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A role for octopamine in coordinating thermoprotection of an insect nervous system

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Abstract

Neural function is susceptible to failure under heat stress but protective measures taken by the central nervous system (CNS) in particular organisms offer a level of protection that allows neural function to be maintained at higher temperatures. We use the migratory desert locust (*Locusta migratoria*), an animal naturally exposed to harsh conditions daily, as a model organism to examine the adaptive physiological mechanisms evolved to protect nervous systems at high temperatures.

The biogenic amine octopamine (OA) generates an arousal response in insects similar to norepinephrine in vertebrates and is released in response to heat stress. It is a known modulator of many behaviours, including walking, flying, and jumping, along with crucial motor patterns for escape and ventilation. OA also regulates physiological processes which include K^+ conductances, Na^+/K^+ ATPase, and possibly the transcription of particular genes. We describe behavioural and physiological modifications that share similarities between OA and heat shock-treated locusts. Notably, there was an increase in failure temperatures and quicker recovery times following hyperthermic failure of the ventilatory neuronal circuit. Furthermore faster extracellular potassium clearance rates of OA-treated locusts corroborate our current model of hyperthermic neural failure and indicate a role for OA in thermoprotection of neural function.

Keywords: Heat shock; Hyperthermia; Octopamine; Neural circuit; Motor pattern generation; Potassium; Na^+/K^+ ATPase; Insect

1. Introduction

The ability of cells and tissues to withstand hyperthermic insults is critical for organismal survival. Animals have evolved physiological mechanisms to cope with fluctuations in body temperature whether this results from environmental alterations, overproduction of metabolic heat or fever associated with infection. Poikilotherms, which do not possess the ability to regulate their internal temperature except by behavioural means, consequently receive daily stresses from ambient temperature fluctuations. These organisms thus provide ideal models to examine the mechanisms by which evolution has adapted physiological processes to cope with extremes in temperatures. Changes occur at the level of the cell and are transiently activated. In the laboratory these changes can be brought on in the locust (*Locusta migratoria*) by conditioning animals with a prolonged sub-lethal thermal stress, heat shock (HS) treatment which entails 3 h at 45 °C followed by a 1 h recovery at room temperature. This allows cells, and indeed whole organisms, to survive stressful situations which would otherwise be lethal.

Adaptive physiological changes occur in the central nervous system (CNS) after hyperthermic stress. This is perhaps because, long before individual cells die, any compromise of the neural circuitry coordinating locomotor behaviours (e.g. jumping, walking or flying) or vital motor patterns (e.g. ventilation and escape) would be strongly selected against. Thus the CNS is an ideal area to examine physiological mechanisms of thermoprotection. Clearly other tissues show HS responses important for survival but CNS function is critical and has been repeatedly shown to have a strong response to hyperthermic stress.

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The thermal operating range of particular neuronal circuits can be extended by as much as 7–10 °C in locusts after HS treatment (Robertson et al., 1996; Barclay and Robertson, 2000) and after cold and anoxia stresses (Newman et al., 2003). An important question is: what are the mechanisms for coordinating different tissues (e.g. muscles used for escape behaviours, or neuronal circuits for escape and ventilation) for an effective protective response at the level of behaviour? It is well established that individual cells and organisms develop a HS response as a result of stress or disease (Morimoto and Santoro, 1998); however, little attention has been given to possible mechanisms for coordinating tissue-specific responses to hyperthermic stress. In invertebrates bioactive substances that can account for diverse coordinated changes in physiology are biogenic amines, particularly but not solely octopamine OA. OA's scope of function is incredibly diverse: it acts as a neurotransmitter (Nathanson, 1979), neuromodulator (Leitch et al., 2003) and neurohormone (Orchard et al., 1982), and is widely accepted to activate a flight or fight response in invertebrates, similar to that of norepinephrine in vertebrates (Hoyle, 1975; Evans, 1985; Roeder, 1999, 2005).

A wealth of literature exists on the functional significance of OA in locusts; in fact a large portion of the OA literature is based upon or derived from studies on the migratory locust (*Locusta migratoria*) or the gregarious African desert locust (*Schistocerca gregaria*). OA modulates many physiological processes. For example it regulates fuel selection during flight, coordinating a change from carbohydrate catabolism to lipid oxidation that is important for long distance flight (Mentel et al., 2003). OA concentrations in the haemolymph increase by a factor of 5 during the initial 10 min of flight (Goosey and Candy, 1980) and this is similar to the increase in circulating concentrations after heat stress (Davenport and Evans, 1984). Peripherally OA increases wing muscle performance during flight (Malamud et al., 1988), and in the hind leg it increases the amplitude of muscle contractions and decreases the relaxation rate (Evans and Myers, 1986; O'Shea and Evans, 1997), essential for effective escape behaviours. In the nervous system OA dishabituates the visual descending contralateral movement detector (DCMD) interneuron from repeated stimuli vital for predator detection (Bacon et al., 1995), and modulates central pattern generators (CPGs) for foregut activity, walking, flying and ventilation (Zilberstein et al., 2004; Sombati and Hoyle, 1984; Stevenson and Kutsch, 1986). OA immunoreactive neurons in the nerve cord appear to possess only peripheral release sites, although there have been reports of central release sites (Watson, 1984). Here we review recent research concerning thermoprotection of the insect nervous system and attempt to integrate it with a possible role OA may play in mitigating thermal stress through behavioural and cellular changes.

2. Similarities between OA modulation and heat shock-induced thermoprotection

2.1. Predator detection and escape

Visually-mediated escape circuitry in the locust generally consists of two parts: first, detection of the predatory strike via the processing of looming stimuli by the lobula giant movement detector (LGMD) interneuron and DCMD (Bacon et al., 1995; Gabbiani et al., 1999, 2002; Gray et al., 2001). Other interneurons such as giant interneurons (GIs) process other modalities e.g. mechanosensory cerci, filiform hairs (Evan and Blagburn, 2001; Goldstein and Camhi, 1991; Widmer et al., 2005). The second part is the activation of neural circuitry for the motor components of escape such as jumping or flying by input from the DCMDs (Burrows and Rowell, 1973) or GIs (Evan and Blagburn, 2001).

After receiving a HS treatment DCMD shows axonal properties that can be characterized as thermoprotective. These include maintained action potential bursting at high temperatures, faster responses to visual looming stimuli, increased membrane excitability and increased action potential amplitude at high temperatures (Money et al., 2005). These changes allow the locust's circuitry for escape to remain functional at high temperatures.

OA neuromodulation results in changes that are similar to the effects of HS. OA application has a general excitatory effect on the responses of mechanosensory cerci, GIs, and filiform hair cells (Evan and Blagburn, 2001; Goldstein and Camhi, 1991; Widmer et al., 2005) sharpening the locusts ability to escape predation. In the visual system of the locust, immunoreactive protocerebral-medulla 4 neurons projecting to the ipsilateral optic lobe have been identified as the source of OA (Stern et al., 1995). Octopamine receptors (OARs) in the visual system are at least three times as dense as in other parts of the CNS (Roeder and Nathanson, 1993). DCMD octopaminergic modulation decreases habituation to repeated stimuli (Bacon et al., 1995), enhancing repeated predator detection. The same observation is apparent after HS treatment in locusts (Money and Robertson, unpublished data) suggesting that OA has been released onto DCMD. Furthermore, OA application increases field potentials of the lobula of honeybees in response to stimuli (Kloppenburg and Erber, 1995), similar to the hyperexcitability of this neural circuit at high temperatures. Haemolymph OA concentrations in the head of the locust increase by 317% after 15 min of stress (Davenport and Evans, 1984). No research group has examined the changes in OA released following HS treatment. However, if more OA is released or its effects are potentiated after HS treatment, this would easily account for the changes in physiology of the visual circuitry of the locust.

The fast extensor tibiae muscle of the locust is responsible for the extension of the hind leg during an escape jump. It is not surprising therefore that it shows adaptive physiological changes after HS that protect the neuromuscular synapse at high temperatures (review: Klose and Robertson, 2004). HS pre-treatment extends

the operating range of synaptic transmission (51 °C after HS vs. 45 °C for controls) and stabilizes excitatory junction potential (EJP) duration and amplitude at high temperatures (Barclay and Robertson, 2000). Recovery of synaptic function after a heat-induced failure is markedly quicker in HS animals (Klose and Robertson, 2004) allowing the circuit to regain function faster after transient debilitating exposures to heat. These physiological changes in temperature thresholds serve as an adaptive trait increasing the chances of survival. As in the visual system of the locust the hind leg is modulated by OA. Haemolymph samples taken from the leg show an increase in OA concentrations (Davenport and Evans, 1984) after stress (7.5–15.5 pg/μL). Changes in thermal operating range could be as a result of differential regulation of OA release in HS-treated animals.

OA-immunoreactive dorsal unpaired median (DUM) neurons and ventral unpaired median (VUM) neurons are situated in several ganglia of the nerve cord (subesophageal ganglia: Bräunig and Burrows, 2004; prothoracic and mesothoracic ganglia: Duch et al., 1999; metathoracic ganglion: Stevenson and Spörhase-Eichmann, 1995). These are the sources of OA for the hind leg; specifically the neurosecretory DUMETi neuron in the metathoracic ganglion (O'Shea and Evans, 1979), which can be identified electrophysiologically by the long duration action potentials (typical of neurosecretory cells), an excitable soma, and a prominent after-hyperpolarisation (Hoyle and Dagan, 1978; Baudoux and Burrows, 1998). OARs have been pharmacologically classified in the hind leg based upon agonist and antagonist binding efficacies and coupling to second messenger pathways. The class 1 receptor (OAR1) expressed in the myogenic bundle is coupled to the IP_3-system which modulates $[Ca^{2+}]$. OAR2a and OAR2b receptors are both coupled to adenylyl cyclase, but differ in that OAR2a modulates transmitter release at the neuromuscular junction of the extensor–tibiae muscle, whereas OAR2b modulates the relaxation rate of twitch tension (Evans, 1981, 1984a,b). Octopaminergic modulation of the neuromuscular junction via OAR2a could be described as a protective mechanism that potentates transmission at high temperatures across the synapse; a site of thermal weakness (Hochachka and Somero, 2002). OAR2b excitation readies the hind leg faster during successive jumps, but this adaptation may have little relevance at high temperatures when relaxation rates are already quicker. Conversely during hypothermic conditions (e.g. at night) which generally decrease muscle relaxation rates because of the slower removal of Ca^{2+} from the sarcomere, modulation by this receptor may provide an adaptive benefit by allowing the locust to make repetitive jumps.

2.2. Ventilation in locusts as a model for examining thermoprotection

Although thermoprotection of neurons and muscle controlling escape from predators is important, the ability to continually ventilate is fundamental for survival. During energy-intensive situations, when animals need a consistent supply of oxygen, ventilatory circuits need to function properly to maintain the transfer of respiratory gasses. This is compounded at extremes in temperature when relative metabolic changes of poikilotherms are much larger than those of homoiotherms which are less at the mercy of temperature fluctuations. The necessity for proper neural circuit function at elevated temperatures has been driven by natural selection. We have shown that exposure to various stresses (e.g. heat, cold or anoxia/hypoxia) results in a subsequent acquired tolerance to heat stress. Locusts (*L. migratoria*) which have received a HS treatment are able to recover function of the ventilatory CPG faster (60 vs. 140 s; Fig. 1) after a subsequent heat-induced failure than unconditioned animals (Newman et al., 2003; Armstrong and Robertson, 2005). Furthermore the temperature at which this circuit can remain functional is higher in heat-shocked animals (Armstrong and Robertson, 2005; Fig. 1).

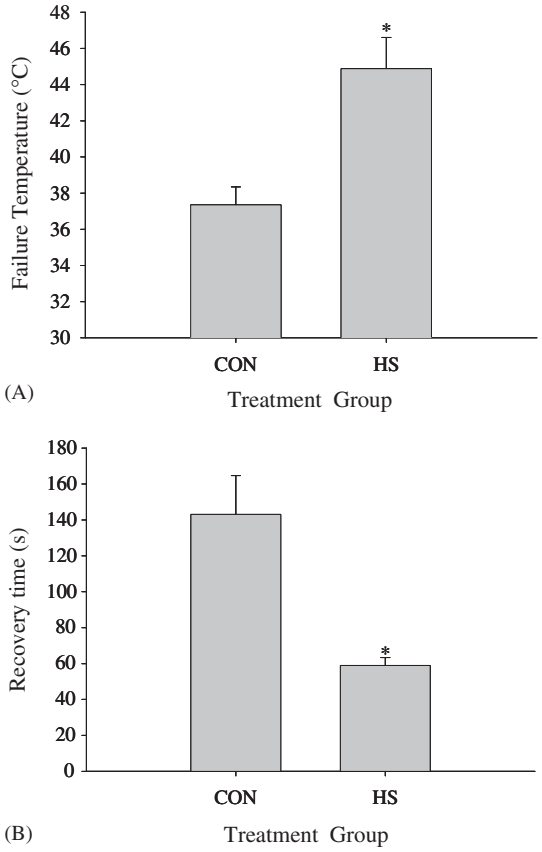

Fig. 1. Mean ± standard error of failure temperature (A) and recovery time (B) of ventilatory motor pattern during a temperature ramp (5 °C/min). CPG was monitored using an EMG electrode positioned on abdominal muscle 161. Locusts in the HS treatment group received 3 h at 45 °C and 1 h recovery at room temperature (~25 °C), before dissection and exposure to the temperature ramp. Control locusts (CON), were left at room temperature for 4 h. * indicate significant differences ($p < 0.05$, *t*-test). Data from Armstrong and Robertson (2004).

In the locust, the CPG controlling abdominal pumping (ventilation) is situated in the metathoracic ganglion (review: Burrows, 1996). If injected with current, interneurons in the CPG can reset the timing of the rhythm (Ramirez and Pearson, 1989a). In the intact unstressed locust, ventilatory motor patterns are erratic with periods of no abdominal movements lasting for minutes, giving the name discontinuous ventilation (review: Lighton, 1994). As the thoracic temperature of a locust is raised, the frequency of rhythmical contraction increases from about 1 Hz to around 3 Hz (Fig. 2). This supplies tissues whose metabolic demands are higher with more oxygen. Moreover, fast ventilation promotes evaporative cooling by increasing the volume of gas passing through the tracheal system (Prange, 1990). Increases in ventilatory frequency at high temperatures arise from several temperature-dependent parameters of synaptic and CPG function. Firstly, the kinetics of ion channels/pumps are temperature sensitive and a rise in temperature increases the open probability of these channels/pumps. As a result of increased open probabilities, the conduction velocities of action potentials increase (Montgomery and Macdonald, 1990). Secondly, the rate of synaptic transmission increases, in part due to an increase in the rate of cycling of neurotransmitter release and reuptake. A third reason for changes in frequency is the excitatory response to neuromodulators. The circulating concentration of OA in locust haemolymph increases over two-fold after a 15 min heat stress (Davenport and Evans, 1984). OA is a known neuromodulator of ventilation increasing its frequency in the dobson fly (*Corydalus cornutus*) and locust (Fig. 3; Bellah et al., 1984; Sombati and Hoyle, 1984; Ramirez and Pearson, 1989b; Armstrong and Robertson, 2004). Recently, work from our lab has shown that OA application thermoprotects the neural circuit for ventilation (Armstrong and Robertson, 2005). There are several possible explanations for OA's role in protecting the CNS from heat stress. Firstly, OA modulation of the rhythm may promote evaporative cooling. Secondly, OA may elicit its thermoprotective effects by augmenting synaptic potentials, thereby increasing the scope for synaptic transmission to accommodate a larger thermal dose (Gerner, 1987). Both of these possibilities appear not to be the case, because short 20 min bath applications do not protect the circuit from heat stress. However, exposures of OA for 1 h or longer significantly protect the ventilatory CPG from heat stress (Fig. 4; Armstrong and Robertson, 2005). This suggests that a time-dependent process is taking place that generates a thermoprotected insect nervous system.

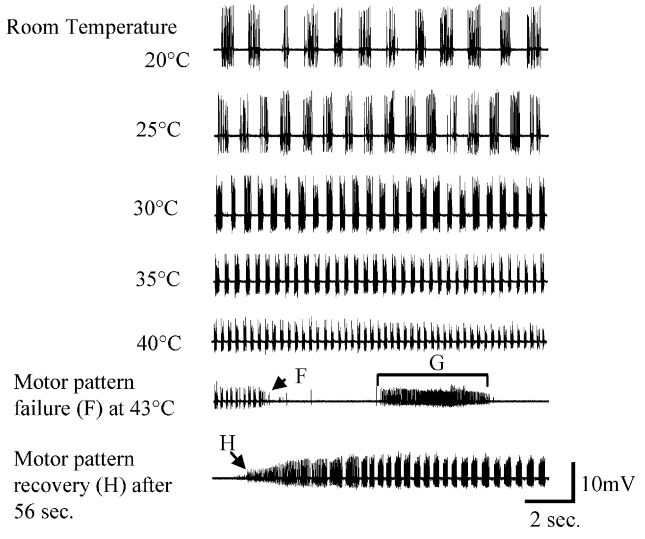

Fig. 2. EMG recordings (abdominal muscle 161) of a locust ventilatory motor pattern during a temperature ramp (5 °C/min). Frequency of contractions triples until failure of motor pattern (F), followed by a burst of electrical activity (G) and its subsequent recovery as temperature is returned to ambient levels (H). Data from Armstrong and Robertson (2005).

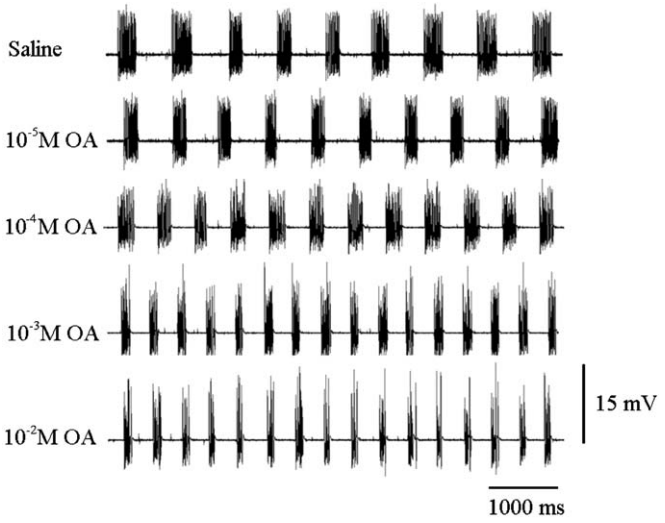

Fig. 3. Exogenous bath application of octopamine (OA) to a semi-intact locust increases ventilatory motor pattern frequency from 1 Hz to about 2 Hz. Nerve 5 of the metathoracic ganglion was cut to allow octopamine to flow into the central nervous system. OA concentrations were successively applied starting with a 10 min bath of standard locust saline (Saline), followed by successive 20 min baths of 10^{-5} M OA, 10^{-4} M OA, 10^{-3} M OA, and 10^{-2} M OA. Data from Armstrong and Robertson (2004).

3. The mechanism of heat-induced CNS dysfunction

To understand the thermoprotective mechanisms that are employed by the insect nervous system, one must first examine the events that lead up to CNS dysfunction. As a consequence of increased temperature increased neural activity results in a rise in $[K^+]_o$. Potassium clearance by glia and reuptake by neurons must concurrently be modulated to maintain the ionic equilibria necessary for neural function at high temperatures, when activity of the CNS is greater. It is possible that K^+ regulatory mechanisms are the first line of defense against thermal stress.

There is a tight correlation between hyperthermic failure of the CNS and a rise in extracellular potassium $[K^+]_o$ and its subsequent restoration during recovery when

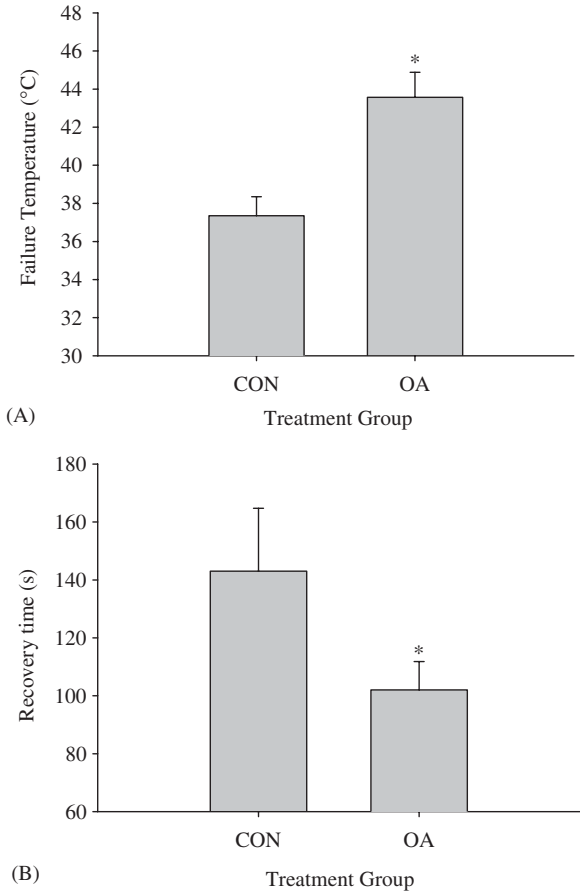

Fig. 4. Mean ± standard error of failure temperature (A) and recovery time (B) of ventilatory motor pattern during a temperature ramp (5 °C/min). CPG was monitored using an EMG electrode positioned on abdominal muscle 161. Locusts in the OA treatment group received a 1 h bath application of octopamine (1×10^{-4} M in locust saline) before temperature ramp. OA treated animals are thermoprotected at high temperatures and recover CNS function faster then controls (CON). Asterisk indicates significant differences ($p < 0.05$, t-test) from CON treatment group which receive a 1 h bath application of standard locust saline. Data from Armstrong and Robertson (2005).

temperature is reduced (Robertson, 2004). Similar results have been observed in rat hippocampal slices after a rise in temperature (Wu and Fisher, 2000). This observation bears striking resemblance to a poorly understood phenomenon observed in vertebrate gray matter which is the generation of waves of slowly propagating depolarization across the neocortex. This is referred to as spreading depression (SD) and similar events can be induced by hypoxia (hypoxic spreading depression-like depolarizations (HSD)) (review: Somjen, 2001, 2002). These share characteristics with the rise in $[K^+]_o$ observed in locust neuropile at thermal failure although the catastrophic rise in $[K^+]_o$ during SD and HSD may have different initiation mechanisms underlying the redistribution of ions across the plasma membrane (Somjen, 2001). In HSD a slow rise in $[K^+]_o$, presumably as a result of Na^+/K^+ ATPase dysfunction due to hypoxia-induced energy limitations, is followed by the true HSD wave across the afflicted hypoxic/ischemic region of neocortex. In normoxic SD conditions, however, the rise in $[K^+]_o$ is abrupt and not preceded by the slow rise in $[K^+]_o$ (Somjen, 2001; Vaillend et al., 2002). This difference highlights a compounding physiological change in energy metabolism in poikilotherms at high temperatures, where an excited nervous system may outstrip the ability of mitochondria to produce the ATP necessary to maintain ionic homeostatic balances (Pörtner, 2002).

We have shown previously that blocking K^+ channels with TEA and bath application with serotonin can increase the thermal operating range of neurons (Wu et al., 2002; Newman et al., 2003). Presumably reduced activity of K^+ channels attenuates the build-up of $[K^+]_o$, thus conferring a level of thermoprotection. Not surprisingly, after HS treatment, recordings from neuronal somata in ganglion slices have revealed a reduction in whole cell K^+ conductance (Ramirez et al., 1999). This suggests that regulation of K^+ channels (reducing K^+ conductance) is a mechanism which neurons employ to protect their CNS at environmental extremes (review: Robertson, 2004).

4. The physiology of octopamine-mediated thermoprotection

4.1. The neural octopamine receptor and its signaling pathways

Along with the three OARs mentioned above (OAR1, OAR2a, and OAR2b) a fourth OA receptor can be distinguished, OAR3, found in the locust CNS. This is the receptor responsible for modulating the ventilatory rhythm, and protecting the CNS from heat stress. In general OA receptors belong to the rhodopsin-like G protein-coupled receptor family (see Palczewski et al., 2000 for structural characteristics of this family of receptors) sharing similarities with the vertebrate α-adrenergic receptor (Evans, 1981). They are characterized by possession of seven transmembrane spanning regions forming a pocket on the extracellular side for ligand binding. Upon binding, a conformational change occurs in the receptor, which in turn is transferred to the heterotrimeric G protein. Several G-proteins exist which elicit different cellular cascades (review: Blenau and Baumann, 2001). The OAR3 is coupled to a stimulatory G protein (G_s) requiring GTP in order to disassociate from the $\lambda\beta$ subunit. Once removed the α_s subunit is free to interact with adenylyl cyclase (Roeder and Gewecke, 1990; Roeder, 1992, 1995), stimulating the production of $[cAMP]_i$ from ATP (Roeder, 1995). Elevated intracellular levels of cAMP activate cAMP-dependent protein kinase A (PKA), which regulates a number of cellular processes including the phosphorylation of heat shock proteins (HSP) in *Drosophila* (Inoue et al., 2000). PKA regulates voltage- and ligand-gated ion channels; specifically, PKA decreases the open-probability of the delayed rectifier type-A K^+-channel in Sprague–Dawley rats (Yuan et al., 2002). In *Aplysia* sensory neurons, slowly activating S-type K^+-channel can be modulated by cAMP analogs therein reducing the outward

current of these channels (Goldsmith and Abrams, 1992). This has also been shown in *Drosophila* (Drain et al., 1994; Wright and Zhong, 1995). Along with ion channels, the Na^+/K^+ ATPase appears to be regulated by PKA and PKC in mammalian cell cultures (Feschenko et al., 2000), and locust K^+ currents and Na^+/K^+ ATPases are regulated by OA (Walther and Zittlau, 1998). It is interesting to note that OA has been used as an agonist of norepinephrine-mediated modulation of mammalian Na^+/K^+ ATPase pump activity (Azzam et al., 2004). As mentioned in the previous section, attenuation of K^+ channels and excitation of the Na^+/K^+ ATPase pump would reduce extracellular build up of $[K^+]_o$, and thus have the effect of reducing the likelihood of circuit failure.

4.2. The role of glia and potassium clearance

As in vertebrates, glial cells form the superficial layer of the insect CNS. This layer is referred to as the sheath of the ganglion and it acts as the invertebrate counterpart of the blood brain barrier. It is responsible for maintaining homeostasis of ionic concentrations in the ganglion and probably plays a role in the immunological control of infection/disease. Bath applications of pharmacological agents, including neuromodulators (such as OA), have difficulty in penetrating the sheath. Although OA permeability through the sheath is low, it has been reported that superficial glial cells are themselves modulated by OA. Schofield and Treherne (1985) reported that exposure to OA reduced potassium transperineurial permeability by 24% in *Periplaneta americana*, and decreased the rate at which $[K^+]_o$ entered into the ganglion. It should be noted that during stress K^+ concentrations in the haemolymph increase (Chapman, 1958), so it is likely that OA is acting as a control mechanisms minimizing entry of K^+ into the ganglia during times of stress. This would limit the build up of K^+ in the CNS. Although the mechanisms by which OA regulates K^+ permeability across the perineurium remains to be discovered, K^+ channels, $K^+/Na^+/2Cl^-$ cotransporter and the Na^+/K^+ ATPase pump are all likely to be involved (Walz and Wuttke, 1999). As mentioned before the Na^+/K^+ ATPase pump is known to be regulated by OA in locusts (Walther and Zittlau, 1998). This pump plays a major role in clearing extracellular potassium. If $[K^+]_o$ in the ventilatory neuropil is artificially raised, accomplished by injecting a tiny bolus of saline (150 nL) containing high K^+ (300 mM vs. 10 mM) this arrests ventilatory CPG function. Presumably this mimics the effect of a temperature-induced failure but at room temperature. After a few minutes the Na^+/K^+ ATPase pump of neurons and glia are able to clear $[K^+]_o$ and the ventilatory CPG recovers its function (Armstrong and Robertson, unpublished data; Fig. 5A and C). We have shown that OA bath application can reduce the time taken to recover motor pattern formation following a K^+ injection into the neuropil (Fig. 5D). This indicates that OA plays a role in regulating $[K^+]_o$ in the insect CNS, and could account for the protective effects generated as a result of octopaminergic neuromodulation.

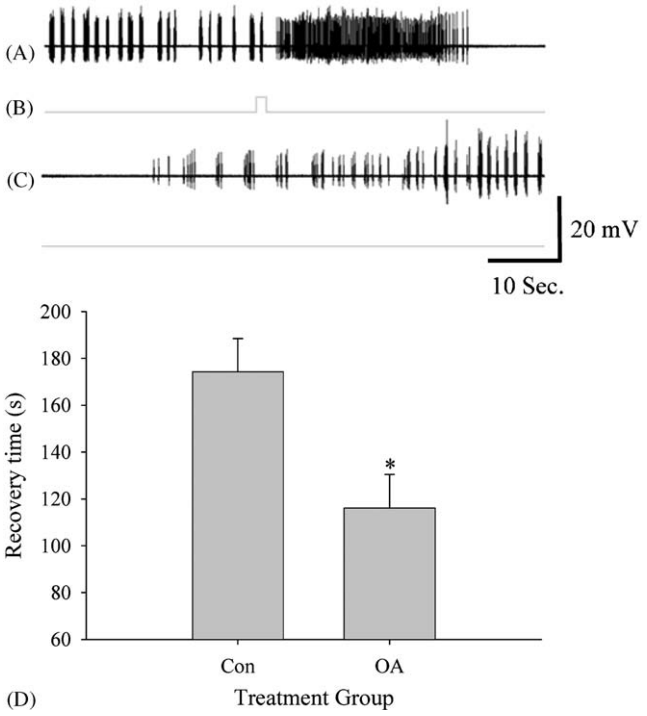

Fig. 5. Nanoliter (150 nL) injection of locust saline containing high potassium (300 mM vs. 10 mM) into the ventilatory neuropil is sufficient to arrest motor pattern formation (A). The square wave in (B) represents the trigger signal for injection time of the bolus. After the microinjection, which raised $[K^+]_o$, recovery of motor pattern function occurs (C). We believe that the time taken to recovery motor pattern function is dependent upon the length of time taken to clear $[K^+]_o$. Following a 1 h bath application of octopamine (1×10^{-4} M OA) recovery time is significantly faster than controls (Con) suggesting that OA-treated animals can better clear extracellular potassium. Data from Armstrong and Robertson (2005).

4.3. Possible role of OA-CREB pathway

In addition to the cytosolic interactions of cAMP-PKA regulation, translocation from the cytoplasm to the nucleus of the activated catalytic subunit of PKA activates at least three transcription factors (De Cesare et al., 1999). These are: cAMP response element binding (CREB) protein, cAMP responsive element modulator (CREM), and activating transcription factor 1 (ATF-1). These transcription factors regulate the expression of specific genes responsible for long-term potentiation (Silva et al., 1998) and circadian rhythms (Foulkes et al., 1997), and are phosphorylated after UV radiation, oxidative and chemical stresses (Deak et al., 1998) and after ischemia in mice hippocampal neurons (Mabuchi et al., 2001). Moreover cAMP dysfunction plays a role in neurodegenerative disorders (Lijima-Ando et al., 2005). An intriguing possibility is that OAR activity leads to the transcription of HSPs. After HS treatment a series of proteins are up-regulated in the locust (Whyard et al., 1986), these include

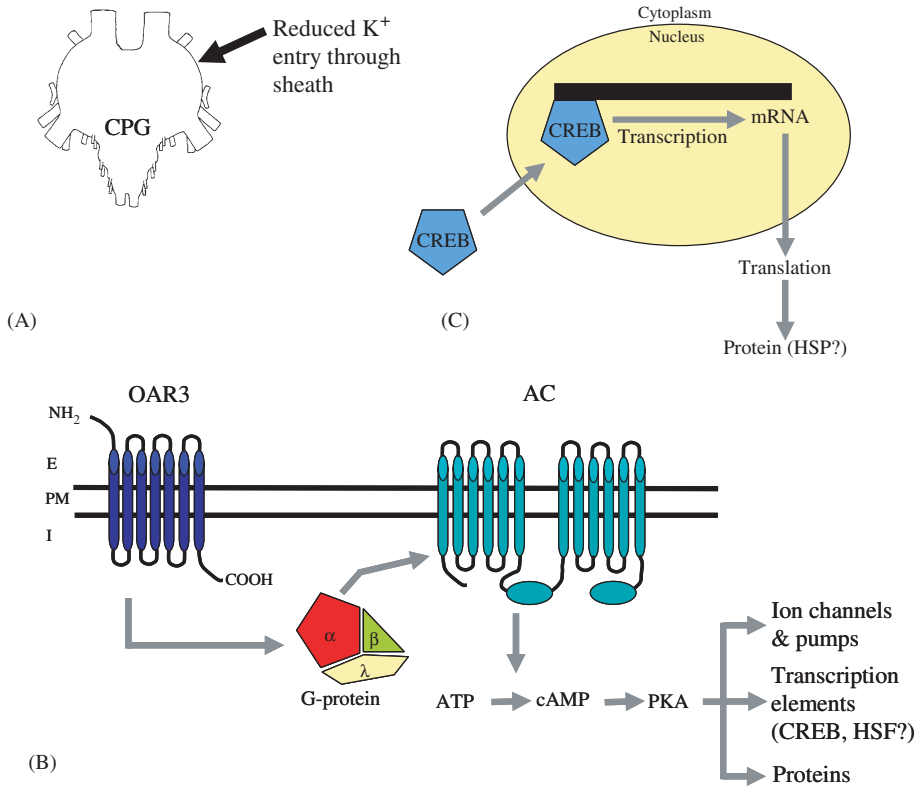

Fig. 6. Diagrammatic representation of the various effects octopamine is known to elicit. A, application of OA to the sheath decreases the K^+ conductance into the metathoracic ganglion. CPG represents the area in the metathoracic ganglia containing the neural circuitry controlling ventilation in the locusts. B. The neural octopamine receptor (OAR3) and its signalling pathway. Once ligand binding occurs, conformational changes in the receptor causes disassociation of the α subunit from the hetrotrimeric G-protein. The α subunit then increases the activity of adenylyl cyclase (AC) which promotes the production of cAMP. The rise in cAMP activates PKA-mediated events; these include ion channel regulation, protein phosphorylation and CREB activity. E, extracellular; PM, plasma membrane; I, intracellular side of the plasma membrane. C, hypothetical CREB regulation of HSP production. Data based on Schofield and Treherne (1985), Blenau and Bauman (2001); Roeder (2005) and Armstrong and Robertson (2005).

proteins with molecular weights of 24, 28, 42, 68, 73, and 81 that are believed to play a role in protection of the nervous system. It is has been observed that the protective effects of OA application can be blocked with antagonists of OAR3, protein synthesis inhibitors and transcription inhibitors, suggesting that OA elicits its thermoprotective effects through an interaction with the genome (Armstrong and Robertson, 2005). It has previously been shown that PKA and PKC antagonists are able to block HSP72 gene expression in human glioblastoma cell lines, and agonists of PKA and PKC are able to promote HSP72 expression levels (Ohnishi et al., 1998). Furthermore *hsp*70 gene expression resulting from hemodynamic stress to the cardiac tissue of rats is regulated by PKA- and PKC-dependent activity (Osaki et al., 1998), and in mice CREB phosphorylation mediates neuroprotection to glutamate and ischemia exposure (Mabuchi et al., 2001). It is possible that OA modulation of the nervous system results not only in a coordinated change in behaviours that are protective, but the nervous system itself is protected from stress as a result of *hsp* and other genes being upregulated after stimulation by OA (Fig. 6). It is important to remember that CREB activity is a result of pleiotropic factors, and that there are other possible signaling pathways that regulate its activity.

5. Conclusions

In its possible role in coordinating thermoprotection octopamine (OA) probably does not act alone. Other biogenic amines and hormones are likely to interact with numerous components of what we have outlined in this paper to produce a thermoprotected insect nervous system. Nevertheless, OA is one of the most abundant biogenic amines in insects with concentrations of dopamine being half that of OA, and with serotonin levels about equal to that of OA (Evans, 1985). Its role is one of the most diverse, regulating metabolism and acting as a neurotransmitter, neuromodulator and neurohormone.

Our research suggests that OA plays a role in protecting the nervous system from heat stress. Octopaminergic modulation influences behaviours crucial for survival such as flying, jumping, ventilation and predator detection. In the CNS, OA reduces potassium conductance and activates the Na^+/K^+ ATPase pump (Walther and Zittlau, 1998), which is important for protection from heat stress in that

these changes in ion regulation would attenuate the build up of $[K^+]_o$. This may account for the thermoprotection we have observed in the locust after OA bath application (Armstrong and Robertson, 2004). We also believe that some of the protective effects observed in the CNS may be a result of gene expression (possible HSP up-regulation) as a result of OA signaling through the PKA–CREB pathway (Armstrong and Robertson, 2005).

With the cloning of the *Drosophila* genome this model organism offers an alternative route for the investigation of the role of OA in coordinating the expression of a selective group of genes. Currently three strains of *Drosophila* exist that will be important in unraveling the role of OA in conferring thermoprotection and, more generally, as a modulator and transmitter of neural processes. The first strain termed *Drosophila melanogaster inactive* (*iav*), isolated because of its reduced locomotor activity (Homyk and Sheppard, 1977) has lower levels of the enzyme tyrosine decarboxylase. As a result these flies have lower levels of tyramine and OA (McClung and Hirsh, 1999). It was recently shown that these flies have impaired thermotolerance (Chentsova et al., 2002). The second strain, *Drosophila virilis* (line 147) is unable to develop stress response to temperature and other stresses (Rauschenback et al., 1987, 1993). While under heat stress (38 °C) these flies have been shown not to have a change in the concentration of tyramine, OA, dopamine or DOPA (Hirashima et al., 2000). The third strain of *D. melanogaster* carries a null mutation in the gene encoding tyramine β-hydroxylase ($T\beta h^{nM18}$) as a result OA levels are reduced but tyramine levels are higher (Monastirioti et al., 1996) and these flies have reduced viability under harsh environmental conditions. These three strains will be critical for unraveling the mechanisms by which OA protects the CNS of insects from heat stress.

Locusts and drosophila are well-adapted to their environments, possessing a strong HS response (Qin et al., 2003) and have evolved physiological mechanisms allowing them to cope with extremes in temperature. Thus the use of these animals, and the *Drosophila* OA mutants, will continue to provide important information about the changes that occur after HS treatment, particularly the role neuromodulators might play in mitigating the rise in $[K^+]_o$ during heat-induced failure of CNS function.

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