Peptidergic counter-regulation of Ca\(^{2+}\)- and Na\(^{+}\)-dependent K\(^{+}\) currents modulates the shape of action potentials in neurosecretory insect neurons

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Abstract

Influx of Ca\(^{2+}\) and Na\(^{+}\) ions during an action potential can strongly affect the repolarization and the fast afterhyperpolarization (fAHP) if a neuron expresses Ca\(^{2+}\)- and Na\(^{+}\)-dependent K\(^{+}\) currents (K,Ca and K,Na). This applies to cockroach abdominal dorsal unpaired median neurons (DUMs). Here the rapid activation of K,Ca depends mainly on the P/Q-type Ca\(^{2+}\) current. Adipokinetic 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 Na\textsuperscript{+} and P/Q-type Ca\textsuperscript{2+} current and the background Ca\textsuperscript{2+} current. Up-
regulation of P/Q-type Ca\textsuperscript{2+} current increases the K,Ca current while enhanced inactivation of
Na\textsuperscript{+} current decreases the K,Na 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 Ca\textsuperscript{2+} and K,Ca current enhanced the hyperpolarization
but had a weak effect on spiking. Down-regulation of Na\textsuperscript{+} and K,Na current decreased
hypermultipolarization and slightly accelerated spiking. Superposition of these modulations
produced an increase in fAHP while the spike frequency remained unchanged. Only when
the up-regulation of the pacemaking Ca\textsuperscript{2+} background current was included in the simulated
modulation the model reproduced 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.

Key words: DUM neuron, adipokinetic hormone, Periplaneta americana
Introduction

Insect adipokinetic hormones (AKHs) mobilize energy reserves from storage organs such as the fat body similar to glucagon in mammals (Van der Horst 2003; Gäde and Auerswald 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 (Witten et al. 1984; Scarborough et al. 1984), one of which, AKH I (pQVNFSNWamide, also called Neurohormone D, Baumann et al. 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 (i) potentiation of a P/Q-type Ca$^{2+}$ current due to channel phosphorylation via protein kinase A (PKA) (Wicher 2001b); (ii) reduction of the fast Na$^{+}$ current, again via PKA (Wicher 2001a); (iii) potentiation of a BK-type Ca$^{2+}$–activated K$^{+}$ (K,Ca) current (Wicher et al. 1994; Derst et al. 2003); (iv) potentiation of a Ca$^{2+}$ background current via $G_{q}$–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 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 which includes a set of ten ion currents described in terms of the Hodgkin-Huxley formalism. Our studies indicate that accelerated spiking is solely due to the change in the 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 *Periplaneta 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 at least 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 NaH₂PO₄, 2 Na-pyruvate, 10 glucose, 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, Germany). 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, 10 HEPES (pH = 7.4), and patch pipettes (resistance > 1.5 MΩ) were filled with solution composed of (in mM): 190 K-gluconate, 5 NaCl, 2 Mg-ATP, 1 CaCl₂, 3 EGTA, 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, 10 HEPES, and bath solution containing (in mM): 60 Na isethionate, 90 choline methylsulfate, 40 TEA-Br, 7 MgCl₂, 1 CdCl₂, 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, 10 HEPES, and the bath solution (in mM): 5 CaCl₂, 190 choline methylsulfate, 10 HEPES; 5 µM tetrodotoxin.

K⁺ currents in DUM neurons were measured with pipette solution containing (in mM): 180 K-gluconate, 10 NaCl, 2 Mg-ATP, 1 CaCl₂, 5 mannitol, 3 EGTA, 10 HEPES. The bath solution to measure K,Ca currents contained (in mM): 190 NaCl, 5 KCl, 5 CaCl₂, 2 MgCl₂, 10 HEPES, and 0.5 µM tetrodotoxin (TTX). K,Ca currents were separated from other currents by applying the BK-channel blocker iberiotoxin (100 nM). The purely 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, 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 ± SEM (n = number of cells). Fitting procedures were performed with the software Prism2 (Graph Pad, San Diego, CA, USA) and with IGOR (WaveMetrics, Lake Oswego, OR, USA).

Chemicals

Salts, 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 iberiotoxin from Calbiochem (Schwalbach, Germany).

Results

Relationship between voltage-gated Ca currents and K,Ca currents in DUM neurons

*Periplaneta* DUM neurons have previously been shown to express pSlo, the α subunit of large conductance K,Ca (BK) channels (Derst et al. 2003). While pSlo channels heterologously expressed in HEK293 cells give rise to a purely non-inactivating K,Ca current, the K,Ca current in DUM neurons is composed of a transient (K,Ca,t) and a sustained (K,Ca,s) component (Wicher et al. 1994; Grolleau and Lapied 1995; Derst et al. 2003). pSlo channels are sensitive to the BK channel blocker iberiotoxin (IbTx; IC₅₀ = 45 nM). The K,Ca channels in DUM neurons seem to be more sensitive to IbTx since both K,Ca current components are blocked by 100 nM IbTx (Derst 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 K,Ca current obtained by subtraction of these currents. There is an initial rapid K,Ca 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 around 2 ms) of the first part of the K,Ca current which is dominated by K,Ca,t. It then reaches a sustained level, i.e. K,Ca,s, some 5 ms after stepping the voltage. Activation of both components of the K,Ca current starts around −50 mV, i.e. at about the same voltage as that of the start of the Ca²⁺ current (Fig. 1B, upper panel).
time-to-peak of the K,Ca current is comparable to that of the voltage-gated Ca\(^{2+}\) current (Fig. 1D).

It should be pointed out that there is no pharmacological tool to separate K,Ca,t and K,Ca,s. However, these components differ in their Ca\(^{2+}\) sensitivity: When voltage-gated currents are blocked and the intracellular [Ca\(^{2+}\)] is raised by perfusing the cell with Ca\(^{2+}\)-rich pipette solution the first current evolving - as seen on repeated application of depolarizing voltage protocols - is K,Ca,t. K,Ca,t. K,Ca,s appears about 1 min later, indicating that K,Ca,s requires a higher [Ca\(^{2+}\)], than K,Ca,t to be activated by depolarization (Derst et al. 2003). The fact that K,Ca,t inactivated in spite of a constant [Ca\(^{2+}\)], indicates that inactivation is an intrinsic channel property perhaps produced by accessory \(\beta\) 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 K,Ca,s to the K,Ca peak current at least at higher depolarizations. Since, however, such error is expected to be small and will not have consequences for the results obtained below we will refer the K,Ca peak current as K,Ca,t.

The Ca\(^{2+}\) current activating positive to –50 mV is composed of three subtypes (Wicher and Penzlin 1997), (i) P/Q-type current sensitive to \(\omega\)-agatoxin IVA (\(\omega\)-AgaTx), (ii) N-type current sensitive to \(\omega\)-conotoxin GVIA (\(\omega\)-CgTx), and (iii) L-type current sensitive to verapamil (Fig. 1B, lower panel). The effect of \(\omega\)-AgaTx (50 nM) and verapamil (10 \(\mu\)M) on Ca\(^{2+}\) 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 Ca\(^{2+}\) channel blockers in order to learn to what extent L-type, N-type and P/Q-type Ca\(^{2+}\) currents might supply the Ca\(^{2+}\) for activation of the K,Ca current. The three Ca\(^{2+}\) currents cause a Ca\(^{2+}\) influx of different size and activation kinetics (Fig. 1B, lower panel, Fig. 1C), and their suppression affected the K,Ca currents differently: \(\omega\)-AgaTx (50 nM) led to reduction of both the transient and the sustained component of K,Ca current (Fig. 2A, C), \(\omega\)-CgTx (1 \(\mu\)M) had no effect on K,Ca current (\(n = 5\), not shown) while verapamil (10 \(\mu\)M) had a clearly weaker effect on the transient component than \(\omega\)-AgaTx and suppressed
the sustained K,Ca current to a similar degree (Fig. 2B, 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 $\omega$-AgaTx (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 since it is reduced by about the same extent by blocking P/Q or L-type channels (Fig. 2C, D).

**Peptidergic up-regulation 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-type and the L-type Ca$^{2+}$ current in DUM neurons (Wicher 2001b). Fig. 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. 3A1 and A2, respectively. AKH I thus up-regulates both the transient and the sustained K,Ca component. This may be plausibly attributed to the up-regulation of the P/Q-type current as this current was seen above to provide Ca$^{2+}$ for the activation of both K,Ca components. The threshold concentration for the AKH I-induced potentiation of the P/Q-type Ca$^{2+}$ current was 1 pM (Wicher 2001b). At this concentration there was already some increase in the K,Ca current (Fig. 3A3) which, however, was mainly restricted to the transient component (Fig. 3B1). Increasing [AKH I] from 1 pM to 10 nM induced progressively larger K,Ca currents (Fig. 3C).
Compared to the K,Ca current under control conditions (isolated with IbTx) the current produced by 10 nM AKH I differed in that the transient component activated at more negative potentials. The non-linearity in the I-V relation for this component around –10 mV (Fig. 3B2) is especially remarkable. It seems to reflect the increase in P/Q-type current by AKH I which is most pronounced at voltages ranging from –20 mV to 0 mV (Wicher 2001b). The possibility that AKH I affects purely voltage-gated K⁺ currents such as the delayed rectifier (I K DR) and the A-type current (I K A) could be ruled out since in the presence of 1 mM Cd²⁺ which completely blocks the Ca²⁺ currents (Wicher and Penzlin 1997) AKH I failed to affect K⁺ currents (n = 5 cells, not shown). Taken together, these results are compatible with the assumption that the up-regulation of the K,Ca current is caused by the increase in P/Q-type current.

The increase in K,Ca current is expected to increase the fast afterhyperpolarization (fAHP) of the action potential as has been previously observed in DUM neurons in the presence of AKH I (Wicher et al. 1994). Conversely, a reduction of K,Ca current was seen to prolong action potentials and to reduce the hyperpolarization (Derst et al. 2003).

**Activation of PKA does not affect I K,Ca**

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 up-regulation of the P/Q-type Ca²⁺ current (Wicher 2001b). In principle the regulation of the K,Ca currents by AKH I might be dual, i.e. be partly due to K,Ca channel phosphorylation. In order 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 Gₛ proteins in HEK293 cells (Wicher, unpublished observation). Application of AKH I (10 nM), after loading the cell with a Ca²⁺-rich pipette solution, had, however, no effect on pSlo currents activated by depolarization (Fig. 4A, left panel). 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 analog 8-bromo-cAMP. Again, there was no change in the properties of pSlo currents (Fig. 4A, right panel). 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 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 down-regulation 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$^+$-dependend 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$^+$-dependend 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). Since K,Na channels are not voltage-gated the I-V relation of the K,Na peak current mirrors the the I-V relation of the Na$^+$ current (Fig. 5D).

**Peptidergic up-regulation 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). Since 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 down-regulation 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 since 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 Ca²⁺- 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 afterhyperpolarization (fAHP) of action potentials?

To simulate the endogeneous spiking of DUM neurons we included ten currents in a one-compartment model of a DUM neuron: One Na⁺ current, four Ca²⁺ currents (the background current, a low-voltage activated current, and two high-voltage activated currents, i.e. P/Q-type and nonP/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,s). The currents were described in terms of the Hodgkin-Huxley formalism (cf. Appendix, Fig. A1 and Tab. 1). Fig. 6 compares recorded spikes from a DUM neuron with the spike pattern generated by the
model, using the parameters given in Tab. 1. The model reproduced the experimentally observed characteristics of the action potential such as threshold, overshoot and afterhyperpolarization as well as the resting firing frequency.

To get insight into the role of the K,Na current and the transient K,Ca current in the regulation of neuronal activity we investigated the effects of varying the size of these currents. The K,Na current was found to be essential for the stability of repetitive activity (Fig. 7A). Both the K,Na and the K,Ca,t current affected the spike frequency (Fig. 7A) and the fAHP (Fig. 7C), though with different efficiency. Upon reduction of the K,Na current there was a robust increase in spike frequency while an increase produced only a weak reduction (Fig. 7A). Changing the K,Ca,t current led to inversely proportional changes in spike frequency. When K,Ca,t was increased by >30% spiking stopped since the action potential threshold was no longer reached (Fig. 7A). On the other hand, spiking did not become irregular even when the K,Ca,t-conductance was reduced by 90%. Reduction of the K,Na conductance by > 40% caused irregular spiking. Reduction of K,Ca,t caused a slight attenuation of the fAHP. Complete block of K,Ca,t was estimated to reduce the AHP by 4 mV which was somewhat less than found experimentally, i.e. after blocking K,Ca with iberiotoxin (9 mV, Wichers 2001b). The dependence of fAHP on I K,Na was similar to that on I K,Ca,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. K,Na and K,Ca, affect spiking. Increasing the Na⁺ conductance unexpectedly produced a rise in frequency (by 100% on increasing G by 30%) and attenuated the AHP (by 4 mV on increase by 30%) while reducing the Na⁺ conductance terminated spiking because the pacemaker activity became too weak (not shown). We then adjusted the Na⁺ conductance solely by changing the inactivation parameters thus imitating the Na⁺ current-modulation caused by AKH I (Fig. 7B, D). Under these conditions the variation of Na⁺ current produced changes more similar to those found for varying the K,Na conductance alone (compare Fig. 7B with 7A). Reduction of Na⁺ conductance caused a steep increase in frequency and a decrease in hyperpolarization until
spiking became irregular at > 40 % reduction (Fig. 7B, D). A rise in Na⁺ conductance did not affect both the spike frequency and the AHP (Fig. 7B, D). Variation of the P/Q Ca²⁺ current had no effect on spike frequency but on AHP (Fig. 7B, D). With G reduced by > 50 % the AHP is only –50 mV, and it saturates at –62 mV when G is increased by > 50 %.

The main conclusions about the role of the K,Na current and the transient K,Ca current in the regulation of spiking are (1) there is a critical size of K,Na current which is required for stable spiking (Fig. 7A, B), (2) the AHP is determined by up- and down-regulation of the K,Ca current by the P/Q-type Ca²⁺ current and by down-regulation of the K,Na current by the Na⁺ current.

Application of 10 nM AKH I produces faster spiking of DUM neurons and affects the action potential shape. The spike frequency increase ranges from 15 to 34 %, the mean is 25.2 ± 4.0 % (n = 7, Fig. 8A). The hyperpolarization increases by 2 to 6 mV, on average by 4.0 ± 1.0 mV (n = 7, Fig. 8B). The question, then, is whether our model reproduces these changes 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 K,Ca currents, down-regulation of Na⁺ current and K,Na current as well as up-regulation of Ca²⁺ background current. In terms of our model (cf. Tab. 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 % which 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).

In order to assess whether the initial size of K,Na and K,Ca currents may affect the simulation of the AKH effect we varied their conductances by ±10 % and repeated the simulation. Variations of the K,Na 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 K,Ca currents were reduced by 10 %. While this variation had no effect on the increase in spike frequency the variation of initial K,Ca 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 up-regulation of the P/Q-type Ca^{2+} current and both K,Ca currents (Fig. 9A). This caused an increase in hyperpolarization (by 5 mV) which is in line with the measured AKH I effect (4 mV) while the spike frequency was hardly affected (reduction by 2 %). Down-regulation of Na^+ current and K,Na current 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,Ca currents were combined with those of Na^+ current and K,Na current 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 up-regulation 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 above (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 up-regulation of the Ca^{2+} background current.

Discussion

Octopamine, the chemical messenger released by the DUM neurons investigated here, is known to up-regulate general activity levels (Roeder 2005; Saraswati et al. 2004). In Drosophila it has recently been shown that, upon 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 (Wicher et al. 2001; Nusbaum et al. 2001; Brezina et al. 2005) 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. Golowasch et al. 1992; Dale 1995). 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,Ca currents (Buchholtz et al. 1992), K,Na currents (Dale 1995) or K,Na and slowly activating K,Ca currents (Dale and Kuenzi 1997). In our model the K,Na current and the K,Ca,t 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_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 persistant 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 persistant Na^{+} current contribute to pacemaking (Grolleau and Lapied 2000).
**How AKH I leads to changes of Ca²⁺- and Na⁺-dependent K⁺ currents**

Since AKH I produces changes in Ca²⁺- and Na⁺ currents the K,Ca and the K,Na current must also change. For I K,Ca we experimentally ruled out the possibility of an additional direct modulation of the respective channels. For I K,Na this is also rather unlikely since 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,Na current. There might be a more sophisticated relationship between Na⁺ and K,Na current than included in our model.

**Modulation of the K, Ca current**

Although three types of voltage-gated Ca²⁺ channels are expressed in DUM neurons (Fig. 1B), only P/Q-type channels appear to provide Ca²⁺ for activation of the transient K,Ca current component. The sustained K,Ca component can additionally be activated by Ca²⁺ 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 while L- and P-type channels activate Ca²⁺-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,Ca,t affects the shape of the action potential. K,Ca,t controls its duration (for further K⁺ currents involved in this process cf. Wicher et al., 2001). In addition K,Ca t determines the size of fAHP (Derst et al. 2003). These roles of the BK current are comparable to those in various mammalian neurones (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 though, 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_{K,Na} (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 following 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,Ca 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 favour of the K,Ca current.

**Why counterregulation of K, Ca current and K, Na current?**

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 since this does not produce a change in spike frequency. In this light it would be sufficient to up-regulate 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-type and P/Q-type) play a central role in triggering transmitter release (Reid et al. 2003). Although we have no evidence that the up-regulation 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 noradrenalin 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 (Raffaelli 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 down-regulation 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 $I_{Ca,\text{back}}$, the low voltage-activated current $I_{Ca,\text{LVA}}$, and the two high voltage-activated currents $I_{Ca,\text{P/Q}}$ (P/Q-type current) and $I_{Ca,\text{nP/Q}}$ (nonP/Q-type current = total HVA current – P/Q-type current). The K\(^+\) currents are the voltage activated delayed rectifier $I_{K,DR}$ and the A-type current $I_{K,A}$, the voltage-independent Na\(^+\)-activated current $I_{K,Na}$, and the Ca\(^{2+}\)-activated current composed of the transient $I_{K,Ca,t}$ and the sustained $I_{K,Ca,s}$. In general, each current $I(t)$ is modeled according to the following equation ($G(t)$ is the conductance, $V_{eq}$ the equilibrium potential, $V_m(t)$ the membrane potential):

$$I(t) = G(t) \cdot \left( V_m(t) - V_{eq} \right)$$  \hspace{1cm} (1)

with

$$G(t) = G_{\text{max}} \cdot m^{\text{exp}}(t) \cdot h^{\text{exp}}(t)$$  \hspace{1cm} (2)

The exponents for $m$ ($m^{\text{exp}}$) and $h$ ($h^{\text{exp}}$) ranged from 0 to 4 and are specified for each of the current. $m(t)$ and $h(t)$ are described by the following 1\(^{st}\) order kinetics:

$$\frac{dm(t)}{dt} = \frac{1}{\tau_m} \cdot \left( m_{\infty}(V_m(t)) - m(t) \right)$$  \hspace{1cm} (3)

and

$$\frac{dh(t)}{dt} = \frac{1}{\tau_h} \cdot \left( h_{\infty}(V_m(t)) - h(t) \right)$$  \hspace{1cm} (4)

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

$$m_{\infty}(V) = \frac{1}{1 + e^{(V_{m,\infty} - V) / \text{Slope}_m}}$$  \hspace{1cm} (5)

and

$$h_{\infty}(V) = \frac{1}{1 + e^{(V_{h,\infty} - V) / \text{Slope}_h}}$$  \hspace{1cm} (6)
The time-constant for m and h were also modeled as voltage-dependent with the following equations:

\[
\tau_m(V) = \frac{1}{e^{\frac{V-V_{m,up}}{\text{Slope}_{m,up}}} + e^{\frac{V-V_{m,down}}{\text{Slope}_{m,down}}}} \quad (7)
\]

and

\[
\tau_h(V) = \frac{1}{e^{\frac{V-V_{h,up}}{\text{Slope}_{h,up}}} + e^{\frac{V-V_{h,down}}{\text{Slope}_{h,down}}}} \quad (8)
\]

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 Gₘₐₓ was corrected, the data given in Tab. 1 correspond to standard conditions (solutions as used for Current Clamp measurements).

The three ion-activated currents, i.e. I_{K,Na} and both the transient and the sustained I_{K,Can} deviated from the above description in the following aspects:

1. To model I_{K,Na}, the actual Na⁺-inward current carried by I_{Na} was calculated and multiplied with an empirical determined scaling factor called sensitivity factor. This approximation appears to be justified at least for the K,Na 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,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 equations 5 and 6 instead of the membrane voltage to calculate m and h, respectively. Since the time-constants of the K,Na current are virtually voltage-independent (Fig. A1, E2 and E3) they were fixed, i.e. equations 7 and 8 did not apply. However, the current had a particular delay of about 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).

(2) To model \( I_{K,Ca,t} \) and \( I_{K,Ca,s} \) we had to make some simplifying assumptions. We first considered these currents purely voltage dependent. To separate the currents we modeled \( K,Ca,t \) with a \( m^2h \)-kinetics and subtracted it from the total \( K,Ca \) current. Under these conditions we determined the voltage dependence of parameters as given in Tab. 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 \( K,Ca \) current we determined the effect of increase and decrease of the \( Ca^{2+} \) current (using 10 mM and 2 mM \( Ca^{2+} \) in the bath solution, respectively) on the \( K,Ca \) current. With this information we calculated a sensitivity factor linking the amplitudes of \( K,Ca,t \) and \( K,Ca,s \) to the \( Ca^{2+} \) current amplitude (Tab. 1). For modeling the \( K,Ca \) 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 \( K,Ca \) 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 \( K,Ca,s \) component (Fig. 2B, D) it is more important for the purpose of this study to investigate the effect of P/Q-type current modulation on \( K,Ca \) currents and spiking. To reproduce the kinetics and voltage dependence of the \( K,Ca \) currents measured in neurons by the modeled currents we had to introduce a delay (~ 1.5 ms) between \( Ca^{2+} \) current and \( K,Ca \) currents.

The background \( Ca^{2+} \) current, \( I_{Ca,back} \), was modeled with a constant, voltage- and ion independent conductance (cf. Wicher 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 microseconds. With a maximum number of 20 000 time steps, this corresponded to a total simulation time ranging from 20 milliseconds to 1 sec. 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,

\[ I_{total}(t) = \sum G_j(t) \cdot (V_m(t) - V_{eq}) \] (9)

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

\[ V_m(t) = \left[ \sum G_j(t) \cdot V_{iEQ} + G_{\text{leak}} \cdot V_{\text{leak}} - I_{\text{inj}}(t) + \frac{C}{\Delta t} V_m(t-1) \right] - \left[ \sum G_j(t) + G_{\text{leak}} + \frac{C}{\Delta t} \right] (10) \]

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

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

\[ \sum G_j(t) \cdot (V_m(t) - V_{eq}) + G_{\text{leak}} \cdot (V_m(t) - V_{\text{leak}}) + I_{\text{inj}}(t) + \frac{C}{\Delta t} (V_m(t) - V_m(t-1)) = 0 \]

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

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’ environment which can be downloaded at no cost from the RSI Website (http://www.rsinc.com/idlvm/).

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References


**Figure legends**

Fig. 1: K,Ca currents in DUM neurons and their relation to Ca$^{2+}$ currents. (A) Separation of the K,Ca current by iberiotoxin (IbTx). Currents activated by the voltage jump indicated were recorded before (Control) and 2 min after application of 100 nM IbTx (upper panel) and were digitally subtracted yielding the IbTx-sensitive K,Ca current (lower panel) which is composed of a transient and a sustained component. (B) I-V relationships for the transient K,Ca current ($I_{K,Ca,t}$), the sustained K,Ca current ($I_{K,Ca,s}$, measured 20 ms after start of depolarisation) and for voltage-gated Ca$^{2+}$ peak current in DUM neurons. K,Ca currents were separated as described above ($n = 7$). All currents were normalized to the maximal Ca$^{2+}$ peak current ($V = 0$ mV). The total voltage-gated Ca$^{2+}$ current comprizes three components shown in the lower I-V relation. The currents shown are those which were blocked by the L-type channel blocker verapamil (10 µM), the N-type channel blocker $\omega$-conotoxin GVIA ($\omega$-CgTx, 1µM) or the P/Q-type channel blocker $\omega$-agatoxin IVA ($\omega$-AgaTx, 50 nM). Currents were normalized as above. (C) Separation of Ca$^{2+}$ current components by $\omega$-AgaTx and verapamil. Upper panel, currents activated by the voltage jump indicated (Control), 1 min after application of $\omega$-AgaTx, and 1 min after application of verapamil (in the continuous presence of $\omega$-AgaTx). Subtraction yields the $\omega$-AgaTx-sensitive P/Q-type current and the verapamil-sensitive L-type current (lower panel). (D) Time to peak for $K_{Ca,t}$ and total Ca current ($n = 7$).

Fig. 2: Block of P/Q-type (A) and L-type (B) Ca$^{2+}$ currents reduces K,Ca currents differently. (A) Example of a K,Ca current and a Ca$^{2+}$ current blocked by $\omega$-AgaTx (50 nM). Currents activated by the voltage jump indicated were recorded before and 1 min after application of $\omega$-AgaTx. The displayed currents are the difference currents, i.e. control – $\omega$-AgaTx. (B) Recordings of K,Ca current and Ca$^{2+}$ current blocked by verapamil (10 µM). Currents activated by the voltage jump indicated were recorded before and 1 min after application of verapamil; displayed are the difference currents. The measurements of Ca$^{2+}$ and K,Ca currents for (A) and (B) had to be carried out in separate cells, and representative traces are co-displayed. (C) I-V relationships for the transient K,Ca current ($I_{K,Ca,t}$) and the sustained
K,Ca current ($I_{K,\text{Ca,s}}$) blocked by $\omega$-AgaTx. Currents were separated as described in (A). $n = 5$. (D) I-V relationships for $K,\text{Ca,t}$ and $K,\text{Ca,s}$ current blocked by verapamil. Currents were separated as described in (A, B). $n = 6$.

Fig. 3: Modulation of $K,\text{Ca}$ currents by the peptide AKH I. (A1) Current traces recorded upon jumps to 0 mV before (Control) and 2 min after application of 10 nM AKH I. (A2) $K,\text{Ca}$ current induced by 10 nM AKH I (difference current of traces shown in A1). (A3) $K,\text{Ca}$ current induced by 1 pM AKH I (difference current: 2 min AKH I – Control); note the onset of the $Ca^{2+}$ inward current on which the $K,\text{Ca}$ current is superimposed. (B1) I-V relationships for transient and sustained $K,\text{Ca}$ current induced by 1 pM AKH I. Currents were separated as described in (A1). $n = 4$. (B2) I-V relationships for transient and sustained $K,\text{Ca}$ current induced by 10 nM AKH I. Currents were separated as described in (A1). $n = 5$. (C) Concentration dependence of AKH I effect on transient $K,\text{Ca}$ current activated by depolarization to $-20$ mV and $+20$ mV. Currents were separated as described in (A1). $n = 4$.

Fig. 4: Test for a direct effect of AKH I on $K,\text{Ca}$ channel. pSlo, the $\alpha$ subunit of Periplaneta $K,\text{Ca}$ channel was coexpressed with the pAKH receptor in HEK293 cells. (A) Sample traces of pSlo currents activated by jumps from $-90$ mV to $+80$ mV before (Control) and 2 min after application of AKH I (10 nM) or 8-bromo-cAMP (8-br-cAMP, 2 $\mu$M). Note that neither AKH I nor 8-br-cAMP affected pSlo currents. (B) Effect of AKH I (10 nM), 8-br-cAMP (2 $\mu$M) and KT5720 (10 $\mu$M) on pSlo currents. Currents measured in the presence of these agents were normalized to Control currents. $n = 5$.

Fig. 5: $K,\text{Na}$ currents and $Na^+$ currents in DUM neurons. (A) Sample traces of currents activated by depolarizations as indicated in (B) before (Control) and 2 min after application of TTX (0.5 $\mu$M). (B) The biphasic current, i.e. the difference of currents shown in (A) results from superposition of $K,\text{Na}$ current and $Na^+$ current. For comparison, a $Na^+$ current is shown which was recorded when $K^+$ currents are suppressed. (C) Voltage dependence of the time to peak for $K,\text{Na}$ and $Na^+$ currents ($n = 5$). (D) I-V relationships for voltage-gated $Na^+$ peak current and for $K,\text{Na}$ peak current in DUM neurons. $K,\text{Na}$ and $Na^+$ currents were separated
by means of TTX as demonstrated in (A, B) \((n = 7)\). The size of Na\(^+\) (and K,Na) currents is thus underestimated, yet this procedure was adequate for comparing the data with the currents suppressed by AKH I. (E) K,Na plus Na\(^+\) current suppressed by 10 nM AKH I (difference current: Control \(-\) 2 min AKH I). (F) I-V relationships for K,Na peak current and for Na\(^+\) peak current suppressed by 10 nM AKH I (Control \(-\) 2 min AKH I, \(n = 7\)).

Fig. 6: Measured (A) and modelled (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 Tab. 1.

Fig. 7: Changes of spike frequency (A, B) and fast afterhyperpolarization, fAHP (C, D), on varying the strengths of the currents I\(_{Na}\), I\(_{K,Na}\), I\(_{Ca}\) and I\(_{K,Ca,t}\). Changes produced by varying either I\(_{K,Na}\) or I\(_{K,Ca,t}\) are shown in A and C. B and D demonstrate what happens when the Na\(^+\) + K,Na current and the P/Q-type Ca\(^{2+}\) and K,Ca currents change concomitantly in the manner observed on application of 10 nM AKH I. \(G_{rel} = 1\) corresponds to the \(G_{max}\) values given in Tab. 1. The change in \(G_{rel}\) of I\(_{Na}\) was produced by changing the inactivation according to the modulation by AKH I, i.e by increasing \(\tau \_\text{Slope up}\) and decreasing \(\tau \_\text{Slope dn}\) (see Results) until the amplitude of I\(_{Na}\) corresponded to a given figure of \(G_{rel}\). A direct change of \(G_{max}\) (with no change in inactivation kinetics) – as performed with the Ca\(^{2+}\) current - would lead to drastically different effects (cf. text). When one set of parameters was varied the others were fixed at \(G_{rel} = 1\). Asterisks indicate instability of spiking.

Fig. 8: Measured (A, B) and calculated (C, D) effect of AKH I (10 nM) on the activity (A, C) and the shape of an action potential (black traces, Control; red traces, 2 min after application of 10 nM AKH I). AKH I accelerates spiking both in the neuron (A) and in the model (C). Also, AKH I leads to stronger hyperpolarization of measured (B) and calculated (D) action potentials. The data used for simulation of the AKH effect are given in the text.

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) Up-regulation of P/Q-type Ca\(^{2+}\) and K,Ca current. (B) Down-
regulation of $\text{Na}^+$ and $\text{K,Na}$ current. (C) Combined modulations of P/Q-type $\text{Ca}^{2+}$ and $\text{K,Ca}$
current and down-regulation of $\text{Na}^+$ and $\text{K,Na}$ current. (D) Like (C), but the up-regulation of
$\text{Ca}^{2+}$ 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).

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 SEM. The curves are fits of Hodgkin-Huxley models to the data described by
parameters given in Tab. 1. Columns show (1) steady-state activation ($m_{\text{act}}$) and inactivation
($m_{\text{int}}$), (2) activation time constant $\tau_m$ and (3) inactivation time constant $\tau_h$ for (A) $\text{Na}^+$ current
(cf. Wicher 2001a), (B) P/Q-type and nonP/Q-type $\text{Ca}^{2+}$ currents, (C) delayed rectifier (DR)
$\text{K}^+$ current, (D) A-type $\text{K}^+$ current (cf. Grolleau and Lapied 1995), (E) $\text{K,Na}$ current (cf.
Grolleau and Lapied 1994) and (F) transient and sustained $\text{K,Ca}$ current ($I_{\text{K,Ca,t}}$ and $I_{\text{K,Ca,s}}$).
Table 1: Parameters for voltage and ion-dependent membrane currents. 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 \( I_{K,DR} \), \( I_{K,A} \) and \( I_{K,Na} \) see also (Grolleau and Lapied 1994, 1995).

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Special Parameters (\( I_{Ca,back} - I_{K,Na} - I_{K,Ca,t} - I_{K,Ca,s} \))

Passive Parameters:

- \( G_{Ca,back} \) [nS]: 0.023
- Memb. Capacity [pF]: 370
- Na-Sensitivity \( I_{K,Na} \): 1.1
- G leak [nS]: 22
- Delay \( I_{K,Na} \) [ms]: 2.3
- V leak [mV]: -50
- Ca-Sensitivity \( I_{K,Ca,t} \): 1.7
Delay $I_{K,Ca,t}$ [ms]: 1.7
Ca-Sensitivity $I_{K,Ca,s}$: 0.5
Delay $I_{K,Ca,s}$ [ms]: 1.7
Fig. 2

A) A-\text{AgaTx} \\
\begin{align*}
  \text{IK,Ca} & \quad 3 \text{nA} \\
  \text{ICa} & \quad 1 \text{nA}
\end{align*}

B) Verapamil \\
\begin{align*}
  \text{IK,Ca} & \quad 3 \text{nA} \\
  \text{ICa} & \quad 1 \text{nA}
\end{align*}

C) \\
\begin{align*}
  I (\text{nA}) & \quad V (\text{mV}) \\
  \text{IK,Ca,t} & \quad \text{IK,Ca,s}
\end{align*}

D) \\
\begin{align*}
  I (\text{nA}) & \quad V (\text{mV}) \\
  \text{IK,Ca,t} & \quad \text{IK,Ca,s}
\end{align*}
Fig. 3

A1  A2  A3

B1  B2

C

\[ \text{[AKH I] (M)} \]

\[ I_{\text{peak}} (\text{nA}) \]

\[ V (\text{mV}) \]

\[ I (\text{nA}) \]
Fig. 4

A

+80 mV
+90 mV
1 nA

5 ms

AKH I

8-br-cAMP

B

[Graph showing bar chart with categories: AKH I, 8-br-cAMP, KT5720]
Fig. 5

A

B

C

D

E

F

I_{K,Na}  I_{Na}
Fig. 6

A
Experiment

B
Model
Fig. 7

A

B

C

D
Fig. 9