Role for Calcium in Heat Shock-Mediated Synaptic Thermoprotection in *Drosophila* Larvae

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ABSTRACT: Chemical synaptic transmission is the mechanism for fast, excitation-coupled information transfer between neurons. Previous work in larval *Drosophila* has shown that transmission at synaptic boutons is protected by heat shock exposure from subsequent thermal stress through pre- and postsynaptic modifications. This protective effect has been, at least partially, ascribed to an up-regulation in the inducible heat shock protein, hsp70. Effects of hsp70 are correlated with changes to intracellular calcium handling, and the dynamics of intracellular calcium regulate synaptic transmission. Consistent with such a relationship, synaptic plasticity increases at locust neuromuscular junctions following heat shock, suggesting an effect of heat shock on residual presynaptic calcium. Intracellular recording from single abdominal muscle fibers of *Drosophila* larvae showed that prior heat shock imparts thermoprotection by increasing the upper temperature limit for synaptic transmission. Heat shock exposure enhances short-term synaptic plasticity and increases its thermal sensitivity. Increasing extracellular calcium levels eliminates the physiological differences between control and heat shock preparations; excess calcium itself induces thermoprotection at elevated concentrations. These data support the hypothesis that stress-induced neuroprotection at the nerve terminal acts, at least partially, through an alteration to the physiological effects of residual presynaptic calcium.

INTRODUCTION

Pre-exposure to elevated temperature (heat shock) has been demonstrated to protect cells, tissues, and organisms during subsequent stressful events (Parsell and Lindquist, 1993; Estruch, 2000; Jolly and Morimoto, 2000). This response has been classically attributed to an up-regulation in the expression of heat shock proteins, cellular protein chaperones acting to maintain protein conformation and function during stress and to prevent the formation of nonfunctional protein aggregates (Craig et al., 1994; Fink, 1999; Hartl and Hayer-Hartl, 2002). The heat shock response is evident in the nervous system, where it improves the survival rate of cells, synapses, and tissues during stressful conditions (Mailhos et al., 1993; Fink et al., 1997; Ohtsuka and Suzuki, 2000) and has been identified as a novel neuroprotectant in several experimental models of nervous system injury (Yenari et al., 1998, 1999). Recent work has shown that prior heat shock not only enhances survival rate, but in fact confers a functional neuroprotection at synapses (Dawson-Scully and Robertson, 1998; Karunanithi et al., 1999; Barclay and Robertson, 2000, 2001; Kelty et al., 2002) that is advantageous for preservation of critical behavioral responses (Barclay and Robertson, 2000). The underlying cellular/molecular mechanisms involved, however, remain unknown.
Heat shock increases short-term synaptic facilitation at the hindleg neuromuscular junction of the locust (Barclay and Robertson, 2001); this is likely to be accomplished through an effect on residual presynaptic calcium levels during high-frequency synaptic transmission (Fisher et al., 1997; Zucker, 1999). The onset and duration of heat shock-induced protection of presynaptic function mirrors those for the up-regulation of hsp70 expression (Karunanithi et al., 1999). As well, transgenic larvae with increased hsp70 expression demonstrate enhanced levels of presynaptic protection (Karunanithi et al., 2002), and exogenous hsp70 attenuated the effects of thermal stress on synaptic parameters in mouse brainstem slice preparations (Kelty et al., 2002), supporting the hypothesis for an involvement of this cellular chaperone. Hsp70 action has also been linked with intracellular calcium handling by a number of biochemical and physiological studies (Smith et al., 1995; Kiang et al., 1998; Nánási et al., 2000; Torrecilla et al., 2000). To test whether heat shock affects short-term synaptic plasticity through an effect on presynaptic calcium, we investigated extracellular calcium concentration and the heat shock response at the Drosophila neuromuscular junction during repetitive presynaptic stimulation.

Using intracellular recording to monitor EJPs in single muscle fibers of larval Drosophila, we show that exposure to prior heat shock imparts thermoprotection by increasing the functional range for synaptic transmission by 5°C. In agreement with previous results (Barclay and Robertson, 2001), we found that heat shock also enhanced the endogenous extent and thermodrillability of synaptic plasticity during tetanic stimulation. Elevation of extracellular calcium in control preparations induced a synaptic thermoprotection similar to that induced by heat shock in other preparations. This treatment nullified the heat shock-induced increase in thermoprotection of synaptic transmission, as the thermoprotective effect of calcium was less pronounced in heat shock preparations than in controls. Therefore, heat shock has a protective effect on short-term synaptic plasticity in larval Drosophila that appears to act via an alteration to the physiological effects of calcium within the presynaptic nerve terminal.

METHODS

Experimental Preparation

Drosophila melanogaster of the Canton-S strain were raised on a standard cornmeal medium at 25°C (60–70% relative humidity). Physiological experiments were conducted on control and heat shock wandering third-instar larvae. Heat shock larvae were exposed to 36°C for 1 h in standard Petri dishes, containing moistened filter paper, that were taped shut to preserve high humidity. Larvae were then allowed to recover at 25°C for .5 h prior to electrophysiological experiments (Karunanithi et al., 1999).

Larvae were dissected to expose the nervous system and body wall muscles (Atwood et al., 1993). The segmental nerve roots were then severed and the central nervous system removed. Experiments were conducted in standard hemolymph-like solution (HL3; Stewart et al., 1994), varying the extracellular calcium concentration as indicated. For all experiments, recordings were initially made at room temperature (15–20°C) and then continuously as the preparation temperature was gradually increased in a ramp-like manner (approximately 5°C/min) until complete failure of synaptic transmission was observed. Preparation temperature was controlled by a Nichrome heating coil around an inlet pipette passing superfusing solution from a reservoir maintained at room temperature. Temperature was monitored with a copper/constantan thermocouple (0.2 mm diameter, BAT-12; Sensortek, Clifton, NJ) placed adjacent to the head of the larvae.

Electrophysiological Recordings

Intracellular recordings were made with glass microelectrodes (filled with 3 mol l⁻¹ KAc; 40–60 MΩ) in ventral abdominal muscle 6, segment 3 of the larvae. An intracellular experimental approach was chosen because, despite muscle contractions, penetrations remained stable over the range of firing frequency and extracellular calcium concentration used in this study. Preparations were selected for analysis provided the intracellular penetration displayed a resting membrane potential more negative than −60 mV. The innervating motor nerve contains two motor axons (Kurdyak et al., 1994); however, over the temperature range used in this experiment the stimulus threshold differential was lost. Therefore we stimulated the severed end of the motor nerve with square voltage pulses (0.3 ms duration) suprathreshold for both motor axons, thus ensuring a consistent recording of the integrated EJP for the whole muscle.

In order to investigate the effects of heat shock on short-term synaptic plasticity of the integrated EJP, a dataset was collected where the motor nerve was stimulated at a high frequency (stimulation protocol: intraburst frequency = 40 Hz; burst repetition rate = 0.1 Hz; burst duration = 250 ms). Stimulation frequency was selected as it is within the physiological range for firing frequency of both motor axons contained within the motor nerve (Barclay et al., 2002) and reliably evoked a quantifiable short-term synaptic plasticity. To manipulate presynaptic calcium levels during short-term synaptic plasticity, datasets were collected at three discrete concentrations of extracellular calcium (1, 2, and 3 mM).
Analysis

For all experiments, the MacLab/4S data acquisition system (AD Instruments) was used to record electrical signals and superfusate temperature simultaneously. Digitized recordings were subsequently analyzed using the Igor Pro 3 software analysis package (Wavemetrics). For each animal, EJPs were analyzed for amplitude (measured from baseline to the event peak), duration (measured at half-maximal amplitude of the event), risetime (measured from the event onset to the event peak), and latency (measured from stimulus artifact to the event onset). An index of short-term synaptic plasticity was calculated as the ratio of the amplitudes of the first and last EJP within each individual stimulus train. Summation of EJPs was corrected for by measuring the amplitude of the final EJP from the peak to an extrapolation of the decay phase of the previous EJP. The results for each parameter were averaged into 5°C bins, such that an individual animal contributed one value only per temperature bin in statistical comparisons. The datasets consisted of recordings from 10 control and nine heat shock animals at 1.0 mM [Ca$^{2+}$/H11001], 11 control and 12 heat shock animals at 2.0 mM [Ca$^{2+}$/H11001], and seven control and eight heat shock animals at 3.0 mM [Ca$^{2+}$/H11001]. Significance ($p < 0.05$) was assessed with a Mann-Whitney rank sum test, t test, or two-way analysis of variance (ANOVA) with the appropriate posthoc test (Tukey test) where applicable. For statistical assessment where conditions of normality and equal variance were not met, the data have been appropriately transformed. All values are reported as mean ± S.E.M.

RESULTS

Heat Shock Increases Intrinsic Membrane Properties

Any effect of heat shock on short-term plasticity (Barclay and Robertson, 2001) could be a consequence of an alteration to the electrophysiological properties of the postsynaptic membrane. It is thus necessary to determine whether heat shock affects the intrinsic properties of the membrane. Prior exposure to heat shock increased both the input resistance [Fig. 1(A)] and time constant of the postsynaptic membrane [Fig. 1(B)]. Input resistance (two-way ANOVA, $F = 5.92$, $df = 1$, $p = 0.02$) and time constant of decay (two-way ANOVA, $F = 12.23$, $df = 3$, $p < 0.01$) were significantly higher in heat shock preparations. Although both parameters were reduced with increasing temperature ($p < 0.01$), their thermosensitivities were unaffected by heat shock.

Thermoprotection of the Synapse Induced by Heat Shock at 1.0 mM [Ca$^{2+}$]

Trains of EJPs were recorded at 1.0 mM [Ca$^{2+}$/H1545] in larval abdominal muscle 6, segment 3 continuously from room temperature until synaptic failure. Characteristic expected changes in EJP parameters were observed in both control and heat shock preparations with an increase in temperature [Fig. 2(A)]; temporal

Figure 1  Heat shock modifies the passive electrical properties of the larval Drosophila postsynaptic membrane. (A) Absolute values of membrane input resistance were increased at all temperatures in heat shock preparations (open circles) in comparison to controls (closed circles). (B) Absolute values of membrane time constant were also increased at all temperatures in heat shock preparations. There are individual significant differences between temperature bins in the 20–25°C range ($p = 0.01$) and the 30–35°C range ($p = 0.04$). The thermosensitivities of both input resistance and time constant were not affected by prior heat shock exposure. Values are means ± S.E.
parameters (latency, risetime, duration) and amplitude were all reduced with increasing temperature \( (p < .01) \). In heat shock preparations, the temperature at which synaptic transmission failed was significantly increased by 4.8°C \( (p < .01) \) and the variability in the upper temperature limit was greatly reduced \( (p < .01) \). In contrast to previous recorded effects of heat shock on excitatory junctional currents at individual synaptic boutons of the *Drosophila* larvae (Karunanithi et al., 1999), heat shock surprisingly did not alter specific parameters of individual EJPs \[ Fig. 3(A) \text{ and data not shown; see also Discussion} \].

**Heat Shock Affects Synaptic Plasticity at the *Drosophila* Neuromuscular Junction**

Heat shock has previously been shown to enhance short-term synaptic plasticity and increase its temperature sensitivity at a facilitating synapse (Barclay and Robertson, 2001). During high-frequency stimulation,
Increasing Extracellular [Ca$^{2+}$] Nullifies the Thermoprotective Effect of Heat Shock

A mechanism for short-term synaptic depression involves a depletion of the readily releasable pool of vesicles, which is known to be influenced by levels of presynaptic calcium (Wang and Kaczmarek, 1998; Dittman et al., 2000; Zucker and Regehr, 2002). To investigate this hypothesis further, the amount of calcium influx at the neuromuscular junction was manipulated by varying the level of extracellular calcium. EJPs were recorded, in control and heat shock preparations, continuously from room temperature until synaptic failure at 1, 2, and 3 mM extracellular [Ca$^{2+}$]. Due to the effects of calcium on quantal content (Augustine and Charlton, 1986), at room temperature EJP amplitude increased with increasing extracellular calcium [Fig. 4(A); two-way ANOVA, $F = 15.573$, $df = 2$, $p < .01$]. The effect of calcium on EJP amplitude was further confirmed by a posthoc Tukey test (1 mM vs. 2 mM [Ca$^{2+}$], $p < .01$; 1 mM vs. 3 mM [Ca$^{2+}$], $p < .01$; 2 mM vs. 3 mM [Ca$^{2+}$], $p = .04$). This was expected, as it has been reported that the strength of synaptic transmission is sensitive to extracellular calcium concentration up to 4 mM [Ca$^{2+}$] (Dawson-Scully et al., 2000). EJP amplitude was not different in heat shock preparations in comparison with controls. All measured EJP parameters were decreased with increasing temperature, regardless of calcium concentration [see, e.g., Fig. 2(A)]. The upper temperature limit for synaptic transmission was increased by raising the calcium concentration in the extracellular solution [Fig. 4(B)]. In control preparations, failure occurred at 37.0°C at 1.0 mM [Ca$^{2+}$], 40.5°C at 2.0 mM [Ca$^{2+}$], and 45.2°C at 3.0 mM [Ca$^{2+}$]; this calcium-induced increase was significant ($p < .01$). Heat shock also increased the failure temperature for synaptic transmission ($p < .01$); however, this thermoprotective effect of heat shock was only evident at lower levels of extracellular calcium. Whereas at 1.0 mM [Ca$^{2+}$] prior exposure to heat shock significantly increased the upper temperature limit of synaptic transmission ($p < .01$), as extracellular calcium concentration was elevated this difference between control and heat shock preparations was abolished. Furthermore, at 1.0 mM [Ca$^{2+}$], the failure temperatures of control preparations were significantly more variable than in heat shock preparations [see, e.g., error bars in Fig. 4(B); $p < .01$] and this difference in variability was also abolished by the increase in extracellular calcium.

Heat Shock Induced Thermoprotection Is Mimicked by Increasing Extracellular Calcium

Calcium concentration altered individual EJP parameters in control preparations (Figs. 5 and 6), mimicking previously published results on the effects of heat shock (Dawson-Scully and Robertson, 1998; Karunanithi et al., 1999; Barclay and Robertson, 2000, 2001). The measured latency of synaptic transmission was significantly reduced by raising extracellular cal-
Due to the effects of calcium concentration on quantal content (Augustine and Charlton, 1986), the amplitude of EJPs was significantly increased with extracellular calcium over the temperature range investigated [Fig. 6(A); two-way ANOVA, $F = 20.72$, $df = 2, p < .01$]. The effect of calcium was confirmed by a posthoc Tukey test (1 mM vs. 2 mM $[\text{Ca}^{2+}]$, $p < .01$; 1 mM vs. 3 mM $[\text{Ca}^{2+}]$, $p < .01$; 2 mM vs. 3 mM $[\text{Ca}^{2+}]$, $p = .03$). To compensate for this effect of calcium concentration, the remaining EJP parameters were normalized with their value at room temperature in order to make unbiased comparisons of the thermostensitivity (i.e., the effect of temperature on an EJP parameter) of synaptic transmission across calcium levels. All EJP parameters were significantly decreased by temperature at all calcium concentrations (two-way ANOVAs, $p < .01$). The thermosensitivities of both EJP amplitude (two-way ANOVA, $F = 11.07$, $df = 2, p < .01$) and duration (two-way ANOVA, $F = 10.06$, $df = 2, p < .01$) were reduced with the elevation of extracellular calcium [Fig. 6(B, C)]. Not all parameters of the EJP were affected; normalized EJP risetime was unaffected by extracellular calcium [Fig. 6(D); $p > .05$].

Increasing extracellular calcium also significantly altered short-term synaptic plasticity in controls (Fig. 7; two-way ANOVA, $F = 4.28$, $df = 2, p = .02$) in a similar manner to heat shock preparations (see, e.g., Fig. 4), although there was no significant interaction between calcium and temperature ($F = 1.06, df = 10, p = .40$). An effect of calcium to increase the thermostensitivity of plasticity, however, was confirmed with linear regression analysis. Plasticity in controls

![Figure 4](image4.png)

Figure 4  Increasing extracellular calcium nullified the thermoprotective effect of heat shock at *Drosophila* synapses. (A) At room temperature, EJP amplitude is significantly increased by increasing extracellular calcium concentration. Amplitudes at all three concentrations are significantly different. (B) The upper temperature limit for synaptic transmission in control preparations was increased by increasing the calcium concentration in the extracellular media, mimicking the effect of prior heat shock. Open circles represent heat shock preparations, closed circles represent control preparations. An increase in the upper temperature limit for successful synaptic transmission by heat shock occurred only at 1.0 mM $[\text{Ca}^{2+}]$ ($p < .01$). Values are means ±S.E.

![Figure 5](image5.png)

Figure 5  Increased extracellular calcium reduced EJP latency in control preparations. Latencies were significantly decreased at 3.0 mM $[\text{Ca}^{2+}]$ (triangles) in comparison to 1.0 mM $[\text{Ca}^{2+}]$ preparations (circles). There are individual significant differences between temperature bins in the 15–20°C range ($p < .01$) and the 25–30°C range ($p = .02$). In order to enhance the visual impact of the effect of extracellular calcium concentration, in this and subsequent figures, only the results for 1.0 and 3.0 mM $[\text{Ca}^{2+}]$ are presented. Values are means ±S.E.
at 1.0 mM [Ca$^{2+}$] was insensitive to temperature ($r^2 = 0.46, p = .14$), with a slope of 0.008 ± 0.004°C$^{-1}$. At 3.0 mM [Ca$^{2+}$] temperature increased the index of plasticity ($r^2 = 0.8, p = .02$), with a slope of 0.013 ± 0.003°C$^{-1}$. Although temperature had a significant effect on plasticity when calcium concentration was elevated, the regression slope was unaffected (analysis of covariance, $t = 1, df = 13, p > .05$).

**DISCUSSION**

In this article we confirm and extend previous results by demonstrating that prior heat shock imparts synaptic thermoprotection by increasing the upper temperature limit for evoked exocytosis, concomitant with an enhancement of endogenous short-term synaptic plasticity and an increase in its thermosensitivity. We show further that increasing extracellular calcium levels negated the thermoprotective effects of heat shock by preventing the heat shock-mediated increase in the upper temperature limit for synaptic transmission. Finally, we demonstrate that elevation of the extracellular calcium concentration itself could induce synaptic thermoprotection, thus mimicking an established effect of heat shock at the synapse. These data support the hypothesis that heat shock acts presynaptically via an effect on residual calcium-dependent mechanisms within the nerve terminal.
Changes to Passive Membrane Properties

Prior exposure to heat shock increased both the membrane input resistance and time constant of decay, without affecting their thermosensitivities (Fig. 1). For each parameter, there was a rightward shift of 5–6°C in the graphical representation of their temperature relationship. Interestingly, this shift approximates the 5°C increase in the upper temperature threshold for synaptic transmission in heat shock larvae. It is most likely that the effects on the passive membrane properties are due to heat shock-modification to the temperature sensitivity of the postsynaptic leak current. Do the effects of heat shock to the passive properties of the membrane, however, account for the subsequent physiological effects on synaptic plasticity?

If the increase in input resistance in heat shock preparations is physiologically meaningful as to have an effect on membrane depolarization, then EJP amplitude should be increased for heat shock larvae. No difference in amplitude, however, was found between control and heat shock preparations. Furthermore, the increase in input resistance is not being compensated for by an equivalent decrease in current, as the opposite has been previously shown (Karunanithi et al., 1999). With similar reasoning, the increase in membrane time constant would imply an effect on EJP duration. This is also not evident in heat shock preparations. Therefore, we conclude that although the passive membrane properties are increased by prior heat shock, it is unlikely that this change is contributing to the effects on synaptic plasticity.

Absence of Effect of Heat Shock on Individual EJP Parameters

The effect of heat shock on EJCs at individual 1b-type boutons of larval Drosophila is to decrease the thermosensitivity of synaptic strength (Karunanithi et al., 1999). We report here, however, that there is no effect of prior heat shock on the amplitude of integrated EJPs reflecting the summed response of the muscle fiber’s boutons [Fig. 3(A)]. A lack of thermoprotection may simply be due to the significant effect at single boutons being masked by variation between all boutons contained in the muscle fiber. There is, however, an alternative, more likely explanation.

In the larva, ventral abdominal muscle 6, segment 3 is innervated by two motor axons contained within the motor nerve: a “fast” axon, generating large EJPs, and a “slow” axon, generating smaller EJPs (Kurdyak et al., 1994), which are physiologically reminiscent of the two excitatory motor axons in other invertebrate preparations, such as the locust hindleg (Burrows, 1996) and crustacean leg (Atwood, 1976). Previous research on the locust hindleg has shown that heat shock has differential effects at the fast and slow extensor tibial neuromuscular junctions (Barclay and Robertson, 2000, 2001). The thermosensitivity of synaptic strength at the fast neuromuscular junction is reduced by heat shock, whereas the absolute strength at room temperature is unaffected. In contrast, heat shock reduces the absolute synaptic strength at the slow neuromuscular junction, but does not affect its thermosensitivity. The Drosophila preparation is limited in that it is prohibitively difficult to separate physiologically synaptic transmission of the two motor axons and assay muscle fiber EJPs at variable temperatures, as stimulus threshold differentials for the two axons established at room temperature (see, e.g., Lnenicka and Keshishian, 2000) disappear as the temperature is raised (J. W. Barclay, unpublished observations). We would argue that different effects of heat shock at “fast” and “slow” neuromuscular junctions, if they were to exist also in the two motor axon types in Drosophila, would offset each other, and thus, mask any effect on integrated EJP amplitude.
Short-Term Synaptic Plasticity and Modification of Residual Calcium

Prior exposure to heat shock was found to alter short-term synaptic plasticity in two ways. At the neuromuscular junction in larval Drosophila, both the endogenous extent of synaptic depression and its thermosensitivity were increased by heat shock [Fig. 3(B)]. A similar response has been previously reported at the locust hindleg neuromuscular junction (Barclay and Robertson, 2001), albeit for short-term facilitation. While there are intrinsic differences between the two insect preparations, it is intriguing that heat shock imparted the same effect regardless of the endogenous direction of synaptic plasticity (i.e., facilitation or depression). A tempting hypothesis is that a primary neurophysiological response to a stressful environment is to enhance the short-term ability to regulate the strength of synaptic connections.

Enhancement of synaptic facilitation would indicate an effect on residual presynaptic calcium levels during repetitive synaptic transmission (Katz and Miledi, 1968; Kamiya and Zucker, 1994); however, transmission at the larval synapse depresses at room temperature. Although there is some evidence implicating the involvement of receptor desensitization at the Drosophila neuromuscular junction during repetitive stimulation (Adelsberger et al., 1997), short-term synaptic depression is believed to be largely attributed to the rapid depletion of the readily releasable pool of vesicles, which is influenced by presynaptic calcium levels (Wang and Kaczmarek, 1998; Dittman et al., 2000; Zucker and Regehr, 2002). Thus, a simple model for all forms of short-term modification of synaptic strength involves presynaptic residual calcium (Zucker, 1999; Dittman et al., 2000; Zucker and Regehr, 2002). Our results would support such a model as a common mechanism for short-term synaptic plasticity, demonstrated by the transition between depression and facilitation at the same neuromuscular junction during exposure to a monotonic increase in temperature in heat shock Drosophila preparations. Although the extent of depression can be affected by the initial release probability, this is unlikely to be a factor here as initial EJP amplitudes were identical in control and heat shock preparations at all temperatures [Fig. 3(A)]. Therefore, the most parsimonious conclusion is that the difference in synaptic depression is a result of heat shock affecting either residual calcium levels or the presynaptic targets/effectors of residual calcium during short-term changes in synaptic strength. This conclusion would agree with the previous work demonstrating an effect of heat shock on residual calcium at a facilitating synapse in the locust (Barclay and Robertson, 2001).

Raising the concentration of extracellular calcium imparts thermoprotection to synaptic transmission in control preparations of Drosophila larvae, mimicking the effect of heat shock [Fig. 4(B)]. Furthermore, it also induced a thermoprotection of EJP amplitude and duration [Fig. 6(B,C)]. These results duplicate established neuroprotective effects of heat shock evident at individual larval Drosophila boutons (Karunanithi et al., 1999) and at locust synapses (Dawson-Scully and Robertson, 1998; Barclay and Robertson, 2000, 2001). Experimentally increasing calcium levels external to the nerve terminal would increase the driving force on calcium, demonstrated by the increase in the absolute strength of synaptic transmission with increased calcium [Figs. 4(A) and 6(A)]. Thus, during a presynaptic depolarization, just prior to vesicular release, there would be an increased amount of calcium entering the nerve terminal. At elevated temperatures this increase in calcium influx could offset the faster closing kinetics of the voltage-dependent calcium channel at the nerve terminal or the increased rate of calcium removal from the active zone (Sabatini and Regehr, 1999). It is unlikely that elevated calcium would prevent a heat-induced failure of action potential conduction, as the extracellular application of divalent cations such as calcium decreases axonal excitability (Frankenhaeuser and Hodgkin, 1957). As increased extracellular calcium negates the thermoprotective effect of heat shock at the synapse and itself imparts thermoprotection of synaptic transmission, it is likely that heat shock is acting through an effect on presynaptic calcium at the nerve terminal.

Our results do not specify the physiological mechanism upstream of the heat shock effect on residual presynaptic calcium. It could be due to altered calcium entry, via a heat shock effect on action potential breadth (Jackson et al., 1991; Byrne and Kandel, 1996; Sugita et al., 1997) as heat shock has been demonstrated to alter K⁺ channel kinetics, and consequently action potential duration (Ramirez et al., 1999; Wu et al., 2001). Alternatively, heat shock could alter presynaptic calcium dynamics via a mechanism independent of spike broadening (Chen and Regehr, 1997). Further experiments will be required to determine whether heat shock preserves calcium levels by affecting calcium entry and/or its removal.

Potential Molecular Mechanisms

An obvious putative mechanism for the effects of heat shock is through inducible chaperones, such as the heat shock proteins (Parsell and Lindquist, 1993). We
have previously shown that the onset and duration for heat shock-induced thermoprotection parallel those of the timeframe for up-regulated hsp70 protein expression (Karunanithi et al., 1999), and the extent of thermoprotection is enhanced in genetically manipulated Drosophila carrying elevated copies of the hsp70 gene (Karunanithi et al., 2002). The results presented in this study give experimental support to an association between residual presynaptic calcium and heat shock-induced thermoprotection. The chaperone function of heat shock proteins requires ATP hydrolysis, which is known to regulated by calcium (Sriram et al., 1997) and exposure to bimoclomol, a chemical inducer of hsp70 that has been shown to affect calcium handling in mammalian ventricular myocardium (Nánási et al., 2000). As a cellular chaperone, hsp70 could act to stabilize proteins intrinsic to the exocytotic machinery from stress-induced denaturation. The ATPase activity of heat shock proteins, and thus their chaperone ability, require activation by members of the DnaJ family of proteins (Kelley, 1998). At the presynaptic nerve terminal, CSP, a member of this family, can activate the ATPase activity of hsp70 (Chamberlain and Burgoyne, 1997) and has chaperone abilities of its own (Chamberlain and Burgoyne, 2000). Recently it has been shown that CSP exists as a trimeric chaperone complex with Hsc70, the heat shock protein cognate, and the SGT protein (Tobaben et al., 2001). It is worth noting that, similar to the results presented here, Drosophila larvae with null mutations in the csp or hsc70 gene have temperature-sensitive impairments of neurotransmitter release (Zinsmaier et al., 1994; Bronk et al., 2001) and that either mutational phenotype can be restored by elevating levels of extracellular calcium (Dawson-Scully et al., 2000; Bronk et al., 2001). One cannot rule out, however, an effect of heat shock through alternative mechanisms, as environmental stress is known to up-regulate a host of different proteins with diverse consequences (Leemans et al., 2000; Zou et al., 2000).

In conclusion, exposure to a prior heat stress alters short-term depression of the Drosophila synapse and its thermosensitivity. This is potentially a neurophysiological response to increase the plasticity of the synapse, thereby increasing its homeostatic potential in the face of increased variability of the external environment. Furthermore, these results support the idea that heat shock acts mechanistically through an alteration to the physiological actions of residual calcium in the presynaptic nerve terminal. Future work, however, is required to determine whether heat shock affects calcium entry and/or removal from the active zone and to characterize any accessory proteins critical for the mechanism of presynaptic alteration.

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