

Long-Term Effects of Prior Heat Shock on Neuronal Potassium Currents Recorded in a Novel Insect Ganglion Slice Preparation

J. M. RAMIREZ,¹ F. P. ELSEN,¹ AND R. M. ROBERTSON²

¹Department of Organismal Biology and Anatomy, The University of Chicago, Chicago, Illinois 60637; and ²Department of Biology, Queens University, Kingston, Ontario K7L 3N6, Canada

Ramirez, J. M., F. P. Elsen, and R. M. Robertson. Long-term effects of prior heat shock on neuronal potassium currents recorded in a novel insect ganglion slice preparation. *J. Neurophysiol.* 81: 795–802, 1999. Brief exposure to high temperatures (heat shock) induces long-lasting adaptive changes in the molecular biology of protein interactions and behavior of poikilotherms. However, little is known about heat shock effects on neuronal properties. To investigate how heat shock affects neuronal properties we developed an insect ganglion slice from locusts. The functional integrity of neuronal circuits in slices was demonstrated by recordings from rhythmically active respiratory neurons and by the ability to induce rhythmic population activity with octopamine. Under these “functional” in vitro conditions we recorded outward potassium currents from neurons of the ventral midline of the A1 metathoracic neuromere. In control neurons, voltage steps to 40 mV from a holding potential of -60 mV evoked in control neurons potassium currents with a peak current of 10.0 ± 2.5 nA and a large steady state current of 8.5 ± 2.6 nA, which was still activated from a holding potential of -40 mV. After heat shock most of the outward current inactivated rapidly (peak amplitude: 8.4 ± 2.4 nA; steady state: 3.6 ± 2.0 nA). This current was inactivated at a holding potential of -40 mV. The response to temperature changes was also significantly different. After changing the temperature from 38 to 42°C the amplitude of the peak and steady-state current was significantly lower in neurons obtained from heat-shocked animals than those obtained from controls. Our study indicates that not only heat shock can alter neuronal properties, but also that it is possible to investigate ion currents in insect ganglion slices.

INTRODUCTION

Brief exposure to high sublethal temperatures (heat shock) has lasting adaptive sequelae for cells and tissues in organisms across all taxa. This is most relevant for poikilotherms, which can be adapted by heat shock to extreme temperatures in their natural environments. There is increasing awareness that for these organisms heat shock has far-reaching consequences for their behavior and population dynamics (Coleman et al. 1995; Feder 1996; Feder and Krebs 1997; Gehring and Wehner 1995; Hofmann and Somero 1996; Norris et al. 1995; Ulmasov et al. 1992). These adaptive effects are primarily mediated by various heat shock or stress proteins. The molecular biology and anatomic distribution of these proteins were well characterized in a variety of animal models (Buchner 1996; Craig et al. 1993; Lindquist and Craig 1988; Mayer and Brown 1994). However, surprisingly little is known as to what extent heat shock affects

neuronal function, particularly the mechanisms that underlie electrical signaling in nervous tissue.

We examined the effect of heat shock on neuronal potassium currents in locusts. These animals are ideal to study heat shock effects on neuronal function for the following reasons. First, in their natural environments, locusts are routinely exposed to ambient temperatures $>40^{\circ}\text{C}$ (Uvarov 1966), and during vigorous activity, such as flight, the temperature in the thorax can be as much as 10°C above ambient (Weis-Fogh 1956). Second, it was demonstrated that locusts exhibit a robust heat shock response, and exposure to 43°C for 3 h induces the production of heat shock proteins (Baldaia et al. 1987; Whyard et al. 1986). Third, it was demonstrated that heat treatment alters neuronal operation in locusts in ways that can be interpreted as being ecologically adaptive (Robertson et al. 1996). For example, it has been shown that heat pretreatment permits tethered flight at higher thoracic temperatures than are normally permissive, and this is a consequence of an extended temperature range of operation in the neuronal circuitry controlling the wingbeat (Robertson et al. 1996). Most interestingly the temperature sensitivity of flight motor rhythms is markedly reduced after nonlethal heat shock. Furthermore, heat shock affects the temperature sensitivities of amplitude potential amplitude and conduction velocity in this system (Gray and Robertson 1998). We tested the idea that heat shock induces lasting modifications of neuronal properties.

To examine the effect of heat shock on ion channel properties we developed a slice preparation obtained from insect nervous tissue. This approach to studying neuronal properties in insects provides an exciting possibility to study adaptive changes in channel properties in neurons that are still embedded in their normal histological environment. We show here that heat shock treatment has a long-term effect on whole cell potassium current recorded from the somata of neurons visualized in a functional slice preparation of the metathoracic ganglion of the locust.

METHODS

Heat shock

Adult locusts (*Schistocerca americana*) were kept in a crowded colony at the University of Chicago. Five days before performing the experiments 50 locusts were isolated from the colony and maintained in the laboratory in cages at room temperature ($\sim 24^{\circ}\text{C}$). All locusts were placed into a ventilated plastic container (1-L capacity) for 3 h. Heat shocked locusts were exposed to 45°C , whereas control locusts were kept at room temperature for the same time period. None of the locusts died as a result of the heat treatment. Heat shocked locusts

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

were maintained for 6–24 h at room temperature before dissection to allow them to recover from the treatment. No difference was noted between the results of animals that recovered for different periods of time.

Slice preparation

Locust slices were prepared by modifying the mammalian slice preparation technique (Ramirez et al. 1996). Locusts were decapitated, and the metathoracic ganglia were removed in saline (containing, in mM, 128 NaCl, 3 KCl, 1.5 CaCl₂, 1 MgSO₄, 24 NaHCO₃, 0.5 NaH₂PO₄, and 30 D-glucose equilibrated with continuous bubbling of carbogen [95% O₂-5% CO₂] to pH 7.4). The dorsal surface of an isolated ganglion was glued onto an agar block with cyanoacrylate glue. This was secured in a vibratome with the rostral end of the ganglion up, and thin slices (100 μm) were sectioned serially from rostral to caudal to expose the rostral portion of the metathoracic ganglion. In most experiments described in this study we used the next slice of 400–600 μm from this rostral boundary extending into the rostral border of the first abdominal neuromere. In only two cases we used a slice obtained from the posterior portion of the metathoracic ganglion and the first fused abdominal neuromere. The slices were immediately transferred into a recording chamber and held in place with an overlying nylon mesh on a titanium frame. The preparation was further stabilized because the overlying mesh covered not only the slice itself but also the polymerized glue that remained partly attached to the slice. The preparation was submerged under a stream of saline (31°C; flow rate 16 ml/min) and stabilized for 10 min before recording neural activity.

Recording technique and analysis

Extracellular recordings were obtained with a suction electrode (low resistance glass pipettes, 0.3 MΩ) and were integrated electronically with a leaky RC circuit (set at a time constant of 20–30 ms). Whole cell patch recordings were obtained with unpolished electrodes that were manufactured from filamented borosilicate glass (Clark GC150F) and had a resistance of 1–2 MΩ when filled with a solution containing (in mM) 140 KCl, 1 CaCl₂, 10 ethylene glycol-bis-β-aminoethyl ether-*N,N,N',N'*-tetraacetic acid, 2 MgCl₂, 4 Na₂ATP, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.2). Locust neurons on the ventral midline of the A1 neuromere of the metathoracic ganglion were recorded with the visual patch-clamp technique with a Zeiss upright microscope in conjunction with infrared Normarski optics. The electrodes with an internal pressure of 30–50 mmHg were positioned onto a soma of a locust neuron with a three-dimensional (3D) piezomicromanipulator (Burleigh). For seal formation, positive pressure in the pipette was released, and gigaseals were formed by slight negative pressure. The membrane was ruptured to achieve whole cell configuration by applying negative pressure pulses. Membrane currents were recorded with an Axopatch 1D amplifier and analyzed with the pClamp 6.0 software in conjunction with a Digidata 1200 interface (Axon Instruments, Foster City, CA). Current response traces were recorded with either off- or on-line leak subtraction, eliminating linear leak current. Serial resistance was always 80% compensated, and the input resistance of all neurons was between 180 and 800 MΩ. All quantitative data are given in mean ± SE, if not indicated otherwise. Statistical significance was assessed with the Student's *t*-test, and significance was assumed for *P* < 0.05. We recorded from 20 neurons in 16 preparations and only quantitatively analyzed those recordings that had an initial seal resistance 2.5 GΩ. All substances used in this study were obtained from Sigma Chemical (St. Louis, MO).

RESULTS

Functional ganglion slice preparation

Previous investigations in *Manduca sexta* (Oland et al. 1996) already indicated that it is possible to preserve the anatomic architecture of an insect ganglion in a slice preparation. We confirmed this finding in our study with 400- to 600-μm transverse slices obtained from the metathoracic ganglion. Characteristic fiber tracts and intraganglionic tracheal supply of the metathoracic ganglion were preserved in the slice preparation (Fig. 1). In addition, the slices were sufficiently thick to preserve not only the 3D arrangement of cell bodies, neuropile, and tracheal supply but also the functional integrity of neuronal circuits (Fig. 2). In slice preparations containing the anterior portion of the metathoracic ganglion, superfusion of the biogenic amine octopamine induced rhythmic neuronal activity. To induce the rhythmicity we used a concentration of 10⁻³ M as was previously described (Ramirez and Pearson 1991a). Suction electrode recordings from the ascending connectives reveal that octopamine activated not just single neurons but a population of neurons (Fig. 2, *top panel*). Previous studies demonstrated that octopamine can release flight rhythms in locust preparations (Ramirez and Pearson 1991a,b; Sombati and Hoyle 1984; Stevenson and Kutsch 1987). Thus it is conceivable that this octopamine-induced rhythm reflects residual rhythmic activity in flight circuitry (see also Wolf et al. 1988). In slices that contained the posterior portion of the metathoracic ganglion and the first fused abdominal ganglion, it was possible to record intracellularly from spontaneously active respiratory rhythmic neurons (Fig. 2, *bottom panel*). The characteristic activation patterns of these neurons were previously described (Ramirez and Pearson 1989), suggesting that this respiratory neuron was inhibited during inspiration and activated during expiration. The finding that neuronal network activity was preserved indicates that neuronal discharge properties remained largely unaffected in the slice preparation. Thus any heat-shock induced alterations in ion channel properties evident under these in vitro conditions may resemble those in intact animals.

Heat-shock induced alteration in potassium currents under control conditions

To characterize ion channel properties, unstained living locust neurons were visualized with infrared Nomarski optics (Figs. 1 and 3A) and routinely voltage clamped with the whole cell patch configuration (Fig. 3, *B* and *C*). All recordings were obtained from cell bodies located in the ventral midline of the A1 neuromere (Fig. 3A, see also bottom right square in Fig. 1). Hyperpolarizing and depolarizing test potentials (duration: 80 ms), incrementing in 10 mV steps, from -80 to 40 mV from a holding potential of -60 mV evoked a series of outward currents (Fig. 3, *B* and *C*). These currents were identified as K⁺ currents because they were blocked by tetraethylammonium chloride (TEA 30 mM) and CsCl (110 mM) (not shown). Figure 3 shows the outward current responses to similar voltage steps in a neuron obtained from a control animal (Fig. 3*B*) and a neuron obtained from a locust that was exposed to a heat shock 24 h before the experiment (Fig. 3*C*). In control neurons the evoked K⁺ outward current consisted of a small rapidly inactivating component and a large slowly inactivating com-

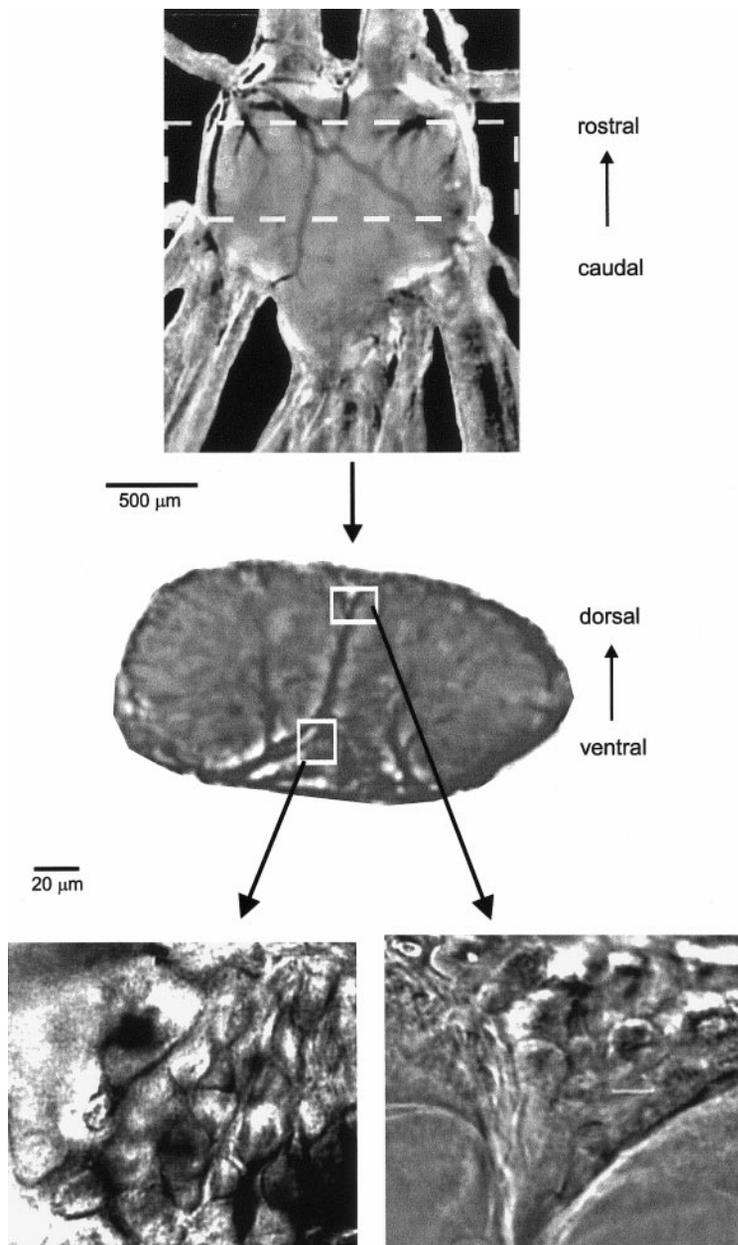


FIG. 1. Functional transverse ganglion slice. Dorsal view of a locust metathoracic ganglion before obtaining a transverse slice at a region indicated by a white square (*top panel*). *Middle panel*: transverse ganglion slice. *Bottom panels*: living insect neurons visualized with infrared-Nomarski optics at the ventral (*left panel*) and dorsal midline (*right panel*).

ponent. The average current amplitude evoked by a depolarizing voltage step from -60 to 40 mV was initially 10.0 ± 2.5 nA (peak, $n = 5$) and after 80 ms 8.5 ± 2.6 nA (steady state, $n = 5$). Thus after 80 ms only 15.5% of the outward current was inactivated (Fig. 3B, *top panel*). In contrast, in heat-shocked neurons most of the K^+ current inactivated rapidly (Fig. 3C, *top panel*). The average peak current amplitude evoked by a voltage step from -60 to 40 mV was initially 8.4 ± 2.4 nA (peak, $n = 5$) and after 80 ms was 3.6 ± 2.0 nA (steady state, $n = 5$). Thus 57% of the outward current was inactivated after 80 ms. The peak outward current was not significantly different in control and heat-shocked neurons (Fig. 3D, *left panel*). However, because in heat-shocked neurons most of the K^+ current inactivated there was a significant difference in the steady-state component of current in control and heat-shocked neurons (Fig. 3D, *right panel*).

At a holding potential of -40 mV most of the current in control animals was still activated when stepping to more

depolarized membrane potentials (Fig. 3B, *bottom panel*). In contrast in all neurons obtained from heat-shocked locusts, most of the outward current was inactivated at a holding potential of -40 mV (Fig. 3C, *bottom panel*), suggesting that outward currents in heat-shocked animals were dominated by an A-type current.

Heat-shock induced alteration in the response to temperature changes

The response to a temperature increase was assessed with a holding potential of -60 mV and the same hyperpolarizing and depolarizing step protocol as described in the previous section. In control animals, the amplitude of the voltage-dependent K^+ current decreased after a temperature step from 31 to 35°C and increased again after a subsequent step to 41°C (Fig. 4A). During each temperature step, the same temperature was maintained for 3 min before obtaining a measurement to ensure that

Octopamine-induced rhythmic activity

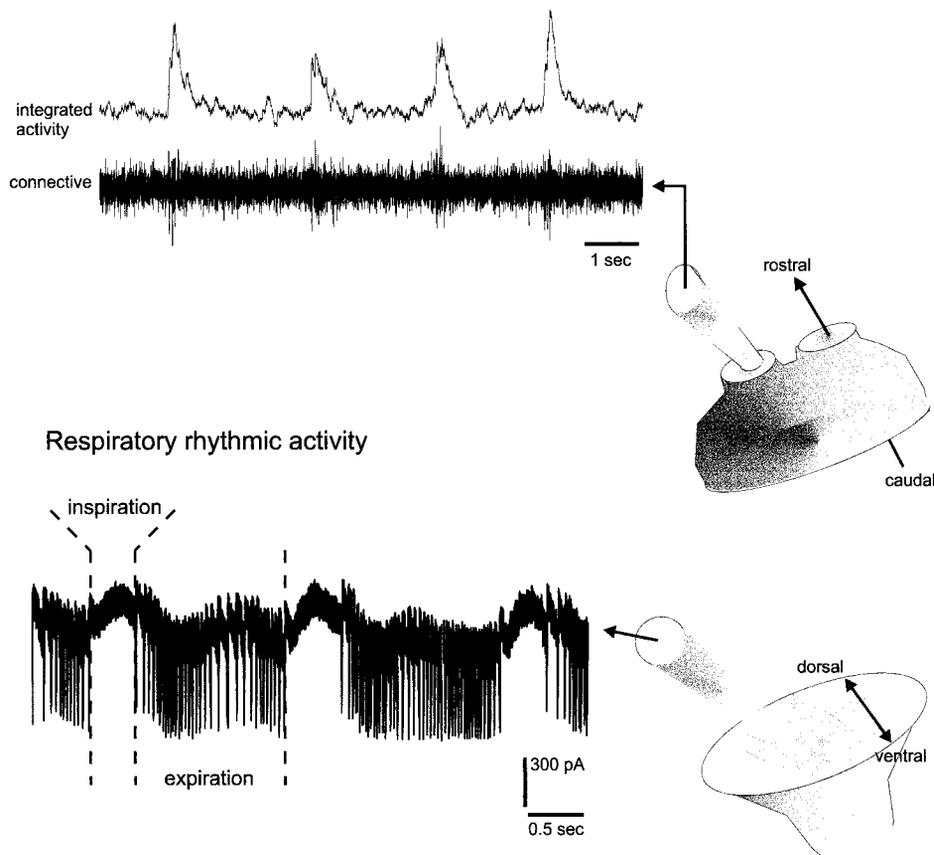


FIG. 2. Functional integrity of neurons within a slice seems to be preserved. *Top panel:* octopamine induces rhythmic activity in the rostral portion of the sliced metathoracic ganglion recorded extracellularly with a suction electrode from the left ascending connective (*bottom trace*). The extracellularly recorded rhythmic activity was integrated in the upper trace. *Bottom panel:* whole cell patch recording from a respiratory neuron obtained in the caudal portion of the metathoracic ganglion. The neuron was held at -50 mV. We assume, based on previous investigations, that the short phase reflects neural inspiration, the longer phase expiration.

the slice equilibrated to the new temperature. The temperature-induced decrease and subsequent increase in the current amplitude are demonstrated by the current-voltage relationship measured at the peak current (Fig. 4B). In the heat-shocked animal the amplitude of the K^+ current continued to decrease in response to temperature steps from 31 to 35°C and to 41°C (Fig. 4, C and D). The current remained reduced for ≥ 10 – 20 min after returning to the starting temperature.

The temperature effect on the K^+ current amplitude evoked by a voltage step from -60 to 40 mV was quantitatively evaluated for five control and five heat-shocked preparations (Fig. 5). When stepping from 31°C to a temperature of 34°C the peak amplitude (Fig. 5A) was not significantly different between control and heat-shocked animals. When stepping to a temperature of 38 and 42°C , the peak amplitude (Fig. 5A) as well as the steady-state amplitude (Fig. 5B) were significantly lower in heat-shocked than in control animals. The outward current after heat shock was presumably reduced because of a shift in the inactivation properties. We assessed the inactivation properties of the K^+ current by applying holding potentials in 10 -mV steps from -90 to -10 mV. These holding potentials were maintained for 2 s before stepping to the same test potential of 40 mV. As demonstrated in Fig. 5C the K^+ current was fully inactivated at a temperature of 36 and 41°C when holding a heat-shocked neuron at -60 mV.

Neither the control nor the heat-shocked animals recovered fully from heat exposure. The current amplitude, obtained 10 min after returning to the control temperature of 31°C , was

variable and not significantly different between heat-shocked and control preparations (Fig. 5, A and B), although it is notable that the variability is greatly increased in control compared with heat-shocked preparations.

DISCUSSION

We have shown that heat-shock pretreatment can lead to long-lasting modifications in K^+ outward currents of neuronal somata. Under control conditions voltage-dependent outward currents consisted of a large component of a slowly inactivating current. After heat shock, outward currents were characterized by a large component of a rapidly inactivating current. This alteration corresponds with a different response to temperature changes, which may be due to a shift in inactivation properties of the rapidly inactivating component. Our finding suggests that, under control conditions, K^+ flows primarily through K^+ channels with delayed-rectifier characteristics. These currents inactivate only very slowly. After heat shock, voltage steps evoke only very little delayed-rectifier current and instead evoke an inactivating K^+ conductance with A-type characteristics. The inactivation properties of this A-type current were altered at higher temperatures because at a holding potential of -70 mV most of the current was fully inactivated at a temperature of 36 and 41°C . This explains why at higher temperatures K^+ outward currents were very reduced in heat-shocked animals.

Despite the dramatic alteration in potassium conductances

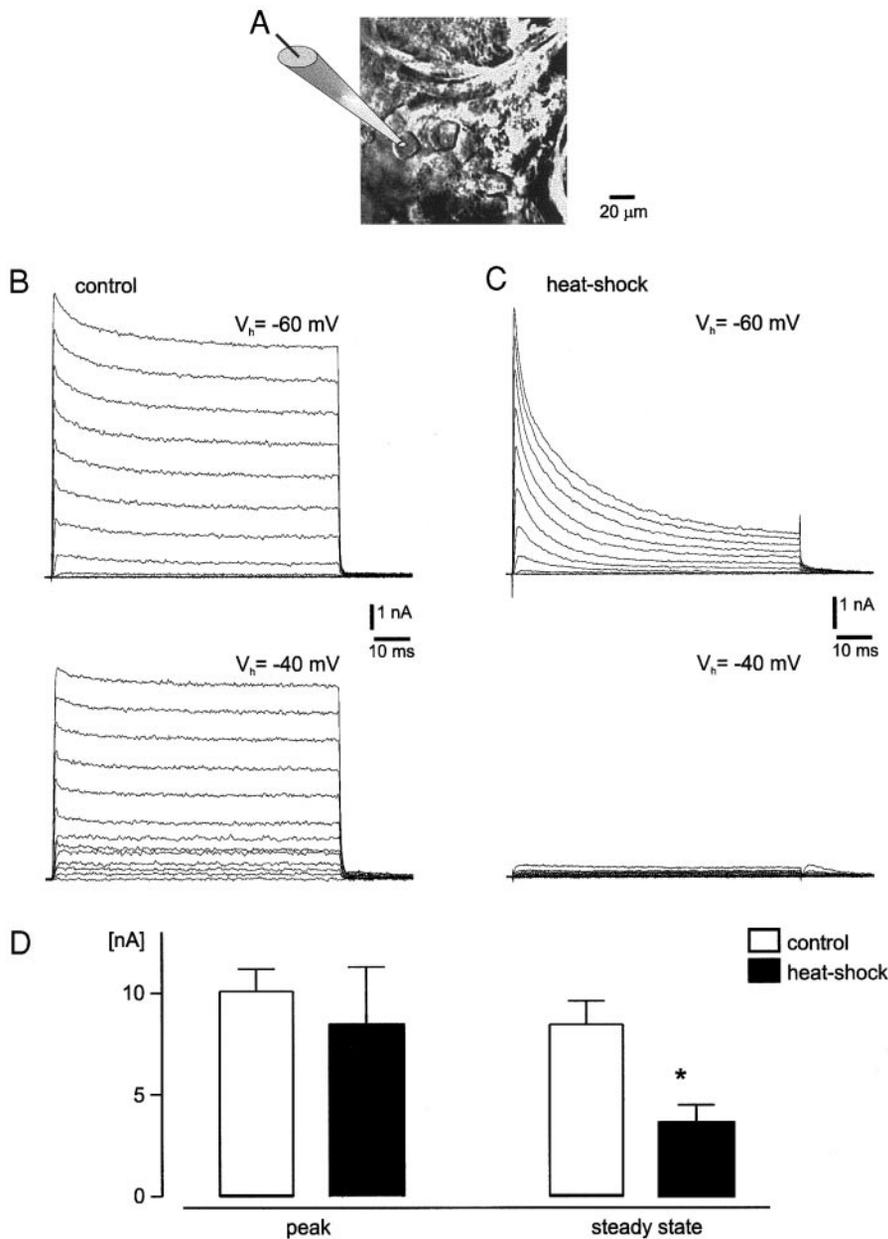


FIG. 3. Voltage-clamp recording from locust neurons (A) in slices obtained from control (B) and heat-shocked animals (C). A: neurons visualized with infrared-Normarski optics. All recordings in this study were obtained from the ventral midline of the A1 metathoracic neuromere. B: recordings were obtained from a neuron of a control animal. Current traces in response to the same voltage protocol (as described in the text) at different holding potentials (-60 mV, top panel; -40 mV, bottom panel). C: recordings were obtained from a neuron of a heat-shocked animal. Current traces in response to the same voltage protocol as shown in B. D: histograms indicating that the peak current was not significantly different between control and heat-shocked animals (left panel), whereas the steady state was significantly different between the two groups of animals (right panel).

the locusts that were heat shocked showed no evidence of neural or behavioral impairment and were alert, walking, and jumping, apparently normally, before dissection. Perhaps these alterations in potassium conductances become behaviorally significant only at higher temperatures. The heat shock treatment as used in this study induced thermotolerance that permits survival of intact animals at temperatures well in excess of 41°C . This increased thermotolerance was similar to thermotolerance shown after the same treatment of a different species of locust (Robertson et al. 1996). Thus the effect on K^{+} conductance as described in this study was evident in slices from thermally protected animals that showed no signs of damage. Whether this alteration in K^{+} conductances might contribute to an increased heat tolerance is another interesting issue that needs further investigation. It is interesting to note, however, that the protective effects on motor pattern generation can be at least partially explained by alterations in the temperature sensitivity of synaptic parameters (Dawson-Scully

and Robertson 1998); the amplitude of postsynaptic potentials reduces less with increases in temperature for heat-shocked animals compared with controls. In parallel with this, intracellularly recorded action potentials are longer in duration and tend to lack an afterhyperpolarization (Wu and Robertson, unpublished observations). With the caveat that these observations were of the inexcitable membranes of cell somata, it is nevertheless a testable hypothesis that the effects on potassium conductances described here result in action potentials with increased durations and protected synaptic transmission thus protecting flight motor patterns. It remains to be determined whether the properties of potassium conductances in excitable membranes are similar to the properties of those described here. In addition to any possible effect on neural signaling the alterations of potassium conductances could have more general beneficial consequences for the health of the animal at stressful temperatures.

A change in voltage-gated K^{+} currents was previously de-

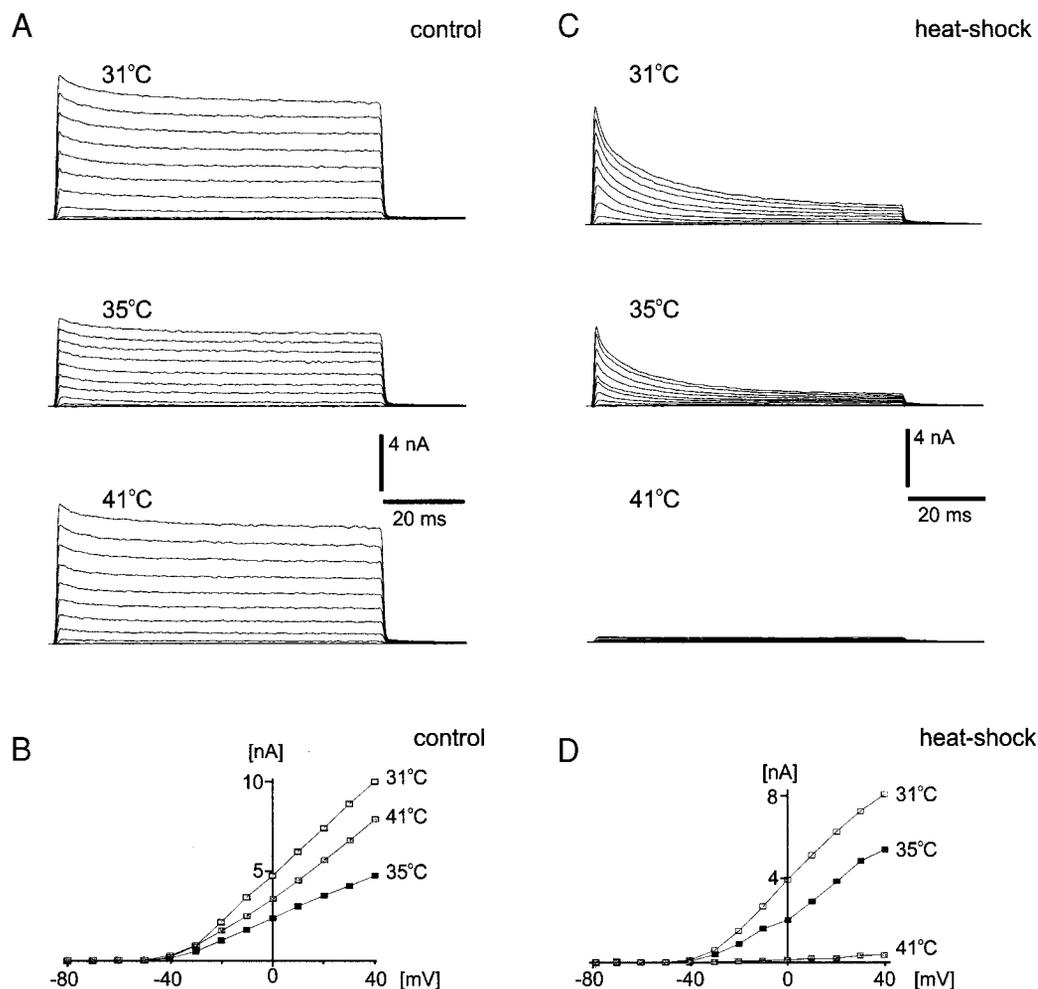


FIG. 4. Heat-shock-induced alterations in the response to temperature changes. *A*: 3 current traces were obtained from a neuron of a control animal in response to the same voltage protocol (as described in the text) at 31, 35, and 41°C. The peak values of these current traces were used to obtain the *I-V* relationship as shown in *B*. *C*: 3 current traces were obtained from a neuron of a heat-shocked animal in response to the same voltage protocol as shown in *A*. The peak values of these currents were used to obtain the *I-V* relationship in *D*.

scribed in various vertebrate and invertebrate neuronal systems. Most commonly such changes were associated with a developmental change. For example only a noninactivating delayed rectifier current was observed in rat embryonic sympathetic neurons; a fast transient current appeared later during postnatal development (Nerbonne et al. 1986). A differential development of a fast-transient and noninactivating delayed rectifier current was also described for tadpole spinal neurons (Ribera and Spitzer 1992). This developmental change was correlated with the temporal regulation of different potassium channel genes (Gurantz et al. 1996). In *Drosophila* flight muscles a fast transient K^+ current appears during development before a delayed rectifier K^+ current (Salkoff and Wyman 1981). This change may be due to a change in calcium sensitivity (Salkoff 1983).

A temperature dependency in the expression of voltage-gated K^+ currents was previously described in insects. In *Drosophila* the relationship between a delayed sustained and a rapidly inactivating current depends on the developmental temperature (Chopra and Singh 1994). A more rapidly occurring alteration in voltage-gated K^+ conductances was demonstrated in locust photoreceptors. These neurons express a delayed

rectifier during the day and an inactivating K^+ conductance during the night (Weckström and Laughlin 1995). These authors suggest that the inactivating conductance is metabolically less demanding, which might be advantageous during the night when the increased dynamic range and frequency response imparted by the sustained current would be unnecessary. In fact, our finding can similarly be interpreted, and the switch we observed in K^+ conductance could be a part of a coordinated physiological response to heat shock, which results in a reduced K^+ outward current. This could protect the locust from a detrimental increase of extracellular K^+ during high temperature exposure. Clearly, this is only one possible interpretation of our finding, and further investigations will be necessary to examine the physiological consequences of the observed alteration in K^+ conductances.

The mechanism that caused the modification of K^+ conductances remains to be determined. This alteration could be due to a direct or indirect effect of heat shock proteins on potassium channels. If this is the case we would expect that HSP70 antibodies might block the observed channel modification by reducing levels of available HSP70. Some support for this hypothesis is provided by previous studies that have described

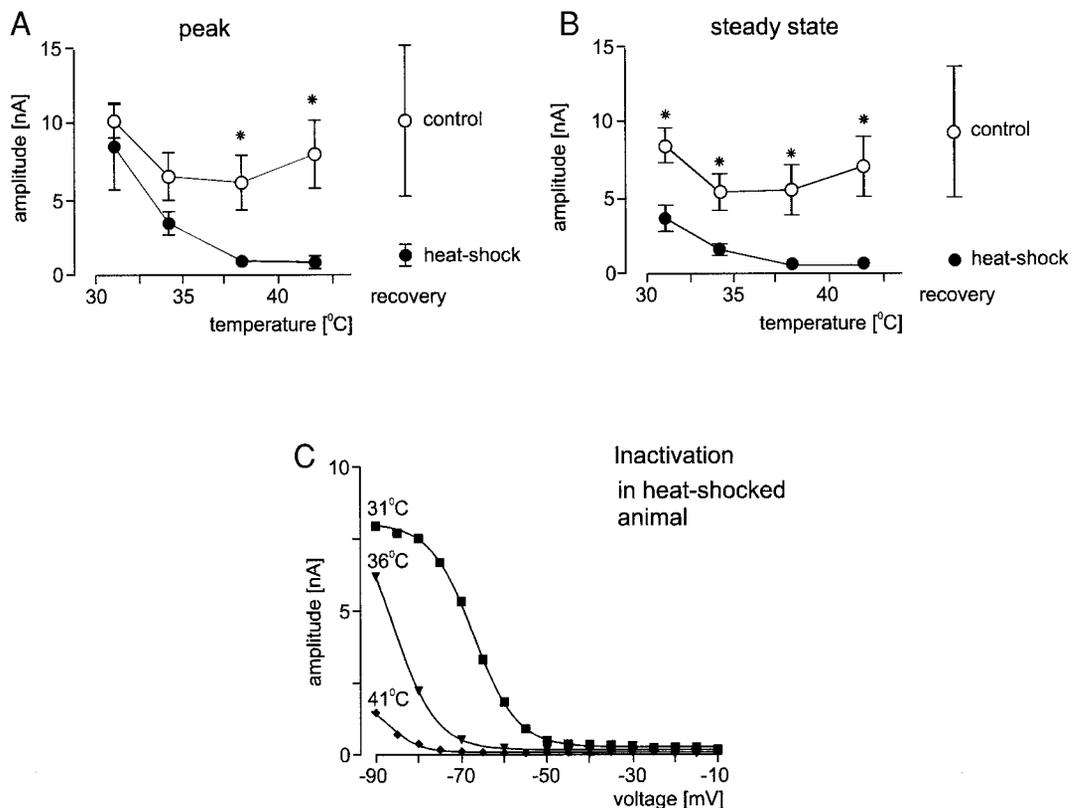


FIG. 5. Heat-shock-induced alterations in the temperature-dependent properties of potassium outward currents. Average peak (A) and steady state amplitude (B) of 5 control (○) and 5 heat-shocked animals (●). *Statistical significance between neuronal responses obtained from control and heatshocked animals. C: inactivation property of a neuron obtained from a heat-shocked animal was shifted at higher temperatures, which can explain the decreased outward current amplitude in neurons obtained from heat-shocked animals.

effects of exogenous application of HSP70 on neuronal calcium flux (Smith 1995) and on calcium-dependent potassium channels in a human promonocyte culture (Negulyaev et al. 1996). Alternatively, the described alterations could also be due to a heat shock-induced release of neuromodulators, which then alters the expression of different types of potassium currents. For locust photoreceptors the diurnal switch in potassium currents was attributed to the release of serotonin (Cuttle et al. 1995). This possibility could be examined with selective antagonists for serotonin. By using the ganglion slice preparation that we describe here, it is possible not only to test these working hypotheses but also to examine how heat shock can condition and protect the operation of neuronal circuitry underlying motor pattern generation in this model system. We predict that the effects of heat shock on neuronal properties we describe are not restricted to locusts but may be also evident in other invertebrates and vertebrates.

Although there are many interesting issues raised by our findings that remain unresolved, we clearly demonstrated that heat shock has long-term effects on neuronal properties. This important aspect of heat shock so far received only very little attention in the literature. The effect we describe is likely to have important consequences for the signaling function and/or the metabolic well-being of neurons in their extracellular environment. Another important outcome of this study is that we demonstrated that the sliced ganglion approach has great utility for the study of the cellular properties of neurons in functional circuits in the locust. We anticipate that such an approach

could easily be modified for use with smaller insects (e.g., *Drosophila*) and other invertebrates (Ermentrout et al. 1998). This would avoid the current necessity to perform detailed cellular investigations in cell cultures containing dissociated and functionally isolated neurons.

This work was in part supported by an award to the University of Chicago's Division of Biological Sciences under the Research Resources Program for Medical Schools of the Howard Hughes Medical Institute to J. M. Ramirez and National Sciences and Engineering Research Council of Canada to R. M. Robertson.

Address for reprint requests: J.-M. Ramirez, University of Chicago, OBA, 1027 East 57th St., Chicago, IL 60637.

Received 27 April 1998; accepted in final form 14 October 1998.

REFERENCES

- BALDAIA, L., MAISONHAUTE, C., PORCHERON, P., AND BEST-BELPOMME, M. Effect of heat shock on protein synthesis in *Locusta migratoria* epidermis. *Arch. Insect Biochem. Physiol.* 4: 225–231, 1987.
- BUCHNER, J. Supervising the fold: functional principles of molecular chaperones. *FASEB J.* 10: 10–19, 1996.
- CHOPRA, M. AND SINGH, S. Developmental temperature selectively regulates a voltage-activated potassium current in *Drosophila*. *J. Neurobiol.* 25: 119–126, 1994.
- COLEMAN, J. S., HECKATHORN, S. A., AND HALLBERG, R. L. Heat shock proteins and thermotolerance: linking molecular and ecological perspectives. *Trends Ecol. Evol.* 10: 305–306, 1995.
- CRAIG, E. A., GAMBILL, B. D., AND NELSON, R. J. Heat shock protein: molecular chaperones of protein biogenesis. *Microbiol. Rev.* 57: 402–414, 1993.

- CUTTLE, M. F., HEVERS, W., LAUGHLIN, S. B., AND HARDIE, S. C. Diurnal modulation of photoreceptor potassium conductance in the locust. *J. Comp. Physiol. [A]* 176: 307–316, 1995.
- DAWSON-SCULLY, K. D. AND ROBERTSON, R. M. Heat shock protects synaptic transmission in flight motor circuitry of locusts. *NeuroReport* 9: 2589–2593, 1998.
- ERMENROUT, B., FLORES, J., AND GELPERIN, A. Minimal model of oscillations and waves in the *Limax* olfactory lobe with tests of the model's predictive power. *J. Neurophysiol.* 79: 2677–2689, 1998.
- FEDER, M. E. Ecological and evolutionary physiology of stress proteins and the stress response. In: *Animals and Temperature*, edited by I. A. Johnston and A. F. Bennet. Cambridge, UK: Cambridge Univ. Press, 1996, p. 79–102.
- FEDER, M. E. AND KREBS, R. A. Natural and genetic engineering of the heat shock protein Hsp70 in *Drosophila melanogaster*: consequences for thermotolerance. *Am. Zool.* 38: 503–517, 1998.
- GEHRING, W. J. AND WEHNER, R. Heat shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara desert. *Proc. Natl. Acad. Sci. USA* 92: 2994–2998, 1995.
- GRAY, J. R. AND ROBERTSON, R. M. Effects of heat stress on axonal conduction in the locust flight system. *Comp. Biochem. Physiol.* 120A: 181–186, 1998.
- GURANTZ, D., RIBERA, A. B., AND SPITZER, N. C. Temporal regulation of Shaker- and Shab-like potassium channel gene expression in single embryonic spinal neurons during K⁺ current development. *J. Neurosci.* 16: 3287–3295, 1996.
- HOFMANN, G. E. AND SOMERO, G. N. Interspecific variation in thermal denaturation of proteins in the congeneric mussels *Mytilus trossulus* and *M. galloprovincialis*: evidence from the heat shock response and protein ubiquitination. *Mar. Biol.* 126: 65–75, 1996.
- LINDQUIST, S. AND CRAIG, E. A. The heat shock proteins. *Ann. Rev. Genet.* 22: 631–677, 1988.
- MAYER, R. J. AND BROWN, I. R. *Heat Shock Proteins in the Nervous System*. London: Academic, 1994.
- NEGULYAEV, Y. A., VEDERNIKOVA, E. A., KINEV, A. V., AND VORONIN, A. P. Exogenous heat shock protein hsp70 activates potassium channels in U937 cells. *Biochim. Biophys. Acta. Bio. Membr.* 1282: 156–162, 1996.
- NERBONNE, J. M., GURNEY, A. M., AND RAYBURN, H. B. Development of the fast, transient outward K⁺ current in embryonic sympathetic neurones. *Brain Res.* 378: 197–202, 1986.
- NORRIS, C. E., DIORIO, P. J., SCHULTZ, R. J., AND HIGHTOWER, L. E. Variation in heat shock proteins within tropical and desert species of poeciliid fishes. *Mol. Biol. Evol.* 12: 1048–1062, 1995.
- OLAND, L. A., MÜLLER, T., KETTENMANN, H., AND HAYASHI, J. H. Preparation of primary cultures and acute slices of the nervous system of the moth *Manduca sexta*. *J. Neurosci. Methods* 69: 103–112, 1996.
- RAMIREZ, J. M. AND PEARSON, K. G. Distribution of intersegmental interneurons that can reset the respiratory rhythm of the locust. *J. Exp. Biol.* 141: 151–176, 1989.
- RAMIREZ, J. M. AND PEARSON, K. G. Octopamine induces bursting and plateau potentials in insect neurones. *Brain Res.* 549: 332–337, 1991a.
- RAMIREZ, J. M. AND PEARSON, K. G. Octopaminergic modulation of interneurons in the flight system of the locust. *J. Neurophysiol.* 66: 1522–1537, 1991b.
- RAMIREZ, J. M., QUELLMALZ, U. J. A., AND RICHTER, D. W. Postnatal changes in the mammalian respiratory network as revealed by the transverse brainstem slice preparation of mice. *J. Physiol. (Lond.)* 491: 799–812, 1996.
- RIBERA, A. B. AND SPITZER, N. C. Developmental regulation of potassium channels and the impact on neuronal differentiation. *Ion Channels* 3: 1–38, 1992.
- ROBERTSON, R. M., XU, H., SHOEMAKER, K. L., AND DAWSON-SCULLY, K. Exposure to heat shock affects thermosensitivity of the locust flight system. *J. Neurobiol.* 29: 367–383, 1996.
- SMITH, P. J. S. AND TYTELL, M. Effects of exogenous heat shock protein (hsp70) on neuronal calcium flux. *Biol. Bull.* 189: 209–210, 1995.
- SALKOFF, L. *Drosophila* mutants reveal two components of fast outward current. *Nature* 302: 249–251, 1983.
- SALKOFF, L. AND WYMAN, R. Outward currents in developing *Drosophila* flight muscle. *Science* 212: 461–463, 1981.
- SOMBATI, S. AND HOYLE, G. Generation of specific behaviours in a locust by local release into neuropil of the natural neuromodulator octopamine. *J. Neurobiol.* 15: 481–506, 1984.
- STEVENSON, P. A. AND KUTSCH, W. A reconsideration of the central pattern generator concept for locust flight. *J. Comp. Physiol. [A]* 161: 115–129, 1987.
- ULMASOV, K. A., SHAMMAKOV, S., KARAEV, K., AND EVGEN'EV, M. B. Heat shock proteins and thermoresistance in lizards. *Proc. Natl. Acad. Sci. USA* 89: 1666–1670, 1992.
- UVAROV, B. *Grasshoppers and Locusts. Anatomy, Physiology, Development, Phase Polymorphism, Introduction to Taxonomy*. London, UK: Cambridge Univ. Press, 1966.
- WEIS-FOGH, T. Biology and physics of locust flight. II. Flight performance of the desert locust (*Schistocerca gregaria*). *Philos. Trans. R. Soc. Lond. [Biol]* 239: 459–510, 1956.
- WECKSTRÖM, M. AND LAUGHLIN, S. B. Visual ecology and voltage-gated ion channels in insect photoreceptors. *Trends Neurosci.* 18 (1): 17–21, 1995.
- WHYARD, S., WYATT, G. R., AND WALKER, V. K. The heat shock response in *Locusta migratoria*. *J. Comp. Physiol. [A]* B156: 813–817, 1986.
- WOLF, H., RONACHER, B., AND REICHERT, H. Patterned synaptic drive to locust flight motoneurons after hemisection of thoracic ganglia. *J. Comp. Physiol. [A]* 163: 761–769, 1988.