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IONIC SPECIES INVOLVED IN THE ELECTRICAL ACTIVITY OF SINGLE ADULT AMINERGIC NEURONES ISOLATED FROM THE SIXTH ABDOMINAL GANGLION OF THE COCKROACH *PERIPLANETA AMERICANA*

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Summary

Adult neurones were obtained by dissociation of the dorsal area of the sixth abdominal (A6) ganglion of the cockroach, and electrical properties were studied with the patch-clamp technique. The neurones showed spontaneous fast action potentials, similar to those recorded with microelectrodes in neurones *in situ* along the dorsal median line of the A6 ganglion. Synthetic saxitoxin (sSTX) at concentrations of $1{\cdot}0\times10^{-8}$ to $1{\cdot}0\times10^{-7}$ mol l^{-1} suppressed the action potential (AP) and induced a dose-dependent hyperpolarization of the resting potential, suggesting that two types of sSTX-sensitive Na^+ channels are present. The resting potential was dependent on the external concentration of both Na^+ and K^+, with a similar sensitivity to each, yielding a slope of about 43 mV per 10-fold change in concentration. The delayed outward rectification present under control conditions was reduced by tetraethylammonium chloride (TEA–Cl, $1{\cdot}0\times10^{-2}$ mol l^{-1}). TEA–Cl or Ca^{2+}-free saline abolished the afterhyperpolarization and increased the overshoot and duration of triggered APs, indicating that a calcium-activated potassium conductance contributes to the falling phase of the AP. At $3{\cdot}0\times10^{-3}$ mol l^{-1}, the Ca^{2+} channel blockers $MnCl_2$, $CoCl_2$ and $NiCl_2$ lengthened the AP. A blocker-dependent increase in the overshoot and threshold of the AP and reduction of the afterhyperpolarization were observed, probably reflecting the relative potencies of these ions in blocking Ca^{2+} channels and thus the Ca^{2+}-activated K^+ conductance. Increasing $MgCl_2$ concentration by $3{\cdot}0\times10^{-3}$ mol l^{-1} had no effect on the AP, indicating that the positive shift of the threshold is due to the blockade of Ca^{2+} channels present at this potential. The results suggest that these isolated neurones are dorsal unpaired median neurones previously studied in a number of insect species.

Introduction

Electrophysiological studies have shown that some insect neurone somata *in situ* are capable of generating action potentials. Spontaneous action potentials have

Key words: single adult aminergic neurone, cockroach, A6 ganglion, dorsally located aminergic neurone, sodium action potentials, patch-clamp.

been recorded from neural somata located near the dorsal surface of cockroach abdominal ganglia (Kerkut *et al.* 1968, 1969; Jego *et al.* 1970; Crossman *et al.* 1971, 1972) and in the octopaminergic dorsal unpaired median (DUM) cells of locust metathoracic ganglia (Hoyle & Dagan, 1978). Overshooting sodium action potentials have been observed in other types of neurones from cicada (Hagiwara & Watanabe, 1956) and *Drosophila* (Ikeda & Kaplan, 1970), and can be induced in the cockroach metathoracic fast coxal depressor (D_f) motoneurone (Pitman *et al.* 1972; Pitman, 1975) and in the locust fast extensor tibiae motoneurone (Goodman & Heitler, 1979) by axotomy or pretreatment with colchicine. Calcium action potentials have also been recorded in insect neurones under certain conditions (Pitman, 1979).

Electrophysiological studies of insect neurones, particularly pharmacological studies, are complicated by the presence of a glial blood–brain barrier (Treherne & Pichon, 1972; Treherne *et al.* 1973; for a review see Abbott *et al.* 1986) surrounding the neurones and restricting the penetration of ions and molecules. Studies are also hampered by glial uptake mechanisms; for example, in the cockroach there is a very efficient glial uptake mechanism that removes γ-aminobutyric acid (GABA) from the synaptic sites (Hue *et al.* 1981).

The development of *in vitro* techniques, such as culture of embryonic cockroach brain, in which the glial cells are degenerating (Beadle *et al.* 1982; Beadle & Hicks, 1985; Dewhurst & Beadle, 1985), has facilitated the investigation of the pharmacology and electrophysiology of insect neurones (Lees *et al.* 1983, 1985; Shimahara *et al.* 1987; Christensen *et al.* 1988). Pinnock & Sattelle (1987) have proposed a novel approach in which adult insect ganglia are dissociated to obtain a heterogeneous population of neurone cell bodies. By this method, isolated adult neurones are rapidly obtained for electrophysiological and pharmacological investigations. The use of adults avoids having to make allowance for the variation in electrical activity which occurs during development (Goodman & Spitzer, 1981*a*,*b*; Lees *et al.* 1985).

In the experiments reported here, patch-clamp recordings were made from adult isolated aminergic neurones obtained after dissociation of the dorsal side of the A6 ganglion of the cockroach *Periplaneta americana*. These neurones, forming a very small population revealed by the non-specific dye Neutral Red (Dymond & Evans, 1979), are located along the dorsal midline of the ganglia. Their electrophysiological and pharmacological properties are not yet well understood in the A6 ganglion, although they are relatively more abundant here than in other ganglia. This paper describes the electrical parameters, and the ionic dependence, of the action potential in the isolated neurones.

Materials and methods

Adult male cockroaches (*Periplaneta americana*), reared at 29°C in the laboratory, were used. Electrophysiological recordings were made from the

somata of neurones revealed by the non-specific dye Neutral Red (see Dymond & Evans, 1979) along the dorsal midline of the sixth abdominal (A6) ganglion.

In situ *recordings*

The dissected abdominal nerve cord and its desheathed A6 ganglion were pinned onto the bottom of a Sylgard-coated Petri dish and superfused with normal cockroach saline. The electrical activity of neurones located along the dorsal median line of the A6 ganglion was recorded through conventional intracellular microelectrodes (tip resistance 30 MΩ when filled with 3 mol l^{-1} KCl) connected to a VF-180 microelectrode amplifier having current injection capability (Biologic, Echirolles, France). All data were stored on video cassettes (see below).

Single-cell studies

Cell isolation

Nerve cell bodies from the A6 ganglion were prepared under sterile conditions according to a modified version of the technique of Pinnock & Sattelle (1987). Briefly, after isolation of the abdominal nerve cord, the A6 ganglia were desheathed and the dorsal median parts were incubated for 1 h in normal saline containing collagenase (type I A, 1 mg ml^{-1}) and hyaluronidase (type IV S, 1 mg ml^{-1}). The ganglia were then rinsed twice in normal saline and mechanically dissociated by repetitive gentle suctions through a Pasteur pipette. Cells were allowed to settle on poly-D-lysine (poly-D-lysine hydrobromide, M_r 70 000–150 000) coating the bottom of a Petri dish filled with normal saline supplemented with foetal calf serum (FCS, 10 % by volume) and gentamycin (20 μg ml^{-1}). All compounds were obtained from Sigma Chemicals (L'Isle d'Abeau Chesnes, France) except FCS (GIBCO, Cergy Pontoise, France). To verify that this technique effectively isolated the expected neurones, control dissociations were carried out on ganglia previously treated with Neutral Red (10 mg l^{-1}), and most of the stained neurones were present among the isolated cells. However, all experiments were carried out on neurones isolated from unstained ganglia.

Electrophysiological recordings

The patch-clamp technique (Hamill *et al.* 1981) was used in the whole-cell configuration to record action potentials in the current-clamp mode. Patch electrodes were pulled from borosilicate capillary tubes (Clark Electromedical Instruments, Reading, UK) with a BB-CH puller (Mecanex, Geneva, Switzerland) and had resistances of 3–4 MΩ when filled with the pipette solution. The Petri dish containing the dissociated cells was placed onto the stage of an inverted microscope (CK2: Olympus, Tokyo, Japan) and the patch electrode was positioned on the cell surface with a three-dimensional hydraulic micromanipulator (MO-103M: Narishige Scientific Instrument Laboratory, Tokyo, Japan). Neurones were chosen for electrophysiological studies only if they had a diameter of 60–70 μm, the same morphology and the same spontaneous activity when the cell membrane under the electrode tip was ruptured after a seal of at least 25 GΩ.

Action potentials recorded with a patch- and cell-clamp amplifier (RK300: Biologic, Echirolles, France) were either spontaneous or evoked by applying a 50 or 100 ms depolarizing current pulse of 0·1–1 nA at 1 Hz with a programmable stimulator (SMP300: Biologic, France). Action potentials and applied current were displayed on a digital oscilloscope (3091: Nicolet Instrument Corporation, Madison, WI, USA) and stored on video cassettes (Sony PCM-701ES digital audio processor and Sony Betamax SL-T50 ME video cassette recorder) for later off-line analysis on the digital oscilloscope.

Solutions

Normal saline contained (in mmol l^{-1}): NaCl, 214; KCl, 3·1; $CaCl_2$, 5; $MgCl_2$, 4; sucrose, 50; Hepes buffer, 10; pH 7·4. Low-Na^+ containing solutions had the same composition as normal saline, except that NaCl was replaced by an equivalent amount of tetramethylammonium chloride (TMA–Cl). For experiments carried out in a high-K^+ medium, extra KCl was directly added to the normal saline. Patch electrodes were filled with a solution containing (in mmol l^{-1}): KCl, 150; $MgCl_2$, 1; NaCl, 10; Hepes buffer, 10; pH 7·4.. During the course of the experiments, the saline superfusing the cell under study was delivered close to the cell through a gravity perfusion system allowing complete solution changes within less than 3 s. All tested compounds were added to the normal saline at the final concentration. Experiments were carried out at room temperature (20°C) and results were expressed as mean ± S.E.

Results

Electrical properties of in situ *and isolated neurones*

To check whether the isolated cells were the neurones located along the dorsal median line of the A6 ganglion, the electrical properties of the isolated cells were compared with those of the *in situ* cells.

Electrical activity recorded with an intracellular microelectrode from *in situ* neurones along the dorsal median line of the A6 ganglion was typically as shown in Fig. 1. Spontaneous overshooting fast action potentials of about 80 mV in amplitude (measured from peak overshoot to peak undershoot) were separated by a long-lasting slow predepolarization phase (100 ms in Fig. 1A) during which the

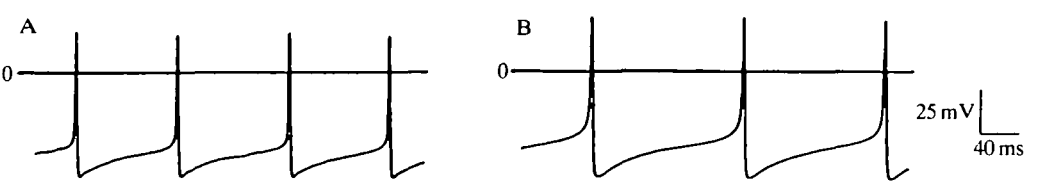

Fig. 1. Spontaneous action potentials recorded with an intracellular microelectrode in an *in situ* neurone located along the dorsal median line of the A6 ganglion (A) and with the patch-clamp technique (whole-cell recording configuration) in a single isolated neurone obtained after dissociation of the dorsal side of the A6 ganglion (B).

Table 1. *Characteristics of spontaneous action potentials*

		RP (mV)	APA (mV)	OS (mV)	Threshold (mV)	APD_{50} (ms)	f (Hz)
In situ recordings	Mean	−46·7	78·7	21·1	−41·1	2·2	10·5
($N = 10$)	S.E.	2·2	1·7	1·0	2·2	0·1	2·4
Isolated cells	Mean	−52·5	107·7	36·7	−35·4	2·3	6·4
($N = 30$)	S.E.	2·4	2·7	2·0	0·8	0·1	1·0

RP, resting potential measured during a period of quiescence; APA, action potential (AP) amplitude; OS, overshoot; threshold, threshold of the spontaneous AP determined as the potential corresponding to the intersection point of the straight lines that could be drawn through the AP upstroke and the predepolarizing phase; APD_{50}, AP duration measured at 50 % of APA; f, spontaneous frequency.

threshold for action potential triggering was reached. The duration of this slow phase depended upon the spontaneous frequency. The resting potential of these cells, measured during a period of quiescence, was relatively low (about −47 mV). Detailed action potential characteristics are given in Table 1. Sometimes, the neurones were not spontaneously active, but action potentials could, nevertheless, be evoked by slightly depolarizing the cells for a few seconds by injecting current through the recording electrode. The evoked action potentials continued after the end of the pulse.

Isolated neurones obtained after dissociation of the dorsal side of the A6 ganglion showed electrical activity, as recorded with the patch-clamp technique in the whole-cell recording configuration, that was generally the same as that of the *in situ* neurones (Fig. 1B; Table 1). Although the isolated neurones exhibited spontaneous fast action potentials of larger amplitude (more than 100 mV) with a more positive overshoot, their resting potentials (−52·5 mV) were very close to those of *in situ* neurones. However, spontaneous frequency and threshold of the action potential were usually lower in isolated neurones than in neurones *in situ*. As with *in situ* recordings, some neurones were not spontaneously active, but a small depolarizing current pulse (0·3–1 nA) applied in the current-clamp mode was sufficient to elicit action potentials which continued after the end of the pulse.

These results suggest that the isolated neurones are identical to those located along the dorsal median line of the A6 ganglion.

Effects of synthetic saxitoxin

To investigate the channel mediating the depolarizing phase of the action potential in these neurones, synthetic saxitoxin (sSTX) was used. This toxin selectively blocks voltage-dependent sodium channels in several types of preparation, especially the cockroach giant axon (Sattelle *et al.* 1979; Pelhate & Sattelle, 1982). When a spontaneously active isolated neurone was superfused with sSTX at $1{\cdot}0\times10^{-7}$ mol l^{-1}, the amplitude of the action potential rapidly decreased and spontaneous activity completely disappeared within 30 s of the application of

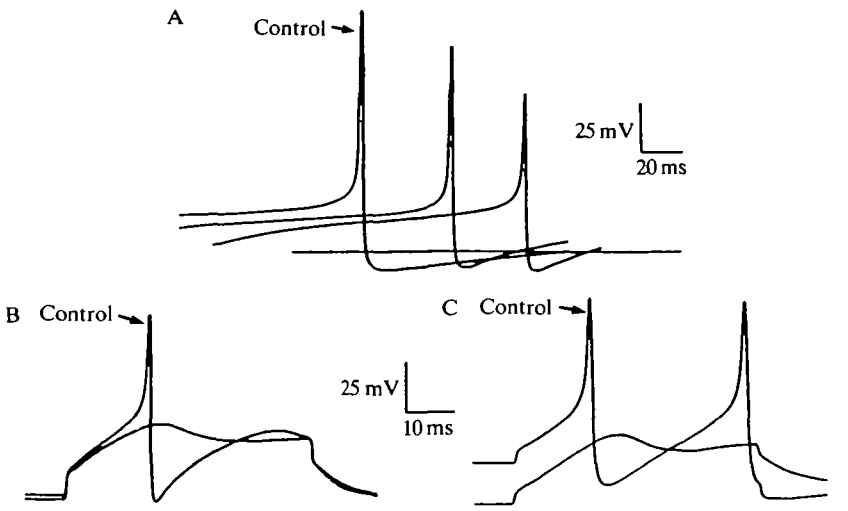

Fig. 2. Typical effects of synthetic saxitoxin (sSTX) on action potentials (APs) recorded in isolated neurones. $1{\cdot}0\times10^{-7}$ mol l^{-1} sSTX progressively decreases the amplitude of the spontaneous APs (A) and finally completely suppresses the APs within 30 s (smooth horizontal tracing). (B,C) Effects of $1{\cdot}0\times10^{-8}$ and $1{\cdot}0\times10^{-7}$ mol l^{-1} sSTX, respectively, on triggered action potentials (0·5 nA for 50 ms). Note the large hyperpolarization in the presence of $1{\cdot}0\times10^{-7}$ mol l^{-1} sSTX.

sSTX (Fig. 2A). The blocking effect of sSTX was completely reversed within 60 s of superfusion with normal saline. Action potentials elicited by applying a depolarizing current pulse were also blocked by sSTX (Fig. 2B,C). At a lower concentration ($1{\cdot}0\times10^{-8}$ mol l^{-1}) sSTX abolished the action potential and slightly increased (i.e. hyperpolarized) the resting potential of the neurone by $4{\cdot}8 \pm 2{\cdot}2$ mV ($N = 7$). However, if the remaining local response was not affected when the sSTX concentration was increased 10-fold (Fig. 2C), an unexpected large hyperpolarization ($22{\cdot}6 \pm 4{\cdot}2$ mV, $N = 7$) was observed. It should be noted that depolarization imposed upon the neurone to bring its resting potential back to the control value and/or increasing the pulse strength were ineffective in reversing the sSTX blocking effect. This apparent difference in sensitivity to sSTX of the fast depolarizing phase of the action potential and of the resting membrane potential suggests that two different types of sSTX-sensitive sodium channels might be present in these neurones. The first would be the classical voltage-dependent channel, responsible for the depolarizing phase of the action potential, whereas the second would be background sodium channels which might be involved in the maintenance of the resting potential at its relatively low level (about −50 mV).

Ionic dependence of the resting potential

Effects of sodium ions

To investigate the extent to which the resting potential was sodium-dependent, TMA–Cl was substituted in equivalent amounts for NaCl in the superfusing saline

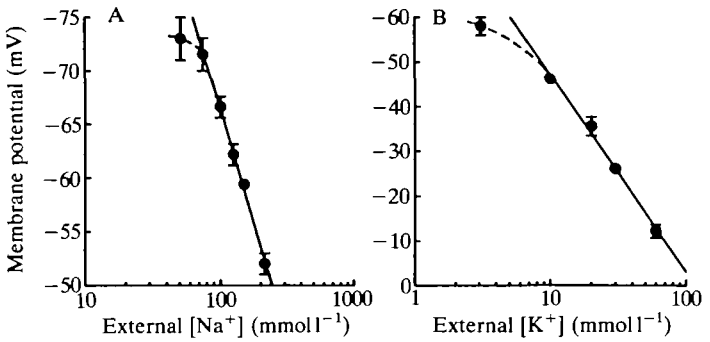

Fig. 3. Ionic dependence of the resting potential of isolated neurones. (A) Effect of external sodium concentration, $[Na^+]_o$, on the resting potential. Each data point represents the mean of five trials each on 5–20 different cells, and error bars represent ±S.E. (B) Dependence of the resting potential on the external potassium concentration, $[K^+]_o$. Points are the means of five trials each in three different cells, and error bars are ±S.E. The solid lines represent the fits to a linear regression with a slope of 42·8 mV per 10-fold change in $[Na^+]_o$ ($r = 0{\cdot}999$) and 44·1 mV per 10-fold change in $[K^+]_o$ ($r = 0{\cdot}997$). The error bars are shown when larger than symbols.

When mean values of the resting potential (obtained in 5–20 different cells) were plotted against the logarithm of the external Na^+ concentration, $[Na^+]_o$, a linear relationship was observed for $[Na^+]_o \geq 75\,mmol\,l^{-1}$ (Fig. 3A). Linear regression through the data points gave a slope of 42·8 mV (correlation coefficient $r = 0{\cdot}999$) per 10-fold change in $[Na^+]_o$, a value that differs by only 15·4 mV from the value that would be expected if the membrane were behaving as an ideal sodium electrode. Below $75\,mmol\,l^{-1}$, the resting potential started to become relatively insensitive to the variations of $[Na^+]_o$. It is noteworthy that the values of hyperpolarization observed at $[Na^+]_o$ of $75\,mmol\,l^{-1}$ (19·5 ± 1·5 mV, $N = 10$) and $51\,mmol\,l^{-1}$ (21 ± 2 mV, $N = 13$) are very close to the level induced by $1{\cdot}0\times10^{-7}\,mol\,l^{-1}$ sSTX (22·6 ± 4·2 mV, $N = 7$). Thus, this resting sodium permeability of isolated neurones might account for the hyperpolarization observed in the presence of sSTX. Moreover, the progressive decrease of the action potential amplitude when $[Na^+]_o$ was reduced is in agreement with the observed blocking effect of sSTX.

The resting potential of *in situ* neurones measured with an intracellular microelectrode was also sensitive to $[Na^+]_o$, but to a lesser extent. In six preparations, the mean hyperpolarization induced by a Na^+-free saline (TMA–Cl substituted) was 13 ± 0·6 mV. For comparison, this value would be obtained in isolated neurones with an external Na^+ concentration of about $100\,mmol\,l^{-1}$.

Effects of potassium ions

The resting potential of isolated neurones was also markedly dependent on the potassium concentration, $[K^+]_o$, of the superfusing saline (Fig. 3B). There was a linear relationship between the measured resting potential and the logarithm of

$[K^+]_o$ above 10 mmol l^{-1}. One remarkable point is that the slope of the linear regression through the data points (44·1 mV, with a correlation coefficient $r = 0{\cdot}997$, per a 10-fold change in $[K^+]_o$) is very close to that obtained for $[Na^+]_o$. Thus, it appears that the resting potential of isolated neurones has a similar dependence on $[K^+]_o$ as on $[Na^+]_o$.

Current–voltage relationship

The current–voltage relationship was determined in isolated neurones, in which action potentials were blocked by $1{\cdot}0\times10^{-7}$ mol l^{-1} sSTX, by measuring the variation of the membrane potential in response to a hyperpolarizing or depolarizing current pulse. In all tested neurones, compensation for the sSTX-induced hyperpolarization was made by applying a constant depolarizing current to bring the resting potential back to the level observed before sSTX treatment. Fig. 4 shows the results obtained in a typical neurone. Under control conditions, all studied neurones showed a delayed outward rectification upon depolarization from resting potential, whereas a linear relationship between the membrane potential and the applied current was observed on hyperpolarization from resting potential. Linear regression through the data points in the negative potential range gives a mean membrane input resistance (R_{in}) of $56{\cdot}9 \pm 7{\cdot}3$ MΩ ($N = 4$). In the more positive potential range R_{in} was decreased to $21{\cdot}5 \pm 3{\cdot}9$ MΩ because of the

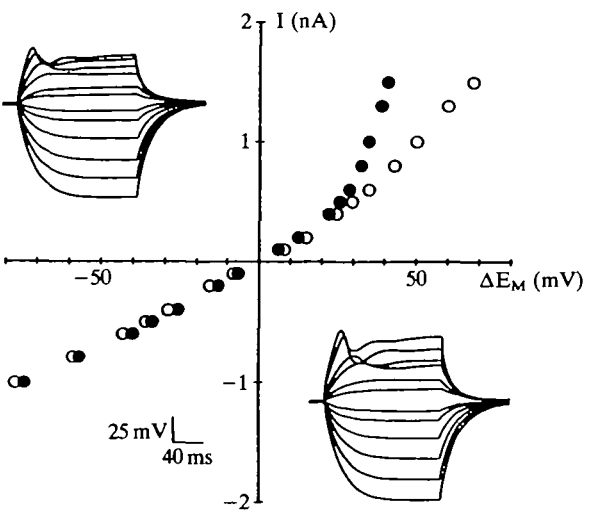

Fig. 4. Current–voltage relationships in an sSTX ($1{\cdot}0\times10^{-7}$ mol l^{-1})-treated isolated neurone under control conditions (filled circles) and after application of $1{\cdot}0\times10^{-2}$ mol l^{-1} tetraethylammonium chloride (TEA–Cl) to the superfusing saline (open circles). Compensation for the sSTX-induced hyperpolarization was made by applying a constant depolarizing current. Membrane potential (abscissa) relative to resting potential is measured at the end of a hyperpolarizing or depolarizing current pulse (ordinate) of 100 ms in duration. The insets show some of the membrane responses to the same applied current pulses before (upper left) and after (lower right) application of TEA–Cl.

delayed outward rectification. Application of the potassium channel blocker tetraethylammonium chloride (TEA–Cl) at $1{\cdot}0\times10^{-2}$ mol l^{-1} reduced the delayed outward rectification by about 60%, but had no apparent effect on the membrane resistance at negative membrane potentials.

Effects of K^+ channel blockers and of calcium-free saline

Because TEA–Cl decreased the delayed outward rectification, its effect was also tested on action potentials evoked by applying a depolarizing current pulse (Fig. 5A). When an isolated neurone was superfused with a saline containing TEA–Cl at $1{\cdot}0\times10^{-2}$ mol l^{-1}, the overshoot of the action potential increased by $+7{\cdot}3\pm1{\cdot}1$ mV ($N=5$) and the falling phase was prolonged. The duration of the action potential (measured at the potential corresponding to 50% of the amplitude of the control action potential) increased from $2{\cdot}1\pm0{\cdot}4$ ms to $4{\cdot}2\pm0{\cdot}7$ ms and the afterhyperpolarization (or undershoot, i.e. the membrane hyperpolarization beyond the cell resting potential) completely disappeared. TEA–Cl did not affect the resting potential, and its effects on the action potential were immediately reversed upon superfusion with normal saline. Among the other potassium channel blockers tested, CsCl had no effect at $1{\cdot}0\times10^{-2}$ mol l^{-1}. The effects of 4-aminopyridine and 3,4-diaminopyridine, which are known to block cockroach axonal potassium channels in the micromolar range (Pelhate & Sattelle, 1982), were unclear (both compounds were used at $1{\cdot}0\times10^{-3}$ and $1{\cdot}0\times10^{-2}$ mol l^{-1}) and await further investigation.

TEA–Cl is known to block calcium-activated potassium channels (Yellen, 1984; Wong & Adler, 1986). The possibility that the K^+ conductance involved in the repolarization of the action potential is calcium-dependent was tested using a saline in which $MgCl_2$ was substituted for $CaCl_2$ in equivalent amounts. In the calcium-free saline, the characteristics of the action potential (Fig. 5B) were similar to those observed in TEA–Cl. There was an increase in the overshoot of $+10{\cdot}2\pm1{\cdot}1$ mV ($N=3$), a prolongation of the falling phase with a duration increase from $2{\cdot}9\pm1{\cdot}3$ ms to $8{\cdot}6\pm1{\cdot}4$ ms, and a suppression of the afterhyperpolarization, without any effects on the cell resting potential.

These effects of TEA–Cl and Ca^{2+}-free saline strongly suggest that potassium

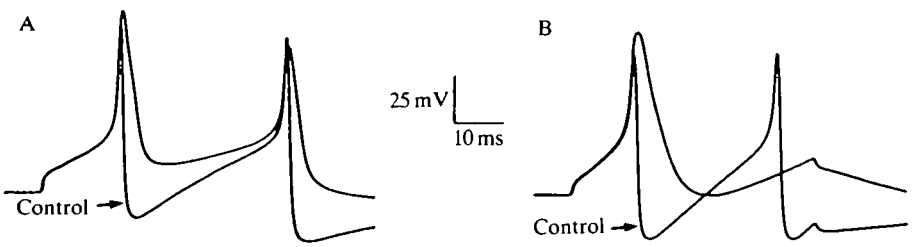

Fig. 5. Typical effects of $1{\cdot}0\times10^{-2}$ mol l^{-1} TEA–Cl (A) and of a Ca^{2+}-free ($MgCl_2$-substituted) saline (B) on action potentials elicited by a 50 ms depolarizing current pulse (0·6 nA) in isolated neurones. A complete reversibility is observed upon superfusion with normal saline.

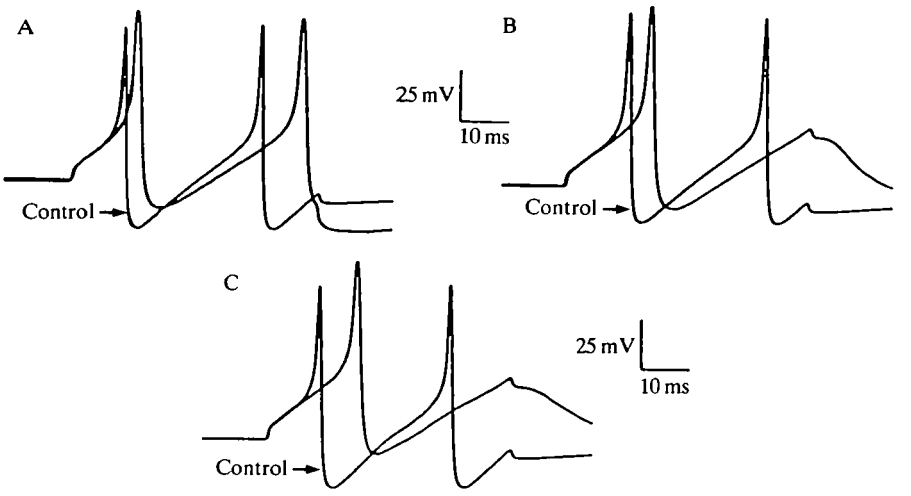

Fig. 6. Effects of the calcium channel blockers $MnCl_2$ (A), $CoCl_2$ (B) and $NiCl_2$ (C) at $3{\cdot}0\times10^{-3}$ mol l^{-1}, on triggered action potentials (0·6 nA for 50 ms) in an isolated neurone. Note the positive shift of the action potential threshold in the presence of the blockers.

ions participate in the falling phase of the action potential and that the underlying potassium conductance is calcium-activated.

Effects of calcium channel blockers

Three different calcium channel blockers ($MnCl_2$, $CoCl_2$ and $NiCl_2$) were used to study the participation of calcium ions in the electrical activity of isolated neurones. The blockers were successively tested on the same isolated neurone at a concentration of $3{\cdot}0\times10^{-3}$ mol l^{-1}. All blockers had qualitatively the same effects (Fig. 6): an increase of the overshoot and duration of the action potential, a reduction of the afterhyperpolarization and a lengthening of the predepolarizing phase, due to a positive shift of the threshold of the action potential. Quantification of the results (Table 2) showed that the changes in action potential overshoot, afterhyperpolarization and threshold depended on the calcium channel blocker used, with the following sequence of effectiveness: $MnCl_2 < CoCl_2 < NiCl_2$. Upon superfusion with normal saline, the effects of the blockers were completely reversed. These effects were not due simply to the elevation of divalent cation concentration, since an increase in $MgCl_2$ concentration by $3{\cdot}0\times10^{-3}$ mol l^{-1} had no effect on the action potential characteristics. This suggests that the modifications of electrical activity observed in the presence of the calcium channel blockers are the consequence of a decreased calcium permeability.

Discussion

The experiments presented above show that the typical electrical activity of

Table 2. *Effects of calcium channel blockers on action potential*

		OS (mV)	Threshold (mV)	APD_T (ms)	RAH (%)
$MnCl_2$	Mean	+6·9	+8·4	+1·6	−38·9
($N=4$)	S.E.	0·3	0·4	0·7	4·6
$CoCl_2$	Mean	+7·5	+10·4	+1·9	−49·3
($N=4$)	S.E.	1·3	0·7	1·1	5·8
$NiCl_2$	Mean	+10·2	+13·2	+1·5	−80·7
($N=4$)	S.E.	0·9	0·8	0·3	2·1

Data correspond to the differences between control values and those in the presence of the blockers used at $3{\cdot}0\times10^{-3}$ mol l^{-1}.

OS, overshoot of the action potential (AP); threshold, threshold of the triggered AP determined as indicated in Table 1 (control value: $-37{\cdot}7\pm2{\cdot}1$ mV); RAH, reduction of the afterhyperpolarization as a percentage of control afterhyperpolarization; APD_T, AP duration measured at the threshold (control value: $4{\cdot}70\pm0{\cdot}24$ ms, $N=4$).

adult isolated neurones obtained after dissociation of the dorsal area of the A6 ganglion of the cockroach *Periplaneta americana*, recorded with the patch-clamp technique, is similar to that recorded *in situ* with intracellular microelectrodes in the neurones located along the dorsal median line. The slight differences in action potential characteristics probably result from variation in the local environment of the cells. Since these cells show spontaneous overshooting action potentials, they appear to correspond to type 4 of the dorsal neurones of this ganglion (Jego *et al.* 1970). The action potentials are similar to those recorded *in situ* in dorsally located nerve cell bodies of thoracic and abdominal ganglia of locusts, cockroaches (Kerkut *et al.* 1968, 1969; Crossman *et al.* 1971, 1972; Lange & Orchard, 1984) and grasshopper embryos (Goodman & Spitzer, 1979). They are also similar to those recorded in neurone somata (freshly isolated or in culture) of *Locusta* thoracic ganglia (Usherwood *et al.* 1980) and in neuronal cultures of the brain of cockroach embryos (Lees *et al.* 1985).

The suppression of the action potential by low concentrations of sSTX ($1{\cdot}0\times10^{-8}$ to $1{\cdot}0\times10^{-7}$ mol l^{-1}) and the decrease in spike amplitude in reduced $[Na^+]_o$ indicate that sodium ions flowing through voltage-dependent Na^+ channels are responsible for the fast depolarizing phase of the action potentials. These results confirm previous observations made in the A6 ganglion *in situ* by Jego *et al.* (1970). The presence of sodium ions in the superfusing saline is essential for the production of action potentials. The unexpected large hyperpolarization induced by sSTX suggested an important participation of sodium ions in maintaining the resting potential, and this was verified by the dependence of the resting potential on $[Na^+]_o$ with a slope of 42·8 mV per 10-fold change in $[Na^+]_o$. The hyperpolarization measured in reduced $[Na^+]_o$ (19·5 mV in 75 mmol l^{-1} and 21 mV in 51 mmol l^{-1}) is similar to that induced by $1{\cdot}0\times10^{-7}$ mol l^{-1} sSTX (22·6 mV),

indicating that most of the resting Na^+ current passes through sSTX-sensitive Na^+ channels. The lower sensitivity to $[Na^+]_o$ of the resting potential of *in situ* neurones (13 mV hyperpolarization in Na^+-free saline) might possibly result from the presence of a glial blood–brain barrier surrounding the neurones and regulating their ionic environment (Abbott *et al.* 1986). It has previously been indicated that sodium ions do not contribute to the maintenance of the resting potential in these neurones (Jego *et al.* 1970) or in motoneurone D_f (Pitman, 1975, 1988). In agreement with results reported by Lees *et al.* (1985) on embryonic cockroach neurones in culture, we found that the resting potential of isolated neurones depended also on $[K^+]_o$, with a slope of 44·1 mV per 10-fold change in $[K^+]_o$. At present we have no explanation for the similar dependence of the resting potential on $[Na^+]_o$ and on $[K^+]_o$. The ratios between sodium and potassium permeabilities, P_{Na}/P_K, calculated from the relationships between the resting potential and $[Na^+]_o$ and $[K^+]_o$ using the Goldman–Hodgkin–Katz equation (assuming that chloride ions are passively distributed at rest and that the intracellular Na^+ and K^+ concentrations remain constant) differ by a factor of two (not shown). This result, together with the apparent insensitivity of the resting potential to variations of $[Na^+]_o$ below 75 mmol l^{-1}, suggests that other ions and mechanisms also contribute to the cell resting potential. Among the possible candidates, chloride ions are likely to be involved, as voltage-dependent background single chloride channels have been observed in cell-attached patches (C. O. Malécot, unpublished observation).

The delayed outward rectification observed in isolated adult neurones, in response to depolarizing current pulses, observed for potentials more positive than about −30 mV, implies an increase in membrane conductance in this range of potentials. Such outward rectification has previously been observed in these neurones (Jego *et al.* 1970) and in other insect neurones (Goodman & Spitzer, 1981*a*,*b*; Lees *et al.* 1985). The sensitivity of this rectification to TEA–Cl shows that, as in other tissues, it is mediated by the activation of a potassium conductance. The prolongation of the falling phase of the action potential in the presence of TEA–Cl or in the absence of external calcium suggests that a calcium-activated potassium conductance is involved in the repolarizing phase of the action potential. Moreover, the suppression of the afterhyperpolarization observed under the same conditions indicates that the afterhyperpolarization is governed by the activation and deactivation of this conductance. In accord with this, ionophoretic injection of calcium into voltage-clamped neurones of cockroach metathoracic ganglion (Thomas, 1984) has been shown to activate an outward current whose reversal potential is dependent on $[K^+]_o$. In the presence of TEA–Cl, the repolarizing phase of the action potential probably depends more on the inactivation of the sodium conductance. The increased overshoot can be explained by the ability of the depolarizing Na^+ current to bring the membrane potential closer to the sodium equilibrium potential when repolarizing currents are decreased. Although the lack of effect of CsCl might suggest that no other potassium conductances are present, a limited single-channel study on cockroach

DUM neurones (Dunbar & Pitman, 1985) has shown the existence of 11 and 34 pS channels conducting potassium ions.

Calcium channel blockers were used to investigate the presence of calcium influx through voltage-dependent calcium channels. The three blockers used ($MnCl_2$, $CoCl_2$ and $NiCl_2$ at $3{\cdot}0\times10^{-3}$ mol l^{-1}) have little effect on the action potential duration compared to the effects of TEA–Cl (although TEA–Cl was used at a higher concentration) or calcium-free saline, but markedly affect the overshoot, afterhyperpolarization and threshold. Increasing the $MgCl_2$ concentration of the normal saline, to the same concentration of divalent cations as in the presence of the blockers, has no effect on action potential characteristics or threshold. Although magnesium ions are less effective than other divalent cations in screening the surface charges near Na^+ channels (Hille *et al.* 1975), the positive shift of the action potential threshold induced by the Ca^{2+} channel blockers is consistent with the presence of voltage-dependent Ca^{2+} channels in these isolated neurones, and the increase of the action potential overshoot and reduction of the afterhyperpolarization might reflect the relative potencies of these ions on the calcium current, and thus the calcium-activated potassium conductance, although the same degree of action potential lengthening was achieved with the three blockers. However, only a voltage-clamp study will clarify this.

The electrophysiological characteristics of the neurones studied in this paper and the staining of the isolated neurones with Neutral Red (indicating their aminergic nature; see Dymond & Evans, 1979) strongly suggest that they might correspond to previously studied DUM neurones in a number of insect species (see text for references). The patch-clamp technique should allow a better understanding of the physiology and pharmacology of this special type of neurone, whose role in the A6 ganglion of the cockroach is still unknown.

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References

Abbott, N. J., Lane, N. J. & Bundgaard, M. (1986). The blood–brain interface in invertebrates. *Ann. N.Y. Acad. Sci.* **481**, 20–41.

Beadle, D. J. & Hicks, D. (1985). Insect nerve culture. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 5, *Nervous System: Structure and Motor Function* (ed. G. A. Kerkut & E. I. Gilbert), pp. 181–211. Oxford: Pergamon Press.

Beadle, D. J., Hicks, D. & Middleton, C. (1982). Fine structure of *Periplaneta americana* neurones in long-term culture. *J. Neurocytol.* **11**, 611–626.

Christensen, B. N., Larmet, Y., Shimahara, T., Beadle, D. J. & Pichon, Y. (1988). Ionic currents in neurones cultured from embryonic cockroach (*Periplaneta americana*) brains. *J. exp. Biol.* **135**, 193–214.

Crossman, A. R., Kerkut, G. A., Pitman, R. M. & Walker, R. J. (1971). Electrically excitable nerve cell bodies in the central ganglia of two insect species, *Periplaneta americana* and *Schistocerca gregaria*. Investigation of cell geometry and morphology by intracellular dye injection. *Comp. Biochem. Physiol.* **40**A, 579–594.

CROSSMAN, A. R., KERKUT, G. A. & WALKER, R. J. (1972). Electrophysiological studies on the axon pathways of specified nerve cells in the central ganglia of two insect species, *Periplaneta americana* and *Schistocerca gregaria*. *Comp. Biochem. Physiol.* **43**A, 393–415.

DEWHURST, S. & BEADLE, D. (1985). Culturing nerve cells and tissues from insects *in vitro*. In *Neurochemical Techniques in Insect Research* (ed. H. Breer & T. A. Miller), pp. 207–222. Berlin, Heidelberg, New York, Tokyo: Springer Verlag.

DUNBAR, S. J. & PITMAN, R. M. (1985). Unitary currents recorded from the soma of identified cockroach neurones using the patch clamp technique. *J. Physiol. Lond.* **367**, 88P.

DYMOND, G. R. & EVANS, P. D. (1979). Biogenic amines in the nervous system of the cockroach, *Periplaneta americana*: association of octopamine with mushroom bodies and dorsal unpaired median (DUM) neurones. *Insect Biochem.* **9**, 535–545.

GOODMAN, C. S. & HEITLER, W. J. (1979). Electrical properties of insect neurones with spiking and non-spiking somata: normal, axotomised, and colchicine-treated neurones. *J. exp. Biol.* **83**, 95–121.

GOODMAN, C. S. & SPITZER, N. C. (1979). Embryonic development of identified neurones: differentiation from neuroblast to neurone. *Nature, Lond.* **280**, 208–214.

GOODMAN, C. S. & SPITZER, N. C. (1981*a*). The mature electrical properties of identified neurones in grasshopper embryos. *J. Physiol. Lond.* **313**, 369–384.

GOODMAN, C. S. & SPITZER, N. C. (1981*b*). The development of electrical properties of identified neurones in grasshopper embryos. *J. Physiol. Lond.* **313**, 385–403.

HAGIWARA, S. & WATANABE, A. (1956). Discharges in motoneurons of cicada. *J. cell comp. Physiol.* **47**, 415–428.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. J. (1981). Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Arch. Eur. J. Physiol.* **391**, 85–100.

HILLE, B., WOODHULL, A. M. & SHAPIRO, B. I. (1975). Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions, and pH. *Phil. Trans. R. Soc. Ser. B* **270**, 301–318.

HOYLE, G. & DAGAN, D. (1978). Physiological characteristics and reflex activation of DUM (octopaminergic) neurons of locust metathoracic ganglion. *J. Neurobiol.* **9**, 59–79.

HUE, B., PELHATE, M. & CALLEC, J. J. (1981). GABA and taurine sensitivity of cockroach giant interneurone synapses: indirect evidence for the existence of a GABA uptake mechanism. *J. Insect Physiol.* **27**, 357–362.

IKEDA, K. & KAPLAN, W. D. (1970). Patterned neural activity of a mutant *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.* **66**, 765–772.

JEGO, P., CALLEC, J. J., PICHON, Y. & BOISTEL, J. (1970). Etude électrophysiologique de corps cellulaires excitables du VIe ganglion abdominal de *Periplaneta americana*. Aspects électriques et ioniques. *C.R. Séanc. Soc. Biol.* **164**, 893–903.

KERKUT, G. A., PITMAN, R. M. & WALKER, R. J. (1968). Electrical activity in insect nerve cell bodies. *Life Sci.* **7**, 605–607.

KERKUT, G. A., PITMAN, R. M. & WALKER, R. J. (1969). Iontophoretic application of acetylcholine and GABA onto insect central neurones. *Comp. Biochem. Physiol.* **31**, 611–633.

LANGE, A. B. & ORCHARD, I. (1984). Dorsal unpaired median neurons, and ventral bilaterally paired neurons, project to a visceral muscle in an insect. *J. Neurobiol.* **15**, 441–453.

LEES, G., BEADLE, D. J. & BOTHAM, R. P. (1983). Cholinergic receptors on cultured neurones from the central nervous system of embryonic cockroaches. *Brain Res.* **288**, 49–59.

LEES, G., BEADLE, D. J., BOTHAM, R. P. & KELLY, J. S. (1985). Excitable properties of insect neurones in culture: a developmental study. *J. Insect Physiol.* **31**, 135–143.

PELHATE, M. & SATTELLE, D. B. (1982). Pharmacological properties of insect axons: a review. *J. Insect Physiol.* **28**, 889–903.

PINNOCK, R. D. & SATTELLE, D. B. (1987). Dissociation and maintenance *in vitro* of neurones from adult cockroach (*Periplaneta americana*) and housefly (*Musca domestica*). *J. Neurosci. Meth.* **20**, 195–202.

PITMAN, R. M. (1975). The ionic dependence of action potentials induced by colchicine in an insect motoneurone cell body. *J. Physiol. Lond.* **247**, 511–520.

PITMAN, R. M. (1979). Intracellular citrate or externally applied tetraethylammonium ions(

produce calcium-dependent action potentials in an insect motoneurone cell body. *J. Physiol. Lond.* **291**, 327–337.

PITMAN, R. M. (1988). Delayed effects of anoxia upon the electrical properties of an identified cockroach motoneurone. *J. exp. Biol.* **135**, 95–108.

PITMAN, R. M., TWEEDLE, C. D. & COHEN, M. J. (1972). Electrical responses of insect central neurons: augmentation by nerve section or colchicine. *Science* **178**, 507–509.

SATTELLE, D. B., PELHATE, M. & HUE, B. (1979). Pharmacological properties of axonal sodium channels in the cockroach *Periplaneta americana* L. I. Selective block by synthetic saxitoxin. *J. exp. Biol.* **83**, 41–48.

SHIMAHARA, T., PICHON, Y., LEES, G., BEADLE, C. A. & BEADLE, D. J. (1987). Gamma-aminobutyric acid receptors on cultured cockroach brain neurones. *J. exp. Biol.* **131**, 231–244.

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.

TREHERNE, J. E. & PICHON, Y. (1972). The insect blood–brain barrier. *Adv. Insect Physiol.* **9**, 257–313.

TREHERNE, J. E., SCHOFIELD, P. K. & LANE, N. J. (1973). Experimental disruption of the blood–brain barrier system in an insect (*Periplaneta americana*). *J. exp. Biol.* **59**, 711–723.

USHERWOOD, P. N. R., GILES, D. & SUTER, C. (1980). Studies of the pharmacology of insect neurones *in vitro*. In *Insect Neurobiology and Pesticide Action* (*Neurotox 1979*), pp. 115–128. London: Society of Chemical Industry.

WONG, B. S. & ADLER, M. (1986). Tetraethylammonium blockade of calcium-activated potassium channels in clonal anterior pituitary cells. *Pflügers Arch. Eur. J. Physiol.* **407**, 279–284.

YELLEN, G. (1984). Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffin cells. *J. gen. Physiol.* **84**, 157–186.