I_A in Kenyon Cells of the Mushroom Body of Honeybees Resembles Shaker Currents: Kinetics, Modulation by K^+, and Simulation

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Pelz, Corinna, Johannes Jander, Hendrik Rosenboom, Martin Hammer, and Randolf Menzel. I_A in Kenyon cells of the mushroom body of honeybees resembles shaker currents: kinetics, modulation by K^+, and simulation. J. Neurophysiol. 81: 1749–1759, 1999. Cultured Kenyon cells from the mushroom body of the honeybee, Apis mellifera, show a voltage-gated, fast transient K^+ current that is sensitive to 4-aminopyridine, an A current. The kinetic properties of this A current and its modulation by extracellular K^+ ions were investigated in vitro with the whole cell patch-clamp technique. The A current was isolated from other voltage-gated currents either pharmacologically or with suitable voltage-clamp protocols. Hodgkin- and Huxley-style mathematical equations were used for the description of this current and for the simulation of action potentials in a Kenyon cell model. Activation and inactivation of the A current are fast and voltage dependent with time constants of 0.4 ± 0.1 ms (means ± SE) at +45 mV and 3.0 ± 1.6 ms at +45 mV, respectively. The pronounced voltage dependence of the inactivation kinetics indicates that at least a part of this current of the honeybee Kenyon cells is a shaker-like current. Deactivation and recovery from inactivation also show voltage dependency. The time constant of deactivation has a value of 0.4 ± 0.1 ms at −75 mV. Recovery from inactivation needs a double-exponential function to be fitted adequately; the resulting time constants are 18 ± 3.1 ms for the fast and 745 ± 107 ms for the slow process at −75 mV. Half-maximal activation of the A current occurs at −0.7 ± 2.9 mV, and half-maximal inactivation occurs at −54.7 ± 2.4 mV. An increase in the extracellular K^+ concentration increases the conductance and accelerates the recovery from inactivation of the A current, affecting the slow but not the fast time constant. With respect to these modulations the current under investigation resembles some of the shaker-like currents. The data of the A current and its modulation by extracellular K^+ were incorporated into a reduced computational model of the voltage-gated currents of Kenyon cells. In addition, the model contained a delayed rectifier K^+ current, a Na^+ current, and a leakage current. The model is able to generate an action potential on current injection. The model predicts that the activation of the delayed rectifier K^+ current is too slow to contribute markedly to repolarization during a single action potential. Because of its fast activation, the A current reduces the amplitude of the net depolarizing current and thus reduces the peak amplitude and the duration of the action potential.

INTRODUCTION

Voltage-gated K^+ currents of the A type are defined by their relatively fast activation and inactivation and by their sensitivity to 4-aminopyridine (4-AP). Since their first description in the somata of Anisodoris neurons (Connor and Stevens 1971a,b) A currents were identified in a large variety of systems (Adams and Galvan 1986; Barry and Nebenne 1996). They have been shown to influence major aspects of electrical activity such as spike broadening during repetitive firing (Ma and Koester 1995, 1996), firing frequency (Byrne 1980a,b; Tierney and Harris-Warrick 1992), or synaptic transmission (Kaang et al. 1992). Mutations affecting A currents lead to malfunctions of the nervous system, as described for the shaker phenotype in Drosophila (Salkoff and Wyman 1981; Tanouye et al. 1981).

In arthropods, the α-subunits of channels underlying A currents are encoded by the shaker or the shal gene. The Drosophila shaker gene encodes a subfamily of K^+ channel components that are produced by alternative splicing (Kamb et al. 1988; Papazian et al. 1987; Pongs et al. 1988; Schwarz et al. 1988). Most of these components give rise to fast-inactivating, voltage-gated K^+ currents (Iverson and Rudy 1990; Iverson et al. 1988; Timpe et al. 1988; Wu et al. 1983). Expression of the shal gene may also yield a fast-inactivating, voltage-gated K^+ current (Pak et al. 1991; Tsunoda and Salkoff 1995).

Immunocytochemical localization of the shaker gene products in the brain of adult Drosophila revealed a nonuniform distribution and indicated a high expression in the mushroom bodies (MBs) (Roget et al. 1997; Schwarz et al. 1990). The MBs are involved in higher functions of the insect brain such as learning and memory, e.g., Drosophila (Davis 1993; de Belle and Heisenberg 1994; Heisenberg et al. 1985) and honeybee (Erber et al. 1980; Menzel et al. 1974). Each MB of the worker honeybee consists of ~170,000 (Withthöft 1967) densely packed and parallel arranged, intrinsic Kenyon cells. Kenyon cells are the third-order interneurons of the olfactory pathway that converge on the MBs with other sensory and modulatory pathways crucial for olfactory learning (Hammer 1993; Hammer and Menzel 1995). The MB Kenyon cells can be taken into primary cell culture (Kreissl and Bicker 1992). Thus native ionic currents can be studied in a well-defined type of neurons in the insect brain.

Descriptions of native neuronal shaker currents in insects are rare. Neuronal shaker currents were first identified in a small subpopulation of neurons that were dissociated from thoracic ganglia of pupal Drosophila (Baker and Salkoff 1990). A detailed kinetic description of a native neuronal shaker current exists for the photoreceptors of Drosophila (Hardie 1991). A number of voltage-dependent ionic currents, Na^+, Ca^{2+}, and K^+ currents, was identified in the Kenyon cells (Schäfer et al. 1994). It was suggested that a prominent A-type K^+ current might be a shaker-like current. Therefore we investigated the
kinetic properties of this A current in detail to enable a comprehensive comparison with shaker and shal currents described in other systems. Our data on the kinetic properties of the A current of honeybee Kenyon cells indicate that it is dominated by a shaker-like current.

Data on the A current was incorporated into a Hodgkin-and-Huxley–style mathematical model that also contained the voltage-gated Na+ current and delayed rectifier K+ current from the honeybee Kenyon cells. The aim of the model was to investigate the role of the A current during action potential generation and its interaction with other currents involved. A rapidly activating K+ current may repolarize the action potential. However, depending on its voltage-operating range and the resting potential of the cell, an A current may also cause a delay in the initiation of an action potential. The model presented predicts that the A current reduces the peak amplitude of the action potential and mediates the repolarization phase.

METHODS

Animals and cell preparation

Pupae of the honeybee, *Apis mellifera*, were collected from the hive between days 4 and 6 of the pupal development, which lasts 9 days under natural conditions. For dissection and culturing of Kenyon cells the original protocol of Kreissl and Bicker (1992) was modified. Brains were removed from the head capsule in Leibowitz L15 medium (GIBCO-Bethesda Research Laboratory) supplemented with sucrose, glucose, fructose, and proline (42.0, 4.0, 2.5, and 3.3 g/l; 500 mosm; pH 7.2). The MBs were dissected out of the brains and incubated in calcium-free saline containing (in mM) 130 NaCl, 5 KCl, 10 MgCl2, 25 glucose, 180 sucrose, and 10 HEPES, 500 mosm, pH 6.7. After transferring the MBs back to L15 medium (2 MBs/100 μl) the cells were dispersed by gentle trituration with a 100-μl siliconized Eppendorf pipette. Cells were then plated in 10 μl medium on poly-L-lysine–coated plastic dishes (Falcon) and allowed to adhere to the substrate for 20 min. Thereafter the dish was filled with 2.5 ml of bee medium; 775 ml of this medium consisted of 100 ml FCS (inactivated, Sigma), 10 ml yeast extract (Sigma), 9.7 g L-15 (GIBCO), 2.66 g glucose, 1.67 g fructose, 2.19 g proline, 25 g sucrose, and 0.5 g PIPES, 500 mosm, pH 6.7. The dishes were kept at 27°C in an incubator at high humidity. Under these conditions the Kenyon cells started to grow processes and survived for ≥2 wk. Cells were used for recordings between 2 and 6 days in culture. Only large Kenyon cells with a soma diameter of ~10 μm and with clearly visible processes were examined but not small Kenyon cells with a diameter of ~6 μm or without processes. The culture also contained a few glia cells, but they were easily recognized because of their large size.

Electrophysiological techniques

Tight-seal whole cell recordings were performed after the methods described by Hamill et al. (1981). All measurements were performed at room temperature. Recordings were made with an Axopatch 200 A amplifier (Axon Instruments). For pulse generation, data acquisition, and data analyses a TL-1 interface in conjunction with pCLAMP software version 6.0 (Axon Instruments) was used. Pipette and membrane capacitance were compensated, and series resistance compensation (80%) was routinely employed. Signals were low-pass filtered with a four-pole Bessel filter at 2 or 5 kHz and digitally sampled at 5–50 kHz depending on the pulse protocol used. Liquid junction potential was corrected, and on-line leakage currents were compensated when necessary. Electrodes were pulled from borosilicate glass capillaries (GC 150–15, Clark, Reading) and had resistances between 3 and 5 MΩ in standard external saline. We used ORIGIN 4.1 (MicroCal) and IGOR pro 3.0 (WaveMetrics) to analyze the data. All data are presented as means ± SE.

Solutions

The standard recording chamber was continuously perfused with saline. The standard external saline consisted of (in mM) 130 NaCl, 6 KCl, 4 MgCl2, 5 CaCl2, 10 HEPES/NaOH, 25 glucose, and 160 sucrose, 500 mosm, pH 6.7. In addition during recording the external saline contained 200 μM quinidine, 50 μM cadmium chloride, and 100 nM TTX. In a few experiments the external solution contained variable concentrations of K+ (2, 6, or 10 mM); in some experiments 4-AP or agitoxin-2 (Alomone Labs) was added.

The pipettes were backfilled with a solution containing (in mM) 20 KCl, 115 K-gluconate, 40 KF, 3 Na3ATP, 3 MgCl2, 10 HEPES/Bis-Tris, 120 sucrose, 5 K-bis-(o-aminophenxy)-N,N,N’,N’-tetraacetic acid, 3 glutathione, and 0.1 GTP-Mg, 500 mosm, pH 6.7. All chemicals were purchased from Sigma unless otherwise stated.

Simulations

The equations originally developed by Hodgkin and Huxley (1952) were modified to derive a set of exponential functions describing the kinetics of the A current (see APPENDIX). These functions were implemented with IGOR pro 3.0, with a least-squares fitting algorithm. The degrees of freedom of the fits were reduced by using fixed steady-state values, which were derived from the steady-state activation and steady-state inactivation. To determine the voltage dependency of the kinetic parameters, the various time constants were fitted with a Boltzmann equation (see APPENDIX). Simulations were run on a SunSPARC workstation with the simulation software package SNNAP (Ziv et al. 1994). The model that was used for simulations under voltage-clamp conditions included only the A current. For the simulation of action potentials the voltage-gated Na+ current, the delayed rectifier K+ current of the honeybee Kenyon cells, and a small leakage current were added to the model. Data from Schäfer et al. (1994) were the basis for the data of the Na+ current and the delayed rectifier current. The total number of recordings available was increased by additional recordings. The parameters necessary for the model were obtained from the original current traces by reevaluation similar to the evaluation described for the A current. We present these parameters in the APPENDIX because the Na+ current and the delayed rectifier current were not in the focus of this study, but they are necessary to document the model.

RESULTS

Isolation of the A current

Kenyon cells of the honeybee express a variety of voltage-gated and calcium-activated ionic currents. To isolate the A current, the Na+ current was blocked by 100 nm TTX, the Ca2+ current by 50 μM cadmium, and the delayed rectifier K+ current by adding 200 μM quinidine to the external solution. Under these conditions a significant part of the delayed rectifier K+ current remains unblocked. Therefore a subtraction procedure was used in addition to separate the delayed rectifier K+ current from the A current. For this in a first pulse protocol the command potential was preceded by a −125-mV prepulse of 3-s duration to completely remove inactivation of the A current (Fig. 1A). In a second pulse protocol the prepulse contained a voltage step to −5 mV (120 ms) to inactivate the A current and a after brief voltage step to −125 mV (26 ms) to deactivate the delayed rectifier K+ current (Fig. 1B). This last step is too short to allow for pronounced recovery from inactivation of the A current. Subtraction of the current traces recorded with these two pulse protocols yielded the pure A current (Fig. 1C). Under
these conditions, depolarizing voltage commands of \(-35\) mV and greater activated a transient outward current in all Kenyon cells recorded. It shows a fast voltage-dependent time course of activation and inactivation. A summary of the derived time constants and steady-state parameters of this A current is given in Table 1.

Approximately 50% of the A current was blocked by 5 mM 4-AP \((n = 14, \text{Fig. } 2)\). Agitoxin-2 did not affect the native A current of the Kenyon cells at concentrations of \(<100\) nM \((n = 6, \text{data not shown})\).

**TABLE 1. Summary of derived time constants and steady-state parameters of A current**

<table>
<thead>
<tr>
<th>Activation</th>
<th>Deactivation</th>
<th>Inactivation</th>
<th>Deactivation (fast)</th>
<th>Deactivation (slow)</th>
<th>(V_{1/2}) activation</th>
<th>Slope</th>
<th>(V_{1/2}) inactivation</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 ± 0.1 ms ((n = 4))</td>
<td>0.4 ± 0.1 ms ((n = 5))</td>
<td>3.0 ± 1.6 ms ((n = 9))</td>
<td>18 ± 3.1 ms ((n = 5))</td>
<td>745 ± 107 ms ((n = 5))</td>
<td>-0.7 ± 2.9 mV ((n = 8))</td>
<td>16.1 ± 0.9</td>
<td>-54.7 ± 2.4 mV ((n = 8))</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>-75 mV</td>
<td>+45 mV</td>
<td>+45 mV</td>
<td>-75 mV</td>
<td>-75 mV</td>
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</tr>
</tbody>
</table>

Values are means ± SE. All measurements were performed at room temperature.
premises as described previously. Inward tail currents were recorded in the range of −105 to −75 mV, and outward tail currents were recorded in the range of −65 to −45 mV (Fig. 4). The decay of the tail current was fitted with a single exponential function. The time constant of deactivation is voltage dependent (Fig. 3); at a test potential of −75 mV its value is 0.39 ± 0.1 ms (n = 5).

Steady-state activation

Steady-state activation of the isolated A current (see Fig. 1) was determined by measuring the peak current amplitude activated by depolarizing voltage steps ranging from −55 to +45 mV. From these values the relative conductance (g/t) was determined by measuring the peak current amplitude activated by depolarizing voltage steps ranging from −55 to +45 mV. From these values the relative conductance (g/t) was calculated. For each cell this normalized curve of the steady-state activation (Fig. 5A) was fitted separately with a Boltzmann function, and the potential at which one-half of the current is activated (V1/2) was determined. The V1/2 values range from +11.9 to −12.1 mV; the mean is −0.7 ± 2.9 mV (n = 8). The value for the factor S, which determines the slope of the curve, is 16.1 ± 0.9. In addition, the curve of the mean conductance-voltage relationship of all cells was fitted with the Boltzmann function (Fig. 5B).

Inactivation

The inactivation time constant was determined by fitting a single exponential function to the falling phase of the isolated A current (see Fig. 1C). This yielded time constants of the inactivation process that were strongly voltage dependent (Fig. 6). A command potential of +45 mV induced a current with an inactivation time constant of 3.0 ± 1.6 ms (n = 9). We also applied a series of double-exponential fits. However, except for the current traces just above the activation threshold where the signal-to-noise ratio is very small and possible minor contaminations with other conductances would be relatively large, we never observed significant improvements in the quality of the fits.

To investigate the kinetics of the recovery from inactivation we determined the time constants by application of double-pulse experiments (Fig. 7). The hyperpolarizing (−125 mV) prepulse before the onset of the double-pulse protocol lasted 5 s. In these experiments the unblocked part of the delayed rectifier K+ current becomes apparent in the noninactivating current at the end of the depolarizing pulses. This current was measured at the end of the first depolarizing pulse and was subtracted from each point of the current trace. This method slightly overestimates the delayed rectifier current. The ratio of the peak current amplitudes elicited by the second and the first depolarizing pulse indicates the extent of recovery at the given time interval. An asymptotic level of 100% of recovery from inactivation is reached within 3 s at an interpulse potential of −125 mV, whereas at interpulse potentials between −110 and −60 mV asymptotic levels of steady-state recovery of <100% were measured.

Because the time course of recovery from inactivation cannot adequately be fitted by a single exponential function, we used a double-exponential function yielding two time constants indicative of the contribution of a slow and a fast process. The ratio of the fast and the slow recovery from inactivation varied only slightly over the range of interpulse potentials, with ∼30 and 70% respectively. Both time constants were voltage dependent (Fig. 6). At an interpulse potential of −75 mV the fast time constant is 18 ± 3.1 ms, and the slow time constant is 745 ± 107 ms (n = 5).

Steady-state inactivation

Steady-state inactivation curves were obtained by measuring the peak currents in response to a test pulse (+45 mV) that was preceded by preconditioning voltage steps to various potentials between −125 and −5 mV (Fig. 8). These preconditioning voltage steps were preceded by a hyperpolarizing prepulse of −125 mV and 3-s duration. We cannot use the subtraction protocol to eliminate the unblocked part of the delayed rectifier K+ currents. Therefore the amplitude of the noninactivating current at the end of the test pulse was subtracted from the current trace. This method introduces a slight error because of
an overestimation of the delayed rectifier current. The peak currents were normalized ($I/I_{\text{max}}$), and the resulting current-voltage relationship of the steady-state inactivation was determined (Fig. 5). The curves of each cell were fitted separately with the Boltzmann function. The measured $V_{1/2}$ values range between -66.7 and -45.9 mV; the means are $-54.7 \pm 2.4$ mV for $V_{1/2}$ and $7.0 \pm 0.2$ for $S$.

Effects of external K$^+$ ions

The K$^+$ concentration of the external solution commonly used in our experiments was 6 mM. To investigate the effects of external K$^+$ ions on the A current, external solutions with K$^+$ concentrations of 2, 6, and 10 mM were used. The respective K$^+$ equilibrium potentials were determined according to the Nernst equation, assuming the K$^+$ concentration within the cell to be identical to that of the pipette filling solution. From current traces evoked with a simple activation pulse protocol (a -125-mV prepulse preceding the test potential from -125 to +45 mV) the conductances under the various K$^+$ concentrations were calculated (Fig. 9A). The conductance is increased by increased K$^+$ concentrations. Scaling the current traces obtained at the various K$^+$ concentrations to the same size reveals that the shape of the traces is not affected ($n = 9$) (Fig. 9B). This shows that there are no K$^+$-dependent changes in the kinetics of A current activation and inactivation. The voltage dependency of steady-state activation and inactivation was also unaffected by the external K$^+$ ion concentration ($n = 6$, data not shown).

We further tested the influence of extracellular K$^+$ concentration on the recovery from inactivation. A plot of the normalized peak current (% recovery) versus the interpulse interval (holding potential -125 mV) shows that a reduced extracellular K$^+$ concentration slows the recovery from inactivation (Fig. 10A). This is due to an increment of the slow time constant (Fig. 10B). Its value increases with a decreasing concentration of external K$^+$ ions from $326 \pm 38$ ms (10 mM K$^+$) to $539 \pm 49$ ms (6 mM K$^+$) to $660 \pm 27$ ms (2 mM K$^+$) ($n = 6$). The fast time constant is not affected.

When repetitive pulses (1 Hz, 20 ms, +45 mV, interpulse potential -85 mV) were applied, the A current cumulatively inactivated because of its slow recovery from inactivation. Because the slow time constant of recovery from inactivation is affected by the concentration of external K$^+$ ions, repetitive pulses result in different degrees of cumulative inactivation depending on the concentration; the lower the K$^+$ concentra-
containing the A current, a voltage-gated Na

1

equations. The percentage of channels that recovers from inactivation before the second pulse depends on the time elapsing (25–3,000 ms) as well as on the interpulse potential (−125 to −60 mV). The recordings shown were conducted with an interpulse potential of −125 mV. For the 2 depolarizing pulses the potential was stepped to +45 mV. The unblocked part of the delayed rectifier K+ currents (measured at the end of the first depolarizing pulse) was eliminated by subtraction before the ratio of recovery was calculated.

The stronger is the reduction of the peak current \((n = 7)\) (Fig. 11). The current traces recorded at a given K+ concentration were normalized to the peak current of the first pulse, so the measurements are independent of the shift of the electromotive force because of the varying external K+ concentrations.

Simulations

For the simulation of the A current in a voltage-clamp situation the same voltage steps as in the physiological experiments \((-125\text{ mV prepulse preceding the test potential from }-55 \text{ to } +45 \text{ mV})\) were applied. Comparison of a recorded whole cell A current with simulated traces of the A current shows reasonable matching (Fig. 12). This confirms the validity of the parameters determined by Hodgkin-Huxley–derived equations.

For the simulation of action potentials we used a model that contained the A current, a voltage-gated Na+ current, a delayed rectifier K+ current, and a leakage current. Before each simulated experiment the cell model was clamped to a holding potential of −70 mV to let it reach a steady state. After termination of the voltage clamp the cell model reached a stable resting potential of −63.2 mV within 21 s in the free running mode (data not shown). On current injection (60 pA) a single action potential was generated (Fig. 13, top). Current injections of <60 pA lead to subthreshold activation of the Na+ current. Injecting currents of >60 pA did not trigger additional action potentials.

The peak of the action potential reached +9.5 mV, which is clearly below the Na+ equilibrium potential. During the action potential the A current reaches its maximum value just 200 ms later than the Na+ current; the time frames of these two currents show marked overlap (Fig. 13, middle). The same holds true for the conductivity of these currents (Fig. 13, bottom). The afterhyperpolarization of the action potential reached −74.2 mV at its maximum and lasted for ∼150 ms.

Discussion

In honeybees it is possible to prepare a pure culture of the intrinsic MB neurons, the Kenyon cells. The A current of these Kenyon cells shows a pronounced voltage dependence of the inactivation time course, which is a distinct property of shaker currents studied in heterologous expression systems (Timpe et al. 1988; Wei et al. 1990; Wittka et al. 1991), transgene giant neurons (Zhao et al. 1995), myotubes (Solc et al. 1987), and photoreceptor cells (Hardie 1991; Hevers and Hardie 1995). By contrast the inactivation kinetics of native shal currents (Tsunoda and Salkoff 1995) as well as shal currents studied in heterologous expression systems (Pak et al. 1991) are relatively voltage independent.

The expression of the shaker gene in the CNS of Drosophila was indicated by means of in situ hybridization (Pongs et al. 1988) and of immunocytochemistry (Rogero et al. 1997; Schwarz et al. 1990). Rogero et al. (1997) detected shaker channels in the neuropil but not in the somata of MBs, and it appears that in most systems shal channels are underlying the somatic current (Baro et al. 1997; Maletic-Savatic et al. 1995; Seridio et al. 1994; Sheng et al. 1992; Song et al. 1998; Tsunoda and Salkoff 1995). Therefore we must take into account that we are recording from a mixture of somatic shal channels and of shaker channels that were incorporated into the space-clamped compartments under cell culture conditions. Nevertheless, the A current of the Kenyon cells appears to be dominated by a single type of channel. Evidences for this derive from the following observations. 1) Fitting the inactivation kinetics with a double-exponential function instead of a single exponential function did not improve the quality of the fit. 2) Elevating extracellular K+ concentrations increased the conductance of the A current. If the A current comprised two components, increasing the conductance of only one current component would result in an altered shape of the normalized trace. However, this was not observed.

The A current of Kenyon cells is modulated by extracellular K+ ions. The whole cell conductance is increased, cumulative
inactivation is decreased, and recovery from inactivation is accelerated at higher external $K^+$ concentration. Fitting the time course of recovery from inactivation requires a function with two exponentials, which indicates two processes that may represent the recovery from N- and C-type inactivation as described for shaker currents (Iverson and Rudy 1990; Iverson et al. 1988). Only the time constant of the slow process is affected by increasing the extracellular $K^+$ concentration.

This kind of modulatory action of $K^+$ is described for several genetically identified shaker and shaker-like currents for different concentration ranges: 2–500 mM (Demo and Yellen 1991), 1 $\mu$M to 20 mM (Pardo et al. 1992), 2–40 mM (Tseng and Tseng-Crank 1992), 2–10 mM (Baukrowitz and Yellen 1995), and 5–150 mM (Levy and Deutsch 1996). In all these systems increasing $K^+$ concentrations accelerates recovery from inactivation. By contrast Jerng and Covarrubias (1997) described a retardation of recovery from inactivation in shal-like mKv4.1 $K^+$ channels in mice in the concentration range of 5–98 mM. The effect of elevating extracellular $K^+$ on invertebrate shal channels was not yet examined.

Agitoxin-2 is a selective and highly potent blocker of shaker and shaker-like currents in heterologous expression systems (e.g., $K_i = 0.64$ nM for shaker B) (Garcia et al. 1994) but did not affect the A current of honeybee Kenyon cells. This may be due to different pharmacological properties of shaker currents in homologous and heterologous expression systems. Zagotta et al. (1989) reported that the expression of shaker B cDNA in Xenopus oocytes gave rise to a current that was sensitive to 50 $\mu$M charybdotoxin, whereas its expression in myotubes resulted in a charybdotoxin-insensitive current. Agitoxin-2 as well as charybdotoxin binds to the outer vestibule of the channel (Durell and Guy 1996).

Schafer et al. (1994) described that the A current was almost completely blocked by 5 mM 4-AP and showed half-maximal steady-state activation at 10.7 mV and half-maximal steady-state inactivation at −42.33 mV. By contrast we find a 50% block of the A current by 5 mM 4-AP, half-maximal steady-state activation at −0.7 mV, and half-maximal steady-state inactivation at −54.7 mV. This contradiction is probably due to the different methods used. 1) We improved the composition of the culture media and used the more physiological pH of 6.7 instead of 7.2 in the culture media and during recording. 2) We allowed the neurons more time to develop in the culture dish. 3) We selected for cells that had grown clearly visible processes. Therefore we assume that we are recording an A current that differs in its underlying channels from the A current described by Schaefer et al. (1994). The A current described in our study is more likely to reflect the axonal or neuritic A current of the Kenyon cells, whereas in the previous study the A current more likely reflects a somatic current.

We observed some variations among different cells in the kinetic parameters of the Kenyon cells A current. This may be due to differences of the time spent in culture or different Kenyon cell types. Although Kenyon cells share a common gross morphology, they may differ with respect to dendritic and axonal morphology, sensory input, and the distribution of transmitters within the MBs (Menzel et al. 1974; Mobbs 1982). Yang et al. (1995) used the enhancer trap technique to distinguish subpopulations of Kenyon cells. However, it is not known whether different subpopulations of Kenyon cells with different voltage-gated currents exist. Preliminary data do not allow to group Kenyon cells according to single biophysical
properties, e.g., half-maximal inactivation of the A current. Rather, there seems to be a continuum of biophysical properties of this current. Moreover, these properties are likely to undergo modulation in a cell type that is involved in learning and memory. Nevertheless, the A current of the Kenyon cells is relatively homogenous with respect to its fast activation and inactivation (in each recording adequately fitted by a single exponential function), its recovery from inactivation (in each recording adequately fitted by a double-exponential function), and its modulation by extracellular $K^+$. 

As described previously, Kenyon cells do not fall into distinct groups with respect to the properties of the A current. Therefore multiple simulations based on single experiments would simply reproduce the bandwidth of the variation among cells. Instead, we present a reduced model of the average Kenyon cell, which has the advantage to reduce errors caused by noise. The reduced model of Kenyon cells is capable of producing single action potentials. The peak of the action potential does not reach the $Na^+$ equilibrium potential in the simulation as well as in soma recordings from cultured Kenyon cells (Kreissl 1992). The simulation shows that the fast-activating A current counteracts the depolarization caused by the $Na^+$ current during the rising phase of the action potential. The A current is mainly responsible for the repolarization because the delayed rectifier current does not contribute markedly to this process because of its slow activation.

The model does not produce a train of action potentials, most likely because the afterhyperpolarization does not reach sufficiently negative values to allow for fast deinactivation of the $Na^+$ current. This is due to the fast inactivation of the A current. In current-clamp recordings from cultured Kenyon cells the afterhyperpolarization is more pronounced, and some of the cells generate trains of action potentials in vitro (Kreissl 1992) and in vivo (Hammer and Menzel 1995). This difference is probably due to the presence of voltage-sensitive $Ca^{2+}$ currents and $Ca^{2+}$-activated $K^+$ currents (Schäfer et al. 1994) in these cells. These currents are usually very slow with respect to the $Na^+$ current (Hille 1992). By contrast the A current under investigation is just slightly slower than the $Na^+$ current. Therefore voltage-sensitive $Ca^{2+}$ currents and $Ca^{2+}$-activated $K^+$ currents should play only a minor role during a single action potential but become more important in a train of action potentials. There are not enough data available from Schäfer et al. (1994) to incorporate the $Ca^{2+}$ current and the $Ca^{2+}$-dependent $K^+$ current into the model. The experiments necessary to model these currents in their full complexity would be beyond the scope of this study.

There is some evidence that shaker currents are involved in the process of olfactory learning in Drosophila (Davis 1996). Cowan and Siegel (1986) reported a shaker mutant line that showed deficiencies in an olfactory learning paradigm. Shaker $K^+$ channels are found at high levels in the MBs of Drosophila (Rogero et al. 1997; Schwarz et al. 1990), a neuropile that is supposed to play an essential role in olfactory learning in insects (de Belle and Heisenberg 1994; Heisenberg et al. 1985; Menzel et al. 1974). The molecular basis of native shaker currents differs from that derived from heterologous expression systems with respect to the heteromultimeric composition, which may include multiple splice variants of the shaker gene (Isacoff et al. 1990; McCormack et al. 1990), products from the
membrane potential, and current. 

\[ g_{\text{Na}} \times (E - E_{\text{ion}}) \times m \times h \]  

(A1)

\[ m; h = 1 / [1 + \exp(-(p - V_m)/s)] \]  

(A2)

\[ \tau(V_m) = (\tau_{\text{max}} - \tau_{\text{min}}) / [1 + \exp([(-p_1 - V_m)/s_1])] \times [1 + \exp([(-p_2 - V_m)/s_2])] \]  

(A3)

\[ I = h_a + h_0 \times \exp(-t/\tau_a) \]  

(A4)

\[ V_{t/2} = -26.4 \text{ mV} \]  

\[ S = 6.1 \]

\[ V_{t/2} = -53 \text{ mV} \]  

\[ S = 5.4 \]

\[ -40 \text{ mV}: 0.25 \text{ ms} \]  

\[ -20 \text{ mV}: 0.27 \text{ ms} \]  

\[ 0 \text{ mV}: 0.19 \text{ ms} \]  

\[ 20 \text{ mV}: 0.09 \text{ ms} \]  

\[ V_{t/2} = -20 \text{ mV}: 2.8 \text{ ms} \]  

\[ 0 \text{ mV}: 0.8 \text{ ms} \]  

\[ 20 \text{ mV}: 0.6 \text{ ms} \]  

\[ 40 \text{ mV}: 0.3 \text{ ms} \]  

\[ V_{t/2} = -15 \text{ mV}: 23 \text{ ms} \]  

\[ 0 \text{ mV}: 13 \text{ ms} \]  

\[ 30 \text{ mV}: 4.3 \text{ ms} \]
60 mV: 3.8 ms
\[ V_{1/2} = -24.6 \text{ mV} \]
\[ S = 19.3 \]

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REFERENCES


