J. exp. Biol. ***197****, 393–398 (1994)*
Printed in Great Britain © The Company of Biologists Limited 1994

SHORT COMMUNICATION

CALCIUM CHANNEL CURRENTS IN CULTURED PARS INTERCEREBRALIS NEUROSECRETORY CELLS OF ADULT *LOCUSTA MIGRATORIA*

ULF BICKMEYER[1], WOLFGANG RÖSSLER[2] AND HERBERT WIEGAND[1]

[1]*Abteilung Neurotoxikologie, Medizinisches Institut für Umwelthygiene an der Heinrich-Heine-Universität Düsseldorf, Auf'm Hennekamp 50, 40225 Düsseldorf, Germany* and [2]*AG Neurobiologie, FB Biologie-Zoologie der Philipps-Universität, Marburg Postfach 1929, 35032, Germany*

Accepted 19 July 1994

The medial neurosecretory cells (MNSCs) of the pars intercerebralis in the brain of insects release various hormonal factors that control essential physiological and developmental functions such as moulting, reproduction and metabolism (Wigglesworth, 1940; Girardie, 1966; Goldsworthy, 1969), and these cells are therefore of considerable biological significance. A culture system for locust embryonic pars intercerebralis neurosecretory cells has recently been developed (Vanhems *et al.* 1993), and Rössler and Bickmeyer (1993) have established an *in vitro* system for growing larval and adult medial neurosecretory cells. Calcium plays an important role in neural physiology: neurosecretion depends on calcium influx into the cells and calcium currents carry the rising phase of action potentials in different types of insect neurones (Orchard, 1976; Pitman, 1979); calcium also mediates other ionic currents (Thomas, 1984). It is therefore of considerable interest to characterize the types of calcium channel currents found in locust neurosecretory neurones.

Voltage-clamp experiments on vertebrate neurones have identified calcium channel currents with different time courses and different sensitivities to pharmacological agents. At least five types of voltage-operated calcium channels have been decribed. Low-voltage-activated calcium currents are described as transient T currents; high-voltage-activated currents (HVA) can be divided into transient and sustained currents through L-, N-, P- and Q-type channels (Nowicky *et al.* 1985; Llinas *et al.* 1989; Wheeler *et al.* 1994). In comparison with investigations of vertebrate calcium channels, little is known about insect voltage-activated calcium currents. Studies carried out on embryonic *Drosophila melanogaster* neurones (Byerly and Leung, 1988), on cockroach brain neurones (Christensen *et al.* 1988), on motoneurones of *Manduca sexta* (Hayashi and Levine, 1992) and on locust thoracic neurones (Pearson *et al.* 1993; Laurent *et al.* 1993) have all described the presence of voltage-operated calcium channels. Pearson *et al.* (1993) and

Key words: insect, calcium channel currents, tetrandrine, medial neurosecretory cells, *Locusta migratoria*.

Hayashi and Levine (1992) found two types of HVA calcium currents which do not fit into the classification scheme devised for vertebrate calcium currents. We have investigated voltage-activated calcium channel currents in cultured locust MNSCs and the effects on them of cadmium, ω-Conotoxin GVIA, dihydropyridines and the plant alkaloid tetrandrine, which is known to affect voltage-operated calcium currents in vertebrate neurones (King *et al.* 1988; Bickmeyer and Wiegand, 1993; Weinsberg *et al.* 1994).

All neurones were taken from adult *Locusta migratoria* of both sexes reared in a crowded colony under constant laboratory conditions. Neurones from the MNSC group were dissected from the tissue and dissociated mechanically after removal of the neural sheath. The preparation of cultures has been described in detail by Rössler and Bickmeyer (1993). Cells were held in culture in serum-free medium containing five parts of Schneider's *Drosophila* medium and four parts of Minimum Essential Medium with Hanks' salts (5+4 medium) containing 25 mmol l^{-1} Hepes supplemented with 0.25 μg ml^{-1} Fungizone and 100 i.u. ml^{-1} penicillin/streptomycin. Dishes were held in a humid incubator chamber at 30 °C in air. Experiments were carried out on neurones maintained in culture for between 4 and 7 days.

Voltage-clamp experiments were carried out using the patch-clamp technique in the whole-cell configuration with a conventional patch-clamp tower (from Luigs and Neumann, Germany). We used fire-polished electrodes with a resistance of 2–5 MΩ. The possible voltage error resulting from series resistance was less than 5 mV in reported experiments.

The pipette solution contained (in mmol l^{-1}): 158 CsCl; 2 $MgCl_2$; 2 NaATP; 0.1 $CaCl_2$; 10 Hepes; 20 tetraethylammonium (TEA^+); 1.1 EGTA. The bath solution contained (in mmol l^{-1}): 155 choline chloride; 2 $MgCl_2$; 10 $BaCl_2$; 10 Hepes; 25 TEA^+.

The CED system hardware and software package was used in combination with the EPC7 patch-clamp amplifier (List Electronics, Germany) for stimulus generation and for analysis and storage of data. For current–voltage relationships, voltage steps with increasing amplitude were applied from a hyperpolarized potential (−80 mV) at 0.33 Hz. All experiments were carried out at room temperature (22±2 °C).

ω-Conotoxin GVIA (special fraction of ω-conotoxins) was purchased from Sigma, nitrendipine and Bay-K-8644 from RBI, USA, and tetrandrine was a gift from the Institute of Health, Beijing, People's Republic of China. Drugs were applied by diluting threefold in a 1 ml bath solution, which was then added to the cells incubating in a 2 ml bath solution.

Voltage-dependent inward currents were normally elicited by voltage steps from a hyperpolarized membrane potential of −80 mV to values more positive than −40 mV (no low-voltage-activated currents). Only HVA calcium channel currents were found. The inward current amplitude depended on the membrane potential, a result similar to those reported by Pearson *et al.* (1993) and Laurent *et al.* (1993).

In a total of 11 experiments in which cells were exposed to Cd^{2+}, some with cumulative treatments, we found a dose-dependent block of calcium channel currents. The current–voltage relationship showed a reduction in amplitude, but with no shift in the positive or negative voltage directions. 1 mmol l^{-1} Cd^{2+} blocks all inward currents (data not shown).

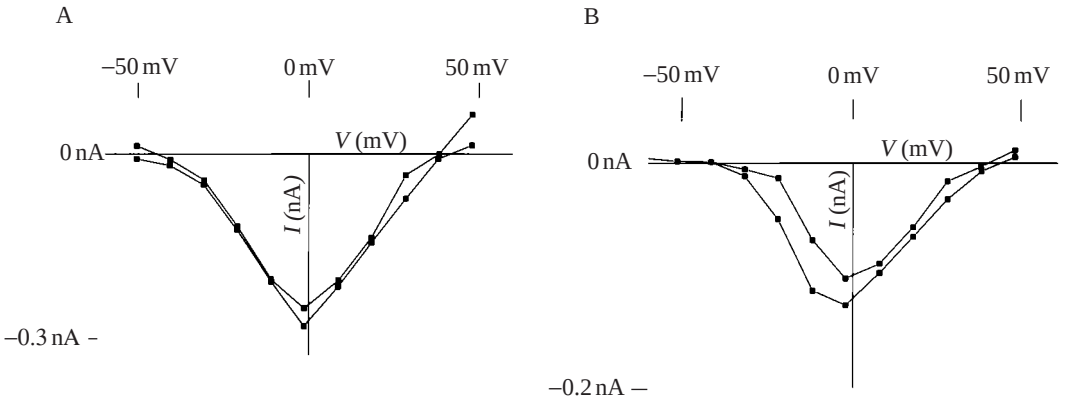

Fig. 1. Effect of 1 μmol l^{-1} nitrendipine on current–voltage relationships of (A) a non-sensitive cell and (B) a cell showing a small effect. The lower traces represent the controls.

ω-Conotoxin has proved a useful tool for differentiating pharmacologically between different HVA calcium currents in vertebrate neurones since N-type calcium channels seem to be preferentially blocked by ω-Conotoxin (Olivera *et al.* 1991; Sher and Clementi, 1991). At concentrations as high as 5 μmol l^{-1} ω-Conotoxin shows no clear effect on calcium currents in MNSCs (N=8), even after several minutes of application (data not shown).

Application of the dihydropyridine calcium channel antagonist nitrendipine showed no consistent effect on calcium channel currents. Although no effect of nitrendipine on current–voltage relationship could be observed in two experiments, in five other experiments a small effect in the current–voltage relationship was found (Fig. 1). The L-type channel agonist Bay-K-8644 failed to increase current amplitudes through HVA channels of MNSCs in a series of four experiments (data not shown). The observation that both agonist and antagonist had apparently little or no effect on calcium channel currents suggests that no L-type currents are present in MNSCs of locusts, thus corroborating the results of Byerly and Leung (1988) and Pearson *et al.* (1993).

Tetrandrine is a bis-benzylisoquinoline isolated from the roots of *Stefania tetrandra* sp. Moore and has been used in traditional medicine in China. One site of action of tetrandrine seems to be on calcium channels and it has been shown to block L-type channels in GH3 pituitary cells (King *et al.* 1988), to reduce calcium currents in nerve terminals (Wiegand *et al.* 1990) and to block all calcium currents in spinal cord cells (Bickmeyer and Wiegand, 1993). In spinal cord neurones, the effects of tetrandrine were maximal after 7 min and the effects were partially reversible by washing cells for about 30 min. Because we could not completely prevent the ‘run down’ of calcium channel currents in MNSCs, we measured the effects of tetrandrine after only 2 min of exposure, a time at which the full effect may not have developed. We were not able to wash out the effects of tetrandrine. In 15 experiments at tetrandrine concentrations of 10–200 μmol l^{-1} we found a dose-dependent reduction of calcium current amplitude; a concentration of 200 μmol l^{-1} blocked all calcium channel currents. Fig. 2A demonstrates the effect of 33 μmol l^{-1} tetrandrine on the peak inward current amplitude. The rate of decay of

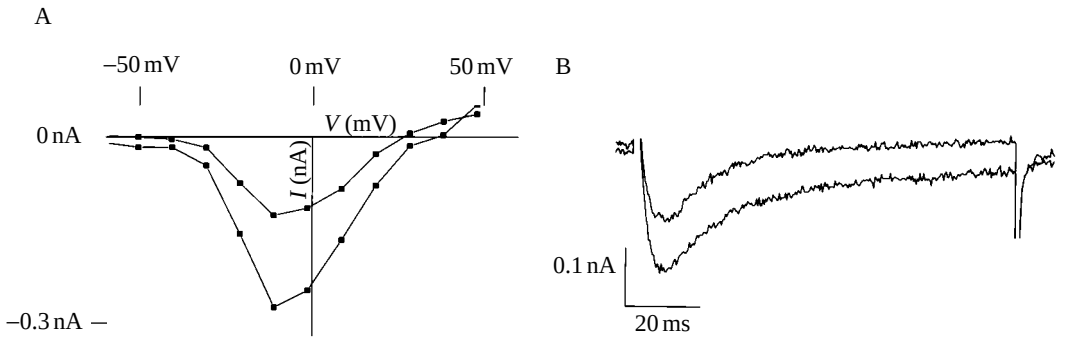

Fig. 2. (A) A representative trace showing the effect of 33 μmol l^{-1} tetrandrine (upper trace) on the current–voltage relationship of a typical cell. (B) Current traces (−80 mV to 0 mV) before (lower trace) and after treatment with tetrandrine (upper trace).

calcium currents was increased under tetrandrine treatment (Fig. 3), an effect similar to that reported by Bickmeyer and Wiegand (1993) in mouse spinal cord neurones, indicating a use-dependent open channel block like that recently demonstrated by Weinsberg *et al.* (1994).

The group of MNSCs of the pars intercerebralis of *Locusta migratoria* represents a rather heterogeneous neurone population with respect to excitability (Rössler and Bickmeyer, 1993), but all the cells we investigated showed voltage-dependent calcium channels. Only HVA calcium currents blocked by high concentrations of cadmium and tetrandrine were found. Common calcium channel antagonists and agonists failed to show a clear effect on MNSCs, indicating some differences between vertebrate and insect calcium channels.

ω-Conotoxin is used for pharmacological classification of HVA calcium currents in vertebrate neurones (Olivera *et al.* 1991), but is ineffective in discriminating MNSC calcium currents, an observation similar to that reported for *Manduca sexta* (Hayashi and Levine, 1992). The HVA currents are not increased by the use of Bay-K-8644 and are only slightly affected by nitrendipine, indicating that no L-type current is present and corroborating data from Pearson *et al.* (1993) from dissociated locust thoracic neurones.

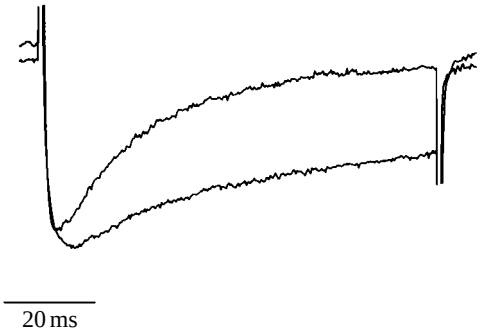

Fig. 3. Normalized current amplitudes of a cell before (lower trace) and after treatment with 50 μmol l^{-1} tetrandrine (upper trace), demonstrating the faster current decay in the presence of tetrandrine.

The fourth pharmacological substance whose effects on MNSCs were examined was tetrandine, which is known to block voltage-operated calcium channels in vertebrate neurones (King *et al.* 1988; Bickmeyer and Wiegand, 1993) and in ventricular cells (Liu *et al.* 1992). In spinal cord and dorsal root ganglion neurones, as well as in bovine chromaffin cells, tetrandrine affects all voltage-operated calcium channels, inducing a faster current decay. In bovine chromaffin cells, tetrandrine has recently been shown to be a use-dependent open-channel blocker (Weinsberg *et al.* 1994). Interestingly, tetrandrine seems to affect calcium channel currents of vertebrate and insect neurones in a similar way, whereas other pharmacological agents fail to be effective, suggesting that the target of tetrandrine in calcium channels may be similar in different animal classes.

Vertebrate calcium channel effectors fail to affect locust MNSCs, so pharmacological substances that affect locust calcium channels may fail to affect vertebrate calcium channels. This may mean that a search for pharmacological substances affecting calcium channels and electrical activity of MNSCs *in vitro* could provide useful information about the regulation of the humoral system in locusts and could, therefore, contribute to the development of pest-controlling substances.

We thank Professor Dr K. Kalmring for supporting this work and Frank Weinsberg and Tanja Kaiser for critical reading of the manuscript and for helpful comments.

References

BICKMEYER, U. AND WIEGAND, H. (1993). Tetrandrine effects on calcium currents in cultured neurones of foetal mice. *NeuroReport* **4**, 938–949.

BYERLY, L. AND LEUNG, H. T. (1988). Ionic currents of *Drosophila* neurones in embryonic cultures. *J. Neurosci.* **8**, 4379–4393.

CHRISTENSEN, B. N., LARMET, Y., SHIMAHARA, T., BEADLE, D. AND PICHON, Y. (1988). Ionic currents in neurones cultured from embryonic cockroach (*Periplaneta americana*) brains. *J. exp. Biol.* **135**, 193–214.

GIRARDIE, A. (1966). Controle de l'activité génitale chez *Locusta migratoria*. Mise en evidence d'un facteur gonadotrope et d'un facteur allaotrope dans la pars intercerebralis. *Bull. Soc. Zool. Fr.* **91**, 423–439.

GOLDSWORTHY, G. J. (1969). Hyperglycaemic factors from the corpus cardiacum of *Locusta migratoria*. *J. Insect Physiol.* **15**, 2131–2140.

HAYASHI, J. H. AND LEVINE, R. B. (1992). Calcium and potassium currents in leg motoneurones during postembryonic development in the hawkmoth *Manduca sexta*. *J. exp. Biol.* **171**, 15–42.

KING, V. K., GARCIA, M. L., HIMMEL, D., REUBEN, J. P., LAM, Y. T., PAN, J. X., HAN, G. Q. AND KACZOROWSKI, G. J. (1988). Interaction of tetrandrine with slowly inactivating calcium channels. *J. biol. Chem.* **263**, 2238–2244.

LAURENT, G., SEYMOUR-LAURENT, K. J. AND JOHNSON, K. (1993). Dendritic excitability and a voltage-gated calcium current in locust nonspiking local interneuron. *J. Neurophysiol.* **69**, 1484–1498.

LIU, Q.-Y., KARPINSKY, E. AND PANG, P. K. T. (1992). Tetrandrine inhibits both T and L calcium channel currents in ventricular cells. *J. cardiovasc. Pharmac.* **20**, 513–519.

LLINAS, R., SUGIMORI, M., LIN, J. W. AND CHERSKY, B. (1989). Blocking and isolation of a calcium channel from neurones in mammals and cephalopods utilizing a toxin fraction (FTX) from funnel web spider poison. *Proc. natn. Acad. Sci. U.S.A.* **86**, 1689–1693.

NOWICKY, M. C., FOX, A. P. AND TSIEN, R. W. (1985). Three types of neuronal calcium channels with different calcium agonist sensitivity. *Nature* **316**, 440–443.

OLIVERA, B. M., IMPERIAL, J. S., CRUZ, L. J., BINDOKAS, V. J. AND ADAMS, M. E. (1991). Calcium channel-targeted polypeptide toxins. *Ann. N. Y. Acad. Sci.* **635**, 114–122.

ORCHARD, I. (1976). Calcium dependent action potentials in a peripheral neurosecretory cell of the stick insect. *J. comp. Physiol.* **112**, 95–102.

PEARSON, H. A., LEES, G. AND WRAY, D. (1993). Calcium channel currents in neurones from locust (*Schistocerca gregaria*) thoracic ganglia. *J. exp. Biol.* **177**, 201–221.

PITMAN, R. M. (1979). Intracellular citrate or externally applied tetraethylammonium produce calcium-dependent action potentials in an insect motoneurone cell body. *J. Physiol., Lond.* **291**, 327–337.

RÖSSLER, W. AND BICKMEYER, U. (1993). Locust medial neurosecretory cells *in vitro*: morphology, electrophysiological properties and effects of temperature. *J. exp. Biol*. **183**, 323–339.

SHER, E. AND CLEMENTI, F. (1991). Omega conotoxin voltage operated calcium channels in vertebrate cells. *Neuroscience* **42**, 301–307.

THOMAS, M. V. (1984). Voltage clamp analysis of a calcium mediated potassium conductance in cockroach (*Periplaneta americana*) central neurones. *J. Physiol., Lond.* **350**, 159–178.

VANHEMS, E., DELBOS, M. AND GIRARDIE, J. (1993). *In vitro* growth of locust embryonic pars intercerebralis neurosecretory cells. *Neuroscience* **52**, 361–368.

WEINSBERG, F., BICKMEYER, U. AND WIEGAND, H. (1994). Effects of tetrandrine on calcium channel currents of bovine chromaffin cells. *Neuropharmacology* (in press).

WHEELER, D., RANDALL, A. AND TSIEN, R. W. (1994). Roles of N-type and Q-type calcium channels in supporting hippocampal synaptic transmission. *Science* **264**, 107–111.

WIEGAND, H., MEIS, S. AND GOTZSCH, U. (1990). Inhibition by tetrandrine of calcium currents at mouse motor nerve endings. *Brain Res.* **524**, 112–118.

WIGGLESWORTH, V. B. (1940). The determination of characters at metamorphosis in *Rhodnius prolixus* (Hemiptera). *J. exp. Biol.* **17**, 201–222.