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Na^+-ACTIVATED K^+ CHANNELS AND VOLTAGE-EVOKED IONIC CURRENTS IN BRAIN STEM AND PARASYMPATHETIC NEURONES OF THE CHICK

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SUMMARY

1. Patch-clamp and computer-modelling techniques were used to study the activation of Na^+-activated K^+ channels ($I_{K(Na)}$) in dissociated neurones from the embryonic chick ciliary ganglion and the embryonic chick brain stem.

2. Numerical solutions of diffusion equations suggested that Na^+ accumulation as a result of Na^+ influx through voltage-sensitive Na^+ channels (I_{Na}) is insufficient to allow for alteration in the gating of $I_{K(Na)}$ channels.

3. Whole-cell recordings using two independent micropipettes were made from chick ciliary-ganglion neurones. These showed that transient outward currents were present only when there were clear indications of incomplete voltage clamp.

4. Single-electrode whole-cell recordings from ciliary-ganglion neurones showed that transient tetrodotoxin (TTX)-sensitive outward currents were present, but only when partial TTX blockade produced significant alterations in the kinetics of I_{Na}. In cells that were properly voltage clamped, there was no effect of TTX on the kinetics of I_{Na} or on voltage-evoked outward currents.

5. Examination of the relationship between peak I_{Na} and the command potential showed that transient outward currents were only present in neurones that showed sharp deviations from the behaviour expected of a cell that is adequately voltage clamped. Transient outward currents were not present in cells that were adequately voltage clamped.

6. Application of TTX to isolated outside-out patches obtained from ciliary ganglion neurones eliminated voltage-evoked inward currents but had no effect on outward currents.

7. Isolated inside-out patches obtained from ciliary-ganglion neurones did not contain $I_{K(Na)}$ channels. These patches usually contained Ca^{2+}-activated K^+ channels ($I_{K(Ca)}$) with a unitary conductance of around 200 pS when $[K^+]_o = 150$ mM and $[K^+]_i = 75$ mM.

8. Two-electrode whole-cell recordings from cultured brain stem neurones showed that transient outward currents were present only when there were clear indications of incomplete voltage clamp.

9. Application of TTX caused blockade of inward but not outward currents in brain stem neurones voltage clamped with a single whole-cell pipette. TTX had no

effect on the kinetics of I_{Na}. Application of TTX to outside-out patches isolated from the same cells blocked only the inward currents.

10. Isolated inside-out patches obtained from brain stem neurones contained $I_{K(Na)}$ channels that could be activated by exposure of the cytoplasmic face of the patch membrane to 75 mM-Na^+. These channels had a predominant conductance state of around 100 pS when $[K^+]_o$ = 150 mM and $[K^+]_i$ = 75 mM. The $I_{K(Na)}$ channels were not activated by 1 mM-Ca^{2+}.

11. $I_{K(Na)}$ channels cannot be activated by Na^+ influx through voltage-sensitive Na^+ channels in cultured chick brain stem neurones and cannot be detected in isolated ciliary-ganglion neurones. Therefore, they are unlikely to alter their gating significantly during the course of a single action potential.

INTRODUCTION

Several classes of potassium currents control the electrical behaviour of excitable cells. Recently a new class of potassium current, the sodium-activated potassium current ($I_{K(Na)}$), has been described in several types of excitable cells. These include isolated cardiac myocytes (Kameyama, Kukei, Sato, Shibisaki, Matsuda & Irisawa, 1984), cultured chick ciliary and trigeminal neurones (Bader, Bernheim & Bertrand, 1985; Haimann, Bernheim, Bertrand & Bader, 1990), crayfish motoneurones (Hartung, 1985), cultured chick brain stem neurones (Dryer, Fujii & Martin, 1989), and various mammalian cortical neurones *in vitro* (Constanti & Simm, 1987; Schwindt, Spain, Foehring, Chubb & Crill, 1988; Foehring, Schwindt & Crill, 1989; Schwindt, Spain & Crill, 1989). These studies have utilized a wide variety of recording techniques. The most direct and reliable are single-channel recordings of $I_{K(Na)}$ channels in isolated inside-out patches (Kameyama *et al.* 1984; Dryer *et al.* 1989; Haimann *et al.* 1990). This approach has revealed complex kinetic behaviour and a variety of conductance states. These experiments have also shown that $I_{K(Na)}$ channels are not particularly sensitive to Na^+ ions. A low level of activity is observed at concentrations of between 10 and 20 mM, which are close to resting intracellular Na^+ concentrations. Much higher concentrations, between 20 and 40 mM, are required to produce a significant increase in activity (Kameyama *et al.* 1984; Dryer *et al.* 1989; Haimann *et al.* 1990).

With macroscopic measurements $I_{K(Na)}$ has been described as an outward K^+ current that is eliminated by perfusion with sodium-free solutions or solutions containing tetrodotoxin (TTX). These currents consist of either a transient outward current that decays completely within 10 ms (Hartung, 1985; Bader *et al.* 1985; Dryer *et al.* 1989; Haimann *et al.* 1990) or a slowly decaying current flowing during the after-hyperpolarization of mammalian cortical neurones (Constanti & Simm, 1987; Schwindt *et al.* 1988; Foehring *et al.* 1989; Schwindt *et al.* 1989).

These macroscopic measurements can be criticized because of the possibility that the observed currents result from inadequate voltage control due to either inadequate space clamp or insufficient series resistance compensation. Moreover, the results of single-channel recordings raise a quantitative dilemma for the interpretation of macroscopic recordings, as it seems unlikely that sufficient sodium can enter a cell through TTX-sensitive sodium channels (I_{Na}) to produce significant changes in the gating of $I_{K(Na)}$ channels (Hartung, 1985; Dryer *et al.* 1989; Martin & Dryer, 1990).

Two possible explanations have been proposed to account for this quantitative disparity. The first suggests that there may be membranous structures, such as plasma membrane folds, that prevent free diffusion of Na^+ away from the plasma membrane (Hartung, 1985). The second suggests that I_{Na} and $I_{K(Na)}$ channels are closely co-localized within the plasma membrane (Dryer *et al.* 1989; Martin & Dryer, 1990).

In the present study I will present the results of calculations that suggest that co-localization of channels, in and of itself, is unlikely to account for the macroscopic currents described previously. This finding has caused me to re-examine these measurements using recording techniques in which voltage control is either assured or independently verifiable. The results indicate that $I_{K(Na)}$ channels, although present within the plasma membrane of chick brain stem neurones, are not activated by Na^+ influx through TTX-sensitive channels and do not alter their gating significantly during the course of a single action potential. The results also indicate that $I_{K(Na)}$ channels are not present in neurones of the chick ciliary ganglion.

METHODS

Computer modelling

Numerical solutions to diffusion equations were obtained with commercially available software (MathCAD, Mathsoft, Inc., Cambridge, MA, USA) and a personal computer. This software makes use of Simpson's rule to compute definite integrals numerically and thus to evaluate the error function complement. The numerical estimation is assumed to converge when the result of an iteration differs by less than 0·1 % from that of the previous iteration. Solutions were obtained for a discrete series of times (increments of 10^{-7} s) at a fixed distance or at a discrete series of distances (increments of 0·25 nm) at a fixed time. Calculations were made for several different values of the sodium-diffusion coefficient.

Cell culture

Chick (*Gallus domesticus*) brain stem cultures were prepared as described previously (Fujii & Berg, 1986; Dryer *et al.* 1989). Chick ciliary ganglia were obtained from embryos on embryonic days 10–14. Ganglia were dissected and placed in a solution nominally free of divalent cations and containing 1 mg ml^{-1} of collagenase (Sigma type II). Ganglia were incubated for 10–30 min at 37 °C. The collagenase was removed by aspiration, and the ganglia were rinsed once in a cell-culture medium consisting of Eagle's Minimal Essential Medium supplemented with 10 % horse serum, 2 mM-glutamine, 50 u ml^{-1} penicillin, 50 μg ml^{-1} streptomycin, and 3 % chick embryo eye extract. The ganglia were resuspended in cell-culture medium and triturated with 8–12 passes through a fire-polished Pasteur pipette. Dissociated neurones were subsequently plated onto polylysine-coated glass cover-slips and allowed to settle for 30 min to 2 h. In most cases neurones were used for recordings within 4 h of plating. Under these conditions the majority of cells were essentially free of neurites, although some process outgrowth could occasionally be detected within 60 min of plating. In a few cases neurones from 8- to 10-day embryos were cultured for 7 days prior to use. Under these conditions, ciliary-ganglion neurones extend fairly elaborate dendritic arborizations (Role & Fischbach, 1987). No obvious differences were observed between acutely isolated and cultured neurones, except that on occasion severe space-clamp problems were encountered in the cultured neurones. Such cells were excluded from further analysis.

Electrical recordings

Whole-cell recordings. Whole-cell recordings were made as described previously (Dryer *et al.* 1989), except that a patch-clamp amplifier with superior series resistance compensation circuits was used (Axopatch 1C, Axon Instruments, Burlingame, CA, USA). The usual electrode resistance was 4–7 MΩ when pipettes were filled with solutions containing (mM): 120 potassium gluconate, 30

NaCl, 3 $MgCl_2$, 1 EGTA, and 10 HEPES, at a pH of 7·4. In most later experiments pipettes were filled with a solution consisting of (mM): 150 KCl, 3 $MgCl_2$, 10 EGTA, and 10 HEPES, at a pH of 7·4. No differences were seen between results obtained with this pipette solution and results obtained with that described above. All experiments were performed at room temperature (21–23 °C). In many cases it was possible to compensate up to 90% of the series resistance without introducing oscillations into the recorded currents.

Double-electrode whole-cell recordings. Once stable single-electrode whole-cell recordings were obtained, a second pipette (8–15 MΩ) was sealed onto the cell and additional suction applied to produce intracellular contact. The second pipette was connected to a standard bridge-balance amplifier and was used simply to monitor the response of the cell to voltage commands applied to the first pipette. In general, sealing of the second pipette to the membrane resulted in some deterioration in the cell, associated with an increase in the resting leak conductance. This arrangement allowed recordings to be made for only a short time, although on one occasion a stable recording was maintained for more than 20 min. This method was only useful for large cells, of greater than 20 μm in diameter, as smaller cells were usually destroyed during sealing of the second pipette.

Outside-out patches. Once stable whole-cell recordings were obtained, it was often possible to form outside-out patches by slowly withdrawing the pipette to a point several hundred microns away from the recorded cell. Surprisingly, this procedure was most successful with large recording pipettes. It yielded patches that invariably contained large numbers of ionic channels. Application of voltage commands to these patches yielded records that, with some signal averaging, closely resembled whole-cell currents. These patches tended to be somewhat unstable, but on occasion it was possible to record unitary channel currents for more than 30 min.

Inside-out patches. Inside-out patches were formed when the pipette was withdrawn following initial seal formation. In the case of ciliary-ganglion neurones, this procedure was usually sufficient to produce inside-out patches as judged by the rapid response to bath application of Ca^{2+} ions (see Fig. 5). In the case of brain stem neurones, it was generally necessary to expose the tip of the pipette to air for about 1 s, to rupture the external surface of vesicles formed during patch excision and to ensure that the cytoplasmic face of the patch membrane was exposed to the bath.

For these experiments the bath salines contained (mM): 10 HEPES, 10 $MgCl_2$, 75 KCl, 10 EGTA, and LiCl or NaCl as described in the text and figure legends. The pH was 7·4 at room temperature. For Ca^{2+}-containing solutions, the EGTA was omitted and 1 mM-$CaCl_2$ was included along with 75 mM-LiCl. The pipette solution consisted of (mM): 150 KCl, 1 EGTA, and 19 HEPES at a pH of 7·4.

Data analysis

Data were filtered at 2–5 kHz with a four-pole Bessel filter and stored as analog signals on videotape for off-line computer analysis. Data were later digitized (25–100 μS/point) and stored prior to further processing and analysis. Data were usually plotted directly from computer files. In some cases analog data from videotape were fed into a digital oscilloscope and dumped to the plotter directly. Data from outside-out patches were usually averaged prior to display. Leak subtractions were performed by storage of responses to voltage pulses equal in magnitude but opposite in polarity to the command pulses. In some cases leak subtraction was performed with smaller voltage pulses. The resulting currents were then multiplied by an appropriate scaling factor to yield the leak currents. The resulting digitized records were then added by a point-by-point addition routine to produce leak subtracted traces. In the case of outside-out patches, care was taken to include only responses to leak pulses that did not contain unitary currents. TTX-sensitive currents were obtained by point-by-point digital subtraction as described elsewhere (Dryer *et al.* 1989).

RESULTS

Analytical Model

Na^+ ions were assumed to diffuse through a single Na^+ channel located on an infinitely long plasma membrane. The single-channel current was assumed to be 1·5 pA, which yields a Na^+ influx (ϕ) of $1{\cdot}45 \times 10^{-17}$ mol s^{-1}. Once Na^+ ions entered the

cytoplasm, they were assumed to diffuse freely into a semi-infinite medium. This assumption is reasonable for a neuronal cell body in the absence of membrane infolding or of buffering, sequestration, or extrusion of Na^+. It would not be appropriate for a structure, such as a node of Ranvier, where the cytoplasmic space has a more restricted diffusional geometry.

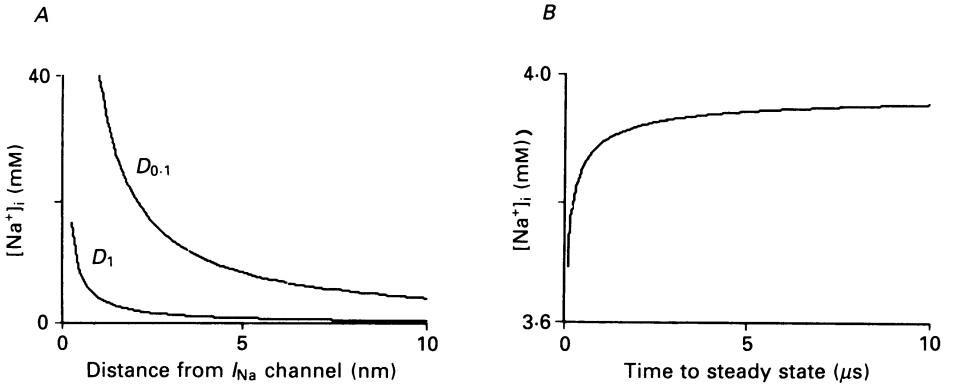

Fig. 1. Theoretical Na^+ concentration as a function of time and radial distance from the mouth of a Na^+ channel. Plots were calculated from text eqn 3. *A*, internal Na^+ concentration plotted against radial distance 1·0 ms after Na^+ channel opening. Plots are shown for two Na^+ diffusion coefficients ($D_1 = 0{\cdot}6 \times 10^{-5}$ cm^2 s^{-1} and $D_{0{\cdot}1} = 0{\cdot}6 \times 10^{-6}$ cm^2 s^{-1}). *B*, internal Na^+ concentration plotted against time after channel opening at a point 1 nm away from the mouth of the Na^+ channel. The calculation assumed a normal Na^+ diffusion coefficient ($D_1 = 0{\cdot}6 \times 10^{-6}$ cm^2 s^{-1}).

According to the diffusion equation as applied to radial flux

$$\frac{\partial C_{Na}}{\partial t} = \frac{D}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C_{Na}}{\partial r}\right), \tag{1}$$

where C_{Na} is the cytoplasmic Na^+ concentration, D is the diffusion coefficient for Na^+, r is the radial distance from the mouth of the Na^+ channel, and t is time.

If one assumes that the channel opens at $t = 0$, then after an ifinitesimal time dt there will be a net influx of $\phi\,\mathrm{d}t$ Na^+ ions. For these boundary conditions the solution to eqn (1) is (Crank, 1975):

$$C_{Na} = \frac{\phi\,\mathrm{d}t}{4(\pi Dt)^{\frac{3}{2}}}\exp(-r^2/4Dt). \tag{2}$$

The cytoplasmic Na^+ concentration produced by longer channel openings can be obtained by integration of eqn (2) with respect to time. The result has been obtained previously (Crank, 1975) and is given by:

$$C_{Na} = \frac{\phi}{2\pi Dr}\mathrm{erfc}\frac{r}{2(Dt)^{\frac{1}{2}}}, \tag{3}$$

where erfc is the error function complement. Equation (3) gives the cytoplasmic Na^+ concentration as a function of time and radial distance from the mouth of the Na^+ channel, on the assumption that the channel remains open throughout the times of interest.

Figure 1*A* shows numerical solutions of eqn (3) at a time $t = 1$ ms after the onset of Na^+ influx, for two different values of D ($D_1 = 0{\cdot}6 \times 10^{-5}$ cm^2 s^{-1} and $D_{0{\cdot}1} = 0{\cdot}6 \times 10^{-6}$ cm^2 s^{-1}). For both diffusion coefficients, the concentration of Na^+ remains below 10 mM at distances of greater than 5 nm. The usually accepted diffusion coefficient for Na^+ in cytoplasm is $0{\cdot}6 \times 10^{-5}$ cm^2 s^{-1} (Kushmerick & Podolsky, 1969), which can be compared to $1{\cdot}0 \times 10^{-5}$ cm^2 s^{-1} for Na^+ diffusion in aqueous solution. Figure 1*A* shows that the Na^+ concentration does not achieve effective levels even when D is assumed to be 10-fold less than the value generally accepted for the cytoplasm. Figure 1*B* shows Na^+ concentration at a distance of 1 nm from the pore and as a function of time after the onset of Na^+ influx, assuming a normal Na^+ diffusion coefficient ($D_1 = 0{\cdot}6 \times 10^{-5}$ cm^2 s^{-1}). This result shows that a steady-state concentration of around 4 mM is achieved within 10 μs after the onset of Na^+ influx. Assuming a diffusion coefficient $D_{0{\cdot}1} = 0{\cdot}6 \times 10^{-6}$ cm^2 s^{-1} and a radial distance of 5 nm, a steady-state concentration of 7 mM is achieved in 25 μs (not shown). These calculations suggest that Na^+ ions cannot accumulate at the mouth of the Na^+ channel in sufficient quantities to allow for significant alteration in the gating of $I_{K(Na)}$ channels described to date, even if the $I_{K(Na)}$ channels are located as close as 5 nm to the mouths of the I_{Na} channels and even if D is assumed to be 10-fold less than its usually accepted value.

This conclusion is based on the assumption that $I_{K(Na)}$ channels in *intact* cells have a sensitivity to Na^+ similar to that observed in excised patches obtained from three different preparations (Kameyama *et al.* 1984; Dryer *et al.* 1989; Haimann *et al.* 1990). This situation would not be expected to pose a problem for Ca^{2+} channels and Ca^{2+}-activated K^+ channels, as the latter can be activated by micromolar concentrations of Ca^{2+} ions (Barrett, Magleby & Pallotta, 1982). This is true even though the unitary currents through most Ca^{2+} channels are of the order of 0·6–0·9 pA (Fenwick, Marty & Neher, 1982) and allowing for some degree of Ca^{2+} buffering. Ca^{2+} diffusion in cytoplasm has been examined in considerable detail by Zucker & Stockbridge (1983) and by Fogelson & Zucker (1985).

The above calculations entail several simplifying assumptions. For example, they do not consider the effects of a clustered distribution of Na^+ channels or restricted diffusional spaces. On the other hand, they ignore factors such as Na^+ extrusion and/or buffering that would tend to decrease effective Na concentrations *further*. As a first approximation, they suggest that Na^+ influx through TTX-sensitive Na^+ channels cannot account for the TTX-sensitive outward currents described previously in recordings from neuronal somata (Bader *et al.* 1985; Hartung, 1985; Constanti & Simm, 1987; Dryer *et al.* 1989; Schwindt *et al.* 1988, 1989; Foehring *et al.* 1989). There are at least two alternative explanations. The first is that patch excision invariably reduces the sensitivity of $I_{K(Na)}$ channels to Na^+. Barring this explanation, one must consider the possibility that the observed macroscopic currents were caused by failure to voltage clamp the cells completely as a result of incomplete series resistance compensation, inadequate space clamp, or both.

Ciliary-ganglion neurones

TTX-sensitive transient outward currents have been described previously (Bader *et al.* 1985) and interpreted as evidence for $I_{K(Na)}$ channels in neurones from the chick ciliary ganglion. Because of the results of the theoretical calculations described above, I have re-examined these currents using several recording techniques.

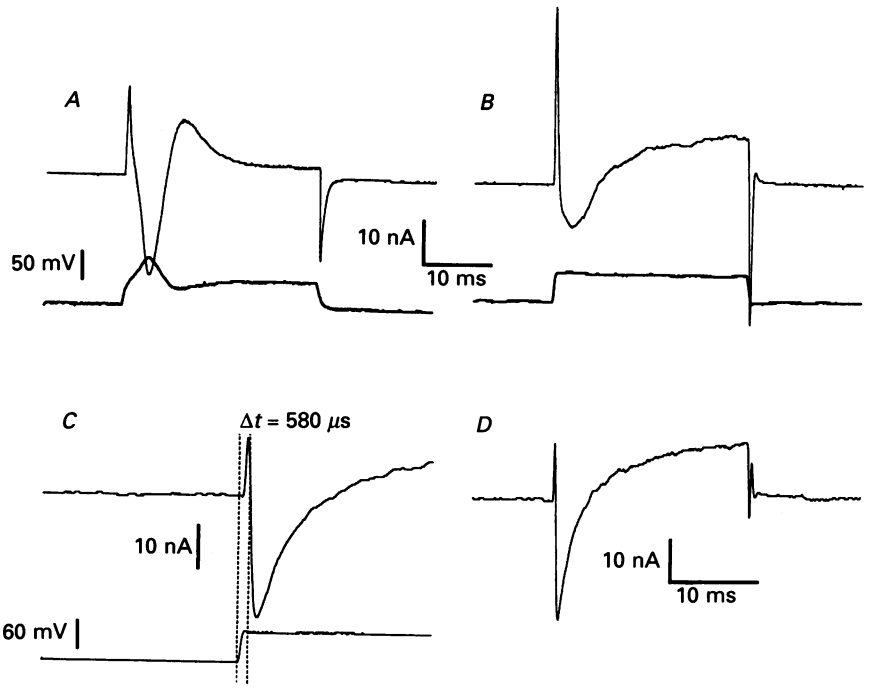

Fig. 2. Double-electrode whole-cell recordings from chick ciliary-ganglion neurones. *A*, membrane currents (upper trace) evoked by a step depolarization to a nominal −10 mV from a holding potential of −60 mV. Lower trace shows resulting voltage response recording from a second, independent micropipette. No series resistance compensation was present. *B*, same cell as in *A*, but after application of series resistance compensation (80% of 7 MΩ). The membrane potential was again stepped to −10 mV from a holding potential of −60 mV. *C*, two-electrode whole-cell recordings from a different cell. Series resistance compensation was present. Upper trace shows membrane currents evoked by depolarizing steps to −10 mV from a holding potential of −70 mV. Lower trace shows voltage response recorded with a second, independent micropipette. The voltage step settles in less than 580 μs. *D*, same recording as *C*, but shown on a slower scale. Note absence of transient outward current.

Figure 2 shows whole-cell currents evoked by a depolarizing command to −10 mV from a holding potential of −60 mV. A second whole-cell pipette was used to monitor passively the resulting voltage change in the recorded cell. Transient outward currents that decayed completely within 10 ms could be detected (Fig. 2*A*, top trace) but only when unclamped action potentials were apparent in the voltage trace (Fig. 2*A*, bottom trace). When adequate series resistance compensation was applied

to the same cell, the transient outward current was eliminated (Fig. 2*B*, top trace), and the voltage trace indicated that the cell was adequately voltage clamped (Fig. 2*B*, bottom trace). In a different cell recorded with adequate series resistance compensation, the voltage command settled within 580 μs, and the peak I_{Na} occurred

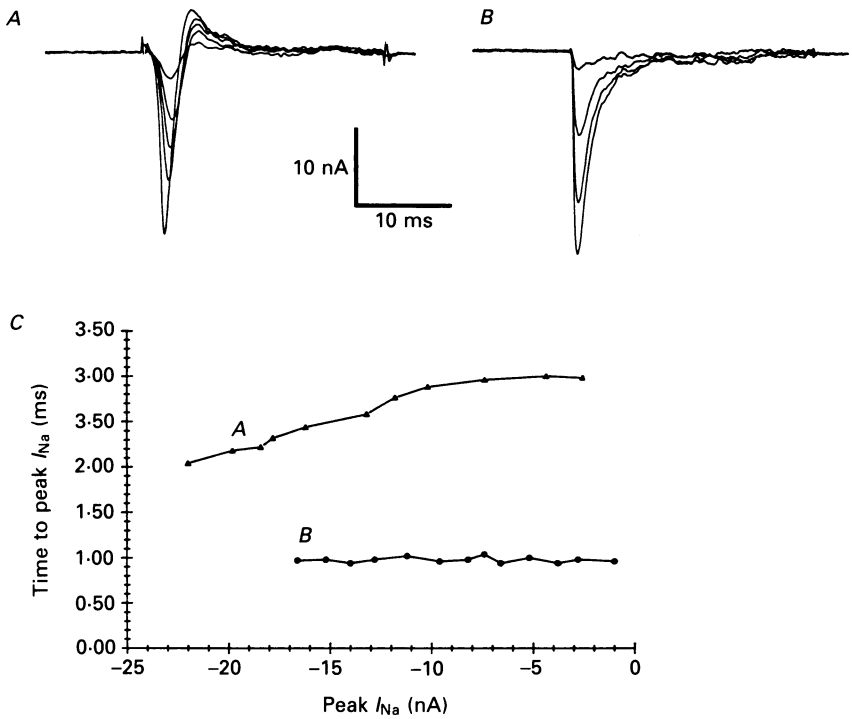

Fig. 3. Effects of TTX on kinetics of Na^+ currents in ciliary-ganglion neurones. Recordings were made with a single micropipette. *A*, selected traces of TTX-sensitive membrane currents during progressive blockade by 1·0 μM-TTX. No series resistance compensation was present. TTX caused a progressive reduction of both inward and transient outward currents. Note shift in the time to peak I_{Na} during progressive blockade. *B*, recording from a different cell with series resistance compensation (80% of 8 MΩ). Only TTX-sensitive inward currents are present, and there is no shift in the time to peak I_{Na}. *C*, time to peak I_{Na} plotted against I_{Na} amplitude for the cells shown in *A* (▲) and B (●).

at around 1 ms after application of the voltage command (Fig. 2*C*). It should be noted that negative capacitance compensation was *not* applied to the passive pipette, and therefore this settling time should be regarded as a conservative estimate of the actual performance of the voltage clamp. In this cell, there was no evidence of a transient outward current (Fig. 2*D*).

If a cell is adequately voltage clamped, then TTX should affect only the amplitude of I_{Na} and should not affect its kinetics. Figure 3*A* shows the TTX-sensitive currents present during a depolarizing step to −10 mV from a holding potential of −60 mV. No series resistance compensation was applied. TTX-sensitive currents were obtained

by point-by-point digital subtraction during progressive TTX blockade as described elsewhere (Bader *et al.* 1985; Dryer *et al.* 1989). Transient TTX-sensitive outward currents were present, but there was a clear shift in the time to peak I_{Na} as TTX blocked inward and outward currents (Fig. 3*A*, *C*). Moreover, under these conditions peak I_{Na} was delayed, the time to reach it ranging from 2 to 3 ms depending on the amplitude of I_{Na} (Fig. 3*C*). In a different cell recorded with adequate series resistance compensation, the time to peak I_{Na} did not depend on the amplitude of I_{Na} (Fig. 3*B*, *C*). Under these conditions the time to peak I_{Na} was close to 1 ms, and no TTX-sensitive outward currents were apparent (Fig. 3*B*). These results suggest that transient TTX-sensitive outward currents are only present in cells that are incompletely voltage clamped.

When neurones are adequately voltage clamped, the relationship between peak I_{Na} and command potential should be a smooth function, with a relatively gradual region of negative slope conductance between −40 and 0 mV (Hodgkin & Huxley, 1952; Fenwick *et al.* 1982). Figure 4 shows the relationship between peak I_{Na} and command potential in a cell recorded without (Fig. 4*A* and *C*) and with (Fig. 4*B* and *C*) series resistance compensation. The pipette solution contained 30 mM-Na^+ for these experiments. In the absence of series resistance compensation, transient outward currents were present following application of large depolarizing commands (to potentials less than −20 mV). However, under these conditions the peak I_{Na} could increase severalfold with changes in command potential of only a few millivolts (Fig. 4*C*). Following application of series resistance compensation, the transient outward currents were eliminated or markedly attenuated, and the relationship between peak I_{Na} and command potential showed the expected region of gradual negative slope conductance (Fig. 4*B*, *C*). In some cells, the effect of incomplete series resistance compensation was particularly severe, as command potentials with distinct 'thresholds' would be found. Under these conditions, repeated pulses to the *same* potential evoked either 'large' or 'small' inward currents, which differed in amplitude by as much as fivefold (data not shown). Transient outward currents were invariably present under these conditions. Readjustments of the clamp amplifier that eliminated these artifactual 'threshold' phenomena invariably reduced or, more typically, eliminated the transient outward currents. In some cases the same effect could be achieved by application of a continuous negative pressure to the recording pipette.

The recordings described above suggest that transient TTX-sensitive outward currents are due to inadequate voltage control to the extent that unclamped action potentials are present. However, in a previous study (Dryer *et al.* 1989), substitution of Li^+ for Na^+ was found to eliminate the transient outward current without affecting the amplitude of the inward current. This was regarded as strong evidence for a macroscopic $I_{K(Na)}$ and would appear to be in contrast to the results described above. However, substitution of Li^+ for Na^+ had no effect on outward currents in ciliary-ganglion cells that were properly voltage clamped according to the criteria described above (not shown). Li^+ substitution typically produced a slight increase in the amplitude of the inward currents, which, given that there was no change in kinetics, was probably due to the greater permeability of I_{Na} channels to Li^+ than to Na^+ (Hille, 1972). More prolonged exposure to Li^+ saline (> 10 min) often caused a reduction in the spike afterhyperpolarization of ciliary-ganglion neurones under whole-cell current-clamp conditions (not shown). This effect might be expected to result in a decrease in the amplitude of the TTX-sensitive transient outward current observed in cells under incomplete voltage clamp. In this regard, it should be noted that Haimann *et al.* (1990)

found that Li^+ substitution produced only an inconsistent reduction of the TTX-sensitive outward currents in trigeminal-ganglion neurones. Moreover, the effects of Li^+ were not reversible, suggesting that Li^+ cannot be acting as proposed previously.

The results described above suggest that TTX-sensitive transient outward currents are only present in ciliary-ganglion neurones that are not completely

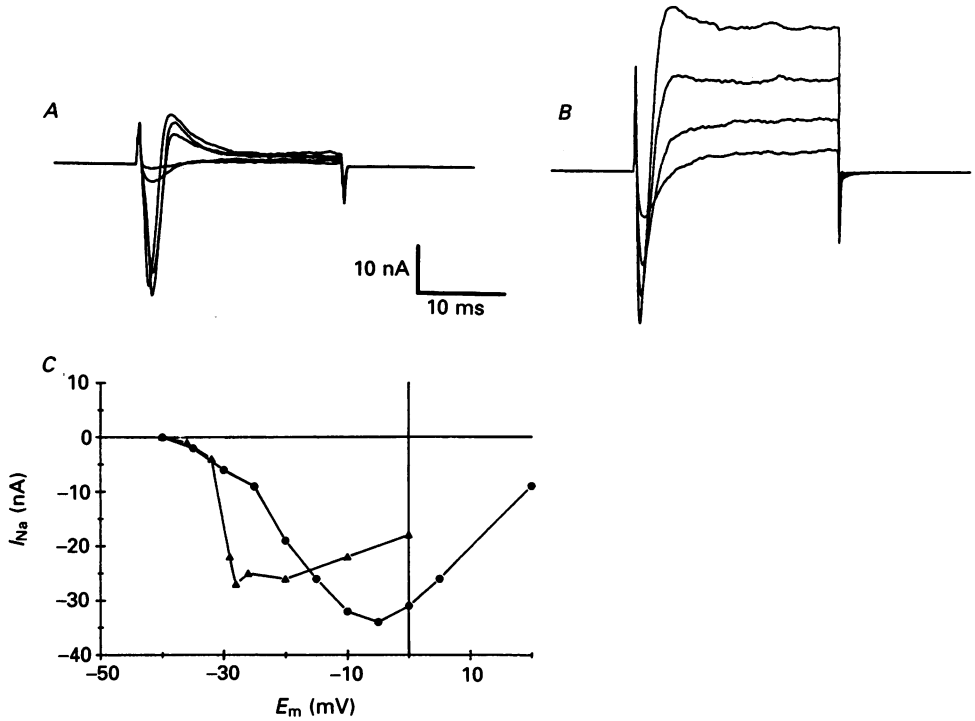

Fig. 4. Relationship between command potential and peak I_{Na} in a ciliary-ganglion neurone. Recordings were made with a single micropipette. *A*, total membrane currents evoked by voltage steps to −36, −32, −29, −28, and −14 mV from a holding potential of −80 mV. No series resistance compensation was present. *B*, total membrane currents in same cell as in *A*, but after application of series resistance compensation (80% of 10 MΩ). Currents were evoked by steps to −25, −20, −15, and −10 mV from a holding potential of −80 mV. *C*, plot of peak I_{Na} *versus* command potential in the same neurone before (▲) and after (●) compensation of series resistance.

voltage clamped. If ciliary-ganglion neurones contain K^+-channels that are activated by Na^+ influx through TTX-sensitive Na^+ channels, then they should be detectable in isolated outside-out patches. In this recording configuration, series resistance compensation should not affect the waveforms of the recorded currents, as they are orders of magnitude smaller than those obtained in the 'whole-cell' configuration. Moreover, in this recording configuration potential problems of inadequate space clamp are eliminated. Figure 5*A* shows a macroscopic current recorded with series resistance compensation (80% of 4 MΩ), evoked by a step depolarization of 60 mV from a holding potential of −70 mV. Shortly after this record was obtained, the pipette was withdrawn from the cell to yield an outside-out patch.

Application of depolarizations of 60 mV to the isolated patch resulted in currents like that shown in Fig. 5*B*. The waveform shown in Fig. 5*B* is the average of eighty leak-subtracted records of currents evoked by depolarizations of 60 mV from a holding potential of −85 mV. Representative individual traces of currents evoked

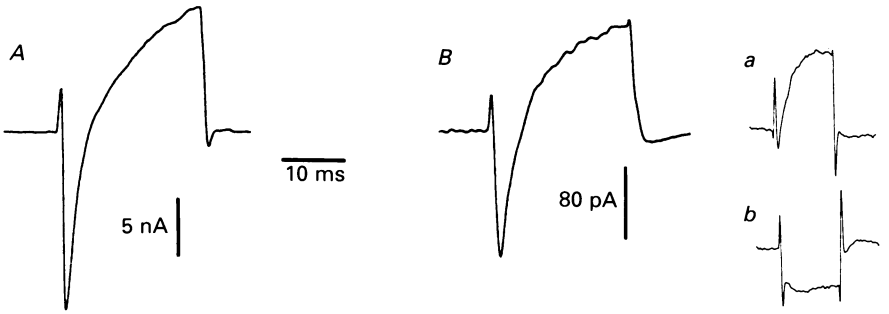

Fig. 5. Membrane currents in a ciliary-ganglion neurone. *A*, single-electrode whole-cell recording of total membrane currents evoked by a voltage step to −10 mV from a holding potential of −70 mV. Series resistance compensation was present (80% of 4 MΩ). *B*, average leak-subtracted membrane currents in an outside-out patch isolated from the same neurone shown in *A*. Main portion of figure shows leak-subtracted average of eighty sweeps evoked by steps to −25 mV from a holding potential of −85 mV. *B*, inset on right side of *B* shows representative single sweep responses evoked by pulses to −25 mV (*a*) and −145 mV (*b*). Negative pulses were used to generate average leakage and capacity currents. Note absence of transient outward currents in either recording configuration.

by commands of +60 mV (top) and −60 mV (bottom) are shown in the inset. Currents evoked in both the whole-cell and outside-out modes consisted of transient inward currents followed by sustained outward currents. No transient outward currents were present.

It should be noted that traces like those shown in Fig. 5*B* could *only* be obtained when a steady DV voltage of −10 to −20 mV was added to both holding and command potentials. This effect has been described previously (Marty & Neher, 1983) and is thought to be due to junctional potential drift caused by the loss of slowly diffusable particles in the cytoplasm. It seems to occur immediately after patch excision. I have often observed a much slower shift in the voltage dependence of ionic currents recorded in the whole-cell mode. This shift is usually complete within 30 min and is likely to be due to similar but much slower diffusion processes.

In several cells it was possible to examine the effect of TTX on averaged currents evoked in outside-out patches. Figure 6*A* shows representative single sweeps caused by step depolarizations of +40 and −40 mV from a holding potential of −79 mV. These recordings were made in normal chick saline. Figure 6*B* shows single sweeps evoked by pulses of +40 and −40 mV following perfusion with solutions containing 0·5 μM-TTX. Figure 6*C* shows the average of 150 leak-subtracted sweeps before (control) and after (TTX) application of TTX. There is no evidence of TTX-sensitive outward currents. This result is best seen in Fig. 6*D*, which shows the difference between the traces shown in Fig. 6*C*.

The results described above indicate that transient TTX-sensitive outward

currents are not present in dissociated ciliary neurones that are properly voltage clamped and cannot be detected in isolated outside-out patches. However, they do not indicate whether or not $I_{K(Na)}$ channels are actually *present* in these cells. For this reason, isolated inside-out patches were exposed to Li^+, Ca^{2+}, and Na^+ ions in an attempt to observe $I_{K(Na)}$ channels directly.

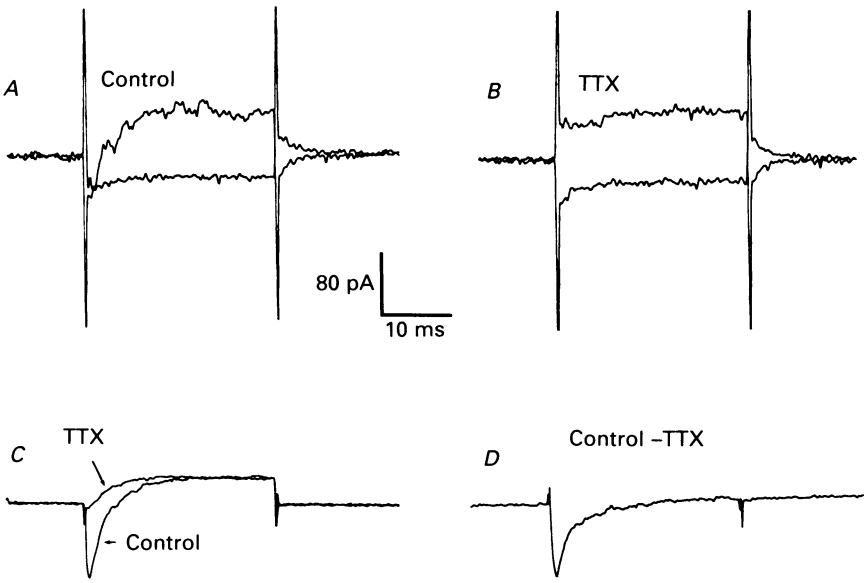

Fig. 6. Effects of TTX on membrane currents recorded in an outside-out patch isolated from a ciliary-ganglion neurone. *A*, representative single sweeps showing currents evoked by voltage steps to −39 and −119 mV from a holding potential of −79 mV. *B*, same as in *A*, but after exposure of patch to saline containing 0·5 μM-TTX. Negative pulses were used to compute average leakage and capacitance currents. *C*, leak-subtracted and averaged currents in the same outside-out patch before (control) and after (TTX) application of 0·5 μM-TTX. 150 sweeps such as those shown in *A* and *B* were used to generate these waveforms. *D*, average TTX-sensitive currents obtained by point-by-point digital subtraction of the traces shown in *C*. Note that TTX blocks only inward currents in this patch.

Typical results are shown in Fig. 7. Figure 7*A* shows a 50 s sweep of currents obtained when the patch was exposed to salines containing 75 mM-Li^+ and 10 mM-EGTA. Very little channel activity was present. No increase in channel activity was observed following exposure to solutions containing 75 mM-Na^+ and 10 mM-EGTA (Fig. 7*B*). However, exposure of the patch to 1 mM-Ca^{2+} in 75 mM-Li^+ evoked a large increase in channel activity (Fig. 7*C*, switch at arrow). This result indicates that the excised patch was actually inside-out and not a closed-off membrane vesicle. In this example, at least two Ca^{2+}-activated K^+ channels were present, each with a unitary amplitude of 15 pA at a membrane potential of −55 mV. Given a calculated E_K of +17 mV, the chord conductance was 208 pS. In other experiments (not shown), Ca^{2+}-activated K^+ channels reversed between +14 and +19 mV and remained active for as long as Ca^{2+} was present. Figure 7*D* shows the effect of

switching back to saline containing 75 mM-Na^+ and 10 mM-EGTA (switch at arrow). The observed channel activity was eliminated. Results similar to these were obtained over a period of several months in more than 120 inside-out patches from isolated ciliary-ganglion neurones and from five patches obtained from cells maintained in

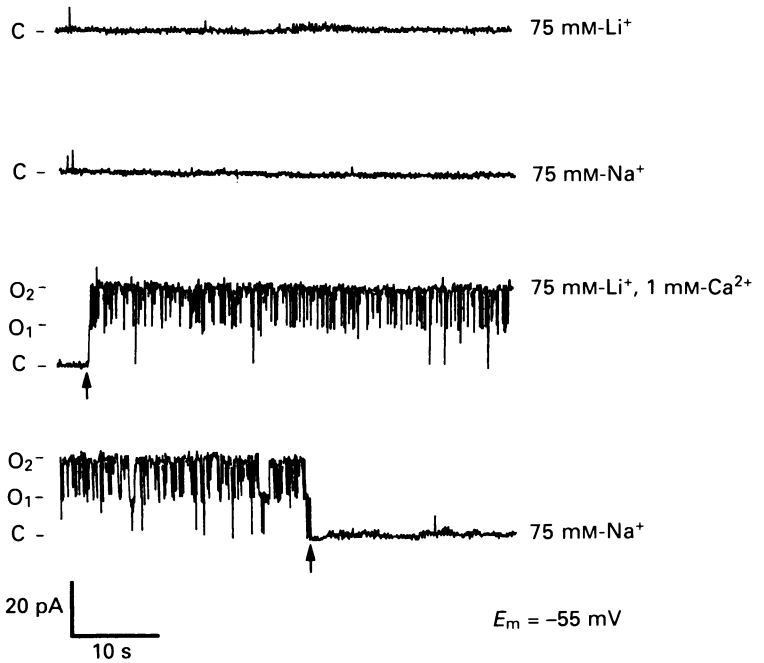

Fig. 7. Unitary K^+ currents in an inside-out patch isolated from a ciliary-ganglion neurone. The cytoplasmic face of the patch membrane was exposed to Li^+, Na^+, or Li^+ and Ca^{2+} in the (mM) concentrations indicated to the right of each trace. The patch membrane was held at −55 mV. Solution switches were made at the end of each trace or at the time indicated by the arrows. K^+ channels with a unitary chord conductance of 208 pS were present when the bath contained 1 mM-Ca^{2+}. The patch was quiescent in the absence of Ca^{2+}. Chord conductance was obtained assuming a (calculated) K^+ equilibrium potential of +17 mV. Closed and open states are indicated to the left of each sweep.

long-term tissue culture. These results suggest that $I_{K(Na)}$ channels are not present in neurones of the chick ciliary ganglion.

Brain stem neurones

Double-electrode whole-cell recordings were obtained from four cultured brain stem neurones as described above. In general, obtaining this recording configuration was much more difficult in brain stem cells than in ciliary-ganglion neurones. One example is shown in Fig. 8, showing total membrane currents evoked by a nominal 50 mV depolarization from a holding potential of −60 mV. As with the ciliary neurones, transient outward currents were present when unclamped action potentials were apparent in the independently recorded voltage trace (Fig. 8*A*). Application of

series resistance compensation (80% of 6 MΩ) eliminated the transient outward current and nearly eliminated the unclamped action potentials (Fig. 8*B*). This result suggests that, as with the ciliary-ganglion neurones, the transient outward currents in cultured brain stem neurones are artifacts.

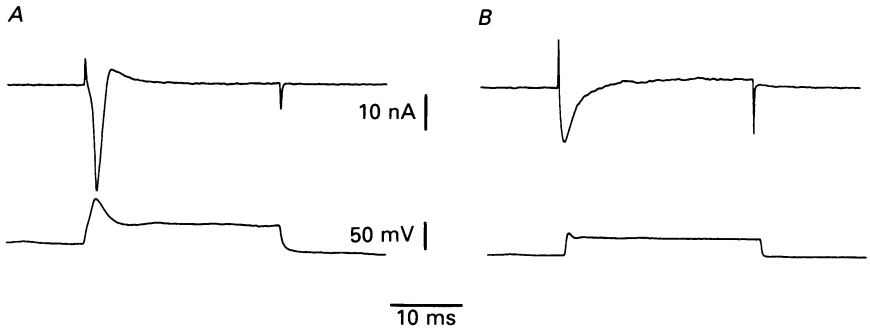

Fig. 8. Double-electrode whole-cell recordings from a cultured brain stem neurone. *A*, total membrane currents (upper trace) evoked by a nominal voltage step to −10 mV from a holding potential of −60 mV. Lower trace shows voltage responses recorded with a second independent micropipette. No series resistance compensation was present. An unclamped action potential is apparent in the voltage trace. *B*, total membrane currents in same cell as *A*, but after application of series resistance compensation (80% of 6 MΩ). Transient outward currents were eliminated (upper trace), and the voltage trace showed markedly improved voltage clamp associated with a small over-damped oscillation.

Several single-electrode experiments similar to those described above for ciliary neurones support this conclusion. For example, it is occasionally possible to observe 'threshold' phenomena, in which repeated pulses to a fixed command potential can lead to inward currents that fluctuate by as much as fivefold. Transient outward currents are invariably present under these conditions (data not shown). Moreover, when transient TTX-sensitive outward currents are present, there are also clear changes in the kinetics of I_{Na} produced by application of TTX (data not shown). In contrast, when brain stem neurones are adequately voltage clamped, there is no indication of transient TTX-sensitive outward currents. An example is shown in Fig. 9. In this cell, whole-cell recordings were made with adequate series resistance compensation (80% of 3 MΩ, Fig. 9*A*). Depolarizing commands of 70 mV were applied from a holding potential of −80 mV. Superfusion with saline containing 1·0 μM-TTX caused a progressive blockade of the fast inward current but had no effect on outward currents or on the kinetics of I_{Na}. Shortly after these records were obtained, the TTX was washed out, and the recording pipette was withdrawn from the cell so as to form a large outside-out patch (Fig. 9*B*). Application of depolarizing commands of 70 mV from a nominal holding potential of −95 mV resulted in inward and outward currents that, after averaging, had waveforms that resembled those obtained in the whole-cell recordings (Fig. 9*B*, control, and top inset). Application of TTX (1·0 μM) eliminated the inward currents but had no effect on the outward currents (Fig. 9*B*, TTX, and bottom inset). Point-by-point subtraction of the averaged records yielded the TTX-sensitive currents in the patch (Fig. 9*B*, control-TTX). These consisted of only inward currents.

The results described above indicate that $I_{K(Na)}$ is not activated by Na^+ influx through TTX-sensitive Na^+ channels in cultured chick brain stem neurones. However, experiments using inside-out patches clearly demonstrate that $I_{K(Na)}$ channels are present in these cells as described previously (Dryer *et al.* 1989) and as

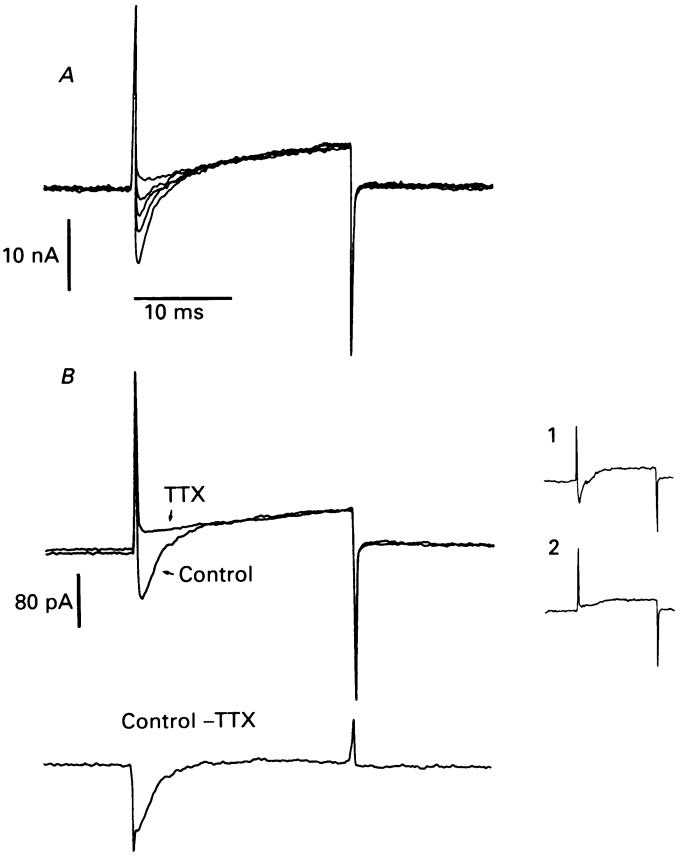

Fig. 9. Effects of TTX on a cultured brain stem neurone. *A*, single-electrode whole-cell recording of total currents evoked by pulses to −10 mV from a holding potential of −80 mV during progressive blockade of I_{Na} by 0·5 μM-TTX. Series resistance compensation was present (80% of 3 MΩ). *B*, averaged response to 150 pulses to −25 mV from a holding potential of −95 mV in outside-out patch isolated from the same cell (top). Responses are shown before (control) and after (TTX) application of 0·5 μM-TTX. TTX-sensitive currents (below) were obtained by point-by-point digital subtraction of the average currents. Representative single sweeps are shown to the right before (1) and after (2) application of 0·5 μM-TTX.

illustrated in Fig. 10. In this experiment an inside-out patch was formed in a saline containing 75 mM-LiCl and 10 mM-EGTA. Very little channel activity was present (Fig. 10*A*). Switching to bath salines containing 75 mM-NaCl and 10 mM-EGTA resulted in the appearance of ionic channels with a unitary current of 9 pA at a membrane potential of −50 mV (Fig. 10*B*). The chord conductance of these channels was 110 pS, which is close to values reported previously for brain stem (Dryer *et al.*

1989) but somewhat less than the main conductance state reported by Haimann *et al.* (1990) for cultured quail trigeminal ganglion neurones. Switching to bath salines containing 75 mM-LiCl and 1 mM-$CaCl_2$ caused these channels to become virtually quiescent (Fig. 10*C*). This result suggests that these channels are not activated by Ca^{2+} and, in fact, represent a unique class of K^+ channel. A similar result has been obtained by Haimann *et al.* (1990).

DISCUSSION

Several previous studies have concluded that $I_{K(Na)}$ channels are activated by Na^+ influx through TTX-sensitive Na^+ channels (Bader *et al.* 1985; Hartung, 1985; Dryer *et al.* 1989; Foehring *et al.* 1989; Schwindt *et al.* 1988, 1989; Haimann *et al.* 1990). In avian neurones this process has been proposed to result in a transient TTX-sensitive outward current that decays within 10 ms and that contributes to the repolarizing phase of the action potential (Bader *et al.* 1985; Dryer *et al.* 1989; Haimman *et al.* 1990). However, studies using isolated inside-out patches have shown that $I_{K(Na)}$ channels are rather insensitive to Na^+ (Kameyama *et al.* 1984; Dryer *et al.* 1989; Haimann *et al.* 1990) and generally require changes in Na^+ concentration of the order of tens of millimolar to alter gating significantly. It is difficult to reconcile these observations quantitatively, unless one assumes that patch excision alters the sensitivity of $I_{K(Na)}$ channels to activation by Na^+.

In the present study I have calculated the expected Na^+ concentration as a function of time and distance from the mouth of a single Na^+ channel, assuming that the channel behaves as a point source of Na^+ ions in an infintely long longitudinal membrane. The calculations suggest that Na^+ concentration is insufficient to activate $I_{K(Na)}$ channels located at a distance greater than 5 nm from the mouth of the Na^+ channel, even if one assumes a cytoplasmic diffusion coefficient 10-fold less than the usually accepted value. Given that the walls of Na^+-channel proteins are probably larger than 5 nm (Guy & Seetharamulu, 1985; Angelides & Nutter, 1984), it is difficult to imagine how Na^+ influx through TTX-sensitive Na^+ channels could activate $I_{K(Na)}$ channels with a sensitivity to Na^+ similar to those described to date, even if they were co-localized in an essentially crystalline array and even in the presence of significant diffusion barriers. For this reason, I have considered a second possibility, namely that previous reports of TTX-sensitive K^+ currents are artifacts caused by failure to voltage clamp the recorded cells adequately.

I have examined two preparations that have been reported to contain TTX-sensitive K^+ currents: isolated neurones from the chick ciliary ganglion (Bader *et al.* 1985) and cultured neurones from the chick brain stem (Dryer *et al.* 1989). The results indicate that in both preparations the presence of a transient TTX-sensitive outward current is an artifact caused by inadequate voltage clamp and was probably caused by incomplete series resistance compensation. That artifacts were present could be ascertained by use of a second, passive voltage-recording electrode, by determination of the relationship between peak I_{Na} and command potential, or by examination of the effects of TTX on the kinetics of I_{Na}. Moreover, averaging a large number of sweeps obtained from isolated outside-out patches yielded waveforms that closely resembled those obtained in the whole-cell configuration *when the cells were properly*

voltage clamped. In these preparations there was no evidence for TTX-sensitive K^+ currents in either recording configuration.

Experiments performed on isolated inside-out patches suggest that dissociated ciliary-ganglion neurones do not even contain $I_{K(Na)}$ channels, although they can be detected readily in cultured brain stem neurones recorded under identical conditions.

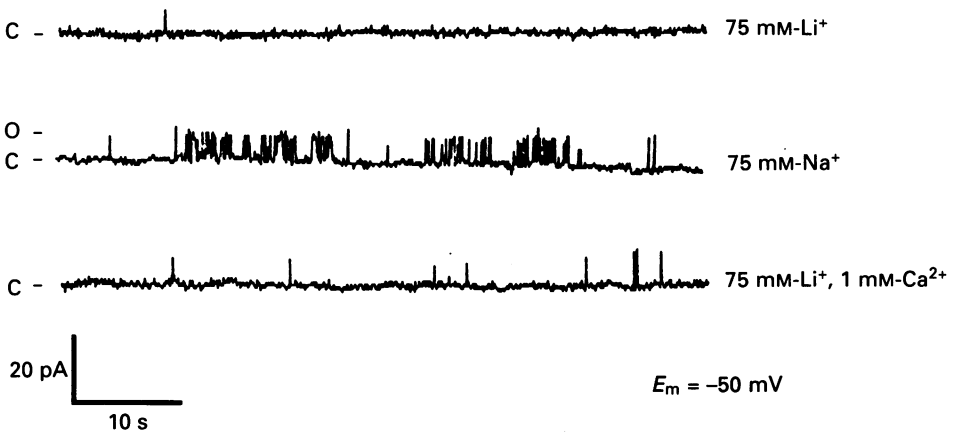

Fig. 10. Unitary K^+ currents in an inside-out patch isolated from a cultured brain stem neurone. The cytoplasmic face of the patch membrane was exposed to Li^+, Na^+, or Li^+ and Ca^{2+} in the (mM) concentrations indicated to the right of each trace. The patch membrane was held at −50 mV. Solution switches were made at the end of each trace. K^+ channels with a unitary chord conductance of 110 pS were present when the bath contained 75 mM-Na^+. The patch was quiescent in the presence of 75 mM-Li^+ or 75 mM-Li^+ and 1 mM-Ca^{2+}. Chord conductance was obtained assuming a (calculated) K^+ equilibrium potential of +17 mV.

Haimann *et al.* (1990) have recently detected single $I_{K(Na)}$ channels in inside-out patches from cultured quail trigeminal ganglion neurones. These workers suggest that these channels are activated by Na^+ influx through TTX-sensitive channels, as they can observe transient TTX-sensitive outward currents in cells that would appear to be adequately voltage-clamped. Substitution of Li^+ for Na^+ did not cause a consistent and reversible reduction in these currents. Because I have not recorded from these cells, I cannot address these results directly. However, the sensitivity of these channels to Na^+ was similar to that of brain stem neurones (Dryer *et al.*1989) and isolated cardiac myocytes (Kameyama *et al.* 1984). Therefore the same sorts of quantitative difficulties arise, and it is unlikely that co-localization of I_{Na} and $I_{K(Na)}$ channels alone can explain these data. It is possible that trigeminal-ganglion neurones have specializations that markedly reduce the mobility of Na^+ ions once they have entered the cytoplasm. Alternatively, it is possible that patch excision decreases the sensitivity of $I_{K(Na)}$ channels to Na^+, possibly as a result of loss of necessary co-regulating factors.

Transient TTX-sensitive outward currents have also been described in crayfish motoneurones (Hartung, 1985). In this case, there was no obvious indication of

inadequate voltage clamp. However, records of Na^+ currents obtained during partial TTX blockade were not shown, so it is not clear whether TTX produced any shift in the kinetics I_{Na}. This is a fairly sensitive indicator of incomplete voltage clamp (see Fig. 3*A* and Bader *et al.* 1985, for examples). The available evidence suggests that $I_{K(Na)}$ channels in invertebrate neurones have a sensitivity to Na^+ that is similar to that of vertebrate preparations. For example, Partridge & Thomas (1976) and Hartung (1985) observed a ouabain-resistant increase in K^+ conductance evoked by intracellular injections of Na^+ and Li^+. Large ionophoretic injection currents were needed to produce these effects, and the calculated cytoplasmic Na^+ concentrations produced by these injections were of the order of 20 mM. Given these results, the observed TTX-sensitive outward currents are difficult to explain without invoking significant barriers to the free diffusion of Na^+ in the cytoplasm.

A somewhat different TTX-sensitive K^+ current has been described in mammalian cortical neurones (Constanti & Simm, 1987; Schwindt *et al.* 1988; Foehring *et al.* 1989; Schwindt *et al.* 1989). In this case, the currents are quite slow and require several seconds to decay completely. One explanation of these data is that they are due to activation of $I_{K(Na)}$ channels. In this case one would predict that the $I_{K(Na)}$ channels would have to be more sensitive to Na^+ than those observed to date in excised patches. A second possibility discussed by Constanti & Simm (1987) is that these currents are space-clamp artifacts caused by failure to control voltage in electrically remote dendritic regions. This could occur if TTX-sensitive currents promoted the active spread of depolarization into the dendrites. This depolarization would then recruit additional voltage- and/or Ca^{2+}-dependent K^+ conductances. Elimination of those active processes by application of TTX would have the effect of reducing the total recorded K^+ conductance, if these additional K^+ channels were located in regions where voltage control was inadequate. In this regard, TTX-sensitive Na^+ currents and various K^+ currents have been described in dendrites of mammalian central neurones (Wong, Prince & Busbum, 1979; Llinás & Sugimori, 1980; Llinás & Yarom, 1981). This notion is supported by the fact that, in olfactory cortical neurones, TTX-sensitive K^+ currents are dependent on the presence of Ca^{2+} in the external saline (Constanti & Simm, 1987). Moreover, the TTX-sensitive outward currents are reduced by agents, such as muscarine and norepinephrine, that also reduce Ca^{2+}-dependent K^+ currents.

In neurones from cat sensorimotor cortex, the TTX-sensitive outward currents can be observed in Ca^{2+}-free salines (Schwindt *et al.* 1988, 1989; Foehring *et al.* 1989). However, these currents could still be a consequence of TTX-sensitive Na^+ channels located in dendrites if voltage-dependent K^+ channels were also present (Llinás & Sugimori, 1980). Single-channel recordings from mammalian cortical neurones are needed to resolve this issue.

The physiological significance of $I_{K(Na)}$ channels in chick brain stem neurones remains unknown. It seems unlikely that they could contribute significantly to the repolarizing phase of the action potential, at least as envisioned previously (Dryer *et al.* 1989; Martin & Dryer, 1990). The sensitivity of these channels to Na^+ suggests that they could contribute to the resting membrane conductance, but the magnitude of this contribution is unknown. One interesting possibility is that these channels might be modulated by neurotransmitters acting through G-protein-linked mem-

brane receptors. If so, $I_{K(Na)}$ would join a growing list of K^+ channels that are active around the resting potential and that exert subtle but long-lasting effects on the electrical behaviour of excitable cells.

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REFERENCES

ANGELIDES, K. J. & NUTTER, T. J. (1984). Molecular and cellular mapping of the voltage-dependent Na^+ channel. *Biophysical Journal* **54**, 31–34.

BADER, C. R., BERNHEIM, L. & BERTRAND, D. (1985). Sodium-activated potassium current in cultured avian neurones. *Nature* **317**, 540–542.

BARRETT, J. N., MAGLEBY, K. L. & PALLOTTA, B. S. (1982). Properties of single calcium-activated potassium channels in cultured rat muscle. *Journal of Physiology* **331**, 211–230.

CONSTANTI, A. & SIMM, J. (1987). Calcium-dependent potassium conductance in guinea pig olfactory cortex *in vitro*. *Journal of Physiology* **387**, 173–194.

CRANK, J. (1975). *The Mathematics of Diffusion*. 2nd edition. Clarendon Press, Oxford.

DRYER, S. E., FUJII, J. T. & MARTIN, A. R. (1989). A Na^+-activated K^+ current in cultured brain stem neurones from chicks. *Journal of Physiology* **410**, 283–296.

FENWICK, E. M., MARTY, A. & NEHER, E. (1982). Sodium and calcium channels in bovine chromaffin cells. *Journal of Physiology* **331**, 599–635.

FOEHRING, R. C., SCHWINDT, P. C. & CRILL, W. E. (1989). Norepinephrine selectively reduces slow Ca^{2+}- and Na^+-mediated K^+ currents in cat neocortical neurons. *Journal of Neurophysiology* **61**, 245–256.

FOGELSON, A. L. & ZUCKER, R. S. (1985). Presynaptic calcium diffusion from various arrays of single channels. *Biophysical Journal* **48**, 1003–1017.

FUJII, J. T. & BERG, D. K. (1986). Preganglionic neurons from the Edinger-Westphal nucleus: Growth and histochemical characterization in cell culture. *Developmental Brain Research* **24**, 1–9.

GUY, H. R. & SEETHARAMULU, P. (1985). Molecular model of the action potential sodium channel. *Proceedings of the National Academy of Sciences of the USA* **83**, 508–512.

HARTUNG, K. (1985). Potentiation of a transient outward current by Na^+ influx in crayfish neurones. *Pflügers Archiv* **404**, 41–44.

HAIMANN, C., BERNHEIM, L., BERTRAND, D. & BADER, C. R. (1990). Potassium current activated by intracellular sodium in quail trigeminal ganglion neurons. *Journal of General Physiology* **95**, 961–980.

HILLE, B. (1972). The permeability of the sodium channel to metal cations in myelinated nerve. *Journal of General Physiology* **59**, 637–658.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology* **117**, 500–544.

KAMEYAMA, M., KUKEI, M., SATO, R., SHIBISAKI, T., MATSUDA, H. & IRISAWA, I. (1984). Intracellular Na^+ activates a K^+ channel in mammalian cardiac cells. *Nature* **309**, 354–356.

KUSHMERICK, M. J. & PODOLSKY, R. J. (1969). Ionic mobility in muscle cells. *Science* **166**, 1297–1298.

LLINÁS, R. & SUGIMORI, M. (1980). Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *Journal of Physiology* **305**, 197–213.

LLINÁS, R. & YAROM, Y. (1981). Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurones *in vitro*. *Journal of Physiology* **315**, 569–584.

MARTIN, A. R. & DRYER, S. E. (1990). Potassium channels activated by sodium. *Quarterly Journal of Experimental Physiology* **74**, 1033–1041.

MARTY, A. & NEHER, E. (1983). Tight-seal whole-cell recording. In *Single-Channel Recording*, ed. SAKMANN, B. & NEHER, E., pp. 107–122. Plenum Press, New York.

PARTRIDGE, L. D. & THOMAS, R. C. (1976). The effects of lithium and sodium on the potassium conductance of snail neurones. *Journal of Physiology* **254**, 551–563.

ROLE, L. & FISCHBACH, G. D. (1987). Changes in the number of chick ciliary ganglion neuron processes with time in cell culture. *Journal of Cell Biology* **104**, 363–370.

SCHWINDT, P. C., SPAIN, W. S. & CRILL, W. E. (1989). Long-lasting reduction of excitability by a sodium-dependent potassium current in cat neocortical neurons. *Journal of Neurophysiology* **61**, 233–244.

SCHWINDT, P. C., SPAIN, W. S., FOEHRING, R. C., CHUBB, M. C. & CRILL, W. E. (1988). Slow conductance changes in neurons from cat sensorimotor cortex *in vitro* and their role in slow excitability changes. *Journal of Neurophysiology* **59**, 450–467.

WONG, R. K. S., PRINCE, D. A. & BUSBUM, A. (1979). Intradendritic recordings from hippocampal neurons. *Proceedings of the National Academy of Sciences of the USA* **76**, 986–990.

ZUCKER, R. S. & STOCKBRIDGE, N. (1983). Presynaptic calcium diffusion and the time course of transmitter release and synaptic facilitation at the squid giant synapse. *Journal of Neuroscience* **3**, 1263–1269.