Ca$^{2+}$ Currents in Central Insect Neurons: Electrophysiological and Pharmacological Properties

DIETER WICHER AND HEINZ PENZLIN
Sächsische Akademie der Wissenschaften zu Leipzig, Forschungsgruppe Neurohormonale Wirkungsmechanismen, D-07743 Jena, Germany

Wicher, Dieter, and Heinz Penzlin. Ca$^{2+}$ currents in central insect neurons: electrophysiological and pharmacological properties. J. Neurophysiol. 77: 186–199, 1997. Ca$^{2+}$ currents in dorsal unpaired median (DUM) neurons isolated from the fifth abdominal ganglion of the cockroach Periplaneta americana were investigated with the whole cell patch-clamp technique. On the basis of kinetic and pharmacological properties, two different Ca$^{2+}$ current types were separated in these cells: mid/low-voltage-activated (M-LVA) currents and high-voltage-activated (HVA) currents. M-LVA currents had an activation threshold of $-50$ mV and reached maximal peak values at $-10$ nV. They were sensitive to depolarizing holding potentials and decayed very rapidly. The decay was largely Ca$^{2+}$ dependent. M-LVA currents were effectively blocked by Cd$^{2+}$, while HVA currents were insensitive to vertebrate LVA channel blockers like flunarizine and amiloride. The currents were, however, potently blocked by $\omega$-conotoxin MVIIIC (1 $\mu$M) and $\omega$-agatoxin IVA (50 nM). The blocking effects of $\omega$-toxins developed fast (time constant $\tau = 15 \text{ s}$) and were fully reversible after wash. HVA currents activated positive to $-30$ mV and showed maximal peak currents at $+10$ mV. They were resistant to depolarizing holding potentials up to $-50$ mV and decayed in a less pronounced manner than M-LVA currents. HVA currents were potently blocked by Ca$^{2+}$ (IC$_{50} = 5 \mu$M) but less affected by Ni$^{2+}$ (IC$_{50} = 40 \mu$M). These currents were reduced by phenylalkylamines like verapamil (10 $\mu$M) and benzothiazepines like diltilazem (10 $\mu$M), but they were insensitive to dihydropyridines like nifedipine (10 $\mu$M) and BAY K 8644 (10 $\mu$M). Furthermore, HVA currents were sensitive to $\omega$-conotoxin GVIA (1 $\mu$M). The toxin-induced reduction of currents appeared slowly ($\tau \sim 120 \text{ s}$) and the recovery after wash was incomplete in most cases. The dihydropyridine insensitivity of the phenylalkylamine-sensitive HVA currents is a property the cockroach DUM cells share with other invertebrate neurons. Compared with Ca$^{2+}$ currents in vertebrates, the DUM neuron currents differ considerably from the presently known types. Although there are some similarities concerning kinetics, the pharmacological profile of the cockroach Ca$^{2+}$ currents especially is very different from profiles already described for vertebrate currents.

INTRODUCTION

In the vertebrate nervous system, at least six different types of voltage-dependent Ca$^{2+}$ channels have been distinguished on the basis of their biophysical and pharmacological properties. According to the voltage range of activation, channels are divided into low-voltage-activated (LVA) channels (T type) (Nowycky et al. 1985) and mid-voltage-activated (MVA) channels (class E channels expressed in Xenopus oocytes) (Ellinor et al. 1993; Soong et al. 1993), taken together as mid/low-voltage-activated (M-LVA) channels on the one hand (Dunlap et al. 1995) and high-voltage-activated (HVA) channels (L, N, P, and Q type) (Fox et al. 1987; Llinas et al. 1992; Randall et al. 1993) on the other hand (for reviews see Miller and Fox 1990; Olivera et al. 1994; Tsien and Tsien 1990).

Ca$^{2+}$ channels in insect neurons were found to have properties different from T-, L-, and N-type channels in vertebrate cells (Bickmeyer et al. 1994a; Byerly and Leung 1988; Pearson et al. 1993; Pelzer et al. 1989). In particular, long-lasting HVA currents (L-type-like) were blocked by phenylalkylamines (as in vertebrates), but not by dihydropyridines (unlike in vertebrates). $\omega$-Conotoxin GVIA (\$\omega$-CgTx GVIA), an antagonist of N-type channels (McCleskey et al. 1987; Williams et al. 1992), was ineffective in various invertebrate neurons (Bickmeyer et al. 1994a; Bindokas and Adams 1989; McCleskey et al. 1987; Sun et al. 1987), but was recently shown to block a component of Ca$^{2+}$ currents in dorsal unpaired median (DUM) neurons of the cockroach Periplaneta americana (Wicher and Penzlin 1994).

Both insect neurons (e.g., locust DUM cells) and vertebrate neurons possess Ca$^{2+}$ channels sensitive to $\omega$-agatoxin I and II (Bindokas and Adams 1989; Bindokas et al. 1991). Furthermore, $\omega$-agatoxin IVA (\$\omega$-Aga IVA), which is a potent blocker of P-type currents (Mintz et al. 1992), was also found to act on Ca$^{2+}$ currents in neurosecretory locust cells (Bickmeyer et al. 1994b). Although there are some similarities, generally differences between voltage-dependent Ca$^{2+}$ currents in insect (or more generally, invertebrate) and vertebrate neurons are stressed in the literature.

Most somata of insect neurons are not capable of firing action potentials. One exception are DUM neurons in the ventral nerve cord. These neurons, e.g., in the terminal ganglion of the cockroach P. americana, are spontaneously active. Their somata exhibit large fast Na$^+$ currents (Lapied et al. 1990). For a maintained repetitive activity, voltage-dependent Ca$^{2+}$ currents were found to be necessary because the activity disappeared in Ca$^{2+}$-free bath solution or in the presence of Ca$^{2+}$ channel blockers like Ni$^{2+}$ (Lapied et al. 1989). Shape of action potential as well as spike frequency can be changed by altering properties of voltage-dependent Ca$^{2+}$ currents that act via Ca$^{2+}$-dependent K$^+$ currents (Wicher and Penzlin 1994; Wicher et al. 1994).

First voltage-clamp investigations revealed, in these neurons, the occurrence of at least two types of voltage-dependent Ca$^{2+}$ currents (Wicher and Penzlin 1994). One is a current unaffected by depolarizing holding potentials ($V_{\text{h}}$) up to $-50$ mV. This current is sensitive to phenylalkylamines
but insensitive to dihydropyridines. The other current is transient and inactivates at depolarized $V_h$.

In this study we investigated electrophysiological and pharmacological properties of voltage-dependent Ca$^{2+}$ currents in DUM neurons isolated from the fifth abdominal ganglion of the cockroach *P. americana*. Two different kinds of currents were separated: MLVA and HVA currents. These currents have different sensitivities to the well-established blockers specific for vertebrate Ca$^{2+}$ channels, ω-CgTx GVIA (McCleskey et al. 1987), ω-Aga IVA (Mintz et al. 1992), and ω-conotoxin MVIIIC (ω-CmTx MVIIIC; Hillyard et al. 1992). The electrophysiological and pharmacological properties of the separated currents were compared with those of currents described in invertebrate and vertebrate preparations.

Some of the results have already been presented in abstract form (Wicher and Penzlin 1995).

**METHODS**

**Cells**

Isolation of cells was performed as described previously (Wicher et al. 1994). Briefly, the fifth and sixth abdominal ganglia of adult cockroaches (*P. americana*) were excised, desheathed, and incubated for 10 min at room temperature in saline composition, in mM: 190 NaCl, 5 KCl, 5 CaCl$_2$, 2 MgCl$_2$, and 10 N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES), pH 7.4 containing 1 mg/ml trypsin. After the enzyme was thoroughly washed off and the ganglia were stored in saline for $\approx$ 1 h, the large DUM cells situated in the dorsal midline of the ganglia were separated with the use of thin metal needles. Viability of cells was assessed by microscopic observation: only those having a bright appearance under phase contrast were used.

The investigations started with the use of terminal ganglion DUM cells. In this ganglion 36 large cells containing octopamine are located in the dorsal midline (Dymond and Evans 1979; Eckert et al. 1992). In the same region eight cells of comparable size were used, solutions had the following compositions. Pipette solution contained (in mM) 100 choline methyl sulphate, 60 CsOH, 10 CsCl, 30 TEA-Br, 2 Mg-ATP, 1 CaCl$_2$, 10 EGTA, and 10 HEPES, pH adjusted to 7.2. Bath solution was composed of (in mM) 190 choline methyl sulphate, 5 CaCl$_2$, 30 CaCl$_2$, 10 EGTA, and 10 HEPES, pH adjusted to 7.2. Most experiments were performed with Ca$^{2+}$-induced rundown of Ca$^{2+}$ currents, Sr$^{2+}$ and Ba$^{2+}$ were occasionally used as charge carriers instead of Ca$^{2+}$ because Ba$^{2+}$, which is most commonly used for this purpose, had the disadvantage that its currents were larger than Sr$^{2+}$ currents and increased the risk of voltage error due to series resistance, the concentration was reduced to 3 mM. When Ca$^{2+}$ or Sr$^{2+}$/Ba$^{2+}$ were used, solutions had the following compositions. Pipette solution was composed of (in mM) 100 choline methyl sulphate, 60 CsOH, 10 CsCl, 30 TEA-Br, 2 Mg-ATP, 1 CaCl$_2$, 10 EGTA, and 10 HEPES, pH adjusted to 7.2. Bath solution was composed of (in mM) 190 choline methyl sulphate, 5 CaCl$_2$ or 5 SrCl$_2$/3 BaCl$_2$, 10 HEPES, and 5 $\times$ 10$^{-4}$ tetrodotoxin. A comparison of Ca$^{2+}$ currents measured in the two different Ca$^{2+}$ solutions revealed no indication of a contribution of Cl$^-$ currents in the Cl$^-$ rich solution. Liquid junction potentials between pipette and bath solutions were corrected. Tetrodotoxin was obtained from Sigma (Deisenhofen, Germany), ω-CgTx GVIA from RBI (Natick, MA), and ω-CmTx MVIIIC and ω-Aga IVA from Alomone Labs (Jerusalem, Israel).

Application or washout of blocking agents was performed by transferring the cell (situated on the pipette tip, which had been inserted into a protecting glass tube) into the blocker-containing or control solution, respectively. A total and fast solution change was then achieved by sucking a small amount of solution into the tube.

Tail current measurements were used to assess the amount of current activation. The tail currents were evoked by depolarization to $V_h$. Tails had a fast activation and deactivation kinetics. Depending on the size of the depolarizing voltage step, the maximum was attained within 100–900 $\mu$s. The maximum of tails was used as measure of current activation and is referred to as “tail” in the text.

Results were given as means ± SD; $n =$ number of cells. The evaluation of statistical significance of differences was performed with the use of Student’s t-test (error probability $P$). For data analysis, including nonlinear fitting procedures, the software Prism 2 (Graph Pad Software, San Diego, CA) was used.

**RESULTS**

**Kinetic properties**

Whole cell recordings of membrane current in DUM neurons from the fifth and the sixth abdominal ganglia of the cockroach *P. americana* revealed prominent voltage-dependent Ca$^{2+}$ currents. Figure 1B shows a family of Ca$^{2+}$ currents obtained by depolarizing command pulses from a $V_h$ of $-90$ mV. The threshold for activation was about $-50$ mV. The currents showed some inactivation, most pronounced on stepping to about $-10$ mV. Peak currents were maximal at 0.5–0.8 MΩ were pulled from borosilicate capillaries (Hilgenberg, Malsfeld, Germany). The series resistance remaining after compensation did not exceed 1.3 MΩ. Furthermore, to minimize voltage error due to series resistance, only cells with maximum peak Ca$^{2+}$ currents $< 7.5$ nA were used for analysis. $V_h$ was $-90$ mV. The pipette solution contained (in mM) 50 choline chloride, 30 CsCl, 60 CsOH, 50 tetraethylammonium (TEA)-Br, 2 Mg-ATP, 1 CaCl$_2$, 10 ethylene glycol-bis(β-aminoethoxy) ether-N,N,N',N'-tetraacetic acid (EGTA), and 10 HEPES. The bath solution for Ca$^{2+}$ current measurements contained (in mM) 160 choline chloride, 30 TEA-Br, 5 CaCl$_2$, 10 HEPES, and 5 $\times$ 10$^{-4}$ tetrodotoxin. This combination of bath and pipette solutions allowed measurement of separated Ca$^{2+}$ currents $\sim 1$ min after breaking into the cell. Within this time, contaminating Na$^+$ and K$^+$ currents disappeared completely.

Most experiments were performed with Ca$^{2+}$ as charge carrier. To prevent Ca$^{2+}$-induced rundown of Ca$^{2+}$ currents, Sr$^{2+}$ and Ba$^{2+}$ were occasionally used as charge carriers instead of Ca$^{2+}$. Because Ba$^{2+}$, which is most commonly used for this purpose, had the disadvantage that its currents were larger than Sr$^{2+}$ currents and increased the risk of voltage error due to series resistance, the concentration was reduced to 3 mM. When Ca$^{2+}$ or Sr$^{2+}$/Ba$^{2+}$ were used, solutions had the following compositions. Pipette solution was composed of (in mM) 100 choline methyl sulphate, 60 CsOH, 10 CsCl, 30 TEA-Br, 2 Mg-ATP, 1 CaCl$_2$, 10 EGTA, and 10 HEPES, pH adjusted to 7.2. Bath solution was composed of (in mM) 190 choline methyl sulphate, 5 CaCl$_2$ or 5 SrCl$_2$/3 BaCl$_2$, 10 HEPES, and 5 $\times$ 10$^{-4}$ tetrodotoxin. A comparison of Ca$^{2+}$ currents measured in the two different Ca$^{2+}$ solutions revealed no indication of a contribution of Cl$^-$ currents in the Cl$^-$ rich solution. Liquid junction potentials between pipette and bath solutions were corrected. Tetrodotoxin was obtained from Sigma (Deisenhofen, Germany), ω-CgTx GVIA from RBI (Natick, MA), and ω-CmTx MVIIIC and ω-Aga IVA from Alomone Labs (Jerusalem, Israel).

Application or washout of blocking agents was performed by transferring the cell (situated on the pipette tip, which had been inserted into a protecting glass tube) into the blocker-containing or control solution, respectively. A total and fast solution change was then achieved by sucking a small amount of solution into the tube.

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Results were given as means ± SD; $n =$ number of cells. The evaluation of statistical significance of differences was performed with the use of Student’s t-test (error probability $P$). For data analysis, including nonlinear fitting procedures, the software Prism 2 (Graph Pad Software, San Diego, CA) was used.

**Electrophysiology**

The ion currents of the isolated neurons were measured at room temperature with the use of the patch-clamp method in the whole-cell configuration (Hamill et al. 1981). Current measurements and data acquisition were performed with an EPC9 patch-clamp amplifier (HEKA Elektronic, Lambrecht, Germany) that was controlled by an ATARI computer (MEGA STe). Data were sampled at 10 kHz and filtered at 2.9 kHz. Capacitive and leak currents were compensated by a cancellation routine provided by E9SCREEN. Remaining uncompensated currents were subtracted with an online P/4 protocol. For off-line data analysis, M2Lab software (Instrutech, Elmont, NY) was used. Pipettes having resistances of
The voltage range in which currents were maximal is rather component activating in the high-voltage range (HVA). Measured at the end of 50-ms command pulses, i.e., after (I-V) millivolts more positive (Fig. 1).

Neurons isolated from the 5th abdominal ganglion. Currents were obtained previously reported for *P. americana*, *V. P. americana,* and *B. americana*. Activation of *B. americana* neuron obtained by voltage jumps from a holding potential but short enough to avoid substantial inactivation. Activation (Fig. 1, inset; Ba²⁺: compare Fig. 3, D and E). The I-V curve for Sr²⁺ peak currents reaches maximum at −10 mV, compared with 0 mV for Ca²⁺ (Fig. 1C), but there is no shift of the whole I-V curve on the voltage axis. Furthermore, with Sr²⁺ solution, the decay of currents activated by low-voltage commands starts later and is less pronounced than with Ca²⁺ (Fig. 1C, inset). Therefore the difference between the maxima of Ca²⁺ and Sr²⁺ I-V curves seems to reflect the influence of time-dependent inactivation on current size. In contrast, the I-V curve for peak of Ba²⁺ currents is shifted by −10 mV toward lower voltages (not shown), which might be attributed to the lower concentration of divalent cations and the lower potency of Ba²⁺ in screening negative surface charges (Frankenhaeuser and Hodgkin 1957; Hille 1992).

The rundown of currents observed in Ca²⁺ solution was substantially decreased in Sr²⁺ and Ba²⁺ solution. This indicates that channel rundown can be partly, although not entirely, accounted for by the influx of Ca²⁺ generated by the repeated activation of Ca²⁺ currents during measurements.

### Activation

Ca²⁺ current activation was estimated from the amplitudes of tail currents elicited on repolarization from short command pulses to *Vₜ*. To achieve full activation, a pulse 5 ms in duration was chosen, which was close to the time to peak but short enough to avoid substantial inactivation. Activation depended on voltage in sigmoidal fashion (Fig. 2AI). The data could be fitted equally well by assuming a model with *m* or *m²* activation kinetics, a situation similar to those previously reported for *Helix* neurons (Akaike et al. 1978).

![Image](http://jn.physiology.org/)

The mean of maximal Ca²⁺ peak currents was 6.7 ± 0.9 (SD) nA (n = 15); the mean cell capacitance was 330 ± 82 pF (n = 26). Assuming a specific membrane capacitance of 1 μF/cm², the maximal current density was 0.2 pA/μm².

### Charge carriers

The dependence of current size, voltage dependence, and kinetics on the charge carrier was investigated by substituting 5 mM Sr²⁺ or 3 mM Ba²⁺ for 5 mM Ca²⁺. In the case of Ba²⁺, an equimolar substitution was avoided after some experiments with 5 mM Ba²⁺ because these currents were too large to be adequately clamped. Generally, currents carried by Sr²⁺ or Ba²⁺ were much larger in size than Ca²⁺ currents and showed less decay (Sr²⁺: Fig. 1C, inset; Ba²⁺: compare Fig. 3, D and E). The I-V curve for Sr²⁺ peak currents reaches maximum at −10 mV, compared with 0 mV for Ca²⁺ (Fig. 1C), but there is no shift of the whole I-V curve on the voltage axis. Furthermore, with Sr²⁺ solution, the decay of currents activated by low-voltage commands starts later and is less pronounced than with Ca²⁺ (Fig. 1C, inset). Therefore the difference between the maxima of Ca²⁺ and Sr²⁺ I-V curves seems to reflect the influence of time-dependent inactivation on current size. In contrast, the I-V curve for peak of Ba²⁺ currents is shifted by −10 mV toward lower voltages (not shown), which might be attributed to the lower concentration of divalent cations and the lower potency of Ba²⁺ in screening negative surface charges (Frankenhaeuser and Hodgkin 1957; Hille 1992).

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\[ I/I_{\text{max}} = 1/(1 + \exp[(V - V_{0.5})S]) \]

where the potential of half-maximal activation *V₀₅* = −18 mV and the slope factor *S* = 8.3 mV (n = 7).

During longer command pulses Ca²⁺ currents show some decay. Activation curves obtained under these conditions are shifted on the voltage axis toward more positive potentials (*V₀₅* = −8 mV, *S* = 8.5 for 50 ms, not shown). Thus two current components can be distinguished according to their activation: an early, transient component activating in the low- or mid-voltage range (M-LVA) and a late, sustained component activating in the high-voltage range (HVA). Because the voltage difference between the figures for *V₀₅* is...
only 10 mV, the denotation ‘‘M-LVA’’ and ‘‘HVA’’ currents has a more operational meaning.

The existence of different Ca\(^{2+}\) currents in DUM neurons can also be discerned in the I-V relationships for the peak and the late current (Fig. 1B) and in the time to peak curve, which shows a hump with a local maximum at ~15 mV for Ca\(^{2+}\) currents and ~25 mV for Ba\(^{2+}\) currents (Fig. 2A2). The much longer time to peak of Ba\(^{2+}\) currents in the low-voltage range is simply attributed to the late start and the low degree of current decay in Ba\(^{2+}\) solution, i.e., with Ca\(^{2+}\) there is some overlap between current activation and inactivation.

**Deactivation**

The deactivation process of currents activated by short pulses (5 ms) occurring on repolarization to the V\(_h\) of ~90 mV can be described with a single-exponential function. The time constant of the deactivating tail currents is largely independent of the command potential and amounts to 977 ± 38 μs (n = 10). In contrast, with longer depolarizations, the description of tail current deactivation requires two-exponential functions when the preceding activating pulses were positive to ~20 mV. The additional occurring deactivating process has a time constant of 724 ± 31 μs (n = 10). Especially for long pulses (50 ms), the latter process is dominating for activations ~0 mV.

These results support the assumption that in DUM cells at least two Ca\(^{2+}\) currents exist that differ in their activation (cf. above: M-LVA and HVA currents).

**Inactivation**

**Steady-State Inactivation.** Depolarizing prepulses led to a reduction of Ca\(^{2+}\) currents that depended on the duration of the conditioning pulse. Prepulses of 100 ms reduced the maximal peak currents to ~40%, whereas 5-s prepulses led to complete inactivation. The voltage dependence of steady-state inactivation is shown in Fig. 2B1. Weak depolarizations already reduced considerably a fast inactivating component (decay time constant τ ~ 6 ms, Fig. 2B, inset), whereas a slow inactivating component (τ ~ 250 ms) was relatively resistant to predepolarizations. The voltage dependence of steady-state inactivation could be fitted by a sum of two Boltzmann equations. Because these processes seem to show some overlap on the voltage axis, a depolarized V\(_h\) does not
lead to a clear separation of one current component. The remaining as well as the inactivated currents are always a superposition of components. With \( \text{Ba}^{2+} \) as charge carrier, the voltage dependence was very similar (Fig. 2B2), indicating that these inactivation processes are voltage dependent and not dependent on \( \text{Ca}^{2+} \) influx.

**Time-dependent inactivation**

**TIME COURSE OF CURRENT DECAY.** The time course of the decay of \( \text{Ca}^{2+} \) currents cannot be described by a single-exponential function. Especially in the voltage range where the decay is most pronounced (about \(-10 \text{ mV}\)), the time course of decay was fitted reasonably well only by a sum of three exponential functions \( A_0 \exp(-t/\tau_k) \) with different decay time constants \( \tau_k \). At \(-10 \text{ mV}\), such a fitting procedure gave the following results: \( A_1 = 2.7 \pm 0.6 \text{ nA}, \tau_1 = 6.3 \pm 2.5 \text{ ms}; A_2 = 3.3 \pm 0.7 \text{ nA}, \tau_2 = 90 \pm 21 \text{ ms}; A_3 = 2.9 \pm 0.5 \text{ nA}, \tau_3 = 250 \pm 45 \text{ ms}; n = 6 \). The inactivation of currents carried by \( \text{Sr}^{2+} \) or \( \text{Ba}^{2+} \) could be fitted with the use of only two time constants. The fast time constant amounted to 20–40 ms (\( \text{Sr}^{2+} \)) and 20–50 ms (\( \text{Ba}^{2+} \)) and the slow time constant was \(-250 \text{ ms}\) for both ions. As with \( \text{Ca}^{2+} \) currents, the faster decaying components of \( \text{Sr}^{2+} \) or \( \text{Ba}^{2+} \) currents dominated in the low-voltage range.

Figure 2B3 shows the voltage dependence of \( \text{Ca}^{2+} \) and \( \text{Ba}^{2+} \) current decay described by the time to half-maximal decay. As outlined above, the fastest decay occurred in the low-voltage range. Although most pronounced with \( \text{Ca}^{2+} \), this is also seen with \( \text{Ba}^{2+} \). Furthermore, in the whole voltage range, \( \text{Ba}^{2+} \) currents are much more slowly decaying than \( \text{Ca}^{2+} \) currents.

**TIME-DEPENDENT INACTIVATION (DECAY) IS LARGELY \( \text{Ca}^{2+} \) DEPENDENT.** During a command voltage step, \( \text{Ca}^{2+} \) currents showed some decay. The largest and fastest decay was observed in the voltage range between \(-20 \) and \( 0 \text{ mV}\). The decay was slower at higher voltages and the extent decreased progressively with increasing depolarization. Both effects are illustrated with command potentials to \(-10, +10, \) and \(+50 \text{ mV}\) in Fig. 3, A and C. When \( \text{Ca}^{2+} \) was substituted for by \( \text{Ba}^{2+} \), the time-dependent decay was strongly reduced (Fig. 3, B and E). This reduction was dramatic in the low-voltage range (compare Fig. 3, D and E). Thus the main part of the observed \( \text{Ca}^{2+} \) current decay is obviously \( \text{Ca}^{2+} \) dependent.

On the other hand, there are also differences in the decay between currents carried by \( \text{Ba}^{2+} \) and \( \text{Sr}^{2+} \). In the low-voltage range the decay of \( \text{Sr}^{2+} \) currents is more pronounced than that of \( \text{Ba}^{2+} \) currents. At \(-20 \text{ mV}\), \( \text{Ba}^{2+} \) currents decayed after 50 ms to 79 ± 4% (n = 8) of the peak current; the corresponding \( \text{Sr}^{2+} \) currents (\(-10 \text{ mV}\)) decayed to 68 ± 9% (n = 9, for comparison: \( \text{Ca}^{2+} \): 45 ± 10%). At higher voltages the differences disappeared. At \( 0 \text{ mV}\), \( \text{Ba}^{2+} \) currents decayed to 82 ± 5% and \( \text{Sr}^{2+} \) currents (\( 0 \text{ mV}\)) to 81 ± 8% (\( \text{Ca}^{2+} \): 66 ± 10%). The differences in the amount of current inactivation between the charge carriers \( \text{Ba}^{2+} \) and \( \text{Sr}^{2+} \) at lower voltages may thus be attributed to differences in the permeability to these ions of channels activated in this potential range.

**Pharmacological properties**

A previous study (Wicher and Penzlin 1994) indicated the occurrence of at least two pharmacologically separable \( \text{Ca}^{2+} \) current components. These investigations were extended to further characterize the components and to compare their properties with those of vertebrate calcium currents.

**INORGANIC IONS.** \( \text{Ca}^{2+} \) currents in DUM neurons were sensitive to the commonly used inorganic blockers \( \text{Cd}^{2+}, \text{Ni}^{2+}, \) and \( \text{Gd}^{3+} \). In vertebrate neurons, LVA, MVA, and HVA currents are found to differ in their sensitivity to \( \text{Ni}^{2+} \) and \( \text{Cd}^{2+} \). HVA and MVA currents are potently blocked by \( \text{Cd}^{2+} \) (Fox et al. 1987; Kasai and Neher 1992; Sather et al. 1993; Soong et al. 1993; Zhang et al. 1993), whereas LVA T-type currents are less sensitive to \( \text{Cd}^{2+} \) (Fox et al. 1987; Miller and Fox 1990). On the other hand, \( \text{Ni}^{2+} \) blocks LVA and MVA currents better than HVA currents (Fox et al. 1987; Sather et al. 1993; Soong et al. 1993; Zhang et al. 1993). To test whether these aspects of the vertebrate classification scheme M-LVA/HVA (Dunlap et al. 1995) are also applicable for \( \text{Ca}^{2+} \) currents in DUM neurons, the effects of \( \text{Ni}^{2+} \)
and Cd\(^{2+}\) in these neurons were investigated. At all potentials tested Cd\(^{2+}\) blocked more effectively than Ni\(^{2+}\). But the sensitivity to Cd\(^{2+}\) and Ni\(^{2+}\) was voltage dependent. This is shown in Fig. 4 for two representative potentials: -10 mV (M-LVA currents dominating) and +20 mV (HVA currents dominating). Current recordings demonstrating the effects of 1 mM Cd\(^{2+}\) and Ni\(^{2+}\) are presented in Fig. 4, A2 and B2. The dose-inhibition curves were well fitted by the equation \(I/I_{\text{max}} = 1/(1+(I_{\text{IC}50}/S))\). In the case of HVA currents, the Hill slope coefficient \(S\) was \(\sim 1\) (Cd\(^{2+}\): \(S = 1.25 \pm 0.2\); Ni\(^{2+}\): \(S = 1.1 \pm 0.1\)), indicating 1–1 binding. For M-LVA currents, however, \(S\) was always <1, indicating multiple binding (Cd\(^{2+}\): \(S = 0.63 \pm 0.1\); Ni\(^{2+}\): \(S = 0.49 \pm 0.05\)). For HVA currents, half-maximal inhibition was obtained with Cd\(^{2+}\) at a half-inactivating concentration \(I_{\text{IC}50}\) of 5.0 ± 0.8 \(\mu\)M, which is in the range typical for HVA calcium currents in vertebrates (e.g., Fox et al. 1987; Kasai and Neher 1992; Sather et al. 1993). For Ni\(^{2+}\), the DUM cell HVA Ca\(^{2+}\) channels have a somewhat higher affinity (\(I_{\text{IC}50} = 40 \pm 5 \mu\)M) than vertebrate HVA channels, e.g., 100 \(\mu\)M Ni\(^{2+}\) blocked only 10% of the current in chick sensory neurons (Fox et al. 1987), and in neuroblastoma-glioma cells the \(I_{\text{IC}50}\) was \(\sim 250 \mu\)M for Ni\(^{2+}\) (Kasai and Neher 1992). In snail neurons, however, the \(I_{\text{IC}50}\) for Ni\(^{2+}\) was 30 \(\mu\)M (Akaike et al. 1978), but in these cells Cd\(^{2+}\) was largely ineffective (\(I_{\text{IC}50} = 3 \text{ mM!}\)).

As is typical for vertebrate LVA and MVA currents, the affinity of DUM M-LVA currents for Ni\(^{2+}\) was higher than in HVA currents: \(I_{\text{IC}50} = 19 \pm 3 \mu\)M. On the other hand, there was also a strong blocking action of Cd\(^{2+}\) that compares with that for HVA currents: \(I_{\text{IC}50} = 9 \pm 2 \mu\)M. Similar dose-response properties were recently found for rBE-II channels expressed in oocytes (Soong et al. 1993). In this system the \(I_{\text{IC}50}\) for Ni\(^{2+}\) amounted to 25 \(\mu\)M, and 10 \(\mu\)M Cd\(^{2+}\) blocked >80% of the current. Similar to DUM cell M-LVA currents, rBE-II currents are transient, inactive at depolarized potentials, and are maximal at depolarizations to -10 mV. Other examples for a Ni\(^{2+}\)/Cd\(^{2+}\) sensitivity similar to DUM cell M-LVA currents are rat cerebellar granule neurons (Ni\(^{2+}\): \(I_{\text{IC}50} = 66 \mu\)M; Cd\(^{2+}\): \(I_{\text{IC}50} = 1 \mu\)M) and doe-1 channels expressed in oocytes (Ni\(^{2+}\); \(I_{\text{IC}50} = 33 \mu\)M; Cd\(^{2+}\); \(I_{\text{IC}50} \sim 1 \mu\)M) (Ellinor et al. 1993).

The effects of Ni\(^{2+}\) and Cd\(^{2+}\) on Ca\(^{2+}\) currents were completely reversible up to concentrations of 0.1 mM. 85 ± 3% of the currents blocked by 0.1 mM Cd\(^{2+}\) and 94 ± 5% of the currents blocked by 0.1 mM Ni\(^{2+}\) recovered within 1 min after wash (\(n = 5\)).

In some experiments the effect of 1 mM Gd\(^{3+}\) on calcium currents in DUM neurons was tested. The inhibiting effect was roughly the same as that of 1 mM Cd\(^{2+}\) (not shown). There was not such a strong block as reported for neuroblastoma-glioma cells (Kasai and Neher 1992), but the action was similar insofar as the inhibition took \(\sim 1\) min to develop.

**Organic compounds**

HVA L-type currents in vertebrates are sensitive to dihydropyridines, phenylalkylamines, and benzothiazepines (e.g., Catterall and Striessnig 1992). Ca\(^{2+}\) currents in invertebrate neurons were shown to be sensitive to phenylalkylamines like verapamil and methoxyverapamil (D600) (Akaike et al. 1978; Pearson et al. 1993; Pelzer et al. 1989; Thomas 1984; Schafer et al. 1994; Thomas 1984; Wicher and Penzlin 1994), but, with the exception of bee Kenyon cells (Schafer et al. 1994), they were never found to be sensitive to dihydropyridines (Bickmeyer et al. 1994a; Byerly and Leung 1988; Pearson et al. 1993; Pelzer et al. 1989; Thomas 1984; Wicher and Penzlin 1994). The benzothiazepine diltiazem in *Drosophila* neurons had no influence on Ca\(^{2+}\) currents that were slightly reduced by phenylalkylamines at high concentrations (Byerly and Leung 1988).

The reduction of peak currents of cockroach DUM cell Ca\(^{2+}\) currents after application of 10 \(\mu\)M verapamil or diltiazem is shown in Fig. 5. Both agents blocked an HVA current component activating at potentials more positive than -30 mV and reaching its maximum between 0 and +10 mV. The difference currents (control registration – registration with blocker; not shown) exhibit substantial decay (\(\tau \sim 90\) ms) in the voltage range between -20 and 0 mV. At more positive potentials the decay becomes slower and less pronounced. The blocker-sensitive currents have large tail currents that deactivate fast (verapamil: \(\tau = 470 \pm 120\) ms, diltiazem: \(\tau = 490 \pm 130\) ms, \(n = 6\)). Because voltage

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**Fig. 4.** Reduction of Ca\(^{2+}\) currents by Cd\(^{2+}\) (A) and Ni\(^{2+}\) (B). A1 and B1: dose-response relationship of Cd\(^{2+}\) block (A1) and Ni\(^{2+}\) block (B1) of peak currents obtained at 2 potentials (-10 mV, +20 mV). The curves were fitted according to the equation \(I/I_{\max} = 1/[1+(I_{\text{IC}50}/S)]\), where \(I_{\text{ion}}\) is Cd\(^{2+}\) or Ni\(^{2+}\). The results for the parameters were as follows. Cd\(^{2+}\): -10 mV, median inhibiting concentration \(I_{\text{IC}50}\) = 9 \(\mu\)M, \(S = 0.63\); +20 mV, \(I_{\text{IC}50}\) = 5 \(\mu\)M, \(S = 0.4\). Ni\(^{2+}\): -10 mV, \(I_{\text{IC}50}\) = 19 \(\mu\)M, \(S = 0.49\); +20 mV, \(I_{\text{IC}50}\) = 40 \(\mu\)M, \(S = 1.1\). Data points are means. Bars: SD. n = 6, A2 and B2: current registrations in the presence and absence of 1 \(\mu\)M Cd\(^{2+}\) (A2) and 1 \(\mu\)M Ni\(^{2+}\) (B2). Currents were obtained by depolarizations to the potentials indicated.
dependence and kinetics of the verapamil-sensitive and the
diltiazem-sensitive currents are very similar, it seems reason-
able to infer that both blockers inhibit the same current. The
effects of verapamil and diltiazem were largely reversible.
One minute after wash, 75 ± 5% of the verapamil-blocked
currents and 80 ± 7% of the diltiazem-blocked currents
recovered.

The third class of compounds known to block vertebrate
L-type currents, the dihydropyridines, had no effect on Ca
currents in DUM neurons. Neither the antagonist nifedipine
(10 μM) nor the agonist BAY K 8644 (10 μM) affected
any current, which is in line with the results of previous
investigations (Wicher and Penzlin 1994).

Although there were no indications for the occurrence of
LVA currents similar to vertebrate T-type currents in DUM
neurons, the effect of 10 μM flunarizine was tested. In all
experiments (n = 10) flunarizine had no effect. Also, amilo-
ride (10–100 μM) did not reduce any Ca
current in DUM
neurons (H. Achenbach, personal communication). Thus the
M-LVA currents found in DUM cells have no similarity with
T-type currents.

**Peptide toxins**

In vertebrate neurons the toxins ω-CgTx GVIA, ω-CmTx
MVIIC, and ω-Aga IVA block more or less specifically
N-, Q-, and P-type channels, respectively (Dunlap et al.
1995; Miljanich and Ramachandran 1995; Olivera et al.
1994). In the case of ω-CgTx GVIA, a blocking effect on
Ca
currents in DUM neurons has already been demon-
strated (Wicher and Penzlin 1994). It seemed interesting to
find out whether the ω-toxins are suitable for a separation
of Ca
current components in these cells.

The toxins were applied to the DUM cells in concentra-
tions that lead to 80–90% block of vertebrate N-, P-, and
Q-type currents: ω-CgTx GVIA, 1 μM; ω-Aga IVA, 50 nM;
ω-CmTx MVIIC, 1 μM. In Fig. 6, A–C, the effects of the
three ω-toxins on peak I–V curves of Ca
currents are shown. Some characteristic differences in the action on
currents between these toxins can be easily discerned. ω-CgTx
GVIA was nearly ineffective in the low-voltage range, but
it reduced HVA currents (Fig. 6A). The I–V curve of the
ω-CgTx-GVIA-sensitive currents has a threshold of ~30 mV
and reaches the maximum at 0 mV. In contrast, ω-CmTx
MVIIC blocked M-LVA currents most potently but only
weakly affected HVA currents (Fig. 6C). The ω-CmTx-
MVIIC-sensitive current component activates positive to
~50 mV and is maximal at ~10 mV. The effect of ω-Aga
IVA was similar to that of ω-CmTx MVIIC but less pro-
nounced (Fig. 6B).

Furthermore, the toxin-sensitive M-LVA and HVA cur-
rents differ in their kinetics. Ca
currents blocked by ω-
CgTx GVIA were hardly decaying (Fig. 6, D1 and D3) and
had pronounced tails with fast deactivation (τ = 510 ± 110
μs, n = 7). On the other hand, currents blocked by ω-CmTx
MVIIC (and ω-Aga IVA) show fast decay (Fig. 6D2).
When Ca
was substituted for by Sr
or Ba
, the amount
of toxin-induced block in the lower-voltage range was in-
creased, whereas the block in the high-voltage range re-
mained of comparable size as with Ca
(Table 1). Sr
and Ba
currents obtained in the lower-voltage range are
slower and less decaying than Ca
currents (Fig. 3).
Thus ω-CmTx MVIIC and ω-Aga IVA seem to block the predepo-
larization-sensitive current component that is transient be-
cause of Ca
-dependent inactivation (compare Fig. 6, D2
and E2). At higher voltages the decay of ω-toxin-sensitive
currents is less pronounced and largely independent of the
charge carrier (Fig. 6, D3–E4).

The toxin-induced current block developed with different
time course for ω-CmTx MVIIC and ω-Aga IVA on the one
hand and ω-CgTx GVIA on the other (Fig. 7A). The
blocking effects in the lower-voltage range appeared quickly
with a time constant of ~15 s. On wash, the currents recov-
ered quickly with a similar time constant (not shown). Espe-
cially in the case of ω-Aga IVA, no strong depolarizing
voltage pulses were necessary to get the relief from block
as described for rat Purkinje cells (Mintz et al. 1992). On
the other hand, in the presence of ω-Aga IVA such pulses
failed to remove the block, which is also in contrast to the
results obtained for the rat neurons. The ω-CgTx-GVIA-
induced current block in the HVA range developed slowly
(time constant > 100 s). The recovery of currents after the
toxin was washed off appeared slow also and was incomplete
after 2 min in most cases.

The above results, summarized in Table 1, point to a
relative specificity of ω-CmTx MVIIC for the rapidly
decaying M-LVA Ca
current component on the one hand
and to a fairly selective block of the slowly decaying HVA
component by ω-CgTx GVIA on the other. But an analysis of
the time course and the voltage dependence of peptide
toxin effects revealed that both the toxins do not act exclusively selectively. As shown in Fig. 7B for Ba^{2+} currents that were obtained by voltage ramps (allowing fast, time-resolved measurements), 1 μM ω-CgTx GVIA also affected M-LVA currents slightly within 10 s after application. After some delay a pronounced reduction of HVA currents developed. Furthermore, with ω-CgTx GVIA at higher concentrations, M-LVA currents were also substantially reduced. The total current at −10 mV was reduced to 64 ± 6% of control when ω-CgTx GVIA was applied at 5 μM.

On the other hand, 1 μM ω-CmTx MVIIC blocked M-LVA currents very significantly within 10 s, but in the following 2 min it caused an additional slight reduction of HVA currents (Fig. 7B).

From the above evaluation of the steady-state inactivation of the current components it is already known that a transient M-LVA Ca^{2+} current component is inactivated by depolarized V_h, whereas the HVA component is not.

With the assumption that ω-CmTx MVIIC and ω-Aga IVA might act on the depolarization-sensitive component, one would expect that the effects of both blockers depend on V_h, whereas the effect of ω-CgTx GVIA is largely independent of V_h. Indeed, when V_h was set to −50 mV and the fast decaying component had vanished, ω-CmTx MVIIC as well as ω-Aga IVA had no or a very little effect (n = 5 experiments, Fig. 8, A2, A3, B2, and B3). To test the pure ω-CgTx GVIA effect on HVA currents, a putative action on M-LVA currents was excluded by the presence of 0.5 μM ω-CmTx MVIIC in the control and test solution (see above). Under these conditions, the blocking effect of ω-CgTx GVIA was, as expected, not affected by a depolarized V_h of −50 mV (Fig. 8, A1 and B1).

Although ω-CmTx MVIIC and ω-Aga IVA on the one side and ω-CgTx GVIA on the other seem to act preferentially on different channel types, their specificity was obviously limited. Therefore it was tested whether there is some

**TABLE 1.** Blocking effect of ω-toxins on currents carried by Ca^{2+}, Sr^{2+}, and Ba^{2+}

<table>
<thead>
<tr>
<th></th>
<th>−10 mV</th>
<th>+20 mV</th>
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<tr>
<td></td>
<td>Ca^{2+}</td>
<td>Sr^{2+}</td>
</tr>
<tr>
<td>ω-CgTx GVIA (1 μM)</td>
<td>79 ± 9</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>ω-Aga IVA (50 nM)</td>
<td>72 ± 10</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>ω-CmTx MVIIC (1 μM)</td>
<td>60 ± 6</td>
<td>45 ± 9</td>
</tr>
<tr>
<td></td>
<td>70 ± 9</td>
<td>66 ± 6</td>
</tr>
<tr>
<td></td>
<td>81 ± 6</td>
<td>81 ± 5</td>
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<tr>
<td></td>
<td>85 ± 5</td>
<td>82 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SD (in % of the control currents) of peak currents remaining 2 min after toxin application for 4–6 cells. ω-CgTx GVIA, ω-conotoxin GVIA, ω-Aga IVA, ω-agatoxin IVA; ω-CmTx MVIIC, ω-conotoxin MVIIC.
The time course of the toxin-induced block could be fitted well with a single-exponential function (time constant \( \tau = 15 \) s). In contrast, the reducing effect of 1 \( \mu M \) \( \omega\)-CgTx GVIA in the high-voltage range (+20 mV) developed considerably more slowly. The exponential function fitted on the data points is described by a time constant of 117 s. B and C: specificity of both the toxins \( \omega\)-CgTx GVIA and \( \omega\)-CmTx MVIC is limited. Fast measurements of instantaneous \( Ba^{2+} \)/V relationships were performed with voltage ramps (100 mV, 100 ms). B: 1 \( \mu M \) \( \omega\)-CgTx GVIA caused a fast reduction of \( Ba^{2+} \) current in the low-voltage range (10 s) that was followed by a slower decrease of high-voltage currents (45 s, 2 min). C: application of 1 \( \mu M \) \( \omega\)-CmTx MVIC resulted in a fast block of low-voltage-activated currents (10 s) but within 2 min there was an additional slight reduction of the high-voltage-activated component.

According to the results given in Figs. 6 and 8, \( \omega\)-CmTx MVIC and \( \omega\)-Aga IVA are most likely affecting the same M-LVA current component. When 1 \( \mu M \) \( \omega\)-CmTx MVIC was applied in the presence of 50 nM \( \omega\)-Aga IVA, the reduction of \( Ba^{2+} \) currents in the low-voltage range was 42 ± 6% (\( n = 4 \)), which was significantly weaker (\( P < 0.001 \)) than the 60% block that was observed without \( \omega\)-Aga IVA (Fig. 9B1). The additional effect of both toxins was in all cases tested less than the sum of the effect of each toxin. These facts indicate that both toxins might act on the same channel.

In the above described experiments with depolarized \( V_h \) it was observed that \( \omega\)-Aga IVA not only blocked a depolarization-sensitive M-LVA component but also a depolarization-insensitive HVA component. Therefore it had to be clar-
ified whether \( \omega \)-Aga IVA might reduce the effect of \( \omega \)-CgTx GVIA in the high-voltage range. In the experiments addressing this question there was no indication for such an occlusion. Although \( \omega \)-Aga IVA blocked a part of HVA currents, the \( \omega \)-CgTx GVIA effect was not significantly reduced in the presence of \( \omega \)-Aga IVA. These results indicate that \( \omega \)-Aga IVA (and possibly \( \omega \)-CmTx MVIIIC, cf. Fig. 7C) might act on HVA channels that are resistant to \( \omega \)-CgTx GVIA.

The \( I-V \) relationship of the current blocked by diltiazem and verapamil is very similar to the \( I-V \) curve of the \( \omega \)-CgTx-GVIA-sensitive current. Because there are no remarkable differences between the kinetic properties like activation and deactivation (large tails) of verapamil-sensitive currents and \( \omega \)-CgTx-GVIA-sensitive current, the assumption that these blockers act on the same target seems to be reasonable. It was, however, impossible to prove this assumption with the use of a combination of verapamil and \( \omega \)-CgTx GVIA. Each of both blockers reduced the HVA current in the presence of the other by 25–35%. Even at a concentration of 100 \( \mu \)M, verapamil did not weaken the blocking potency of \( \omega \)-CgTx GVIA. Therefore the possibility that several ion channels are present in DUM cells that contribute to the total HVA current cannot be ruled out.

**Discussion**

**\( \text{Ca}^{2+} \) currents in cockroach DUM cells and invertebrate neurons**

In DUM neurons from cockroach abdominal ganglia, large \( \text{Ca}^{2+} \) currents were obtained by depolarizing pulses. The maximal current density, with \( \text{Ba}^{2+} \) as charge carrier—which was approximately twice that of \( \text{Ca}^{2+} \) currents—compared with that of \( \text{Ba}^{2+} \) currents in somata of locust neurons (Pearson et al. 1993). The maximum of the \( I-V \) curve in DUM cells was within the range reported for the other invertebrate neurons (between −5 and +20 mV). The electrophysiological and pharmacological analysis of the total \( \text{Ca}^{2+} \) current in cockroach DUM neurons resulted in the separation of M-LVA currents and HVA currents (see below). A summary of the kinetic and pharmacological properties of the M-LVA and HVA \( \text{Ca}^{2+} \) currents is shown in Table 2.

Many invertebrate \( \text{Ca}^{2+} \) currents investigated so far have electrophysiological properties similar to those of vertebrate HVA currents (Akaike et al. 1978; Hayashi and Levine 1992; Pearson et al. 1993).

Concerning activation threshold and kinetics, invertebrate \( \text{Ca}^{2+} \) currents show a heterogeneity comparable with that of vertebrate currents. Activation of \( \text{Ca}^{2+} \) currents in cockroach DUM neurons started at −50 mV (Fig. 1). Such a low-threshold activation was also found in neurons of snails (Akaike et al. 1978), locusts (Bickmeyer et al. 1994a; Laurent et al. 1993), and bees (Schäfer et al. 1994). In other preparations, including snail neurons (Barnes et al. 1994; Brezina et al. 1987) and various insect neurons (Byerly and Leung 1988; Hayashi and Levine 1992; Pearson et al. 1993), activation started at potentials positive to −40 mV.

Fast decay of \( \text{Ca}^{2+} \) currents as seen in DUM neurons had also been observed in snail neurons (Akaike et al. 1978),
effect could be reversed by a train of strongly depolarizing pulses (Bickmeyer et al. 1994b). This relief was not observed in \(\omega\)-Aga-IVA-sensitive DUM cell M-LVA currents, and, additionally, these currents were rapidly blocked by 50 nM \(\omega\)-Aga IVA.

Taking the comparison between the electrophysiological properties of Ca\(^{2+}\) currents in cockroach DUM cells and other invertebrate neurons together, one can state that, on the basis of the limited availability of data, the cockroach Ca\(^{2+}\) currents are more or less similar to other invertebrate Ca\(^{2+}\) currents. From the pharmacological point of view, the results obtained for the DUM cell currents concerning the effects of phenylalkylamines and dihydropyridines are in line with previous findings in many other invertebrate preparations. It seems to be typical for neuronal invertebrate Ca\(^{2+}\) channels that phenylalkylamine-sensitive channels lack the dihydropyridine sensitivity. For the effects of \(\omega\)-toxins on cockroach M-LVA and HVA currents there is no parallel in other invertebrate neurons.

In general, however, there is presently a lack of information about electrophysiological and pharmacological characterization of invertebrate Ca\(^{2+}\) currents. The data on invertebrate Ca\(^{2+}\) currents available from literature are not comprehensive enough to allow a detailed comparison with the DUM cell currents. One can expect that the application of \(\omega\)-toxins or other more specific agents will allow to introduce a typology of Ca\(^{2+}\) currents structured in a manner comparable with that in vertebrates.

**Ca\(^{2+}\) currents in cockroach DUM cells and vertebrate neurons**

In vertebrates Ca\(^{2+}\) currents are categorized according to the voltage range of their activation into HVA and LVA currents. Currents activating in an intermediate voltage range and showing some properties of LVA currents were previously found when class E channels were expressed in *Xenopus* oocytes (Soong et al. 1993). Ca\(^{2+}\) channels carrying LVA currents and MVA currents are assumed to belong to one family of \(\alpha_1\) subunits called M-LVA (Dunlap et al. 1995).

According to this scheme, we referred to the currents separated in our investigations as M-LVA and HVA currents (for kinetic and pharmacological properties see Table 2). This classification does not mean that we consider two channel types as responsible for total Ca\(^{2+}\) current in DUM neurons. It might be, for example, that the total HVA current is a superposition of currents flowing through several channel types (see RESULTS).

M-LVA currents in DUM neurons activated at potentials positive to \(-50\) mV and inactivated during depolarized prepotentials. These currents showed a very rapid decay when Ca\(^{2+}\) was the charge carrier. Experiments with Ba\(^{2+}\) (Fig. 3) revealed that this decay was mainly Ca\(^{2+}\)-dependent. Such Ca\(^{2+}\)-dependent decay is a common property of Ca\(^{2+}\) channels in vertebrates and invertebrates that is not restricted to LVA currents (Neely et al. 1994). Cd\(^{2+}\) blocked M-LVA currents more effectively than Ni\(^{2+}\), but the difference in blocking efficacy was less pronounced than for the HVA currents. M-LVA currents were insensitive to the vertebrate T-type blockers flunarizine and amiloride, but they were affected by \(\omega\)-Aga IVA and, most potently, by \(\omega\)-CmTx-MVIIC.

In vertebrates the LVA T-type currents have a very low activation threshold and are sensitive to predepolarizations (Carbone and Lux 1987; Nowycky et al. 1985). T-type currents are, in contrast to M-LVA currents, only mildly blocked by Cd\(^{2+}\). Furthermore, the insensitivity of M-LVA currents to amiloride and flunarizine shows that there is hardly a similarity between vertebrate T-type currents and DUM cell M-LVA currents.

A type of Ca\(^{2+}\) currents bearing some similarities with M-LVA currents was obtained when class E channels were expressed in *Xenopus* oocytes. One of these channels comprising the \(\alpha_1\)-subunit rbE-II (Soong et al. 1993) carried a current that activated positive to \(-50\) mV (in 4 mM Ba\(^{2+}\)) and was maximal at \(-10\) mV. Like M-LVA, the rbE-II current was strongly blocked by Cd\(^{2+}\) but it was also sensitive to Ni\(^{2+}\) (IC\(_{50}\) = 28 \(\mu\)M). Furthermore, \(\omega\)-CgTx GVIA did not affect the rbE-II current, but 200 nM \(\omega\)-Aga IVA blocked 33% of the current (\(\omega\)-CmTx MVIIC was not tested). As in observed in DUM cell Ca\(^{2+}\) currents, the \(\omega\)-Aga-IVA-induced block did not reverse on a train of strong depolarizations. The inactivation rate of rbE-II Ba\(^{2+}\) currents (\(\tau \approx 340\) ms) is, on the other hand, slower than that of M-LVA currents (\(\tau \approx 20\)–50 ms with 3 mM Ba\(^{2+}\)), but the decay depends, as demonstrated by Sather et al. (1993), on the coexpressed \(\beta\)-channel subunits.

Another vertebrate M-LVA current is the doe-1 current, which is also connected with a class E channel (Ellinor et al. 1993; Zhang et al. 1993). The sensitivity of doe-1 currents to Ni\(^{2+}\) and Cd\(^{2+}\) compared with that of the cockroach M-LVA current. But doe-1 currents were insensitive to \(\omega\)-CmTx MVIIC and \(\omega\)-Aga IVA and were only slightly reduced by \(\omega\)-CgTx GVIA (5 \(\mu\)M).

Also, vertebrate HVA currents have some pharmacological properties in common with DUM cell M-LVA currents. P- and Q-type currents are sensitive to \(\omega\)-CmTx MVIIC and \(\omega\)-Aga IVA (Hillyard et al. 1992; Mintz et al. 1992; Sather et al. 1993; for review see Olivera et al. 1994). Class A channels expressed in *Xenopus* oocytes are sensitive to \(\omega\)-CmTx MVIIC (IC\(_{50}\) < 0.15 \(\mu\)M) and \(\omega\)-Aga IVA (IC\(_{50}\) = 0.2 \(\mu\)M; Sather et al. 1993) and insensitive to \(\omega\)-CgTx GVIA (Mori et al. 1991). In contrast to the fast block of M-LVA currents, it took \(\sim 20\) min for 1.5 \(\mu\)M \(\omega\)-CmTx MVIIC to achieve maximal block of \(\alpha_{AI}\)-currents in *Xenopus* oocytes (Sather et al. 1993). Approximately the same time was necessary for the 1.5 \(\mu\)M \(\omega\)-CmTx-MVIIC-induced inhibition of hippocampal synaptic transmission (Wheeler et al. 1994). The time course of toxin action is concentration dependent (Ellinor et al. 1994; Sather et al. 1993; Wheeler et al. 1994) and depends further on the specificity of peptide binding on the channel proteins. Ellinor et al. (1994) demonstrated that the time constant of \(\omega\)-CgTx-induced block was raised 20-fold when, instead of N-type channels, mutants with changes in the putative \(\omega\)-CgTx-binding site were investigated. The \(\omega\)-CmTx MVIIC effect in DUM cells is interesting insofar as the block was fast—which indicates a specific binding to the channels—but, on the other hand, there was a quick recovery from block that was in contrast, e.g., to the irreversible action of \(\omega\)-CgTx in N-type channels.

Similarly to P-type currents (Mintz et al. 1992), but in
contrast to DUM cell M-LVA currents, the block of class A currents by $\omega$-Aga IVA could be relieved by strong depolarization. The corresponding $\alpha_{1A}$-currents are referred to as HVA type. In 2 mM Ba$^{2+}$ they activate positive to $-30$ mV, and the peak I-V curve is maximal at $-5$ mV (Sather et al. 1993). This voltage dependence is similar to that of M-LVA Ba$^{2+}$ currents (3 mM), but $\alpha_{1A}$-currents inactivate considerably more slowly. Taking all the differences between P/Q currents and cockroach M-LVA currents together, it is less probable that M-LVA currents are related to vertebrate P- or Q-type currents.

HVA currents in cockroach DUM neurons activated at potentials positive to $-30$ mV and were resistant to predepolarizations from negative to $-50$ mV. For potential steps positive to $+10$ mV, even with Ca$^{2+}$ as charge carrier, current decay was slow and less pronounced. These currents were effectively blocked by Cd$^{2+}$. The IC$_{50}$ of 5 $\mu$M is in the range known from many vertebrate Ca$^{2+}$ channels. Another result that is typical for HVA currents is the much lower current blocking efficacy of Ni$^{2+}$. DUM cell HVA currents were reduced by verapamil and diltiazem, which are known to block vertebrate L-type channels. But, L-type channels are identified by their sensitivity to dihydropyridines, which did not affect the cockroach HVA currents. Another agent that reduced HVA currents is the specific blocker of vertebrate N-type channels, $\omega$-CgTx GVIA. The effect of this toxin was in most cases, at least partly reversible.

Vertebrate HVA N-type currents are moderately sensitive to depolarized $V_b$. They are strongly and irreversibly blocked by $\omega$-CgTx GVIA (Boland et al. 1994; Fox et al. 1987; McCleskey et al. 1987; Nowycky et al. 1985; Williams et al. 1992). Rather as an exception, in some objects also a reversible block of N-type currents by $\omega$-CgTx GVIA was observed (Plummer et al. 1989; Sher and Clementi 1991). Furthermore, N-type currents are sensitive to $\omega$-CmTx MVIIC (Hillyard et al. 1992), but insensitive to $\omega$-Aga IVA (Olivera et al. 1994). Although the DUM cell HVA currents were affected by $\omega$-CgTx GVIA, these currents cannot be considered as N like, because this toxin sensitivity is the only one common property with N-type currents, e.g., the pretreatment with $\omega$-CmTx MVIIC did not occlude the block of $\omega$-CgTx GVIA. In vertebrates, however, both the toxins selectively block N-type channels.

On the other hand, there is no similarity with P- or Q-type currents, which are sensitive to $\omega$-Aga IVA and $\omega$-CmTx MVIIC but not to $\omega$-CgTx GVIA (Mori et al. 1991; Sather et al. 1993).

Vertebrate L-type currents have a high threshold for activation, they are resistant to predepolarization, and they show large tails on repolarization. They are, furthermore, more sensitive to Cd$^{2+}$ than to Ni$^{2+}$. They are blocked by dihydropyridines, phenylalkylamines, and benzothiazepines. A reversible block by $\omega$-CgTx GVIA as seen for the DUM cell HVA currents was also reported for L-type currents in some vertebrate preparations (Aosaki and Kasai 1989; Wang et al. 1992) and in class D channels expressed in Xenopus oocytes (Williams at al. 1992). Some Ca$^{2+}$ currents described in invertebrates have properties comparable with those of L-type currents (with the exception of missing dihydropyridine sensitivity). The HVA currents found in DUM neurons share this similarity to the L type. The reason why, nevertheless, DUM cell HVA currents should not be considered as L-type-like current is the missing dihydropyridine sensitivity, which is the most important pharmacological criterion in identification of L-type currents.

Summarizing the results of the comparison between the M-LVA and HVA Ca$^{2+}$ currents in cockroach DUM cells and various types of vertebrate Ca$^{2+}$ currents, we conclude that the insect currents, although showing some similarities with vertebrate currents, have no identity or strong similarity with any known vertebrate Ca$^{2+}$ current. Therefore we propose the existence of Ca$^{2+}$ channels in cockroach DUM cells that are different from the channels characterized so far in vertebrates. It remains unclear whether these new channels occur only in invertebrates.

The $\omega$-toxins tested proved to be useful tools to separate M-LVA and HVA currents. On the other hand, this utility was limited due to the limited target specificity of the toxins.

**Role of Ca$^{2+}$ currents in cockroach DUM neurons**

The somata of most cockroach DUM neurons are electrically excitable. The discharge pattern of these cells is a beating, not a bursting. An essential condition for repetitive activity is a Ca$^{2+}$-dependent K$^+$ current, $I_{K,ca}$, which is responsible for afterhyperpolarization (Laped et al. 1989; Wicher et al. 1994). Therefore one important role of voltage-dependent Ca$^{2+}$ currents is — via controlling $I_{K,ca}$ — the control of afterhyperpolarization.

Furthermore, when the neuronal activity is modulated, Ca$^{2+}$ channels are a possible target for the modulator. Neurohormone D, a peptide belonging to the adipokinetic hormone family, increases the spike frequency of DUM neurons, which is accompanied by changes in the shape of action potentials, e.g., a stronger and shorter afterhyperpolarization (Wicher et al. 1994). The peptide was shown to enhance a transient component of $I_{K,ca}$ that was most probably caused by a peptide-induced potentiation of a transient Ca$^{2+}$ current (Wicher and Penzlin 1994).

As in vertebrates (Takahashi and Momiyama 1993; Uchitel et al. 1992; Wheeler et al. 1994), a major role of the non-L-type currents in the release of transmitters and other mediators from nerve endings is conceivable for the DUM cells investigated here, which are thought to secrete octopamine (Dymond and Evans 1979; Eckert et al. 1992).

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Address for reprint requests: D. Wicher, Sächsische Akademie der Wissenschaften zu Leipzig, PF 100322, D-07703 Jena, Germany.

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