Peptidergic Counter-Regulation of $\text{Ca}^{2+}$- and $\text{Na}^{+}$-Dependent $\text{K}^{+}$ Currents Modulates the Shape of Action Potentials in Neurosecretory Insect Neurons

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¹Saxon Academy of Sciences, Department of Neurohormones, Jena; ²Institute of Physiology, Department of Neuroendocrinology, Philipps University Marburg, Marburg; and ³Max-Planck Institute of Neurobiology, Department of Systems and Computational Neurobiology, Martinsried, Germany

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Wicher, Dieter, Jens Berlau, Christian Walther, and Alexander Borst. Peptidergic counter-regulation of $\text{Ca}^{2+}$- and $\text{Na}^{+}$-dependent $\text{K}^{+}$ currents modulates the shape of action potentials in neurosecretory insect neurons. J Neurophysiol 95: 311–322, 2006. First published September 21, 2005; doi:10.1152/jn.00904.2005. Influx of $\text{Ca}^{2+}$ and $\text{Na}^{+}$ ions during an action potential can strongly affect the repolarization and the fast afterhyperpolarization (fAHP) if a neuron expresses $\text{Ca}^{2+}$- and $\text{Na}^{+}$-dependent $\text{K}^{+}$ currents ($K_{\text{Ca}}$ and $K_{\text{Na}}$). This applies to cockroach abdominal dorsal unpaired median neurons (DUMs). Here the rapid activation of $K_{\text{Ca}}$ depends mainly on the P/Q-type $\text{Ca}^{2+}$ current. Adipokine hormones (AKHs)—insect counterparts to mammalian glucagon—mobilize energy reserves but also modulate neuronal activity and lead to enhanced locomotor activity. Cockroach AKH I accelerates spiking and enhances the fAHP of octopaminergic DUM neurons, and it is generally held that enhanced release of the biogenic amine from these and other neurons may lead to general arousal. AKH I modulates the voltage-gated $\text{Na}^{+}$ and P/Q-type $\text{Ca}^{2+}$ current and the background $\text{Ca}^{2+}$ current. Up-regulation of P/Q-type $\text{Ca}^{2+}$ current increases the $K_{\text{Ca}}$ current, whereas enhanced inactivation of $\text{Na}^{+}$ current decreases the $K_{\text{Ca}}$ current. We quantified the hormone-induced changes in ion currents in terms of Hodgkin-Huxley models and simulated the resulting activity of DUM neurons. Up-regulation of P/Q-type $\text{Ca}^{2+}$ and $K_{\text{Ca}}$ current enhanced the hyperpolarization but had a weak effect on spiking. Down-regulation of $\text{Na}^{+}$ and $K_{\text{Na}}$ current decreased hyperpolarization and slightly accelerated spiking. Supposition of these modulations produced an increase in fAHP while the spike frequency remained unchanged. Only when the up-regulation of the pacemaking $\text{Ca}^{2+}$ background current was included in the simulated modulation did the model reproduce the experimentally observed AKH-I-induced changes. The possible physiological relevance of this dual effect is discussed in respect to transmitter release and synaptic integration.

INTRODUCTION

Insect adipokinetic hormones (AKHs) mobilize energy reserves from storage organs such as the fat body similar to glucagon in mammals (Gaede and Auerswald 2003; Van der Horst 2003). As found recently, they also enhance locomotor activity in response to starvation (Lee and Park 2004). In cockroach (Periplaneta americana), there are two AKH peptides (Scarborough et al. 1984; Witten et al. 1984), one of which, AKH I (pQVNFSNPWamide, also called Neurohormone D) (Baumann et al. 1984; Baumann and Penelin 1984) accelerates spiking and alters the shape of action potentials in abdominal dorsal unpaired median (DUM) neurons. These DUM neurons are of general interest inasmuch as they release the biogenic amine octopamine. The octopaminergic system of insects, comparable to the noradrenergic system of mammals, plays an important role in arousal and affects general activity levels (e.g., Roeder 2005).

Previous investigations revealed that AKH I modulates a whole set of ion currents in the DUM neurons (Wicher et al. 2001). AKH I leads to potentiation of a P/Q-type $\text{Ca}^{2+}$ current due to channel phosphorylation via protein kinase A (PKA) (Wicher 2001b); reduction of the fast $\text{Na}^{+}$ current, again via PKA (Wicher 2001a); potentiation of a BK-type $\text{Ca}^{2+}$-activated $\text{K}^{+}$ ($K_{\text{Ca}}$) current (Derst et al. 2003; Wicher et al. 1994); potentiation of a $\text{Ca}^{2+}$ background current via $\text{G}_{\text{i}}$-mediated stimulation of phospholipase C (PLC) (Wicher et al. 2004). This latter current contributes to pacemaker depolarization and spontaneous firing of the abdominal DUM neurons. Other currents investigated, such as voltage-dependent $\text{K}^{+}$ currents, were not affected by the peptide (Wicher et al. 1994).

The present study analyzes how the complex modulation of several currents leads to the increase in spike frequency and to the altered shape of the action potential. To simulate spiking of DUM neurons, we developed a one-compartment model that includes a set of 10 ion currents described in terms of the Hodgkin-Huxley formalism. Our studies indicate that accelerated spiking is solely due to the change in the $\text{Ca}^{2+}$ background current, whereas the increase in fast afterhyperpolarization (fAHP) of action potentials (Wicher et al. 1994) requires the change in all other modulated currents.

METHODS

Cell isolation

Isolation of dorsal unpaired (DUM) neurons from the fifth abdominal ganglion of the cockroach P. americana was performed as described previously (Wicher et al. 1994). Briefly, the ganglia were excised, desheathed, and incubated for 10 min at room temperature in saline (for composition see bath solution used for spike recordings) containing 0.5 mg/ml trypsin (type II, Sigma, Deisenhofen, Germany) and 0.5 mg/ml collagenase (type I, Sigma). After washing off the enzyme, the ganglia were stored in saline for $\approx 1$ h. Prior to the measurements, the somata of DUM neurons were separated using thin metal needles.

Electrophysiology

Ion currents in HEK293 cells and in DUM neurons were measured at room temperature using whole cell patch-clamp with appropriate
compensation of series resistance and of capacitive and leakage currents. Pipettes having resistances of 2–4 MΩ (HEK cells) or 0.5–0.8 MΩ (DUM cells) were pulled from borosilicate capillaries. Current measurements and data acquisition were performed using an EPC9 patch-clamp amplifier controlled by PULSE software (HEKA Elektronik).

HEK CELLS. For experiments with Periplaneta Slowpoke (pSlo) channels, the bath solution contained (in mM) 135 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 0.33 Na₂HPO₄, 2 Na-pyruvate, 10 glucose, and 10 HEPES. The pipette solution contained 140 KCl, 4 NaCl, 2 Mg-ATP, and 10 HEPES. The free Ca²⁺ concentration was adjusted to 150 μM by adding CaCl₂ and measuring [Ca²⁺] with calcium-sensitive electrodes (KWIK tips; WPI, Berlin). The pH of bath and pipette solution were adjusted to 7.4 and 7.3, respectively.

DUM NEURONS. Spiking of neurons was recorded under current-clamp conditions without current injection. The bath solution contained (in mM) 190 NaCl, 5 KCl, 5 CaCl₂, 2 MgCl₂, and 10 HEPES (pH = 7.4), and patch pipettes (resistance >1.5 MΩ) were filled with solution composed of (in mM) 190 K-glucuronate, 5 NaCl, 2 Mg-ATP, 1 CaCl₂, 3 EGTA, and 10 HEPES (pH = 7.25). Between recordings (duration: 1 s) the cells were held at −70 mV under voltage clamp.

Na⁺ currents were separated as described (Wicher 2001a) with pipette solution composed of (in mM) 5 NaCl, 100 choline methylsulfate, 30 TEA-Br, 3 CsCl, 60 CsOH, 2 Mg-ATP, 1 CaCl₂, 5 EGTA, and 10 HEPES and bath solution containing (in mM) 60 Na isethionate, 90 choline methylsulfate, 40 TEA-Br, 7 MgCl₂, 1 CdCl₂, and 10 HEPES. For Ca²⁺ current measurements (Wicher and Penzlin 1997), the pipette solution contained (in mM) 100 choline methylsulfate, 30 TEA-Br, 8 CsCl, 60 CsOH, 2 Mg-ATP, 1 CaCl₂, 5 EGTA, and 10 HEPES, and the bath solution (in mM) had 5 CaCl₂, 190 choline methylsulfate, and 10 HEPES and 5 μM tetrodotoxin.

K⁺ currents in DUM neurons were measured with pipette solution containing (in mM) 180 K-glucuronate, 10 NaCl, 2 Mg-ATP, 1 CaCl₂, 5 mannitol, 3 EGTA, and 10 HEPES. The bath solution to measure K_ca currents contained (in mM) 190 NaCl, 5 KCl, 5 CaCl₂, 2 MgCl₂, and 10 HEPES and 0.5 μM tetrodotoxin (TTX). K_ca currents were separated from other currents by applying the BK-channel blocker ibotenic acid (100 μM). The voltage-gated delayed rectifier (DR) and the A-type current were measured with the above bath solution containing in addition 2 CdCl₂. To isolate the DR, the A-type current was depressed by 3 mM 4-aminopyridine. The bath solution to measure K_na currents contained (in mM) 190 NaCl, 5 KCl, 1 CaCl₂, 2 MgCl₂, 2 CdCl₂, and 10 HEPES. K_na currents, together with Na currents, were separated by comparing recordings performed in the absence and presence of the Na⁺ channel blocker TTX (0.5 μM). The pH of bath solutions was adjusted to 7.4 and that of pipette solutions to 7.25. Liquid junction potentials were compensated.

Application or washout of blocking agents was performed by transferring the cell (attached to the pipette tip) within a glass tube into the various solutions. A complete and fast solution change within a few milliseconds was achieved by sucking a small amount of solution into the tube.

Data analysis

Results are given as means ± SE (n = number of cells). Fitting procedures were performed with the software Prism2 (Graph Pad, San Diego, CA) and with IGOR (WaveMetrics, Lake Oswego, OR).

Chemicals

Salt, tetraethylammonium, tetrodotoxin, and verapamil were purchased from Sigma (Deisenhofen, Germany). ω-Agatoxin IVA and ω-conotoxin GVIA were obtained from Alomone Labs (Jerusalem, Israel) and ibotenic acid from Calbiochem (Schwalbach, Germany).

RESULTS

Relationship between voltage-gated Ca currents and KCa currents in DUM neurons

Periplaneta DUM neurons have previously been shown to express pSlo, the α subunit of large conductance KCa (BK) channels (Durst et al. 2003). While pSlo channels heterologously expressed in HEK293 cells give rise to a purely non-inactivating KCa current, the KCa current in DUM neurons is composed of a transient (KCa,t) and a sustained (KCa,s) component (Durst et al. 2003; Grolleau and Lapied 1995; Wicher et al. 1994). pSlo channels are sensitive to the BK channel blocker ibotenic acid (IbTx; IC₅₀ = 45 nM). The KCa channels in DUM neurons seem to be more sensitive to IbTx because both KCa current components are blocked by 100 nM IbTx (Durst et al. 2003). This is illustrated in Fig. 1A, which shows sample traces of K⁺ currents, measured prior and after application of IbTx and the KCa current obtained by subtraction of these currents. There is an initial rapid KCa current rise (the time to peak ranges from 7 ms at −20 mV to 3 ms at ≥20 mV) and a fast decay (decay to 50% takes ~2 ms) of the first part of the KCa current which is dominated by KCa,t. It then reaches a sustained level, i.e., KCa,s, some 5 ms after stepping the voltage. Activation of both components of the KCa current starts around −50 mV, i.e., at about the same voltage as that of the start of the Ca²⁺ current (Fig. 1B, top). The time-to-peak of the KCa current is comparable to that of the voltage-gated Ca²⁺ current (Fig. 1D).

It should be pointed out that there is no pharmacological tool to separate KCa,t and KCa,s. However, these components differ in their Ca²⁺ sensitivity: When voltage-gated currents are blocked and the intracellular [Ca²⁺] is raised by perfusing the cell with Ca²⁺-rich pipette solution, the first current evolving—as seen on repeated application of depolarizing voltage protocols—is KCa,t. KCa,s appears ~1 min later, indicating that KCa,s requires a higher [Ca²⁺] than KCa,t to be activated by depolarization (Durst et al. 2003). The fact that KCa,t inactivated in spite of a constant [Ca²⁺] indicates that inactivation is an intrinsic channel property perhaps produced by accessory β subunits. It cannot be excluded that in our present measurements there is some overlap of currents, i.e., that there is a small contribution of KCa,s to the KCa peak current at least at higher depolarizations. Because, however, such errors is expected to be small and will not have consequences for the results obtained in the following text, we will refer the KCa peak current as KCa,t.

The Ca²⁺ current activating positive to −50 mV is composed of three subtypes (Wicher and Penzlin 1997): P/Q-type current sensitive to ω-agatoxin IVA (ω-AgTx), N-type current sensitive to ω-conotoxin GVIA (ω-CgTx), and L-type current sensitive to verapamil (Fig. 1B, bottom). The effect of ω-AgTx (50 nM) and verapamil (10 μM) on Ca²⁺ current is illustrated in Fig. 1C; the concentrations used have been previously shown to block the respective currents within 1 min (Wicher and Penzlin 1997).

We applied the Ca²⁺ channel blockers to learn what extent L-, N-, and P/Q-type Ca²⁺ currents might supply the Ca²⁺ for activation of the KCa current. The three Ca²⁺ currents cause a Ca²⁺ influx of different size and activation kinetics (Fig. 1, B, bottom, and C), and their suppression affected the KCa currents differently: ω-AgTx (50 nM) led to reduction of
both the transient and the sustained component of $K_{Ca}$ current (Fig. 2, A and C), $\omega$-CgTx (1 $\mu$M) had no effect on $K_{Ca}$ current ($n = 5$, not shown), whereas verapamil (10 $\mu$M) had a clearly weaker effect on the transient component than $\omega$-Agatoxin and suppressed the sustained $K_{Ca}$ current to a similar degree (Fig. 2, B and D). The lack of effect of N-type channel block might indicate a spatial separation of N-type Ca$^{2+}$ channels and $K_{Ca}$ channels so that Ca$^{2+}$ entering through N-type channels hardly diffuses to $K_{Ca}$ channels. On the other hand, the weak inhibition of the transient $K_{Ca}$ current component by the block of the L-type channel may be largely due to the rather slow activation kinetics of L-type channel. As illustrated in Fig. 2B, the peak of L-type current is attained after the peak of the verapamil-sensitive $K_{Ca}$ current. The rise in intracellular [Ca$^{2+}$] due to the L-type current might thus overlap with the intrinsic inactivation of the channel conducting $K_{Ca,t}$. By contrast, the P/Q-type current activates more rapidly and reaches the peak earlier than the $K_{Ca}$ current sensitive to $\omega$-Agatoxin (Fig. 2A). Thus the fast activating P/Q-type current seems to supply most of the Ca$^{2+}$ required for the activation of the transient $K_{Ca}$ current component. On the other hand, the sustained $K_{Ca}$ current may rely on Ca$^{2+}$ entering via both types of Ca$^{2+}$ channels because it is reduced by about the same extent by blocking P/Q- or L-type channels (Fig. 2, C and D).

Peptidergic upregulation of $K_{Ca}$ current

The Periplaneta adipokinetic hormone AKH I, a peptide released from the corpora cardiaca, is known to enhance $K_{Ca}$ currents (Wicher et al. 1994). AKH I also potentiates the P/Q-type Ca$^{2+}$ but not the N- and L-type Ca$^{2+}$ currents in DUM neurons (Wicher 2001b). Figure 3 demonstrates the AKH-induced effect on $K_{Ca}$ currents: the total outward current produced by a voltage step to 0 mV before and after application of 10 nM AKH I, and the difference current representing the peptide-induced $K_{Ca}$ current are shown in Fig. 3A, 1 and 2, respectively. AKH I thus upregulates both the transient and the sustained $K_{Ca}$ component. This may be plausibly attributed to the upregulation of the P/Q-type current as this current was seen in the preceding text to provide Ca$^{2+}$ for the activation of both $K_{Ca}$ components. The threshold concentration for the
Increasing [AKH I] from 1 pM to 10 nM induced progressively the possibility that AKH I affects purely voltage-gated K$_{\text{Ca}}$ currents at voltages ranging from –20 to 0 mV (Wicher 2001b). The increase in P/Q-type current by AKH I that is most pronounced was mainly restricted to the transient component (Fig. 3A1, n = 10). The AKH I-induced potentiation of the P/Q-type Ca$^{2+}$ current was seen to prolong action potentials and to reduce the hyperpolarization (Derst et al. 2003).

**Activation of PKA does not affect I$_{\text{KCa}}$**

Some Slo channels are known to be modulated by phosphorylation via protein kinases such as PKA (Schubert and Nelson 2001). AKH I in fact activates PKA, which is a necessary step in the upregulation of the P/Q-type Ca$^{2+}$ current (Wicher 2001b). In principle the regulation of the K$_{\text{Ca}}$ currents by AKH I might be dual, i.e., be partly due to K$_{\text{Ca}}$ channel phosphorylation. To evaluate whether PKA can affect the pSlo channel, we coexpressed pSlo together with the Periplaneta AKH receptor (pAKHR) in HEK293 cells. Stimulation of heterologously expressed pAKHR with AKH I was previously shown to increase [cAMP] via activating G$\alpha_\text{i}$ proteins in HEK293 cells (Wicher, unpublished observation). Application of AKH I (10 nM), after loading the cell with a Ca$^{2+}$-rich pipette solution, had, however, no effect on pSlo currents activated by depolarization (Fig. 4A, left). There was neither a change in the size of currents nor in the kinetics. In another series of experiments, we induced a rise in [cAMP] by bath application of the membrane-permeable cAMP analogue 8-bromo-cAMP. Again, there was no change in the properties of pSlo currents (Fig. 4A, right). To exclude the possibility that PKA might be fully activated under control conditions, we tested whether inhibition of PKA would affect pSlo currents. Application of 10 μM KT5720 (10 μM) on pSlo currents. Currents measured in the presence of these agents were normalized to control currents. n = 5.
KT5720, which was previously shown to completely abolish the AKH I effect on P/Q-type Ca\(^{2+}\) current (Wicher 2001b), did not change pSlo currents. These results, which are summarized in Fig. 4B, do not support the possibility that PKA regulates pSlo. Recently direct binding of a PKA and a Src tyrosin kinase to domains in the C-terminus of the *Drosophila* Slo channel (dSlo) and phosphorylation of the dSlo channel protein by both kinases has been demonstrated (Wang et al. 1999). However, no difference in peak current amplitude nor in voltage dependence of dSlo gating was observed after coexpression of dSlo with either protein kinase (Wang et al. 1999). On the other hand, the free catalytic subunit of PKA binds to dSlo and leads to downregulation of channel activity (Zhou et al. 2002). This modulation, however, does not involve phosphorylation of the only consensus PKA-substrate site in the C-terminal domain of dSlo.

**Relationship between voltage-gated Na currents and K\(_{Na}\) currents in DUM neurons**

DUM neurons express Na\(^{+}\)-dependent K\(^{+}\) channels (Wicher et al. 1994; Grolleau and Lapied 1994). Therefore blocking the Na\(^{+}\) current with tetrodotoxin (TTX) leads to disappearance of a transient outward current component (Fig. 5A), and the total TTX-sensitive current is the sum of the Na\(^{+}\) current and the Na\(^{+}\)-dependent K\(^{+}\) (K\(_{Na}\)) current (Fig. 5B). Due to the lack of a specific blocker, it is impossible to separate the K\(_{Na}\) current from the Na\(^{+}\) current (Grolleau and Lapied 1994). Although the TTX-sensitive outward current is contaminated with the Na\(^{+}\) current, we will refer to it as the K\(_{Na}\) current. It activates very rapidly, its peak following that of the Na\(^{+}\) current within \(<1\) ms (Fig. 5C). Because K\(_{Na}\) channels are not voltage-gated, the I-V relation of the K\(_{Na}\) peak current mirrors the I-V relation of the Na\(^{+}\) current (Fig. 5D).

**Peptidergic upregulation of K\(_{Na}\) current**

AKH I accelerates the inactivation of the Na\(^{+}\) current in DUM neurons, thereby reducing both peak size and duration and thus also the net Na\(^{+}\) influx (Wicher 2001a). Because activation of the K\(_{Na}\) current requires a high intracellular Na\(^{+}\) concentration (Dryer 1994), any reduction of Na\(^{+}\) influx should entail a reduction of the K\(_{Na}\) current. An example of a current suppressed by AKH I (i.e., the difference of the current measured under control conditions and in presence of AKH I) is shown in Fig. 5E. The relation between the AKH-I-sensitive Na\(^{+}\) current and the AKH-I-sensitive K\(_{Na}\) current (Fig. 5F) resembles that between the TTX-sensitive Na\(^{+}\) current and K\(_{Na}\) current under control conditions (Fig. 5D). Thus the reduction of K\(_{Na}\) peak current was proportional to the reduction of Na\(^{+}\) peak current, and the downregulation of K\(_{Na}\) current by AKH I, therefore, is probably solely due to the reduction of the Na\(^{+}\) influx. A test of a possible effect of PKA on the K\(_{Na}\) channel (Slo2 or slack) (Yuan et al. 2003) could not be performed because this channel has not yet been cloned in *Periplaneta*. Furthermore, an experimental analysis of the role of the peptidergic modulation of Na\(^{+}\) current and K\(_{Na}\) current on action potential shape is impaired by the lack of tools to separate the currents: Li\(^{+}\) permeates Na\(^{+}\) channels but fails to activate K\(_{Na}\) channels in some preparations. By contrast, in DUM neurons Li\(^{+}\) also activates the K\(_{Na}\) current, and it can thus not be used as tool to separate the Na\(^{+}\) current from K\(_{Na}\) currents (Grolleau and Lapied 1994).

**Modeling the effect of peptidergic counter-regulation of Ca\(^{2+}\)- and Na\(^{+}\)-dependent K\(^{+}\) currents on the action potential shape**

We performed a modeling study of the differential modulation of the K\(^{+}\) currents with the aim of answering two main questions: Does modulation of these currents affect the pacemaker depolarization and does superposition of the modulatory
effects explain quantitatively the observed increase in fast AHP (fAHP) of action potentials?

To simulate the endogeneous spiking of DUM neurons, we included 10 currents in a one-compartment model of a DUM neuron: one Na current, four Ca currents (the background current, a low-voltage activated current, and 2 high-voltage activated currents, i.e., P/Q-type and non-P/Q-type current), and five K currents (the delayed rectifier, K_DR, the A-type current, K_A, the K_Na current and the K_Ca currents, K_Ca,t and K_Ca,a). The currents were described in terms of the Hodgkin-Huxley formalism (cf. APPENDIX, Fig. A1 and Table 1). Figure 6 compares recorded spikes from a DUM neuron with the spike pattern generated by the model, using the parameters given in Table 1. The model reproduced the experimentally observed characteristics of the action potential such as threshold, overshoot, and AHP as well as the resting firing frequency.

To get insight into the role of the K current and the transient K current in the regulation of neuronal activity, we investigated the effects of varying the size of these currents. The K current was found to be essential for the stability of repetitive activity (Fig. 7A). Both the K current and the K current affected the spike frequency (Fig. 7A) and the fAHP (Fig. 7C) although with different efficiency. On reduction of the K current, there was a robust increase in spike frequency, whereas an increase produced only a weak reduction (Fig. 7A). Changing the K current led to inversely proportional changes in spike frequency. When K current was increased by >30%, spiking stopped because the action potential threshold was no longer reached (Fig. 7A). On the other hand, spiking did not become irregular even when the K current conductance was reduced by 90%; Reduction of the K current conductance by >40% caused irregular spiking. Reduction of K current caused a slight attenuation of the fAHP. Complete block of K current was estimated to reduce the AHP by 4 mV, which was somewhat less than found experimentally, i.e., after blocking K with iberiotoxin (9 mV) (Wicher 2001b). The dependence of fAHP on

TABLE 1. Parameters for voltage- and ion-dependent membrane currents

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Measured currents were fitted by Hodgkin-Huxley models (see APPENDIX). The listed parameters describe the fitted curves in Fig. A1. The steady-state data for I_{Na} were taken from Wicher (2001a) and for I_{Ca} LVA from Grolleau and Lapied (1996), the kinetic data for I_{Ca} LVA were estimated from the current traces shown in the latter paper. For the data describing I_{KDR}, I_{K_A}, and I_{KNa}, see also Grolleau and Lapied (1994, 1995). Special Parameters (I_{Ca,back} = I_{K_Na} - I_{K_Ca,t} - I_{K,Ca,a} G_{Ca,back}, ns: 0.023, Na-Sensitivity I_{Na,Na} 1.1, Delay I_{K,Na}, ms: 2.3, Ca-Sensitivity I_{K,Ca}, 1.7, passive parameters Membrane capacity, pF: 370, G_{Na}, ns: 22, V_{leak}, mV: -50, Delay I_{K,Ca,a}, ms: 1.7, Ca-Sensitivity I_{K,Ca,a}, 0.5, Delay I_{K,Ca,a}, ms: 1.7.

FIG. 6. Measured (A) and modeled (B) spiking of a DUM neuron. A: current-clamp recording (no current injected). B: calculated activity produced by the model described in the APPENDIX using the parameters given in Table 1.

I_{ KNa} was similar to that on I_{ KCa,t} but considerably steeper (Fig. 7B).

We further investigated how the combined variation of the inward currents, the Na current and the P/Q-type Ca current and the coupled K currents, i.e., KNa and KCa, affect spiking. Increasing the Na conductance unexpectedly produced a rise
in Na downregulation of the K current thus imitating the Na slope up and decreasing 1.0 mV (Fig. 8D). This means the following changes: G (Ca P/Q) from 209 to 287 nS, τ h slope up (Na) from +11 to +13 mV, τ h slope dn (Na) from −22 to −18 mV, and G (Ca, back) from 0.023 to 0.07 nS. These changes produced indeed an accelerated spiking. In the example shown in Fig. 8C, the increase in spike frequency amounts to 25%; this is in accordance with the mean AKH I effect. Furthermore, the hyperpolarization is increased by 3 mV (Fig. 8D). The model also predicts a slight reduction in overshoot (by 3 mV, Fig. 8C), which was, however, not observed with DUM neurons (Fig. 8A).

To assess whether the initial size of KNa and KCa currents may affect the simulation of the AKH effect, we varied their conductances by ±10% and repeated the simulation. Variations of the KNa current did not affect the spike frequency increase. When the initial G was increased by 10% the only effect was a slight reduction of hyperpolarization-increase by 1 mV. A similar effect on hyperpolarization was seen when the initial KCa currents were reduced by 10%. While this variation had no effect on the increase in spike frequency, the variation of initial KCa currents by +10% led to an AKH-induced acceleration by 34% which matches the upper limit of the AKH I effect observed in DUM neurons. Thus the model sufficiently and stably reproduces the changes in neuronal activity by AKH I.

We finally simulated a situation where AKH I would only lead to upregulation of the P/Q-type Ca2+ current and both KCa currents (Fig. 9A). This caused an increase in hyperpolarization (by 5 mV), which is in line with the measured AKH I effect (4 mV), whereas the spike frequency was hardly affected (reduction by 2%). Downregulation of Na+ current and KNa current and whether this requires that in fact all observed AKH I-induced changes in ion currents are implemented, i.e., up-regulation of P/Q-type Ca2+ current and KCa currents, down-regulation of Na+ current, and KNa current as well as up-regulation of Ca2+ background current. In terms of our model (cf. Table 1), this means the following changes: G (Ca P/Q) from 209 to 287 nS, τ h slope up (Na) from +11 to +13 mV, τ h slope dn (Na) from −22 to −18 mV, and G (Ca, back) from 0.023 to 0.07 nS. These changes produced indeed an accelerated spiking. In the example shown in Fig. 8C, the increase in spike frequency amounts to 25%; this is in accordance with the mean AKH I effect. Furthermore, the hyperpolarization is increased by 3 mV (Fig. 8D). The model also predicts a slight reduction in overshoot (by 3 mV, Fig. 8C), which was, however, not observed with DUM neurons (Fig. 8A).

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accelerated spiking (by 7%) and slightly reduced the hyperpolarization (by 2 mV; Fig. 9B). Another effect was a slight reduction of the overshoot (by 3 mV). When the modulations of P/Q-type Ca\(^{2+}\) current and \(K_{\text{Ca}}\) currents were combined with those of Na\(^{+}\) and \(K_{\text{Na}}\) currents, the change of the hyperpolarization was in accord with that observed experimentally (increase by 3 mV; Fig. 9C). The spike frequency, however, remained virtually unaffected (increase by 2%). When in addition the upregulation of Ca\(^{2+}\) background current was implemented, the result was an increase in spike frequency by 25% with no further effects on the action potentials i.e., the observed AKH I effects were now correctly reproduced as already mentioned in the preceding text (Fig. 9D).

In conclusion, the modulation of two voltage-gated currents (P/Q-type Ca\(^{2+}\) current and Na\(^{+}\) current) together is involved in shaping the action potential, but it does not affect spike frequency. In line with previous findings (Wicher et al. 2004), the acceleration of spiking by AKH I solely relies on the upregulation of the Ca\(^{2+}\) background current.

**DISCUSSION**

Octopamine, the chemical messenger released by the DUM neurons investigated here, is upregulated to general activity levels (Roeder 2005; Saraswati et al. 2004). In *Drosophila*, it has recently been shown that, on starvation, AKH release produces enhanced locomotor activity (Lee and Park 2004). Conceivably, the physiological relevance of the AKH I-induced modulation of spiking lies in the enhanced release of octopamine both to local targets (e.g., respiratory muscles) and to the hemolymph thereby contributing to a rise of systemic octopamine level.

Neuropeptides, in general, affect not a single but several ionic currents in the excitable cells they act on, e.g., neurons and skeletal muscles of invertebrates (Brezina et al. 2005; Nusbaum et al. 2001; Wicher et al. 2001) or neurons of vertebrates (e.g., Pena and Ramirez 2004). In the present study on cockroach DUM neurons, we have completed knowledge of the neuro-modulatory actions on ion currents of the hormone AKH I, and we have tested, in a model of this neuron, how the interplay of these effects leads to the observed changes in firing and time course of action potentials.

To understand the role of different ion currents in the discharge patterns of neurons, various computational models have been developed for well studied examples from invertebrates and vertebrates (e.g., Dale 1995; Golowasch et al. 1992). This approach has also been used in analyzing the modulation of currents by chemical messengers (e.g., Harris-Warrick et al. 1995). Neuronal models designed to simulate spontaneous activity either implement \(K_{\text{Ca}}\) currents (Buchholtz et al. 1992), \(K_{\text{Na}}\) currents (Dale 1995), or \(K_{\text{Na}}\) and slowly activating \(K_{\text{Ca}}\) currents (Dale and Kuenzi 1997). In our model, the \(K_{\text{Na}}\) current and the \(K_{\text{Ca}}\) current, two rapidly activating currents contributing to action potential repolarization, act in parallel and are both subject to modulation. In contrast to various other models, ours lacks the hyperpolarization-activated cation current, \(I_{\text{h}}\). This type of current, which has not been found in cockroach DUM neurons (Grolleau and Lapied 2000) but in *Drosophila* CNS (Marx et al. 1999) and in bee olfactory receptor neurons (Gisselmann et al. 2003), is present in some but not all spontaneously active insect neurons (Wicher et al. 2001). Generally, for the neurons to spontaneously spike in the tonic mode, only some persistent inward current plus a low-threshold Ca\(^{2+}\) current seem to be required. In the case of cockroach DUMs, a Ca\(^{2+}\) background current, a low-voltage-activated Ca\(^{2+}\) current and a persistent Na\(^{+}\) current contribute to pacemaking (Grolleau and Lapied 2000).

**How AKH I leads to changes of Ca\(^{2+}\)- and Na\(^{+}\)-dependent K\(^{+}\) currents**

Because AKH I produces changes in Ca\(^{2+}\) and Na\(^{+}\) currents, the \(K_{\text{Ca}}\) and the \(K_{\text{Na}}\) current must also change. For \(I_{\text{KCa}}\), we experimentally ruled out the possibility of an additional direct modulation of the respective channels. For \(I_{\text{KNa}}\), this is also rather unlikely because our model calculations returned realistic results without implementing any direct modulation of this current. The observed slight discrepancy with respect to the amplitude of the action potential (i.e., reduction in overshoot by 3 mV) indicates that in the real neurons the effect of Na\(^{+}\) current reduction on overshoot is compensated for by the reduction of \(K_{\text{Na}}\) current. There might be a more sophisticated relationship between Na\(^{+}\) and \(K_{\text{Na}}\) current than included in our model.

**FIG. 9.** Simulation of the effects on spiking obtained by AKH-I-induced modulations of ion currents. Black traces, activity under control conditions; red traces, activity changed due to the indicated modulations. A: upregulation of P/Q-type Ca\(^{2+}\) and \(K_{\text{Ca}}\) current. B: downregulation of Na\(^{+}\) and \(K_{\text{Na}}\) current. C: combined modulations of P/Q-type Ca\(^{2+}\) and \(K_{\text{Ca}}\) current and downregulation of Na\(^{+}\) and \(K_{\text{Na}}\) current. D: like C, but the upregulation of Ca\(^{2+}\) background current is included. In this situation, i.e., when all AKH-I-induced modulations are implemented, the model reproduces the experimentally observed effects (for further explanations, cf. text).
Modulation of the \( K_{Ca} \) current

Although three types of voltage-gated \( Ca^{2+} \) channels are expressed in DUM neurons (Fig. 1B), only P/Q-type channels appear to provide \( Ca^{2+} \) for activation of the transient \( K_{Ca} \) current component. The sustained \( K_{Ca} \) component can additionally be activated by \( Ca^{2+} \) influx through L-type channels (Fig. 2). Coupling of BK channels to L- and Q-type channels has been reported for rat chromaffin cells (Prakriya and Lingle 1999). In mouse sympathetic neurons, however, BK channels are linked to N-type channels, whereas L- and P-type channels activate \( Ca^{2+} \)-activated Cl\(^{-} \) currents (Martinez-Pinna et al. 2000). In hippocampal neurons, N-type channels activate BK channels, L-type channels activate small conductance (SK) \( K_{Ca} \) channels and P/Q-type channels fail to activate either type of channel (Marrion and Tavalin 1998).

Blocking the BK currents in DUM neurons under current-clamp conditions demonstrated that only the rapidly activating, transient component \( K_{Cat} \) affects the shape of the action potential. \( K_{Cat} \) controls its duration (for further \( K_{Ca} \) currents involved in this process, cf. Wicher et al. 2001). In addition \( K_{Cat} \) determines the size of the fAHP (Durst et al. 2003). These roles of the BK current are comparable to those in various mammalian neurons (Faber and Sah 2002). The sustained \( K_{Ca} \) component, according to the predictions of our model, controls the interspike interval similar to the purely voltage-dependent \( K \) currents \( K_{A} \) and \( K_{DR} \), although, of course, in \( Ca^{2+} \)-dependent fashion (not shown).

Modulation of the \( K_{Na} \) current

\( K_{Na} \) channels require a relatively high intracellular \( Na^{+} \) concentration (~50 mM) to become activated. In some preparations, prolonged discharges or long-lasting depolarizations are required to activate \( I_{KNa} \) (Dryer 1994). Co-localization of \( Na^{+} \) and \( K_{Na} \) channels may, however, allow a sufficient rise in \([Na^{+}]\) to activate the \( K_{Na} \) current already during a single action potential (Koh et al. 1994). In hippocampal CA1 neurons, for example, the \( K_{Na} \) current is responsible for the fAHP after a single action potential (Liu and Stan Leung 2004).

In bursting neocortical neurons, the \( K_{Na} \) current is the main cause of the postexcitatory hyperpolarization (Franceschetti et al. 2003). In these cells, the \( K_{Cat} \) current, too, makes an albeit small contribution to the hyperpolarization. In DUM neurons, the fAHP is about equally dependent on \( K_{Na} \) and \( K_{Ca} \) current, the former activating slightly more rapidly than the latter (which accords to the somewhat different activation kinetics of the \( Na^{+} \) and the \( Ca^{2+} \) currents). The modulations of the P/Q-type \( Ca^{2+} \) current and the \( Na^{+} \) current by AKH I change the initial proportion of \( K_{Ca} \) to \( K_{Na} \) current in favor of the \( K_{Ca} \) current.

Why counterregulation of \( K_{Ca} \) and \( K_{Na} \) currents?

Would one considers acceleration of spiking as the main effect of AKH I on the DUM neurons, then it remains enigmatic why a whole combination of currents (P/Q-type \( Ca^{2+} \) current, \( Na^{+} \) current, \( K_{Ca} \) and \( K_{Na} \) current) is modulated by the peptide because this does not produce a change in spike frequency. In this light, it would be sufficient to upregulate the \( Ca^{2+} \) background current to get the observed acceleration of spiking. Spike frequency, however, is not the sole parameter involved in controlling secretion. It is known from vertebrate neurons that presynaptic voltage-gated \( Ca^{2+} \) currents (N and P/Q types) play a central role in triggering transmitter release (Reid et al. 2003). Although we have no evidence that the upregulation of P/Q-type current in the soma of DUM neurons observed with AKH I also occurs in the release zones of its terminals, this would be a possible means to enhance octopamine release. That a modulator may produce the same changes in the soma and the terminals of a neuron is shown by the example of neurons in rat superior cervical ganglion where application of norepinephrine reduces both neurotransmission and somatic \( Ca^{2+} \) currents (Stephens and Mochida 2005).

Another important principle involved in the control of transmitter release is the regulation of the BK channel-mediated fAHP. On the one hand, the fAHP can limit transmitter release (Raffaaelli et al. 2004). On the other hand, it is necessary for an effective repolarization of action potentials, thus supporting the terminal’s capacity to produce full-size action potentials on repetitive firing (e.g., Sausbier et al. 2004). In the case of the DUM neurons, it seems possible that the downregulation of \( Na^{+} \) current and \( K_{Na} \) current is necessary to limit the effect of \( Ca^{2+} \) and \( K_{Ca} \) current modulation on hyperpolarization.

Conclusion

We have demonstrated a considerable complexity behind the “simple” increase in a neuron’s firing rate induced by a hormone. Such complexity may be of functional relevance in the terminals of the neuron. Alternatively—or additionally—it could enable the neuron to respond differently to a (more or less) constant synaptic input. Such kind of mechanism was, for example, found for dopamine modulation in lobster stomatogastric neurons (Harris-Warrick et al. 1995). It might also be involved in the adaptation of insect neurons to different behavioral situations as observed, for example, in locust thoracic DUM neurons—ranging from patterned activity to complete inhibitory silencing (Pflüger 1999).

APPENDIX

Modeling spike activity of DUM neurons

We simulated a one-compartment model with each current being described by a Hodgkin-Huxley formalism (Yamada et al. 1998). Beside the leak current, there are 10 currents implemented: One \( Na^{+} \) current (\( I_{Na} \)), four \( Ca^{2+} \) currents, and 5 \( K^{+} \) currents. The \( Ca^{2+} \) currents are the voltage-independent background current \( I_{Ca,back} \), the low-voltage-activated (LVS) current \( I_{Ca,LVS} \), and the two high-voltage-activated currents \( I_{Ca,P/Q} \) (P/Q-type current) and \( I_{Ca,nP/Q} \) (non-P/Q-type current = total \( HVA \) current –P/Q-type current). The \( K^{+} \) currents are the voltage-activated delayed rectifier \( I_{KDR} \) and the A-type current \( I_{KA} \), the voltage-independent \( Na^{+} \)-activated current \( I_{KNa} \), and the \( Ca^{2+} \)-activated current composed of the transient \( I_{KCa,t} \) and the sustained \( I_{KCa,s} \). In general, each current \( I(t) \) is modeled according to the following equation [\( G(t) \) is the conductance, \( V_{eq} \) the equilibrium potential, \( V(t) \) the membrane potential]

\[ I(t) = G(t) \cdot (V(t) - V_{eq}) \]  \hspace{1cm} (A1)

with

\[ G(t) = G_{max} \cdot m^{h_{exp}}(t) \cdot h^{h_{exp}}(t) \]  \hspace{1cm} (A2)

The exponents for \( m \) (\( m_{exp} \)) and \( h \) (\( h_{exp} \)) ranged from 0 to 4 and are specified for each of the current. \( m(t) \) and \( h(t) \) are described by the following first-order kinetics.
The voltage dependence of the steady-state values for \( m \) and \( h \) are calculated according to the following equations:

\[
\frac{dm(t)}{dt} = \frac{1}{\tau_m} \cdot (m_\infty(V_m(t)) - m(t)) \tag{A3}
\]

and

\[
\frac{dh(t)}{dt} = \frac{1}{\tau_h} \cdot (h_\infty(V_m(t)) - h(t)) \tag{A4}
\]

The voltage dependence of the steady-state values for \( m \) and \( h \) are calculated according to the following equations:

\[
m_\infty(V_m) = \frac{1}{1 + e^{V_m - h_{\text{m0}}}} \tag{A5}
\]

and

\[
h_\infty(V_m) = \frac{1}{1 + e^{V_m - h_{\text{h0}}}} \tag{A6}
\]

The time constants for \( m \) and \( h \) were also modeled as voltage dependent with the following equations:

\[
\tau_m(V_m) = \frac{1}{e^{V_m - m_{\text{tau}}}} + \frac{1}{e^{V_m - m_{\text{tau}}} + e^{V_m - m_{\text{tau}}}} \tag{A7}
\]

and

\[
\tau_h(V_m) = \frac{1}{e^{V_m - h_{\text{tau}}}} + \frac{1}{e^{V_m - h_{\text{tau}}} + e^{V_m - h_{\text{tau}}}} \tag{A8}
\]

This description results in a total of 18 parameters specifying each of the currents. These parameters given in Table 1 were determined by describing the various currents in terms of Hodgkin-Huxley models (Fig. A1). The currents to be fitted according to the above equations were separated as outlined in METHODS. For technical reasons, i.e., to get an adequate voltage clamp, the currents were measured using solutions with reduced concentration of permeating ions. For example, the Na\(^+\) current was measured with a bath solution containing 60 mM Na\(^+\) (instead of 190 mM in standard bath solution). In such cases, the maximum conductance \( G_{\text{max}} \) was corrected, the data given in Table 1 correspond to standard conditions (solutions as used for current-clamp measurements).

The three ion-activated currents, i.e., \( I_{\text{KNa}} \) and \( I_{\text{KCa}} \), and the sustained \( I_{\text{KCa}} \) were separated as outlined in METHODS. First, to model \( I_{\text{KNa}} \), the actual Na\(^+\)-inward current carried by \( I_{\text{KNa}} \) was calculated and multiplied with an empirical determined scaling factor called sensitivity factor. This approximation appears to be justified at least for the \( K_{\text{Ca}} \) peak current, which was found to be proportional to the Na current (Fig. 5D). Moreover, using this approximation, we were able to simulate a \( K_{\text{Na}} \) current with amplitude and kinetic properties similar to the current obtained in the cells. The Na\(^+\)-inward current times the sensitivity factor was used in Eqs. A5 and A6 instead of the membrane voltage to calculate \( m \) and \( h \), respectively. Because the time constants of the \( K_{\text{Na}} \) current are virtually voltage independent (Fig. 1E and 3F), they were fixed, i.e., Eqs. A7 and A8 did not apply. However, the current had a particular delay of 2 ms with respect to the Na-inward current. The sensitivity factor and the delay were adjusted to reproduce time course and size of the modeled current according to the measured current at different voltages (for size, cf. Fig. 5D).

Second, to model \( I_{\text{KCa}} \) and \( I_{\text{KCa}} \), \( I_{\text{KCa}} \) and \( I_{\text{KCa}} \), we had to make some simplifying assumptions. We first considered these currents purely voltage dependent. To separate the currents, we modeled \( K_{\text{Ca}} \) with a \( m^2 h \) kinetics and subtracted it from the total \( K_{\text{Ca}} \) current. Under these conditions, we determined the voltage dependence of parameters as given in Table 1 and Fig. A1, F1–F3. These parameters, of course, reflect a “mean” Ca\(^2+\) supply through voltage-gated Ca\(^2+\) channels. To link the Ca\(^2+\) to the \( K_{\text{Ca}} \) current, we determined the effect of increase and decrease of the Ca\(^2+\) current (using 10 and 2 mM Ca\(^2+\) in the bath solution, respectively) on the \( K_{\text{Ca}} \) current. With this information, we calculated a sensitivity factor linking the amplitudes of \( K_{\text{Ca}} \) and \( K_{\text{Ca}} \) to the Ca\(^2+\) current amplitude (Table 1). For modeling the \( K_{\text{Ca}} \) currents, we made a further simplifying assumption in that we calculated these currents from the actual Ca\(^2+\) current. According to the results shown in Fig. 2, we coupled the \( K_{\text{Ca}} \) currents to the P/Q-type Ca\(^2+\) current. Although this neglects the fact that also the L-type Ca\(^2+\) current provides Ca\(^2+\) for the activation of the \( K_{\text{Ca}} \) component (Fig. 2, B and D), it is more important for the purpose of this study to investigate the effect of P/Q-type current modulation on \( K_{\text{Ca}} \) currents and spiking. To reproduce the kinetics and voltage dependence of the \( K_{\text{Ca}} \) currents measured in neurons by the modeled currents, we had to introduce a delay (–1.5 ms) between Ca\(^2+\) current and \( K_{\text{Ca}} \) currents. The background Ca\(^2+\) current, \( I_{\text{Ca,bkg}} \), was modeled with a constant, voltage- and ion-independent conductance (cf. Wiché et al. 2004).

The total number of parameters including leak conductance, leak potential and membrane capacitance amounted to 156. The simulation could be run in either the current- or voltage-clamp mode at a variable time-resolution ranging from 1 to 50 μs. With a maximum number of 20,000 time steps, this corresponded to a total simulation time ranging from 20 ms to 1 s. All parameters could be saved in a parameter file in ASCII format, which allowed for easy editing.

In voltage-clamp, the total current, i.e., the sum of all voltage- and ion-activated currents, was calculated as leak-subtracted and without capacitive current

\[
I_{\text{tot}}(t) = \sum G_i(V_m(t) - V_{\text{leak}}) \tag{A9}
\]

In current clamp, the actual membrane voltage \( V_{\text{m}} \) at time \( t \) was updated from its previous value at time \( t-1 \) according to

\[
V_{\text{m}}(t) = \left[ \sum G_i(V_{\text{m}}(t) - V_{\text{leak}}) + G_{\text{leak}}(V_{\text{m}}(t) - V_{\text{leak}} - \frac{C}{\Delta t} \cdot V_{\text{m}}(t-1)) \right] / \left[ \sum G_i(t) + G_{\text{leak}} + \frac{C}{\Delta t} \right] \tag{A10}
\]

This equation can be derived from fact that the sum of all currents (active, leak, injected and capacitive) equal 0

\[
I_{\text{tot}}(t) + I_{\text{leak}}(t) + I_{\text{leak}}(t) + C \frac{\Delta V(t)}{\Delta t} = 0
\]

\[
\sum G_i(t) \cdot (V_{\text{m}}(t) - V_{\text{leak}}) + G_{\text{leak}} \cdot (V_{\text{m}}(t) - V_{\text{leak}}) + I_{\text{leak}}(t) + \frac{C}{\Delta t} \cdot (V_{\text{m}}(t) - V_{\text{leak}} - V_{\text{m}}(t-1)) = 0
\]

\[
V_{\text{m}}(t) \cdot \left[ \sum G_i(t) + G_{\text{leak}} + \frac{C}{\Delta t} \right] = \sum G_i(t) \cdot V_{\text{m}}(t) + G_{\text{leak}} \cdot V_{\text{m}}(t) - I_{\text{leak}}(t) + \frac{C}{\Delta t} \cdot V_{\text{m}}(t-1) = 0
\]

The simulation software was written in IDL programming language (RSI Research Systems, Boulder, CO) with a menu-driven GUI. The compiled code including the parameter list is available on request from the authors and can be run under the “IDL virtual machine”

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**FIG. A1.** Description of DUM cell ion currents in terms of Hodgkin-Huxley models. Current separation was performed as described in METHODS. Symbols represent means of 5–9 cells, bars denote SE. The curves are fits of Hodgkin-Huxley models to the data described by parameters given in Table 1. Columns show steady-state activation (\( m_{\infty} \)) and inactivation (\( m_{\infty} \)); activation time constant \( \tau_m \) and inactivation time constant \( \tau_h \) for Na\(^+\) current (A) (cf. Wiché 2001a), P/Q- and non-P/Q-type Ca\(^2+\) currents (B), delayed rectifier (DR) K\(^+\) current (C), A-type K\(^+\) current (D) (cf. Grošleau and Lapiéd 1995), K\(_{\text{Na}}\) current (E) (cf. Grošleau and Lapiéd 1994), and transient and sustained K\(_{\text{Ca}}\) current (F; \( I_{KCa,t} \) and \( I_{KCa,s} \)).
environment, which can be downloaded at no cost from the RSI Website (http://www.rsinc.com/udlv2/).

**GRANTS**

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**REFERENCES**


