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UNITARY K^+ CURRENTS IN GROWTH CONES AND PERIKARYON OF IDENTIFIED *HELIX* NEURONES IN CULTURE

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Summary

1. Unitary potassium (K^+) currents of several different conductances have been recorded from the growth cones of isolated C1 neurones from *Helix* aspersa. The isolated neurones were maintained in culture for up to 1 week. Similar unitary currents were recorded in the growth cones of other isolated *Helix* neurones.

2. The activity of one type of unitary K^+ current recorded from the growth cones of the C1 neurone and other neurones was very similar to that described for the S-channel of the perikarya of *Aplysia* sensory neurones. Another type of unitary K^+ current showed fast flickering and reduced amplitude when the membrane was held at large positive potentials, which is suggestive of channel block by some agent.

3. The conductances of the K^+ channels in the growth cones of isolated C1 neurones were generally smaller than those recorded in this and in previous studies from the perikarya of C1 neurones *in situ*. However, unitary K^+ currents recorded from the perikaryon of the C1 neurone, and from other identified neurones, in culture also had lower conductances than those recorded *in situ*. The mean resting potential of the isolated neurones was smaller than those from neurones *in situ*. This and other results suggested that reduced intracellular K^+ concentration in the isolated neurones might be an important factor in deciding the conductance of the recorded channels.

Introduction

Molluscan neurones have been shown by intracellular recording and voltage-clamp techniques to possess several types of K^+ currents which show different voltage-dependences, and different susceptibilities to blockers such as tetraethyl-ammonium (TEA^+), 4-aminopyridine (4-AP) and barium (Thompson, 1977; Hermann and Gormann, 1979, 1981*a*,*b*). The advent of the patch-clamp technique has enabled direct recording of such currents through single channels. By this means it has been shown that K^+ channels with various conductances are present in the perikarya of molluscan neurones (Barnes, 1987; Cottrell *et al.* 1984; Brezina

Key words: K^+ channels, growth cones, multiple conductances, *Helix* neurones.

et al. 1987; Kazachenko and Geletyuk, 1984). Most existing data on single K^+ channel currents have been obtained from neuronal perikarya because they are most accessible in intact or acutely isolated preparations of neurones. It has been assumed that ionic conductances present in neuronal perikarya have similar properties to those which may be present in neuronal terminals. Direct recording of ionic currents in neuronal terminals is only possible in a small number of preparations in which large numbers of synaptic terminals are exclusively present in a small area, from synaptosomes which may be isolated mechanically (Mason and Dyball, 1986; Lemos *et al.* 1986) and from unusually large synapses such as that found in the squid (see e.g. Llinás *et al.* 1981). Growth cones may provide a useful alternative model of the ionic currents which may be present in neuronal terminals since, in some cases, they share some of the properties of neuronal terminals; for example, transmitter release (Young and Poo, 1983). Growth cones may also provide a model of dendritically located membrane currents which would be important in determining the efficiency of peripherally located synaptic input.

Dissociated cultures of molluscan neurones have obvious growth cones which are accessible to experiment without damage to the rest of the cell. Unitary and macroscopic currents have been recorded from growth cones in a variety of different neurones, or neurone-like cells, in culture (Belardetti *et al.* 1986; Lipscombe *et al.* 1988; Cohan *et al.* 1985; Streit and Lux, 1987; Marom and Dagan, 1987). We have used cultured neurones to study unitary outward current channels present in growth cones of identified neurones of the land snail *Helix aspersa*.

Materials and methods

The method of culturing was based on that used by Wong *et al.* (1981). Neurones were dissected in *Helix* saline containing (mmol l^{-1}): NaCl, 80; KCl, 5; $MgCl_2$, 5; $CaCl_2$, 7; Hepes, 20; buffered to pH 7.4 with NaOH. The antibiotic saline contained 500 units ml^{-1} penicillin, 0.5 mg ml^{-1} of streptomycin (GIBCO) and 1.25 μg ml^{-1} Fungizone (GIBCO). The L-15 medium (GIBCO special order) lacked inorganic salts, and all the other usual components were at half-strength. Inorganic salts were added to achieve the same concentrations as for *Helix* saline except that the NaCl concentration was reduced to 62 mmol l^{-1} to compensate for the osmotic value of the L-15, and 100 units ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin and 0.75 mg ml^{-1} Fungizone were added (defined medium). Conditioned medium was produced by exposing the desheathed *Helix* circumoesophageal ganglia (4 ml^{-1}) in defined medium for 72 h at 22°C, then filtering the resultant solution through 0.22 μm pore size Gelman filters.

An acid-cleaned 22 mm×22 mm glass coverslip was placed in each culture dish and exposed to the lectin concanavalin A (Sigma), 2 mg ml^{-1} in distilled water, for 2 h. Some coverslips were coated with poly-L-lysine (Sigma, M_r 300 000) as an alternative; however, neurones grown on this substratum were not used for recording in this study. The dish was rinsed three times with *Helix* saline, exposed

to conditioned medium for 24 h at 22 °C, then rinsed with saline, after which 1.5 ml of defined medium was added.

Adult *Helix aspersa* (6–8 g) were obtained locally and maintained in the laboratory at 18–20 °C. The shells were removed and the snails immersed in 25 % Listerine (Warner Lambert Co.) in *Helix* saline for 20 min and then saline containing antibiotics and fungicide for 30 min. The snails were removed to a sterile area under a laboratory hood where the circumoesophageal ganglionic ring was removed and placed in antibiotic saline.

The ganglia were pinned out in a sterile dish filled with Sylgard (Dow-Corning Corporation). The outer connective tissue layers were removed, using sharpened no. 5 forceps, until the neuronal perikarya could be clearly seen. The ganglia were then treated with 0.5 % Pronase (British Drug House, 80 000 PUK units g^{-1}) in *Helix* saline at 22 °C for 1 h and then washed with 30 ml of saline. The final layer of softened connective tissue was removed using sharpened forceps. The exposed perikarya were treated with 0.1 % Trypsin (GIBCO) in *Helix* saline for 90 min, rinsed as before, then treated with 0.1 % Trypsin inhibitor (British Drug House) for 30 min. Individual perikarya with varying lengths of axon were separated from the ganglia using steel pins (A1, Watkins and Doncaster) and the axon severed using either iridectomy scissors or by looping monofilament thread (Ethicon, 13 μm) around it (Fuchs *et al.* 1981). Neurones were sucked into a fire-polished pipette (tip diameter 0.5 mm) and transferred to a 30 mm diameter culture dish containing defined medium. The dish was not moved for 18 h to facilitate attachment of cells to the substratum, before being transferred to a humidified incubator at 22 °C.

Recordings from the C1 neurone *in situ* were made from isolated cerebral ganglia pinned to Sylgard-coated recording dishes. After desheathing, the neurones were exposed to a *Helix* physiological solution containing 0.1 % Trypsin (Sigma type IX) for 15 min to render the membrane clean enough for 'giga-seal' formation.

Recordings from isolated neurones in culture were made in defined medium. In most cases the patch pipette also contained defined medium or physiological solution. The high-K^+ pipette solution (A) contained (mmol l^{-1}): KCl, 80; $CaCl_2$, 2; $MgCl_2$, 5; Hepes, 20 (pH adjusted to 7.5 with KOH). Experiments on the *in situ* C1 perikaryon used a physiological solution containing (mmol l^{-1}): NaCl, 80; KCl, 5; $CaCl_2$, 7; $MgCl_2$, 5; Hepes, 20 (with pH adjusted to 7.5 with NaOH).

Unitary currents were recorded by the patch-clamp technique using an L/M EPC-7 amplifier manufactured by List-Electronic. In all recordings in this study membrane potentials were maintained at steady values while recordings were made. Kinetic analysis of unitary current data was performed on an IBM PC-AT computer using software supplied by Mr J. Dempster (University of Strathclyde, Glasgow) with low-pass filtering at 800 Hz. Unitary current amplitudes were measured manually on a Nicolet 3091 digital storage oscilloscope. Sections of recording used for kinetic analysis were 5–60 s in length, depending on the activity of the channel. Slope conductances were estimated from the unitary current

amplitudes with holding membrane potentials of greater than 0 mV, over the range that the I/V relationship could be approximated by a straight line. In some cases, to estimate the permeability of the ion channels, theoretical fits using the Goldman, Hodgkin and Katz (GHK) equation (Hodgkin and Katz, 1949) were made to the data by eye.

Some experiments were carried out with simultaneous recording of unitary currents from cell-attached patches and intracellular recording from the same cells to monitor membrane potential continuously. A Neurolog NL-102 d.c. bridge preamplifier was used for intracellular recording, and high-resistance (>30 MΩ) electrodes which were filled with 1 mol l^{-1} potassium acetate (pH 7.4). The patch pipette contained only 100 mmol l^{-1} KCl for these experiments. Unitary current amplitudes were measured at a series of holding potentials and the reversal potential could be found by extrapolation or interpolation from I/V plots. When more than one channel was present on a patch, the reversal potentials obtained for each channel were generally found to agree fairly closely, so a mean value was taken for that cell.

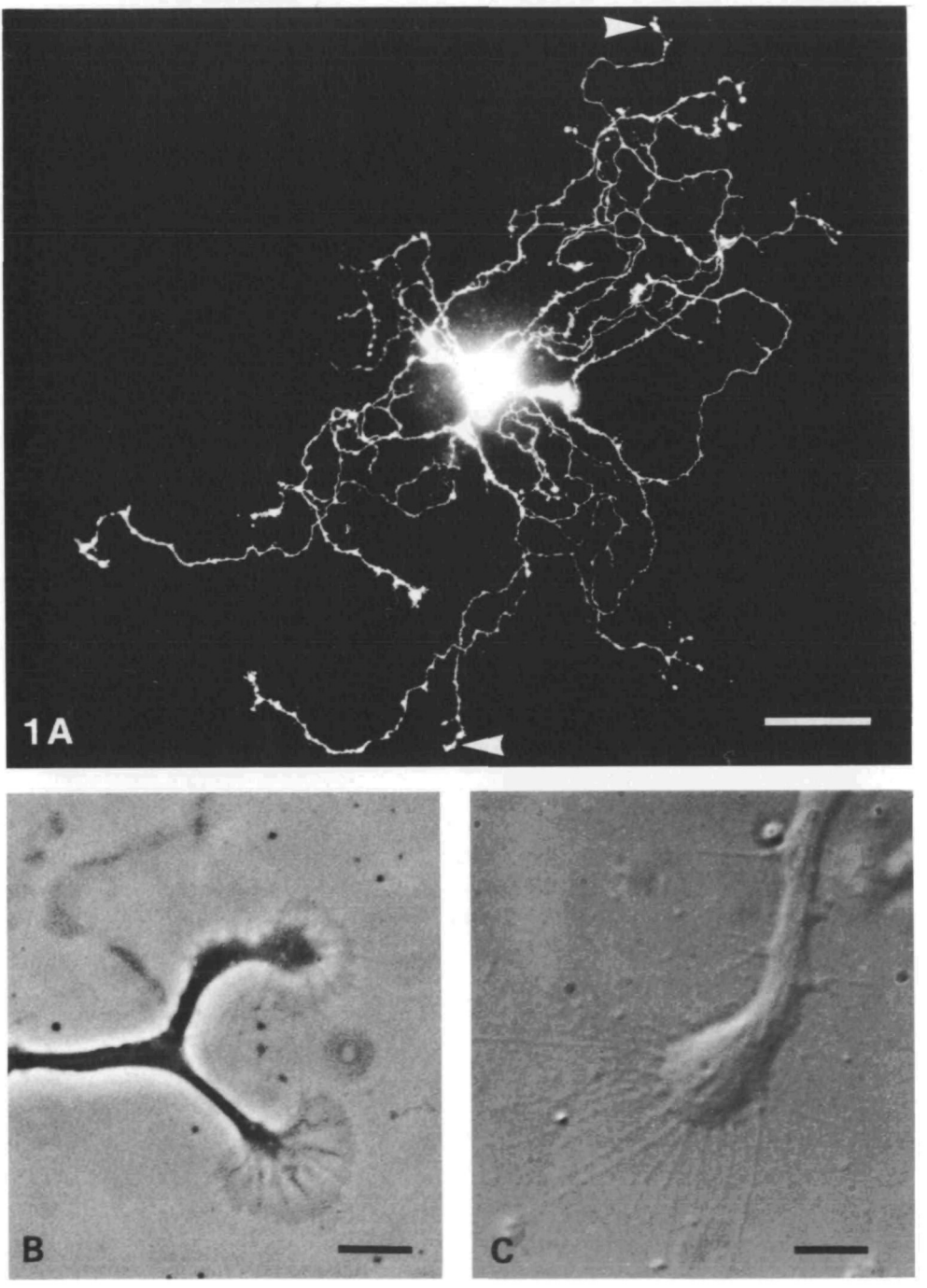
Results

Recordings have been made of unitary outward current channels in cell-attached patches on growth cones of identified neurones in culture (for location of identified neurones see Cottrell and Macon, 1974). Most recordings were made on C1 neurones from the cerebral ganglion (20 successful patches) after maintenance in culture for 3–5 days. Extensive neurite outgrowth usually occurred by day 3 in culture (Fig. 1A). Growth cones of the C1 neurone, and the A neurone (from the buccal ganglion) were similar in appearance. They were up to about 8 μm in width when plated onto a substratum of concanavalin A and were phase-dark and hence very flat (Fig. 1B,C). Growth cones of neurones plated onto a substratum of polylysine were larger in area than those of neurones developing on concanavalin A and the neurites were slower in extending. All recordings from neurones in culture in the present study were made from neurones developing on concanavalin A. Growth cones remained attached to the rest of the cell during recording, but in most cases they were spatially well isolated from the perikaryon. Similar unitary currents were seen in a further 17 satisfactory patches recorded from C1 neurone growth cones which had been isolated from the rest of the cell by sectioning of the neurite with a broken micropipette.

With defined growth medium in the recording pipette and in the recording

Fig. 1. (A) Fluorescence image of a C1 neurone grown in culture for 4 days. Recordings were made from the growth cones marked with arrows and the cell was subsequently injected with Lucifer Yellow, fixed in 4% phosphate-buffered paraformaldehyde (pH 7.4), and mounted in methylsalicylate (scale bar, 200 μm). (B) Phase contrast (scale bar, 10 μm), and (C) Nomarski (scale bar, 5 μm) photomicrographs of typical C1 neurone growth cones developing on polylysine and Concanavalin A, respectively.

chamber, several sizes of unitary outward currents were seen when the patch was held at a steady depolarized level. These currents were apparently independent, with various combinations of different sizes being seen in each patch (Fig. 2A). Since in most experiments the cell-attached recording configuration was used, the



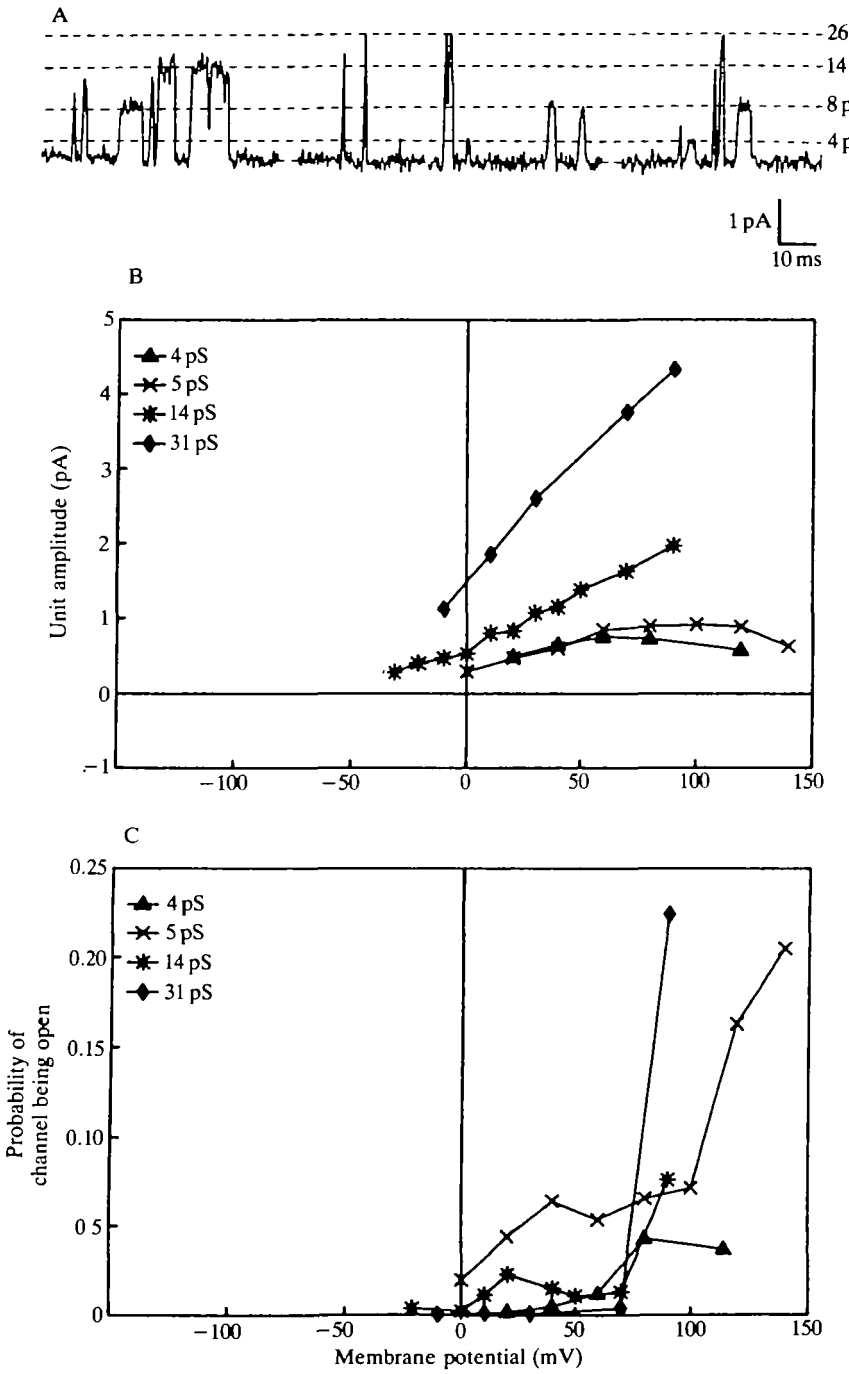


Fig. 2. (A) Examples of strongly voltage-dependent unitary currents present in a single growth cone patch at a holding potential of +90 mV. The dashed lines show conductance levels of 4, 8, 14 and 26 pS. (B) Amplitude and (C) probability of being open for voltage-dependent unitary currents plotted against holding potential for the same four channels present in different C1 growth cone patches.

absolute patch holding potential depended on the resting potential inside the growth cone. In some cases the membrane potential was directly measured when the patch ruptured after recording. For 12 growth cones, eight of them on the C1 cell, membrane potentials of up to −49 mV with a mean of −31±9.1 mV were recorded (values lower than −20 mV were rejected since they were unrepresentative and probably due to gross damage to the growth cone). The mean resting potential of C1 neurones in intact ganglia is −59.8±8 mV (N=38, Barnes, 1987), and that of 14 other unidentified suboesophageal neurones in the present investigation was −59.7±7.9 mV. The mean membrane potential measured for isolated *Helix* neurones in culture by both intracellular and patch-clamp recording was lower, being −47.5±12.0 mV (N=29, P<0.01), although they appeared to be in good condition. The lower membrane potentials recorded at the growth cones compared with the perikaryon of cultured neurones probably reflects mechanical damage occurring in the process of recording. Further, as it was not known what effects the pipette solution had on the membrane potential, the largest membrane potential value was taken as soon as possible after breakdown of the patch of membrane. Where the absolute membrane potential was not known for a particular growth cone, either an estimated value was used, or the value obtained from a previous recording on the same neurone was used.

The reversal potential of the unitary currents recorded with defined growth medium in the patch pipette was not accurately measurable, but was estimated from I/V plots to be between −50 and −70 mV. With the high-K^+ solution (A) in the recording pipette the unitary currents reversed at more depolarized membrane potentials of 0 to +20 mV, which strongly indicates that the channels are predominantly permeable to K^+.

Voltage-dependent unitary currents

Most of the currents showed pronounced voltage-dependence and a very low probability of being open at potentials less depolarized than 0 mV. Examples of voltage-dependent unitary outward currents recorded from a growth cone of the C1 neurone held at a membrane potential of +90 mV are shown in Fig. 2A. Currents with conductances of 4, 8, 14 and 26 pS were present. The low probability of being open and the large depolarization required for activation may be due to partial inactivation of the channels, because steady depolarizations were used rather than short command steps. In addition, any low-conductance channels that were present and active at negative membrane potentials became lost in the noise due to the asymmetric K^+ distribution across the patch. Unitary inward currents were often seen at negative membrane potentials when the K^+ driving force was increased at these potentials by using a solution containing a high concentration of K^+ in the recording pipette. Fig. 2B shows the amplitudes of four different voltage-dependent unitary outward currents plotted against holding potential. The probability of being open for the same channels is plotted against holding potential in Fig. 2C. Gating of channels was erratic in some cases with occasional periods of

relatively high activity and periods when no or very few openings were seen (e.g. 31 pS channel in Fig. 2C).

S-type channels

In five C1 growth cone patches, unitary current activity was observed which was only weakly dependent on the holding potential at potentials positive to the resting level (Fig. 3A). These channel currents had a conductance of 20 pS and remained active even at membrane potentials as negative as −100 mV. The probability of being open was characteristically very high (>0.8) for long periods (several minutes), although the channel appeared to switch occasionally between this high probability mode of gating to a much lower probability mode ($P<0.1$). The

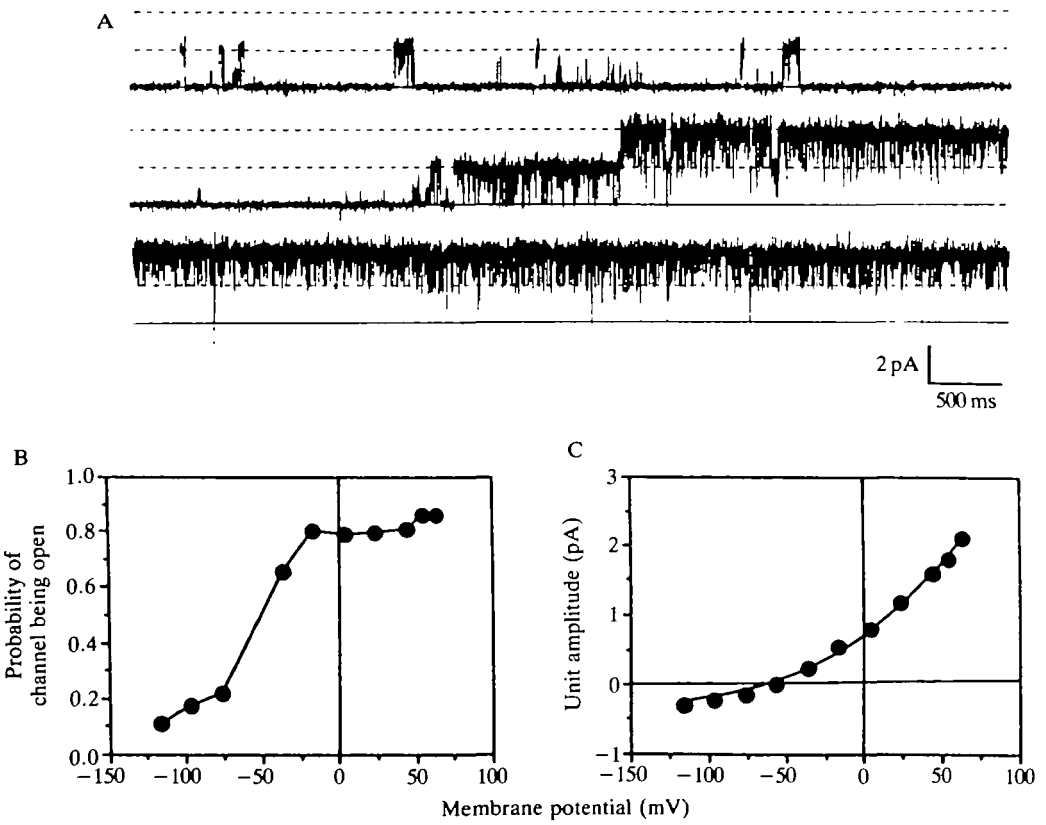


Fig. 3. Activity resembling that of the S-type channel of *Aplysia* neurones recorded from growth cones of the *Helix* C1 neurone. (A) Continuous recording showing sudden transition of this channel from a low to a high probability mode of gating. Two channels were present on this patch and both became active at about the same time. (B) Probability of being open plotted against membrane potential for another patch containing a single channel of this type. (C) Unit amplitude for the same S-type channel as in B plotted against holding potential. The data have been fitted by a theoretical curve based on a permeability of 12.7×10^{-14} cm^{3}s^{-1} and an internal K^+ concentration of 62 mmol l^{-1}.

transition was abrupt and frequently affected more than one channel of the same type (Fig. 3B). When in the high probability mode of gating, conductance sublevels were seen with an amplitude of 50–60 % of the main conductance level. The amplitude of these sublevels increased in proportion to that of the main conductance state and no inward currents were seen during periods of low activity, demonstrating that the sublevels were not due to simultaneous opening of inward current channels. The properties of these unitary currents strongly resemble those which have been ascribed to S-type K^+ channels on *Aplysia* neurones, where they have been reported as having permeabilities of $8\times10^{-14}\,cm^3\,s^{-1}$ (Siegelbaum *et al.* 1982) and $13\times10^{-14}\,cm^3\,s^{-1}$ (Brezina *et al.* 1987). Fig. 3C shows unit amplitude for the S-type channel recorded from growth cones of the *Helix* C1 cell plotted against holding potential. The data were best fitted by a theoretical curve calculated from the GHK equation based on a permeability value of $12.7\times10^{-14}\,cm^3\,s^{-1}$ and a value for the internal K^+ concentration of $62\,mmol\,l^{-1}$. Thus the K^+ concentration within the growth cones may be lower than that directly measured in *Helix* neurone perikarya *in situ* ($98\,mmol\,l^{-1}$, Alvarez-Leefmans and Gamino, 1982). This may be a real difference and not just due to mechanical damage of the growth cones, because the conductance of this channel was similar in all patches in which the channel activity was seen.

Channels displaying fast flickering

In patches from three growth cones, unitary currents showing fast flickering and reduced amplitude were recorded when the potential of the patch was held more positive than +60 mV (Fig. 4A). This type of effect generally indicates that a channel block phenomenon is occurring. As can be seen from Fig. 4A, the amplitude of these unitary currents obeyed the GHK equation up to +60 mV, beyond which they appeared to be increasingly attenuated because of rapid flickering between open and closed states so that mean open time was decreased while opening frequency was increased (Fig. 4B–D). The unit amplitudes for this current were best fitted with a theoretical curve calculated for a permeability value of $5.7\times10^{-14}\,cm^3\,s^{-1}$, and an internal K^+ concentration of $60\,mmol\,l^{-1}$. Such channel activity was probably also present on other growth cone patches but was obscured by the opening of other, larger currents at such positive potentials.

Comparison of currents at growth cones with those of perikaryon

We have measured unitary current amplitudes from a large number of cell-attached patches under similar recording conditions to compare the conductances of channels present (i) on the growth cones of the C1 neurone in culture, (ii) on the perikaryon in culture and (iii) on the perikaryon *in situ*. Unitary current amplitudes recorded from C1 growth cones are plotted in Fig. 5A. A range of conductances is present but the largest is clearly less than can be recorded from the C1 neurones *in situ*, where currents of more than 40 pS are frequently seen (Fig. 5B). In contrast, unitary currents recorded from the perikaryon of C1 neurones in culture showed a maximum conductance of about 30 pS, as in the

growth cone patches (Fig. 5C). The discrepancy could be explained if the internal K^+ concentration of the C1 neurone were lower when the cell was maintained in isolation in culture. If this were the case, then the measured conductance for K^+ channels of the C1 neurone in culture would be reduced, even though the same

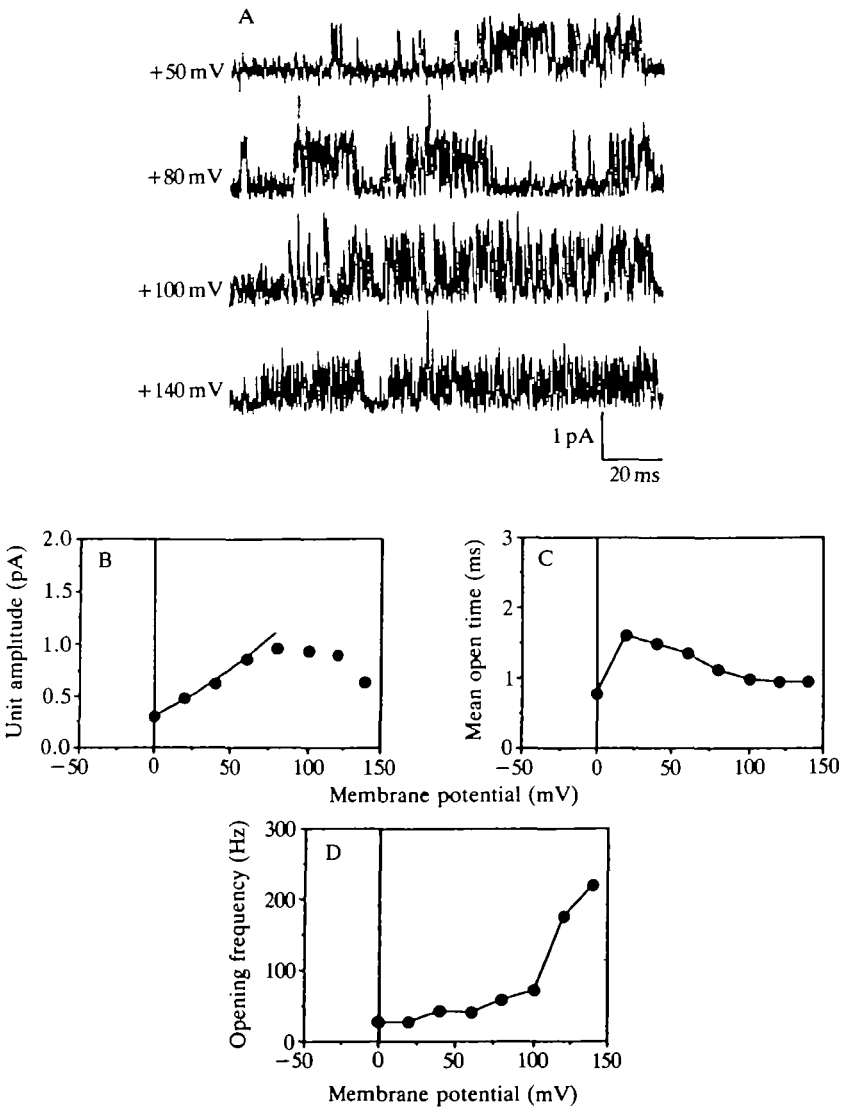


Fig. 4. Unitary currents showing fast flickering and reduced amplitude at large positive membrane potentials on C1 growth cones. (A) Traces showing openings of this channel at four different holding potentials, noted as membrane potential by each trace. (B), (C) and (D), respectively, show unit amplitude, mean open time and opening frequency plotted against holding potential for the same patch. The unit amplitudes (B) have been fitted by a theoretical curve calculated for a permeability value of $5.7\times10^{-14}\,cm^3\,s^{-1}$ and an internal K^+ concentration of $60\,mmol\,l^{-1}$.

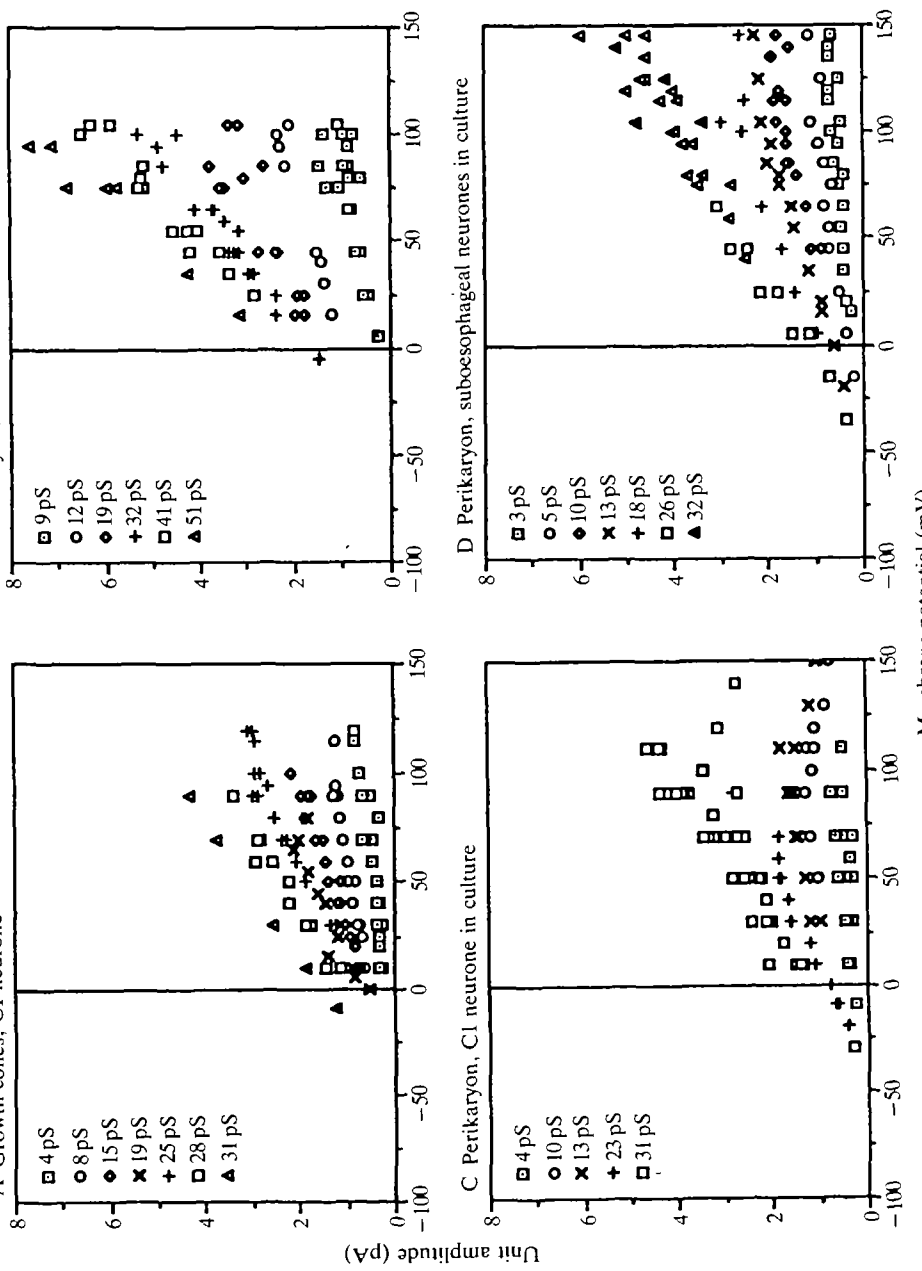


Fig. 5. Amplitudes of unitary outward currents recorded from cell-attached patches on growth cones or perikaryon with defined medium or normal Ringer in the patch pipette. Channels have been roughly categorized according to conductance or voltage-dependence. (A) C1 cell growth cones up to 4 days in culture (13 patches). (B) C1 cell *in situ* in intact brain (eight patches). (C) C1 cell perikaryon, up to 4 days in culture (16 patches). (D) Perikaryon of suboesophageal neurones after 7 days in culture (eight patches).

channels with the same permeabilities were present in both situations. It may also be that the largest channels present on the neurones *in situ* are not present or are much less active in the cultured neurones. Both these explanations could cause the lower neuronal resting potential seen in cultured neurones.

Ionic conductances in the perikaryon might be affected by the presence or absence of other neurones in their immediate environment. C1 neurones were generally plated in isolation with only a small number of other neurones included by accident. Similar results, however, were obtained with cell-attached patches recorded from the perikarya of unidentified suboesophageal neurones with about 20 neurones in the same culture dish (Fig. 5D). Another factor which might have explained our results was the harsh trypsinization used in the dissociation procedure for the cultures.

Recording from the C1 cell *in situ* requires applications of 0.15% Trypsin for 15 min to enable gigaseals to be obtained on the cell surface. Dissociation of the cells for culturing also required Trypsin at a similar concentration but applied for a much longer period (2 h). Trypsin may enter myocardial cells and cause cytoskeletal disruption (Masson-Pevet *et al.* 1976). It also directly enhances Ca^{2+} currents when applied intracellularly in these cells (Hescheler and Trautwein, 1988). In *Helix* neurones, tetrodotoxin sensitivity of Na^+ currents is reduced by application of Trypsin (Lee *et al.* 1977). We therefore tested C1 cells in the intact brain with trypsinization for 2 h at the higher concentration of 0.5%. Large (>50 pS), channels were still readily recorded from these cells (nine patches).

Reversal potential measurements

The reversal potentials of unitary currents in cell-attached patches have been measured with only 100 mmol l^{-1} KCl in the patch pipette and with continuous monitoring of the neurone resting potential by an intracellular pipette. With KCl only in the recording pipette the reversal potential of cation channels should be directly dependent on the intracellular concentration of K^+. With simultaneous intracellular recording, patch membrane potentials can accurately be placed on an absolute scale. Under these conditions the K^+ reversal potential was measured for unidentified suboesophageal neurones in culture and *in situ*. The mean reversal potential of the unitary currents on neurones *in situ* was found to be lower than that of neurones in culture ($P<0.05$, see Table 1). The reversal potential values overlapped to some extent, which suggests that a reduced intracellular K^+ concentration may not entirely explain the absence of the largest conductance events in all cells in culture. All neurones from which recordings were made appeared healthy and had extending neurites. Anomalously low values (e.g. −18 mV) may be partly explained by leakage of K^+ into the cell from the intracellular pipette, although this problem should be minimized by the use of high-resistance (>30 MΩ) electrodes.

Discussion

There are clearly several different types of K^+ channels on growth cones of the

Table 1. *Reversal potential values with 100 mmol l^{-1} KCl in the patch pipette*

In situ
Reversal potential (mV) −7, 0, −3.5, −2.5, −18, +1, −7, +1.5, −4, +4, +4
Mean −2.86 mV
S.D. 6.34 mV
S.E. 1.91 mV
$[K^+]_i$ 112 mmol l^{-1}
In culture
Reversal potential (mV) +9, +7, +1, +4.5, +6.5, −6, −7, 0, +10, +16, +6.5, +23.5, +18, −12
Mean +5.5 mV
S.D. 9.9 mV
S.E. 2.65 mV
$[K^+]_i$ 80 mmol l^{-1}
t-value=2.432 ($P< 0.05$)

Helix C1 neurone as well as on the perikaryon of this cell. We have shown that the growth cone K^+ channels display unitary currents with lower conductance values than those on the *in situ* C1 cell perikaryon. K^+ channel activity recorded from the perikaryon of the C1 neurone in culture was similar to that at the growth cones, suggesting that isolation and maintenance under the conditions described leads to a change in properties of the neurones. The difference may be partly explained by a lowering of the intracellular K^+ concentration, as is suggested by the reversal potential determinations and other results. Further clarification of this might be obtained by the use of ion-sensitive electrodes inserted into the cells. There may also be a change in the properties of the large-conductance channels in the cultured neurones so that they are much less active in the cultured neurones. Properties of these neurones may be modified *in vivo* by the presence of appropriate synaptic connections or possibly some factor (or factors) circulating in the haemolymph. Alternatively, some agent used in the culturing of the neurones may affect the cells. Further experiments are required to determine the reason for the difference.

Experiments of Cohan *et al.* (1985) have demonstrated non-specific cation channels with a conductance of 70 pS in growth cones of *Helisoma*. They were not present in growth cones that had stabilized and stopped migrating. We observed some channel activity in all our patches, even in growth cones which had apparently stabilized and stopped migrating.

Stewart *et al.* (1989) have described ionic currents present in identified leech neurones in culture and have shown the presence of different K^+ currents in 'whole cell' and unitary current recordings (Bookman and Dagan, 1987). Transient and delayed K^+ currents were also present at axonal growing tips of isolated leech neurones in short-term culture (Garcia *et al.* 1989). We have not yet carried out a detailed characterization of the K^+ channels recorded from the

growth cones of the C1 neurone. A detailed description of the individual channel types, and their identification with the different components of the 'whole-cell' current, will require the use of specific blocking agents to enable their separation. We have, however, observed and described two channels which display distinct patterns of activity.

Channels of the delayed rectifier have been reported to be susceptible to channel block by Na^+ and other monovalent cations at highly depolarized potentials (Latorre and Miller, 1983). Noise analysis experiments on *Helix* neurones by Reuter and Stevens (1980) gave a value for the unitary conductance of the delayed rectifier of 2.4 pS, and they saw no voltage-dependence of the channel conductance. This value is lower than that for the channel we describe which showed fast flickering at highly depolarized potentials and is probably a different channel.

It is interesting to note that S-type channels may be present in growth cones of the *Helix* C1 neurone. These channels are susceptible to modulation by 5-hydroxytryptamine (5-HT) and the invertebrate cardioexcitatory peptide Phe-Met-Arg-Phe-NH_2 (FMRFamide) in *Aplysia* neurones (Siegelbaum *et al.* 1982; Brezina *et al.* 1987). In *Aplysia* these channels are also apparently present on the growth cones, since whole-cell currents in isolated growth cones may be modulated by 5-HT (Siegelbaum *et al.* 1986). Modulatory effects of 5-HT and FMRFamide on unitary K^+ currents observed in the *Helix* C1 neurone were on channels with a larger permeability than the S-type channels ($25\times10^{-14}\,cm^3\,s^{-1}$, Cottrell *et al.* 1984; Barnes, 1987). Additional experiments are in progress to determine if any of the channel currents that we have recorded from the growth cones of the C1 neurone are sensitive to neuromodulators and to attempt to discriminate further between the different types of K^+ channel.

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