leg by increasing failure temperature by approximately 5 °C (Robertson et al., 1996; Barclay and Robertson, 2000; Wu et al., 2002). A functional explanation may lie in the importance of this particular motor activity to the organism's survival. Ventilation is very sensitive to small changes of the excitatory state of the insect central nervous system (Miller, 1971) and the critical nature of the ventilatory mechanism is illustrated by the observation in locusts that changes in ventilation are often noted as the first behavioral response to a stimulus (Hustert, 1975). It is possible that the ventilatory mechanism is substantially protected in its pre-stressed condition, leaving little room for a further protection against short-term heat exposure. If so, then this may reflect the nature of the neural circuitry and suggests that its operation relies less on vulnerable processes such as synaptic transmission than does locomotor circuitry. For example, synaptic transmission in the flight system has demonstrated thermoprotection when heat-shocked animals were exposed to a subsequent temperature ramp to circuit failure (Wu et al., 2002).

A mechanistic explanation that is not mutually exclusive with the above arises from consideration of the possible protective mechanism and the nature of the heat ramp. When exposed to periods of extreme, though sublethal, temperatures, organisms experience profound alterations in the cellular environment such as changes in the patterns of protein synthesis. Most notably, in response to stress, most organisms synthesize heat shock proteins (HSPs) that delay cell and tissue death and presumably allow continued behavior. For example, studies on hsp-deficient mutant strains of microorganisms and eukaryotic cells have demonstrated that some HSPs are required for both the innate ability to survive at high temperature and the acquisition of thermotolerance (Feder and Hofmann, 1999). As molecular chaperones, HSPs prevent protein aggregation and refold damaged proteins (Feder and Hofmann, 1999), a time-dependent process. *L. migratoria* are native to the hot arid regions of Africa where daily temperatures regularly exceed 40 °C and they synthesize HSPs in response to high temperature exposure (Whyard et al., 1986; Qin et al., 2003). The heat shock response is sensitive to rates of heating (DiDomenico et al., 1982; Tomanek and Somero, 2000). The intensity of a heat stress is a function of the rate of temperature increase and the length of time at the increased temperature, and not just by the absolute temperature increase (Hochachka and Somero, 2002). Indeed, it is possible to define a thermal dose for tissues based on exposure times at particular temperatures (Gerner, 1987). In our experiment, animals experiencing

a ramp-like temperature increase were not subjected to extreme temperatures for more than 1 or 2 min before the circuit failed, therefore any protective mechanism activated by prior stress may not have had enough time to have an effect, e.g. a time-dependent protective process involving protein interactions may have been ineffective against a rapidly increasing temperature. On the other hand, heat-shocked animals that were exposed to prolonged high temperatures displayed thermotolerance thus suggesting a heat shock-mediated response had sufficient time to protect the ventilatory mechanism. The existence of cross-tolerance (thermoprotection from prior anoxia or cold shock) supports the contention that the different stressors activate a single pathway such as the heat shock response. Future experiments will determine the extent to which the thermoprotective mechanisms in neuronal circuitry are time-dependent and/or dependent on the nature of the stress.

4.3. The role of serotonin

The results show that the effects of heat shock were slightly different in the two experimental protocols (with and without serotonin comparison) when exposed to sustained high temperature (i.e. in Fig. 5 no heat shock preparations failed while held at 43 or 47 °C, whereas in Fig. 7 33% of heat shock preparations failed while held at 45 °C). Although we do not yet know the nature of these differences, one possibility may be seasonal differences during data collection. Original experiments were conducted during the summer months, whereas those comparing the effects of serotonin were conducted through the winter. This may point to interesting areas for future investigation but does not alter the main finding that serotonin mimics a thermoprotective effect of heat shock.

We tested the role of serotonin in mediating protection because much circumstantial evidence suggests this. Serotonin is a neuromodulator present in the insect hemolymph and it is known to increase the amplitude and duration of action potentials in the fast extensor tibiae motorneuron of locusts which increases the amplitude of synaptic potentials in postsynaptic neurons (Parker, 1995). In comparison, heat shock alters the parameters of the EPSPs in locust flight interneurons by decreasing the thermosensitivity of EPSP amplitude when exposed to subsequent thermal stress (Dawson-Scully and Robertson, 1998). Both the effects of heat shock and serotonin may be attributable to changes in potassium currents. Following a heat shock, potassium currents in locust neurons are decreased (Ramirez et al., 1999), action potential durations in flight motorneurons are increased (Wu et al., 2001) and artificially increasing action potential duration using the potassium channel blocker TEA makes them more thermotolerant (Wu et al., 2001). Serotonin has a well-described effect to increase action potential duration in *Aplysia* via cAMP and protein kinase A-mediated phosphorylation of potassium conductances (Camardo et al., 1983). Moreover serotonin also decreases potassium conductances of neuronal somata in locust ganglion slices (J.K. Lee and R.M. Robertson personal communication).

The putative protective mechanism outlined above is not time-dependent and thus would be expected to increase the failure temperature in response to a ramp increase of temperature such as has been shown for flight motor rhythms (Robertson et al., 1996). We did not observe this for ventilatory motor rhythms in our stress pre-treated animals. Nevertheless serotonin is also known to increase HSP70 levels in some preparations (e.g. Koumenis et al., 1995), serotonin antagonists can blunt a pharmacologically induced increase in HSP70 (O'Neill et al., 1998) and lastly, during prolonged stress, serotonin receptor synthesis is induced in degenerating mouse neurons (Singh et al., 1996). These findings indicate a role for serotonin in protective stress responses. We found that the application of serotonin did have a thermoprotective effect by mimicking an effect of heat shock to decrease the percentage of preparations in which ventilatory pattern generation fails during a period of maintained high temperature, and to increase the percentage of preparations that recover after such failure. We also found that the effect of serotonin treatment was not additive with the heat shock effect. These results suggest that serotonin can activate a time-dependent mechanism of thermoprotection and that prior heat shock and serotonin are acting via the same pathway. These ideas can be tested in future experiments with agonists and antagonists of specific serotonin receptors.

5. Conclusion

We provide evidence that exposure to a prior environmental stressor better enables the ventilatory control circuit in locusts to recover after heat-induced failure and to do so faster than control animals. There was a significant difference in the time required for heat-shocked animals' ventilatory rhythms to fail while their internal temperature was being held at a sub-lethal temperature, which adds to the argument that a prior stressor affords the ventilatory mechanism some degree of protection. Desiccation at prolonged high temperatures is a considerable threat to locusts' survival (Makings, 1987) and they may derive a greater benefit from increased thermotolerance to extended, more ecologically relevant, heat exposures than to relatively rapid increases of temperature past failure. Also, serotonin treatment mimics the effects of heat shock, suggesting that this neuromodulator plays a role in the development of thermoprotection. We suggest that investigations of stress-mediated thermoprotection of ventilatory pattern generation in locusts will be a profit-

able research avenue for discovering the cellular and sub-cellular mechanisms of this phenotypic neural plasticity.

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