Functional Parameters of Voltage-Activated \( \text{Ca}^{2+} \) Currents From Olfactory Interneurons in the Antennal Lobe of \textit{Periplaneta americana}

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First published November 14, 2007; doi:10.1152/jn.00719.2007. Toward our goal to better understand the physiological parameters that mediate olfactory information processing on the cellular level, voltage-activated calcium currents \( (I_{\text{Ca}}) \) in olfactory interneurons of the antennal lobe from adult cockroaches were analyzed under two conditions: 1) in acutely dissociated cells (in vitro) and 2) in an intact brain preparation (in situ). The study included an analysis of modulatory effects of potential inorganic and organic \( \text{Ca}^{2+} \) channel blockers. \( I_{\text{Ca}} \) was isolated and identified using pharmacological, voltage, and ion substitution protocols. \( I_{\text{Ca}} \) consisted of two components: transient and sustained. The decay of the transient component was largely \( \text{Ca}^{2+} \) dependent. In vitro, \( I_{\text{Ca}} \) had an activation threshold of \(-50 \text{ mV} \) with a maximal peak current at \(-7 \text{ mV} \) and a half-maximal voltage \( (V_{0.5\text{act}}) \) for tail-current activation of \(-18 \text{ mV} \). In situ these parameters were significantly shifted to more depolarized membrane potentials: \( I_{\text{Ca}} \), activated at \(-40 \text{ mV} \) with a maximal peak current at \(8 \text{ mV} \) and a \( V_{0.5\text{act}} \) for tail-current activation of \(-11 \text{ mV} \). The sensitivity of \( I_{\text{Ca}} \) to the divalent cations \( \text{Cd}^{2+} \), \( \text{Co}^{2+} \), and \( \text{Ni}^{2+} \) was dose dependent. The most effective blocker was \( \text{Cd}^{2+} \) with an IC\(_{50} \) of \( 10^{-5} \text{ M} \) followed by \( \text{Ni}^{2+} \) \( (\text{IC}_{50} = 3.13 \times 10^{-7} \text{ M}) \) and \( \text{Co}^{2+} \) \( (\text{IC}_{50} = 1.06 \times 10^{-7} \text{ M}) \). The organic channel blockers verapamil, diltiazem, and nifedipine also blocked \( I_{\text{Ca}} \) in a dose-dependent way and had differential effects on the current waveform. Verapamil blocked \( I_{\text{Ca}} \) with an \( \text{IC}_{50} \) of \( 1.5 \times 10^{-4} \text{ M} \) and diltiazem had an \( \text{IC}_{50} \) of \( 2.87 \times 10^{-4} \text{ M} \). Nifedipine blocked \( I_{\text{Ca}} \) by \( 33\% \) at a concentration of \( 10^{-4} \text{ M} \).

I N T R O D U C T I O N

The first-order synaptic relay in olfactory systems of vertebrate and invertebrate animals have striking similarities of glomerular and neuronal organization, suggesting that olfactory information is processed through similar mechanisms in these evolutionarily remote animals (Hildebrand and Shepherd 1997; Strausfeld and Hildebrand 1999; Wilson and Maenin 2006). One experimental system that has served very successfully as a model to understand olfactory information processing is the first-order olfactory relay or antennal lobe (AL) of insects (see Laurent 1999; Wilson and Maenin 2006). As an important step toward the long-term goal to better understand also the cellular mechanisms that mediate olfactory information processing we characterized the physiological and biophysical properties of voltage-activated \( \text{Ca}^{2+} \) currents in olfactory interneurons from the ALs of adult \textit{Periplaneta americana}.

Calcium plays a critical role in the control of a variety of neuronal processes such as synaptic release, membrane excitability, enzyme activation, and activity-dependent gene activation (Augustine et al. 2003; Berridge 1993). A main source of cytoplasmic \( \text{Ca}^{2+} \) that contributes significantly to the dynamics of intracellular \( \text{Ca}^{2+} \) signals is the \( \text{Ca}^{2+} \) influx through voltage-gated \( \text{Ca}^{2+} \) channels (VGCCs). Multiple types of voltage-gated \( \text{Ca}^{2+} \) channels, characterized by different functional properties, are usually differentially distributed in functionally specialized subcellular compartments of the neuron and contribute to its whole cell \( \text{Ca}^{2+} \) current. Characterized by their physiological and biophysical phenotypes the following voltage-gated \( \text{Ca}^{2+} \) channel types can be presently distinguished in vertebrates: low-voltage–activated (LVA) channels (T-type, \( \text{Ca}_{3.1}–\text{Ca}_{3.3} \)) and high-voltage–activated (HVA) channels (L-, N-, P/Q-, R-type, \( \text{Ca}_{1.1}–\text{Ca}_{1.4}, \text{Ca}_{2.1}–\text{Ca}_{2.3} \)).

A comparison of calcium channel gene sequences indicates that in certain structural aspects insect VGCCs might resemble the vertebrate VGCCs (Littleton and Ganetzky 2000). Despite the similarities of \( \text{Ca}^{2+} \) channel sequences in invertebrates and vertebrates, invertebrate channels differ greatly in their pharmacological profile. For instance, one of the characteristics of L-type channels in vertebrates is their sensitivity to 1,4-dihydropyridines (e.g., nifedipine), whereas most invertebrate channels with homologous “L-type–like” sequences lack this feature (for review see Jeziorski et al. 2000; Wicher et al. 2001).

In the insect CNS, VGCCs can be separated electrophysiologically into LVA or mid-LVA (M-LVA) and HVA calcium channels (Grolleau and Lapied 1996; Wicher and Penzlin 1997). It has been demonstrated that the LVA current in dorsal unpaired median (DUM) neurons of \textit{P. americana} could be further bisected into two components according to their sensitivity to \( \text{Ni}^{2+} \) ions: a transient (LVA) and a maintained LVA current (mLVA; Grolleau and Lapied 1996). In DUM neurons, LVA currents start to activate at \(-80 \text{ mV} \), M-LVA at \(-50 \text{ mV} \), and HVA currents at \(-40 \text{ mV} \). M-LVA and HVA currents were further characterized by their differential sensitivity to inorganic ions (\( \text{Ni}^{2+}, \text{Cd}^{2+} \)) and peptide toxins (conotoxins and agatoxins; Wicher and Penzlin 1997). Differential sensitivity of HVA currents in embryonic brain neurons from \textit{P. americana} to peptide toxins suggests two current components resembling the vertebrate P/Q- and R-type channels (Benquet et al. 1999).

Organic \( \text{Ca}^{2+} \) channel blockers that selectively block specific \( \text{Ca}^{2+} \) channel types in vertebrates also modify \( \text{Ca}^{2+} \) currents in insects. Earlier studies have demonstrated that \( \text{Ca}^{2+} \) currents in insect neurons are reduced by phenylalkylamines (PAAs; e.g., verapamil; Wicher and Penzlin 1997), benzothia-
zepines (BZTs; e.g., diltiazem; David and Pitman 1995), 1,4-dihydropyridines (DHPs; e.g., nifedipine; Schäfer et al. 1994), and amiloride (Baines and Bate 1998). Investigations in DUM neurons of *P. americana* showed that HVA currents are affected by verapamil and diltiazem, but not by nifedipine and amiloride (Wicher and Penzlin 1997). However, nifedipine partially blocked barium currents in embryonic brain neurons of *P. americana* (Benquet et al. 1999). In motoneurons of *P. americana* Ca\(^{2+}\) current components could be separated by their sensitivity to nifedipine (Mills and Pitman 1997). Detailed analyses, however, of dose–response relationships and effects of organic channel blockers on biophysical properties of Ca\(^{2+}\) currents in insect neurons are still lacking.

The goal of this study was 1) to characterize voltage-activated Ca\(^{2+}\) currents from olfactory interneurons of the antennal lobe in vitro and in situ and 2) to investigate the effects of some Ca\(^{2+}\) channel blockers that have been shown to be effective in vertebrates. The focus for the latter was on verapamil, diltiazem, and nifedipine, all of which selectively block vertebrate L-type channels and are members of different chemical classes. Such detailed knowledge on these readily available blockers could be helpful to block specific components of the calcium currents to better analyze their physiological function in neuronal information processing.

**METHODS**

**Animals and materials**

*P. americana* were reared in crowded colonies at 27°C under a 13:11 h light/dark photoperiod regimen and reared on a diet of dry dog food, oatmeal, and water. All experiments were performed with adult animals of both sexes. Before dissection the animals were anesthetized by CO\(_2\) or cooling (4°C) for several minutes. For cell culture they were then adhered in a plastic tube with adhesive tape and the heads were immobilized using dental modeling wax (S-U Modelierwachs, Schuler-Dental, Ulm, Germany) with a low solidification point (57°C). For in situ experiments the animals were placed in a custom-built holder, and the head was immobilized with dental wax (S-U Modelierwachs, Schuler-Dental, Ulm, Germany) with a low solidification point (57°C). For in situ experiments the animals were placed in a custom-built holder, and the head was immobilized with dental wax. The antennae were placed in small tubes on a plastic ring that was later used to transfer the preparation to the recording chamber.

All chemicals, unless stated otherwise, were obtained from Appli-chem (Darmstadt, Germany) or Sigma–Aldrich (Taufkirchen, Germany) with a per-analysis purity grade.

**Cell culture**

To examine the electrophysiological properties of isolated antennal lobe neurons, cells were dissociated and cultured using modified protocols reported previously (Grolleau and Lapied 1996; Hayashi and Hildebrand 1990; Kirchhof and Mercer 1997). The head capsule of anesthetized animals was opened and the antennal lobes were dissected with fine forceps. Typically, ALs from eight animals were enzyme treated (papain, P4762, Sigma, 0.3 mg ml\(^{-1}\)) were fashioned from borosilicate glass (0.86 mm OD, 1.5 mm ID, GB150-8P, Science Products, Hofheim, Germany) with a low solidification point (57°C). For in situ experiments the animals were placed in a custom-built holder, and the head was immobilized with dental wax. The antennae were placed in small tubes on a plastic ring that was later used to transfer the preparation to the recording chamber.

During the experiments, if not stated otherwise, the cells were superfused constantly with saline solution containing (in mM): 185 NaCl, 4 KCl, 6 CaCl\(_2\), 2 MgCl\(_2\), 35 \(\text{D-glucose, 10 HEPES, and 5% fetal bovine serum (S-10, c.c.pro, Neustadt, Germany), adjusted to pH 7.2 (with NaOH), which resulted in an osmolarity of 420 mOsm.}

**Intact brain preparation**

The intact brain preparation was based on an approach described by Kloppenburg et al. (1999a,b), in which the central olfactory network was left intact. Shortly before the experiment, the head capsule of the anesthetized animal was opened by cutting a window between the two compound eyes and the bases of the antennae. The brain with antennal nerves and antennae attached was dissected from the head capsule and pinned with fine wire in a Sylgard-coated (Dow Corning, Midland, MI) recording chamber containing “normal” saline (see following text). To gain better access to the recording site and facilitate the penetration of pharmacological agents into the tissue, the brain was enzyme treated (papain, P4762, Sigma, 0.3 mg ml\(^{-1}\) and 1-cysteine, 30090, Fluka/Sigma, 1 mg ml\(^{-1}\) dissolved in “normal” saline) for about 3 min at room temperature before the AL was desheathed using fine forceps. The AL neurons were visualized with a fixed-stage upright microscope (BX51WI, Olympus) using a \(\times 40\) water-immersion objective (UMPLFL \(\times 40, 0.8\ NA, 3.3\ mm\ WD,\ Olympus\) and IR-DIC optics (Doedt and Zieglgänsberger 1994).

**Whole cell recordings**

Whole cell recordings were performed at 24°C following the methods described by Hamill et al. (1981). Electrodes (tip resistance between 3 and 5 M\(\Omega\)) were fashioned from borosilicate glass (0.86 mm OD, 1.5 mm ID, GB150-8P, Science Products, Hofheim, Germany) with a temperature-controlled pipette puller (PIP5, HEKA Elektronik, Lambrecht, Germany), and filled with a solution containing (in mM): 190 CsCl, 10 NaCl, 1 CaCl\(_2\), 2 MgCl\(_2\), 10 HEPES, and 10 EGTA adjusted to pH 7.2 (with NaOH), resulting in an osmolarity of 415 mOsm.

During the experiments, if not stated otherwise, the cells were superfused constantly with saline solution containing (in mM): 185 NaCl, 4 KCl, 6 CaCl\(_2\), 2 MgCl\(_2\), 10 HEPES, and 5 glucose. The solution was adjusted to pH 7.2 (with NaOH) and to 430 mOsm (with glucose).

To isolate the Ca\(^{2+}\) currents we used a combination of pharmacological blockers and ion substitution that has been shown to be effective in other insect preparations (Kloppenburg and Hörner 1998; Kloppenburg et al. 1999b; Schäfer et al. 1994). Transient voltage-gated sodium currents were blocked by tetrodotoxin (TTX, 10\(^{-6}\) to 10\(^{-4}\) M, T-550, Alomone, Jerusalem, Israel). 4-Aminopyridine (4-AP, 4 \(\times 10^{-3}\) M, A78403, Sigma) was used to block transient K\(^{+}\) currents (I\(_{\text{K,AP}}\); nomenclature adapted from Connor and Stevens 1971) and tetraethylammonium (TEA, 30 \(\times 10^{-3}\) M, T2265, Sigma) blocked sustained K\(^{+}\) currents (I\(_{\text{K,TEA}}\)) as well as Ca\(^{2+}\)-activated K\(^{+}\) currents (I\(_{\text{K,CA}}\)). In addition the pipette solution did not contain potassium.

Whole cell voltage-clamp recordings were made with an EPC9 patch-clamp amplifier (HEKA Elektronik) that was controlled by the
program Pulse (version 8.63, HEKA Elektronik) running under Windows. The electrophysiological data were sampled at intervals of 100 μs (10 kHz), except the 5-ms tail current measurements were sampled at 20 kHz. The recordings were low-pass filtered at 2 kHz with a four-pole Bessel filter. Compensation of the offset potential and capacitance were performed using the “automatic mode” of the EPC9 amplifier. The liquid junction potential between intracellular and extracellular solution (see Neher 1992) of 4.8 mV [calculated with Patcher’s PowerTools plug-in from http://www.mpibpc.gwdg.de/abteilungen/140/software/index.html for Igor Pro (WaveMetrics, Portland, OR)] was also compensated. To remove uncompensated leakage and capacitive currents, a p/6 protocol was used (see Armstrong and Bezanilla 1974). Voltage errors due to series resistance (Rs) were minimized using the Rs compensation of the EPC9. Rs was compensated between 30 and 70% with a time constant (τ) of 2 μs. Stimulus protocols used for each set of experiments are provided in the results.

**Organic Ca**

channel modulators

The following organic Ca\(^{2+}\) channel modulators that modify L-type and T-type VGCCs in vertebrate preparations were used in this study: (+)-verapamil (V4629, Sigma), (+)-cis-diltiazem (D2521, Sigma), nifedipine (N7634, Sigma), amiloride (A7410, Sigma), and (±)-BAY K 8644 (B-350, Alomone). (±)-Verapamil and (+)-cis-diltiazem were applied in concentrations ranging from 10\(^{-3}\) to 10\(^{-6}\) M. Nifedipine was dissolved in dimethyl sulfoxide (DMSO; D8418, Sigma) and then added to the saline for final concentrations from 10\(^{-4}\) to 10\(^{-6}\) M. BAY K 8644 was tested at a concentration of 10\(^{-3}\) M. First, it was dissolved in DMSO and then stored in aliquots at −20°C. The aliquot was added to the saline shortly before the experiments were conducted. The DMSO concentration of both the nifedipine- and the BAY K 8644–containing salines was 0.5%. At this concentration it had no obvious effect on I\(_{\text{ca}}\), and was also added to the “control”, although it increased the osmolarity by about 50 mOsm. Due to its photosensitivity (see product sheets from Sigma and Alomone, respectively), all experiments with nifedipine and BAY K 8644 were performed in the dark. Amiloride was dissolved in gently heated saline and used at a concentration of 10\(^{-3}\) M. Drug-containing as well as control saline were bath applied at a rate of 4–7 ml min\(^{-1}\).

**Data analysis**

The data from the dose–response experiments were fit with a hill equation of the form

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + \left(\frac{IC_{50}}{[C]}\right)^n} \tag{1}
\]

where \(I\) is the peak amplitude of \(I_{\text{ca}}\) at a −5-mV test pulse from \(V_h = −60 mV\) in the presence of different concentrations of drugs ([C]), and \(I_{\text{max}}\) is the peak amplitude of the control. \(IC_{50}\) is the concentration where half of \(I_{\text{ca}}\) is blocked and \(n_H\) is the Hill coefficient. Steady-state tail-current activation and steady-state inactivation data were fit using a first-order (\(n = 1\)) Boltzmann equation

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + e^{\left(V_{\text{V_t}} - V_{\text{V_0.5}}\right)/h}} \tag{2}
\]

where \(I_{\text{max}}\) is the maximal current, \(V\) is the voltage of the test pulse, \(I\) is the current at voltage \(V, s\) is the slope factor, and \(V_{0.5s}\) is the voltage at which half-maximal activation occurs. To convert peak current to peak conductance we assumed an \(E_{\text{ca}} = 160 mV\) (estimated with MaxChelator, http://www.stanford.edu/~cpatton/maxc.html; Patton et al. 2004). For analysis of electrophysiological data we used the software Pulse (version 8.63, HEKA Elektronik), Igor Pro 4 (WaveMetrics, including the Patcher’s PowerTools plug-ins), and Sigma Stat (Systat Software, Erkrah, Germany). All calculated values are expressed as means ± SD. Significance of differences between mean values were evaluated with Mann–Whitney rank-sum tests or paired t-tests. Significance was accepted at \(P \leq 0.05\).

**Results**

Whole cell voltage-clamp recordings were used to characterize voltage-dependent Ca\(^{2+}\) currents of olfactory interneurons in antennal lobe interneurons of P. americana under two conditions: in acutely dissociated cells (in vitro) and in an intact brain preparation (in situ). The study was expanded by an in vitro analysis of modulatory effects of potential inorganic and organic Ca\(^{2+}\) channel blockers. The recorded neurons were identified as antennal lobe interneurons by the position of their cell bodies within the antennal lobe. They were not unequivocally classified as local interneurons or projection (output) neurons. Besides the receptor neurons the main neuronal components of the AL are the local interneurons (~500) and the projection neurons (~150; numbers from Bocck and Ernst 1987). It can be expected that the different physiological and computational tasks of these neurons are reflected in the biophysical properties, which might cause a certain variability of the pooled data. If not stated otherwise, the membrane potential was clamped at −60 mV, which is in the range of the normal resting potential of these neurons in situ (−57.2 ± 9.2 mV; n = 28). Typically, depolarizing voltage steps evoked a combination of inward and outward currents in AL neurons. When voltage-gated Na\(^{+}\), K\(^{+}\), and H currents were reduced by ion substitution, pharmacological reagents, and appropriate voltage protocols, we recorded inward currents that had the characteristics of typical Ca\(^{2+}\) currents. Our recording conditions were designed to drastically minimize, or even abolish, outward currents through K\(^{+}\) channels and in most, but not all, recordings we did not observe outward currents. For recordings in which residual outward currents at strong depolarizations were detected we could not rule out that the K\(^{+}\) currents were not completely blocked and that there was some residual K\(^{+}\) in the neurons. However, we think it is more likely that the observed outward currents were carried by Cs\(^{+}\) through Ca\(^{2+}\) channels, which are not completely impermeable to Cs\(^{+}\) (Hess et al. 1986). This scenario was supported by our observation that no residual outward currents could be detected when \(I_{\text{ca}}\) was blocked by Cd\(^{2+}\) (data not shown).

To analyze \(I_{\text{ca}}\) outward currents were minimized by substituting K\(^{+}\) in the pipette solution with Cs\(^{+}\) and adding 3 × 10\(^{-2}\) M TEA and 4 × 10\(^{-3}\) M 4-AP to the extracellular solution. The remaining inward current consisted of a transient very fast activating/inactivating component and a more slowly activating component. Steady-state tail-current activation and steady-state inactivation data were fit using a first-order (\(n = 1\)) Boltzmann equation

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + e^{\left(V_{\text{V_t}} - V_{\text{V_0.5}}\right)/h}} \tag{2}
\]

where \(I_{\text{max}}\) is the maximal current, \(V\) is the voltage of the test pulse, \(I\) is the current at voltage \(V, s\) is the slope factor, and \(V_{0.5s}\) is the voltage at which half-maximal activation occurs. To convert peak current to peak conductance we assumed an \(E_{\text{ca}} = 160 mV\) (estimated with MaxChelator, http://www.stanford.edu/~cpatton/maxc.html; Patton et al. 2004). For analysis of electrophysiological data we used the software Pulse (version 8.63, HEKA Elektronik), Igor Pro 4 (WaveMetrics, including the Patcher’s PowerTools plug-ins), and Sigma Stat (Systat Software, Erkrah, Germany). All calculated values are expressed as means ± SD. Significance of differences between mean values were evaluated with Mann–Whitney rank-sum tests or paired t-tests. Significance was accepted at \(P \leq 0.05\).
Depolarizations (1 s; amount of inactivation during a depolarizing voltage pulse was 5 mV in Fig. 2, C1). Ca2+ in vitro

The characteristics of ICa are shown in Fig. 3. The I/V relationship of the peak currents was determined by increasing

Charge carrier

Experiments with varying extracellular Ca2+ concentrations confirmed that Ca2+ was the charge carrier of the investigated inward current. Low extracellular Ca2+ concentrations acted quickly to reduce the inward current reversibly (n = 5; Fig. 2A). This reduction of ICa was concentration dependent. When EGTA containing Ca2+-free extracellular solution was applied, the inward current was completely abolished (data not shown).

When extracellular Ca2+ was substituted with Ba2+ (Fig. 2, B–E), the maximal ICa was enhanced by about 20% (Fig. 2, B and D), indicating that the channels are more permeable to Ba2+ than to Ca2+, as described for Ca2+ channels in other cell types (Hille 2001). Simultaneously the current–voltage (I/V) relation shifted by about 10 mV (n = 4) to more hyperpolarized membrane potentials (Fig. 2D), which might result from changes in the surface charge of the cell membrane (Fedulova et al. 1985). The resulting voltage-dependent differences between ICa and IBa are demonstrated by steps to −20 and −5 mV in Fig. 2, C1 and C2, respectively. Furthermore, the amount of inactivation during a depolarizing voltage pulse was reduced, which became especially obvious during long-lasting depolarizations (1 s; −5 mV; Fig. 2E). These results suggest that inactivation during a voltage step is, at least in part, a Ca2+-dependent mechanism.

I_Ca in vitro

The characteristics of ICa are shown in Fig. 3. The I/V relationship of the peak currents was determined by increasing

FIG. 1. Whole cell recording in vitro of voltage-activated inward currents. A depolarizing voltage step (50 ms) from a holding potential of −60 to −5 mV evoked a combination of inward and outward currents. Outward currents were blocked by substituting K+ in the pipette solution with Cs+ and adding 3 × 10−3 M tetraethylammonium (TEA) and 4 × 10−3 M 4-aminopyridine (4-AP) to the extracellular solution. The remaining inward current consisted of a transient very fast activating/inactivating component and a more slowly inactivating component. A: the fast transient component was a tetrodotoxin (TTX)-sensitive Na+ current, whereas the other components of the inward current were identified as voltage-activated Ca2+ conductances, ICa (see Fig. 2). B: ICa consisted of an inactivating and sustained component, which did not completely inactivate even after long (1 s) depolarization.

FIG. 2. Ca2+ is the charge carrier of the TTX-insensitive voltage-activated inward currents. All experiments are in vitro recordings. The cells were held at −60 mV. A: experiments with varying extracellular Ca2+ concentrations indicated that Ca2+ was the main charge carrier of the investigated inward current. A1: inward currents elicited by 50-ms voltage steps to −5 mV were reduced reversibly by decreasing the extracellular Ca2+ concentration from 6 × 10−3 to 2 × 10−3 M (n = 5). A2: time course of peak inward current during a decrease in extracellular calcium concentration, from 6 × 10−3 to 2 × 10−3 M. B: voltage-activated inward currents when Ca2+ was the main charge carrier (ICa) and when Ca2+ was substituted with Ba2+ (IBa). Each series represents current responses to increasing voltage steps between −60 and 50 mV in 5-mV increments. When extracellular Ca2+ was substituted with Ba2+, the peak amplitude was enhanced by about 20%. C: currents elicited by voltage steps to −20 mV (C1) and −5 mV (C2) demonstrate the difference between ICa and IBa at different command potentials. D: the current–voltage (I/V) relation of IBa was shifted by about 10 mV to more hyperpolarized membrane potentials compared with ICa (n = 4). E: ICa and IBa during a long-lasting depolarization (1 s; −5 mV) demonstrating decreased inactivation for IBa.
voltage steps (50 ms, 5 mV) between −60 and 40 mV from a holding potential of −60 mV (Fig. 3A). The voltage dependence of activation of $I_{Ca}$ was determined from tail currents that were evoked by 5-ms voltage steps from −80 mV holding potential to 40 mV in 5-mV increments (Fig. 3B). The $I/V$ relations were fit to a first-order Boltzmann equation (Eq. 2; Fig. 3, G and I). Steady-state inactivation of $I_{Ca}$ was measured from a holding potential of −60 mV. Prepulses (500 ms) were delivered in 5-mV increments from −95 to −5 mV, followed by a 50-ms test pulse to −5 mV, and the peak currents were determined (Fig. 3C). The $I/V$ relations were fit to a first-order Boltzmann equation (Eq. 2; Fig. 3, H and I).
During a depolarizing voltage step $I_{Ca}$ activated relatively quickly and decayed during a maintained voltage step (Fig. 3A). The current waveforms and $I/V$ relations for activation were typical for $I_{Ca}$ but varied between cells (Fig. 3, D–F). In vitro $I_{Ca}$ started to activate with voltage steps more depolarized than $-40$ mV (Fig. 3D). The mean peak currents reached a maximum amplitude ($I_{max}$) of $1.7 \pm 0.6$ nA (Fig. 3D2) at $-6.5 \pm 3.8$ mV ($n = 65$; Fig. 3E) and decreased during more positive test pulses as they approached the calcium equilibrium potential (Fig. 3D). Given a mean whole cell capacitance of $20 \pm 500$ pA, the current density was $42.6 \pm 14.3$ pA/pF.

Fig. 4. Calcium currents ($I_{Ca}$) in the intact brain preparation (in situ). For details see legend of Fig. 3. A–C: current traces for steady-state activation, steady-state activation of tail currents, and steady-state inactivation, respectively. The voltage step for steady-state inactivation was 5 mV. D: voltage dependence of peak $I_{Ca}$ of 22 neurons. D1: data from single neurons. D2: the averaged data. The current is activated at command potentials more depolarized than $-40$ mV with a maximum around $8 \pm 7.8$ mV ($n = 22$). The mean peak amplitude ($I_{max}$) is $1.2 \pm 0.4$ nA. E: current density/voltage ($I/V$) relation. The mean maximal current density was $42.6 \pm 14.3$ pA/pF. F: $I/V$ relation of peak $I_{Ca}$ normalized to the maximal current of each cell, $I_{max}$. On average the mean maximal current ($I_{max}$) of $1.2 \pm 0.4$ nA is reached at a membrane potential ($E_{max}$) of $8 \pm 7.8$ mV. G: $I/V$ relation of tail currents normalized to the maximal tail current of each cell. The mean maximal tail current is $1.8 \pm 0.4$ nA ($n = 13$). H: $I/V$ relations for steady-state inactivation of 8 neurons. I: mean $I/V$ relations of steady-state inactivation of peak $I_{Ca}$ (filled squares) and tail-current activation (filled circles). The Boltzmann fits have the following parameters: Tail-current activation: $V_{0.5 \text{act}} = -10.5 \pm 6$ mV; $s_{\text{act}} = 7.5 \pm 1.8$; $n = 13$. Steady-state inactivation: $V_{0.5 \text{inact}} = -19.9 \pm 6.7$ mV; $s_{\text{inact}} = 8.7 \pm 1.9$; $n = 8$. 

During a depolarizing voltage step $I_{Ca}$ activated relatively quickly and decayed during a maintained voltage step (Fig. 3A). The current waveforms and $I/V$ relations for activation were typical for $I_{Ca}$ but varied between cells (Fig. 3, D–F). In vitro $I_{Ca}$ started to activate with voltage steps more depolarized than $-50$ mV (Fig. 3D). The mean peak currents reached a maximum amplitude ($I_{max}$) of $1.7 \pm 0.6$ nA (Fig. 3D2) at $-6.5 \pm 3.8$ mV ($n = 65$; Fig. 3E) and decreased during more positive test pulses as they approached the calcium equilibrium potential (Fig. 3D). Given a mean whole cell capacitance of...
29.6 ± 10.6 pF (n = 65), this corresponds to a mean current density of 52.8 ± 18.1 pA/pF (Fig. 3F).

The activation and inactivation kinetics during a voltage step are voltage dependent (Fig. 3, A and B); the time to peak current and the time constant for the decay during a voltage pulse decreased when voltage steps of increasing amplitude were applied. These parameters, however, were not analyzed quantitatively. The tail currents that are independent of the changing driving force during the series of voltage pulses had a maximum amplitude of 1.9 ± 0.4 nA (n = 21). This corresponds to a mean maximal conductance \( G_{\text{max}} \) of 12.6 ± 2.7 nS and a mean current density of 63.6 ± 13.8 pA/pF. The \( I/V \) relation of the tail currents was fit by a first-order Boltzmann equation with a mean voltage for half-maximal activation \( V_{0.5\text{act}} \) of −17.8 ± 3.3 mV (s = 6.0 ± 2.2; n = 21; Fig. 3, G and I).

Steady-state inactivation started in vitro at prepulse potentials around −60 mV and increased with the amplitude of the depolarizing prepulse (Fig. 3, C, H, and I). The \( I/V \) relationship was fit with a first-order Boltzmann equation (Eq. 2) with a voltage for half-maximal inactivation \( V_{0.5\text{inact}} \) of −24.2 ± 3.7 mV (s = 8.7 ± 2; n = 10; Fig. 3, H and I).

\( I_{\text{Ca}} \) in situ

\( I_{\text{Ca}} \) recorded in situ (Fig. 4) showed characteristics similar to those in vitro. However, the \( I/V \) relationships had a larger variability and the means were shifted significantly to more depolarized membrane potentials. The mean voltage for activation of the maximal peak current was shifted by 14.4 mV (P < 0.001). The voltage for half-maximal tail-current activation \( V_{0.5\text{act}} \) was shifted by 7.3 mV (P < 0.001). In situ, \( I_{\text{Ca}} \) started to activate at command potentials more depolarized than −40 mV (Fig. 4D). The mean peak currents reached a maximum amplitude \( I_{\text{max}} \) of 1.2 ± 0.4 nA (n = 22; Fig. 4D) at 8 ± 7.8 mV (Fig. 4E). Based on a mean whole cell capacitance of 28.8 ± 7.8 pF, this corresponds to a mean current density of 42.6 ± 14.3 pA/pF (Fig. 4F). The tail currents had a mean maximum of 1.8 ± 0.4 nA and a mean voltage for half-maximal activation \( V_{0.5\text{inact}} \) of −10.5 ± 6 mV (s = 7.5 ± 1.8; n = 13; Fig. 4, G and I). In situ steady-state inactivation started at prepulse potentials around −60 mV and had a mean voltage for half-maximal inactivation \( V_{0.5\text{inact}} \) of −19.9 ± 6.7 mV (s = 8.7 ± 1.9; n = 8; Fig. 4, H and I).

**Fig. 5.** The divalent inorganic ions Cd\(^{2+}\) (A), Ni\(^{2+}\) (B), and Co\(^{2+}\) (D) reduce \( I_{\text{Ca}} \) in a concentration-dependent way. A: effect of 10\(^{−5}\) M Cd\(^{2+}\) on \( I_{\text{Ca}} \). A1: \( I_{\text{Ca}} \) evoked by a 50-ms voltage step to −5 mV from a holding potential of −60 mV before (control), during, and after (wash) application of 10\(^{−5}\) M Cd\(^{2+}\). A2: time course of Cd\(^{2+}\) (10\(^{−3}\) M) induced reduction of peak \( I_{\text{Ca}} \). The black bar indicates the time of Cd\(^{2+}\) application. B: effect of 10\(^{−3}\) M Ni\(^{2+}\) on \( I_{\text{Ca}} \). B1: \( I_{\text{Ca}} \) before (control), during, and after (wash) application of 10\(^{−3}\) M Ni\(^{2+}\). B2: time course of Ni\(^{2+}\) (10\(^{−3}\) M) induced reduction of peak \( I_{\text{Ca}} \). The black bar indicates the time of Ni\(^{2+}\) application. C: effect of 2 × 10\(^{−3}\) M Co\(^{2+}\) on \( I_{\text{Ca}} \). C1: \( I_{\text{Ca}} \) before (control), during, and after (wash) application of 2 × 10\(^{−3}\) M Co\(^{2+}\). C2: time course of Co\(^{2+}\) (2 × 10\(^{−3}\) M) induced reduction of peak \( I_{\text{Ca}} \). The black bar indicates the time of Co\(^{2+}\) application. D: dose-response curves for the aforementioned blockers. Curves are fit by a Hill function (Eq. 1) with the following parameters: cadmium: \( IC_{50} = 10^{−3}\) M; \( n_H = 0.87; \) nickel: \( IC_{50} = 3.13 \times 10^{−4}\) M; \( n_H = 1.01; \) cobalt: \( IC_{50} = 1.06 \times 10^{−3}\) M; \( n_H = 1.04; \) h ≥ 3 for all data points.
In a series of in vitro experiments we analyzed the effect of several inorganic ions and organic substances on $I_{Ca}$, which block or enhance voltage-gated calcium currents in vertebrate preparations. $I_{Ca}$ was blocked by the divalent cations Cd$^{2+}$, Ni$^{2+}$, and Co$^{2+}$. We also found that verapamil, diltiazem, and nifedipine, which all belong to different chemical classes (phenylalkalamine, benzothiazepine, and 1,4-dihydropyridine, respectively) and are known to selectively block vertebrate L-type channels, differentially modify $I_{Ca}$. Amiloride (10$^{-3}$ M), a T-type channel blocker, and (±)-BAY K 8644 (10$^{-4}$ M), a 1,4-dihydropyridine and L-type channel agonist, had no effect (data not shown).

**INORGANIC IONS.** The divalent cations Cd$^{2+}$, Co$^{2+}$, and Ni$^{2+}$ blocked $I_{Ca}$ in a dose-dependent way. Figure 5, A–C shows single example experiments for each of the blockers and Fig. 5D shows the dose-inhibition curves for all three cations. $I_{Ca}$ was elicited by a 50-ms voltage pulse to −5 mV from a holding potential of −60 mV. The dose-inhibition curves were well fit with a Hill equation (Eq. 1). The most effective blocker was Cd$^{2+}$ with a half-inactivating concentration (IC$_{50}$) of 10$^{-5}$ M (Hill coefficient $n_H = 0.87$) followed by Ni$^{2+}$ (IC$_{50}$ = 3.13 × 10$^{-5}$ M; $n_H = 1.01$) and Co$^{2+}$ (IC$_{50}$ = 1.06 × 10$^{-4}$ M; $n_H = 1.04$).

**ORGANIC CA$^{2+}$ CHANNEL BLOCKERS.** Verapamil, diltiazem, and nifedipine effected $I_{Ca}$ with different concentration dependencies. For verapamil and diltiazem we were able to measure dose-inhibition curves and determine the concentrations for full and half-maximal block of the peak current (IC$_{50}$). Nifedipine was soluble only in concentrations below its IC$_{50}$. If not stated otherwise, any further experiments were conducted at the respective IC$_{50}$ or, in the case of nifedipine, at the maximal usable concentration (10$^{-4}$ M). To compare the voltage dependence for steady-state activation and inactivation we compared...
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VERAPAMIL. The phenylalkamine verapamil was tested at concentrations ranging from $10^{-6}$ to $10^{-3}$ M. Example experiments demonstrating the verapamil effect are shown in Fig. 6A and the dose–response curve is given in Fig. 6A2. An obvious effect of verapamil started at $10^{-3}$ M and a nearly complete block was achieved at $10^{-2}$ M (Fig. 6). The effects reached a steady state within 3–5 min. The dose–inhibition curve was well fit by a Hill equation (Eq. 1) with a half-maximal block of $I_{\text{Ca}}$ at $1.5 \times 10^{-4}$ M ($n_H = 1.55$; Fig. 6A2).

Verapamil increased the decay rate of $I_{\text{Ca}}$ during depolarizing voltage pulses in a dose-dependent manner with a maximum effect at concentrations between $10^{-4}$ and $2.5 \times 10^{-4}$ M. For example, at the IC50 verapamil decreased the time constant for the decay (from a monoexponential fit) during a voltage pulse to $-5$ mV from 16.8 ± 4.2 to 4.2 ± 1 ms ($P = 0.016$; $n = 4$; Fig. 6B and inset).

The voltage dependence of the peak- and tail-current activation was not modified by verapamil (Fig. 6, C1, C2, and D). However, the voltage dependence for steady-state inactivation of $I_{\text{Ca}}$ was changed (Fig. 6E): The mean voltage for half-maximal inactivation ($V_{0.5\text{inact}}$) was shifted to hyperpolarized membrane potentials from $-27 ± 3.3$ mV ($s = 7.7 ± 2.2$) to $-36.4 ± 2.1$ mV ($s = 0.5 ± 1.5$; $P = 0.005$; $n = 9$).

DILTIAZEM. The benzothiazepine diltiazem was tested at concentrations ranging from $10^{-6}$ to $10^{-3}$ M. Example experiments demonstrating the effect of diltiazem are shown in Fig. 7A and the dose–response curve is given in Fig. 7A2. The effects reached a steady state within 3–5 min. A clear diltiazem effect started at $10^{-4}$ M. The dose–inhibition curve was well fit by a Hill equation (Eq. 1) with a half-maximal block of $I_{\text{Ca}}$ at a concentration of $2.87 \times 10^{-4}$ M ($n_H = 1.06$; Fig. 7A2). The $I_{\text{Ca}}$ block was accompanied by a change in the waveform of $I_{\text{Ca}}$ (Fig. 7B and inset). During a depolarizing voltage pulse diltiazem increased the rate of inactivation. The mean time constant ($\tau$) of the decay of $I_{\text{Ca}}$ (from a monoexponential fit) during a voltage pulse to $-5$ mV was significantly increased from $17.3 ± 2.1$ to $28.2 ± 6.5$ ms ($P = 0.003$; $n = 6$) when the IC50 of diltiazem ($3 \times 10^{-5}$ M) was applied. This effect was

**FIG. 7.** Effect of diltiazem on $I_{\text{Ca}}$. In all experiments except the dose-inhibition curve diltiazem was applied near its IC50 ($2.5 \times 10^{-4}$ M; see A). The cells were held at $-60$ mV. For details see the legend of Fig. 6. A: the reduction of $I_{\text{Ca}}$ by diltiazem is dose dependent. Diltiazem was applied in the range of $10^{-6}$ to $10^{-2}$ M. A2: the curve is a fit to a Hill function (Eq. 1) with $IC_{50} = 2.84 \times 10^{-3}$ M and $n_H = 1.06$. B: current traces of $I_{\text{Ca}}$ before and during application of diltiazem. Inset: the inactivation of $I_{\text{Ca}}$ during a sustained voltage step was decreased during diltiazem application. The decay time constant ($\tau$) (from a monoexponential fit) of $I_{\text{Ca}}$ changed significantly from $17.3 ± 2.1$ ms (control) to $28.2 ± 6.5$ ms ($P = 0.003$; $n = 6$) during application of diltiazem. C: IV relations of $I_{\text{Ca}}$ from 6 neurons before (squares) and during application of diltiazem (triangles). The diltiazem-sensitive current started to activate at more hyperpolarized potentials than the controls (for quantitative analysis see Fig. 6E). D and E: IV relation for tail-current activation and steady-state inactivation of $I_{\text{Ca}}$ under control conditions (solid squares) and in diltiazem (solid triangles). The Boltzmann fits have the following parameters. D: tail-current activation was significantly changed by diltiazem ($P < 0.001$; $n = 8$): Control: $V_{0.5\text{act}} = -20.3 ± 2.3$ mV; $s_{\text{act}} = 5.4 ± 1.3$; $D$: $V_{0.5\text{act}} = -5.5 ± 1.0$; $s_{\text{act}} = -23.7 ± 1.9$ mV. E: steady-state inactivation was significantly changed by diltiazem ($P < 0.001$; $n = 7$): Control: $V_{0.5\text{inact}} = -22.3 ± 2.6$ mV; $s_{\text{inact}} = 9.2 ± 1.6$. Diltiazem: $V_{0.5\text{inact}} = -30.8 ± 2.3$ mV; $s_{\text{inact}} = 7.1 ± 1.3$. The cells were held at $-60$ mV. For details see the legend of Fig. 7.
even more prominent with higher concentrations of diltiazem (data not shown).

The $I_{\text{Ca}}$ component that was not blocked by diltiazem at or above its $IC_{50}$ was activated at slightly, but significantly more hyperpolarized potentials (Fig. 7, C2 and D). Accordingly, the mean $V_{0.5\text{act}}$ for steady-state activation of the tail currents (from a first-order Boltzmann fit) was shifted from $-20.3 \pm 2.3$ mV ($s = 5.4 \pm 1.3$) in the control to $-23.7 \pm 1.9$ mV ($s = 5.5 \pm 1$; $P < 0.001$; $n = 8$) during diltiazem application (Fig. 7D). In addition, diltiazem shifted the voltage for half-maximal voltage for inactivation ($V_{0.5\text{inact}}$; from a first-order Boltzmann fit) to more hyperpolarized membrane potentials from $-22.3 \pm 2.6$ mV ($s = 9.2 \pm 1.6$) in the control to $-30.8 \pm 2.3$ mV ($s = 7.1 \pm 1.3$; $P < 0.001$; $n = 7$) during diltiazem application (Fig. 7E).

NIFEDIPINE. The 1,4-dihydropyridine nifedipine was tested in concentrations from $10^{-6}$ to $10^{-4}$ M, in which range it was relatively easy to dissolve. Example experiments demonstrating the effect of nifedipine are shown in Fig. 8A1 and the mean dose–response data are given in Fig. 8A2. An obvious effect of nifedipine on $I_{\text{Ca}}$ was detectable at $10^{-5}$ M (Fig. 8A). The maximal usable concentration of nifedipine ($10^{-4}$ M) blocked about 33% of $I_{\text{Ca}}$ (Fig. 8A). The remaining experiments with nifedipine were carried out with a concentration of $10^{-4}$ M. At this concentration nifedipine reduced $I_{\text{Ca}}$ without significantly changing the waveform and the voltage dependence for activation and inactivation (Fig. 8, B–E).

NONSPECIFIC EFFECTS OF $\text{Ca}^{2+}$ ORGANIC BLOCKERS. Verapamil, diltiazem, and nifedipine had to be used in the millimolar range to yield a significant block. To test whether these substances cause effects on other channels we tested all blockers at their $IC_{50}$ on the voltage-activated whole cell sodium and potassium currents. The main components of the potassium current are $I_{\text{A}}$ and $I_{K(V)}$ in these neurons. To record the sodium and potassium currents $K^+$ was used instead of Cs$^+$ in the pipette solution. The normal extracellular solution was used without TTX, 4-AP, and TEA, whereas the change in osmolarity was compensated with NaCl. We did not perform a detailed biophysical analysis, but all three organic blockers clearly had substantial effects on the voltage-activated sodium and potassium currents (Fig. 9). All blockers drastically reduced the amplitude and/or waveform of the sodium and potassium currents. Verapamil seemed to preferentially block the sustained component [$I_{K(V)}$] of the potassium current (Fig. 9C). However, the effect of verapamil on the isolated $I_{K(V)}$ (Fig. 9D) indicated that verapamil increased the inactivation of $I_{K(V)}$ and/or might act as an open channel blocker for $I_{K(V)}$.

**DISCUSSION**

This study is an initial step to obtain detailed information about physiological and biophysical properties of $I_{\text{Ca}}$ in inter-
neurons from the adult insect olfactory system and to test pharmacological tools to manipulate $I_{Ca}$. The insect AL serves as an important model for olfactory information processing on the network level (Hildebrand and Shepherd 1997; Laurent 1999; Strausfeld and Hildebrand 1999; Wilson and Mainen 2006). Together with future research, this work aims to better understand the role of Ca$^{2+}$ currents in mediating olfactory information processing on both the cellular and the subcellular levels.

$I_{Ca}$ recorded in vitro from isolated somata of adult AL olfactory interneurons activated at membrane potentials above approximately $-50$ mV with a maximum current around $-5$ mV. $I_{Ca}$ consisted of both relatively fast activating/inactivating and noninactivating components. In situ the $I$/$V$ relation for steady-state activation/inactivation was shifted to more depolarized membrane potentials. Interestingly, in a similar study of honeybee antennal motoneurons, differences between in vitro and in situ recordings were not observed (Kloppenburg et al. 1999b). After taking into account the different experimental conditions between in vitro and in situ experiments, however, such a shift of parameters was not unexpected. One reason might be imperfect voltage control across the whole neuron in the intact brain preparations, in which the neuronal arborizations are still intact, and/or differences in voltage dependence of calcium channels that are localized in distal regions of the neurons. Previous studies in other insect and invertebrate preparations have demonstrated neuritic or axonal localizations of calcium channels (Duch and Levine 2002; Haag and Borst 2000; Kloppenburg et al. 2000, 2007). However, given the complex morphology of these neurons it can be assumed that a major part of the measured in situ currents originates from the cell body. Nevertheless, both the in vitro and in situ potentials of $I_{Ca}$ are well in the range of Ca$^{2+}$ currents described in other insect preparations including Drosophila neurons (Byerly and Leung 1988; Saito and Wu 1991), honeybee Kenyon cells (Schäfer et al. 1994), Manduca motor neurons (Hayashi and Levine 1992), embryonic cockroach neurons (Benquet et al. 1999a), cockroach DUM neurons (Heidel and Pflüger 2006), locust thoracic neurons (Laurent et al. 1993; Pearson et al. 1993), cricket giant interneurons (Kloppenburg and Hörner 1998), and honeybee antennal motoneurons (Kloppenburg et al. 1999b). The average current density of about 50 pA/pF in vitro was in the same range as that found in vitro for honeybee projection neurons (Grünewald 2003) and cockroach DUM neurons (Heidel and Pflüger 2006).

In this study we presented averaged data from a large number of experiments to characterize the parameter space of $I_{Ca}$ in AL interneurons. It is the first stage to characterize $I_{Ca}$ of adult AL interneurons in detail and to get pharmacological tools to manipulate $I_{Ca}$. The variability of the data, however, should not be ignored. It might be due to differential expression of Ca$^{2+}$ channel types in different cell types. This hypothesis can be tested by recordings from neurons that are unequivocally identified by single-cell labeling.

In vertebrates, Ca$^{2+}$ currents are usually classified into low-voltage–activated (LVA or T-type, with activation starting above approximately $-70$ mV) and high-voltage–activated (HVA, activation starting above approximately $-30$ mV) classes. Subtypes such as L-, P/Q-, N-, and R-type are defined by biophysical and pharmacological properties (Ertel et al. 2000; Hille 2001; Triggle 2006). In accordance with previous studies, we found that the pharmacological classification of vertebrate calcium currents is difficult to transfer to $I_{Ca}$ of insects (for review see King 2007). The $I_{Ca}$ in AL interneurons exhibits some characteristics that are typical for some HVA channel types: Ba$^{2+}$ is a better charge carrier than Ca$^{2+}$,
inactivation is Ca\textsuperscript{2+} dependent and relatively slow compared with LVA channels, \(I_{\text{Ca}}\) is more sensitive to Cd\textsuperscript{2+} than to Ni\textsuperscript{2+}, and \(I_{\text{Ca}}\) is reduced by the L-type blockers verapamil, diltiazem, and nifedipine. However, the activation range of \(I_{\text{Ca}}\) is more hyperpolarized than traditional HVA channels and thus resembles currents with L-type properties that have more mid-voltage-activated ranges (Johnson et al. 2003; Wicher and Penzlin 1997). Despite these “L-type” like characteristics (±)-BAY K 8644 did not modify \(I_{\text{Ca}}\), indicating that \(I_{\text{Ca}}\) in AL interneurons differs from vertebrate L-type calcium currents pharmacologically. Amiloride, a vertebrate T-type channel blocker, did not affect \(I_{\text{Ca}}\), whereas amiloride does inhibit calcium currents in different Drosophila preparations [embryonic central neurons (Baines and Bate 1998); larval muscles (Gielow et al. 1995)].

Often the organic Ca\textsuperscript{2+} channel blockers act more potently on vertebrate cells than on invertebrate neurons and, in previous studies, it has been argued that the \(I_{\text{Ca}}\) blocker concentrations, which are needed to inhibit calcium currents in insect neurons, are too high to achieve any specific effects on calcium channel subtypes (Benquet et al. 2002). Our studies confirmed these concerns. All three blockers—verapamil, diltiazem, and nifedipine—dramatically affected voltage-activated sodium and potassium currents. Similar effects on voltage-activated potassium currents have been described previously (e.g., Caballero et al. 2004; DeCourcy 1995; Trequattrini et al. 1998). Unfortunately, these findings limit the use of the tested blockers for many experimental application. However, some blockers, e.g., diltiazem, reduce a specific component of \(I_{\text{Ca}}\) and thus might be useful for experiments, in which a certain component of \(I_{\text{Ca}}\) is analyzed. To specifically block \(I_{\text{Ca}}\) or its components without affecting other ionic currents different (classes of) substances have to be tested. In this regard spider toxins might provide a more specific tool for \(I_{\text{Ca}}\) characterization (King 2007).

Verapamil

The verapamil-induced block of \(I_{\text{Ca}}\) in AL interneurons is in agreement with studies in cockroach DUM neurons (Wicher and Penzlin 1997), embryonic cockroach brain neurons (Benquet et al. 1999), cockroach motoneurons (Mills and Pitman 1997), and locust thoracic neurons (Pearson et al. 1993). Compared with cockroach DUM and motoneurons the \(I_{\text{Ca}}\) in AL interneurons seems to be less sensitive to verapamil. However, only Benquet et al. (1999) tested different concentrations of verapamil, and this dose-inhibition curve was in the same range as for \(I_{\text{Ca}}\) in this study, although with a slightly lower value for half-maximal inhibition (IC\textsubscript{50}). Although verapamil did not affect the I/V relationship for activation, it shifted the voltage dependence for the steady-state inactivation of \(I_{\text{Ca}}\) to more hyperpolarized potentials. It was previously reported that this is an effect of drugs that bind to and stabilize the inactivated state of ion channels (Bean et al. 1983; Gomora et al. 2001).

Verapamil, especially at concentrations between \(10^{-4}\) and \(2.5 \times 10^{-4}\) M, decreased the inactivation rate of \(I_{\text{Ca}}\) in a dose-dependent manner. Considering that in vertebrates various Ca\textsuperscript{2+} channel subtypes differ in their inactivation kinetics (Budde et al. 2002; Fox et al. 1987), this finding could indicate that \(I_{\text{Ca}}\) in AL interneurons consists of current components with different inactivation kinetics. However, it also has been argued that such an increase in the decay rate could be caused by the blocking mechanism of verapamil (“open channel blocker”; Johnson et al. 1996).

Diltiazem

Similar to the diltiazem-induced block of \(I_{\text{Ca}}\) described here, a reduction of \(I_{\text{Ca}}\) by diltiazem has been demonstrated in cockroach DUM neurons (Wicher and Penzlin 1997) and Drosophila muscles (Gielow et al. 1995). However, dose-dependent effects of diltiazem were not previously investigated in detail.

The current that was not blocked by diltiazem activated and inactivated at more hyperpolarized potentials compared with the control. This finding suggests that a current component activating in the high-voltage range is inhibited by diltiazem, which would be in agreement with previous findings in DUM neurons (Wicher and Penzlin 1997). Compared with our verapamil results, the shift to more negative potentials is less pronounced during the application of diltiazem. Diltiazem, in contrast to verapamil, increased the \(I_{\text{Ca}}\) decay time constant during a depolarizing voltage pulse. Either diltiazem preferentially blocks a current component with fast inactivation kinetics or the drug modifies the gating behavior of a single calcium channel type. Thus diltiazem might be a valuable tool to separate different components of the whole cell \(I_{\text{Ca}}\) of AL interneurons in P. americana.

Nifedipine

Nifedipine reduced the maximal conductance of \(I_{\text{Ca}}\) without affecting the voltage dependence for steady-state activation/inactivation and the decay rate during a voltage step. Thus nifedipine seems not to block a single specific component of \(I_{\text{Ca}}\) as observed in cockroach motoneurons (Mills and Pitman 1997) and in Drosophila larval muscle (Gielow et al. 1995). However, in this context it is important to consider that most neurons in the present study mainly expressed high-voltage-activated (HVA) Ca\textsuperscript{2+} currents, meaning that mostly the effect of nifedipine on HVA Ca\textsuperscript{2+} channels was examined.

This study provides a detailed biophysical analysis of calcium currents in insect olfactory interneurons. In addition, calcium current pharmacology demonstrated substance-specific effects on \(I_{\text{Ca}}\) for some organic blockers, but also revealed strong nonspecific effects of all tested organic blockers on other voltage-activated currents. The current analysis lays groundwork for our present and future studies in the intact brain to further analyze the basis and role of intracellular Ca\textsuperscript{2+} dynamics in olfactory information processing, with a focus on cell-type-specific differences.

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