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A Na^+-ACTIVATED K^+ CURRENT IN CULTURED BRAIN STEM NEURONES FROM CHICKS

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SUMMARY

1. Patch-clamp techniques were used to study the properties of a Na^+-activated K^+ current ($I_{K(Na)}$) in neurones cultured from embryonic chick brain stem.

2. With whole-cell clamp, a depolarizing voltage command evoked an inward current that was followed by an outward current with two components, the first transient, the second sustained.

3. Tetrodotoxin (TTX, 1 μM) eliminated the inward current and the transient component of the outward current, without affecting the sustained outward current. In addition, the transient outward current was attenuated when all external Na^+ was replaced by Li^+, suggesting that it was activated specifically by Na^+ entry into the cell.

4. The time course of the transient outward current was obtained by subtracting records obtained in Li^+ solution from those obtained in Na^+ solution. There was significant overlap between the decay of the inward current and the onset of the transient outward current.

5. When, just after the peak of the transient outward current, the membrane was stepped to progressively more hyperpolarized levels, the tail currents associated with the current reversed polarity near the calculated K^+ equilibrium potential.

6. 4-Aminopyridine (4-AP, 4 mM) abolished the transient outward current and approximately half of the sustained late current. Tetraethylammonium (TEA, 2 mM) had no effect on the transient current, but reduced the sustained current slightly.

7. Inside-out patches, made in LiCl bathing solutions, contained channels that were activated by exposing the cytoplasmic face of the patch to Na^+. Channel activity continued as long as Na^+ was present.

8. The single-channel currents reversed at the K^+ equilibrium potential, and were associated with a main conductance that depended upon K^+ concentration (about 50 pS with $[K^+]_o$ = 150 mM, $[K^+]_i$ = 5 mM, and 100 pS when $[K^+]_i$ was increased to 75 mM).

9. The open probability of the channels increased with increasing cytoplasmic

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Na^+ concentration. At $[Na^+]_i = 150$ mM (the maximum concentration tested), channels were open almost continuously. Open probability was considerably less at 50 mM, and still measurable at 20 mM.

10. The magnitude of $I_{K(Na)}$ and its overlap with the inward Na^+ current indicate that these channels contribute significantly to the repolarizing phase of the action potential. In addition, the relation between channel activity and Na^+ concentration suggests that the channels may make a measurable contribution to membrane conductance at resting intracellular Na^+ concentrations.

INTRODUCTION

Several K^+ currents are known to contribute to the repolarization of excitable cell membranes during the falling phase of the action potential. These include the voltage-dependent 'delayed rectifier' current (Hodgkin & Huxley, 1952; Armstrong & Hille, 1972) and various Ca^{2+}-dependent K^+ currents (Meech & Standen, 1975; Adams, Constanti & Brown, 1982; Pennefather, Lancaster, Adams & Nicoll, 1985). Other K^+ currents, including 'A-currents' (Connor & Stevens, 1971; Madison & Nicoll, 1984) and 'M-currents' (Constanti & Brown, 1981; Adams, Brown & Constanti, 1982), play a role in regulating the excitability and repetitive firing characteristics of neurones.

Recently, an additional K^+ current that appears to contribute to the repolarizing phase of the action potential has been reported in both vertebrate (Bader, Bernheim & Bertrand, 1985) and invertebrate (Hartung, 1985) neurones, and in isolated ventricular myocytes (Kameyama, Kakei, Sato, Shibisaki, Matsuda & Irisawa, 1984). This is a K^+ current activated by intracellular Na^+ ($I_{K(Na)}$). We have observed a similar current in brain stem neurones cultured from embryonic chicks. The macroscopic currents were studied with whole-cell current recording techniques. In addition, we used isolated membrane patches from the same cell type to study single-channel K^+ currents that were activated when Na^+ (20–150 mM) was applied to the cytoplasmic face of the patch. Such K^+ channels presumably were responsible for the macroscopic currents observed in the whole-cell experiments. Some of the preliminary results have been reported previously (Dryer, Fujii & Martin, 1988*a*, *b*).

METHODS

Preparation

Neurones were isolated from the midbrains of 17-day-old chick embryos and grown in culture as described previously (Fujii & Berg, 1986). Briefly, small cubes of tissue containing the Edinger–Westphal nucleus and adjacent areas were dissected and incubated for 20 min at 37 °C in salt solutions free of Ca^{2+} and Mg^{2+} and containing 0·05% trypsin. After removal of the trypsin and trituration with a fire-polished pipette, cells were plated onto collagen-coated polystyrene Petri dishes. Cells were plated in Eagle's minimal essential medium supplemented with 5% chick embryo extract, 10% heat-inactivated horse serum, 50 U/ml penicillin and 50 μg/ml streptomycin. To minimize the growth of non-neuronal cells after the first 5 days, the embryo extract was replaced with 3% chick embryo eye extract. The medium was changed every 2–3 days thereafter. Recordings were made from medium to large neurones (25–50 μm in diameter) 2–5 weeks after plating.

Recording and data analysis

Patch-clamp electrodes were pulled in two stages from borosilicate glass, coated to near the tip with Sylgard resin to reduce electrode capacitance, and fire-polished to a final resistance of 4–10 MΩ. Seals between the electrode tips and the cell membranes of well over 1 GΩ were made by gentle suction; for whole-cell recordings further suction ruptured the membrane patch within the pipette to provide access to the cell interior. For whole-cell recording, the pipette solution consisted of (mM): 120 potassium gluconate, 30 NaCl, 3 $MgCl_2$, 1 EGTA, 10 HEPES, at a pH of 7·4. The bathing solution consisted of (mM): 145 NaCl, 5·3 KCl, 5·3 $CaCl_2$, 0·8 $MgCl_2$, 5·0 glucose, 13 HEPES (pH 7·4) and 1 mg/ml bovine serum albumen (BSA). All potentials were corrected to allow for the liquid junction potential between the electrode and bath solutions. This was determined by balancing the amplifier output to zero with pipette solution in both the electrode and the bath, and then noting the voltage offset that developed when the bath was changed to normal bathing solution (cf. Fenwick, Marty & Neher, 1982). The correction, determined in this way, was −11 mV. All experiments were at room temperature (*ca.* 22 °C).

With whole-cell recording, the resting membrane potential of the cell was taken as the applied electrode potential necessary to reduce the recorded current to zero. Responses to applied voltage pulses were recorded with the maximum possible series resistance compensation, which, from one cell to the next, ranged between 10 and 30 MΩ. Compensation affected the amplitudes and time courses of both inward and outward currents. In some records there were indications of incomplete voltage clamping, due to inadequate series resistance compensation and/or inadequate space clamp, indicating the necessity for some caution in interpreting the current waveforms (see Results). Currents recorded from the patch clamp amplifier were filtered with an 8-pole Bessel filter (f_c = 1850 Hz) and stored as analog signals on videotape. Records were later digitized (140–200 μs/point) and stored for further processing and analysis, or played into a chart recorder for display.

Inside-out patches were formed by withdrawing the pipette after the initial seal formation and exposing the tip to air for about one second. Air-exposure disrupted the external surface of any vesicle formed during patch excision, thereby insuring that the cytoplasmic face of the patch membrane was exposed to the bathing solution. For these experiments, the pipettes contained (mM): 150 KCl, 1·0 EGTA, 13 HEPES at a pH of 7·4. The bathing solution (seen by the cytoplasmic face of the patch) consisted of (mM): 150 LiCl, 5·0 KCl, 10 $MgCl_2$, 5·0 glucose, 1·0 EGTA, 13 HEPES (pH 7·4) and 1 mg/ml BSA. For these conditions, the liquid junction potential correction, added to all potential measurements, was −15 mV. In some experiments part of the LiCl in the bathing solution was replaced by osmotically equivalent concentrations of glucosamine-HCl and/or KCl, and by varying concentrations of NaCl when it was desired to evoke channel activity. A Perspex ring in the Petri dish reduced the volume of the bathing solution to about 0·2 ml and perfusion was at about 1·0 ml/min. Responses to changes in the bathing solution usually stabilized within 1 min.

RESULTS

Whole-cell currents

Whole-cell records were obtained from forty-two cells. Cells were considered satisfactory if their resting potentials were greater in magnitude than −55 mV and if they produced brisk inward currents in response to a step voltage command to −31 mV from a holding potential of −71 mV. In twelve cells in which it was measured, the input resistance was 1·4 ± 0·2 GΩ (mean ± S.D.). As shown in Fig. 1 (inset), application of suprathreshold voltage commands from the holding potential evoked an inward current, presumably due to Na^+ influx, followed by a more prolonged outward current, lasting for the duration of the depolarizing pulse. In these records, the upper trace indicates the voltage protocol, *not* records of the actual voltage applied to the cell. The initial part of the outward current contained a large transient component which, after its peak, decayed towards a steady-state level over

a period of about 6 ms. After application of TTX, the inward Na^+ current was abolished, as expected. Unexpectedly, the transient component of the outward current was abolished as well. Subtraction of records obtained after TTX action was complete from those obtained initially resulted in the 'TTX-sensitive current' shown in Fig. 1. This consisted of the inward current, with a total duration of about 2 ms, followed by the transient outward current.

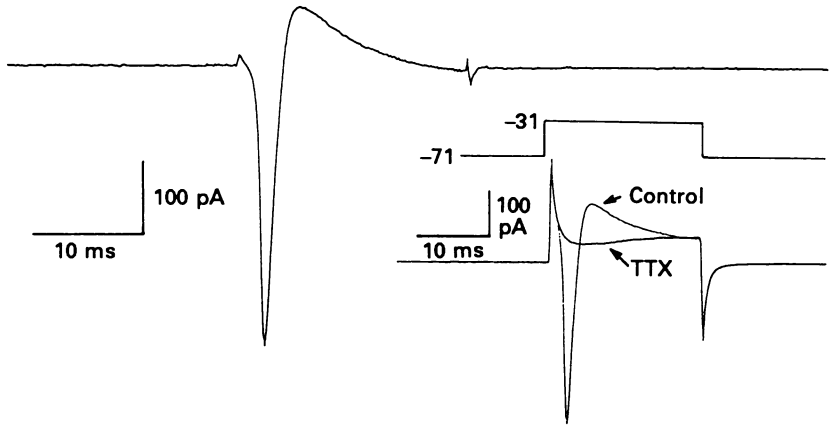

Fig. 1. TTX-sensitive current in a brain stem neurone. Inset: currents produced by depolarizing the cell from −71 to −31 mV (Control) consisted of capacitative transients at the beginning and end of the pulse, plus a fast inward current through the cell membrane, followed by slower outward current. The outward current had a large transient component, lasting almost to the end of the pulse. After application of 1 μM-tetrodotoxin (TTX), the inward current and the transient phase of outward current were abolished. Main record: TTX-sensitive current, obtained by digital subtraction of the record after TTX application from the control record. In this and all subsequent figures the voltage trace (inset) shows pulse protocol (mV), not the actual voltage applied to the electrode.

Experiments such as that illustrated in Fig. 1 suggested that depolarization produced a transient outward current that either was directly sensitive to TTX or was activated by prior Na^+ entry. An additional possibility was that the transient component of the outward current record was associated with incomplete clamping of the action potential, due to inadequate series resistance compensation or to poor space clamping of the cell. In either case, the unclamped voltage change might well have produced a diphasic current in the clamped region, which would then have added to the recorded clamp current to produce records such as those shown in Fig. 1*A*. This possibility has been discussed by Bader *et al.* (1985). In some cells, there were clear indications that the clamp was not adequate. However, in the experiments described below, we found that the transient outward current could be abolished by a procedure that would not have been expected to alter the clamping conditions, namely replacing extracellular Na^+ with Li^+.

Several inorganic cations are capable of permeating voltage-activated Na^+ channels. In particular, Li^+ has been shown to permeate the channels somewhat more

easily than Na^+ (Hille, 1972). The effect on the TTX-sensitive currents of substituting Li^+ for Na^+ in the bathing solution is shown in Fig. 2*A*. The inward current was of the usual magnitude, but the transient outward current was no longer present. Figure 2*B* (lower traces) shows total currents (i.e. currents with no subsequent subtractions) that were obtained in another experiment before (Control) and after

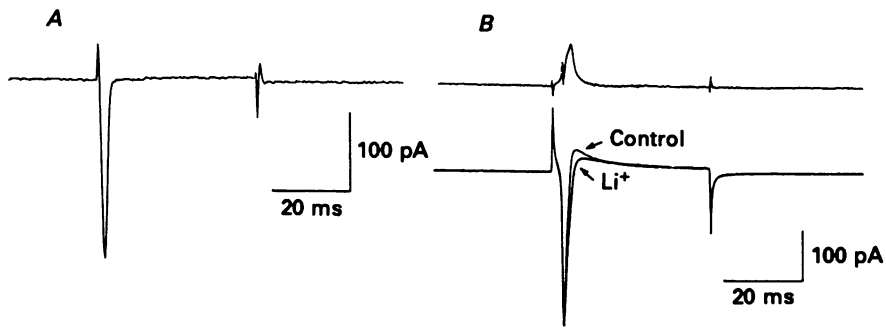

Fig. 2. *A*: TTX-sensitive current (obtained by subtraction as in Fig. 1) from a neurone bathed in Li^+ solution. Note absence of transient outward current. *B*: lower records, response of a different neurone to a 25 mV depolarizing pulse, from a holding potential of −71 mV, in normal bathing solution (Control), and 10 min after replacing Na^+ in the bathing solution with Li^+. In lithium solution almost all of the transient outward current was abolished. Upper record, Li^+-sensitive current obtained by subtraction of the two lower records (Control − Li^+).

Li^+ substitution. In the Li^+ solution, the magnitude of the inward current was unchanged and its duration prolonged. In contrast, only a small fraction of the transient outward current remained (a component possibly related to incomplete clamping). The current obtained by subtraction of the second record from the first is shown in the upper trace. If one assumes that the kinetics of the inward current was unaltered by the ion substitution (Hille, 1972), the upper trace then represents the magnitude and time course of the Na^+-dependent outward current.

One possibility not yet considered is that the TTX-dependent outward currents were activated by Ca^{2+} fluxes (rather than Na^+ fluxes) through TTX-sensitive channels. This hypothesis would be difficult to reconcile with the effect of Li^+ substitution (Fig. 2*B*) unless such channels were blocked by Li^+ as well. In any case, six of the forty-two cells were examined in extracellular solutions in which Ca^{2+} had been omitted and 1 mM-EGTA added. Results from those cells were indistinguishable from those bathed in the normal extracellular solution.

In order to determine the ionic nature of the TTX-sensitive outward current, experiments were done to determine its reversal potential, as shown in Fig. 3. The pulse protocols consisted of a depolarization to −41 mV from the holding potential, followed by successively increasing steps to less depolarized, and then to hyperpolarized levels. After addition of TTX, the identical voltage protocols were again applied to the cell, and the corresponding records were subtracted from those obtained initially, to give the family of current records shown. The TTX-sensitive 'tail currents' following the repolarizing steps were successively reduced in amplitude

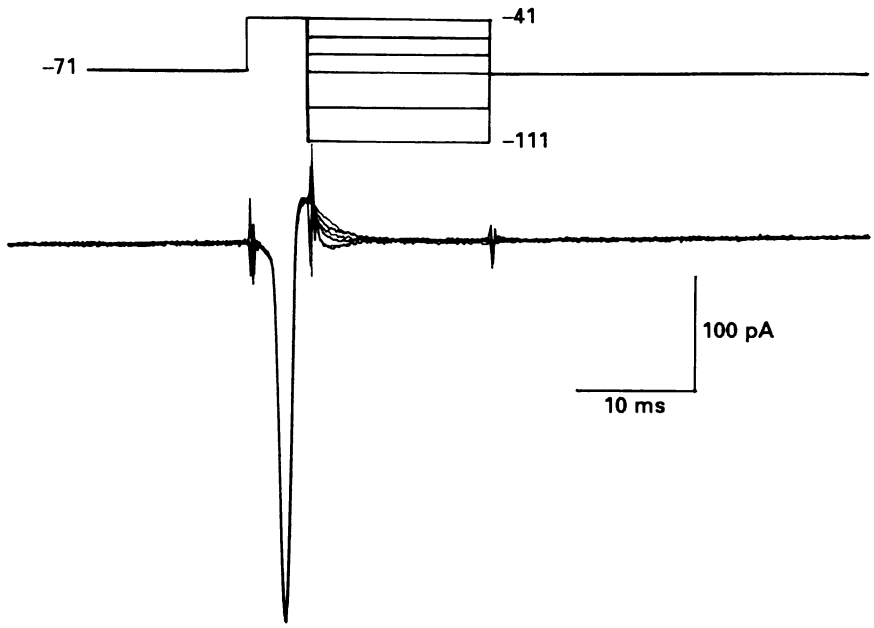

Fig. 3. Reversal of TTX-sensitive outward current. Initial step from −71 to −41 mV produced inward and outward currents (lower records), as in Fig. 1. Just after the peak of the outward current, the membrane potential was stepped back to various levels, as indicated in the upper pulse protocol traces. The resulting tail currents were all outward, except for the last two (nearly overlapping), produced by steps to −91 and −111 mV, which reversed to inward. Estimated reversal potential was about −80 mV, near the K^+ equilibrium potential.

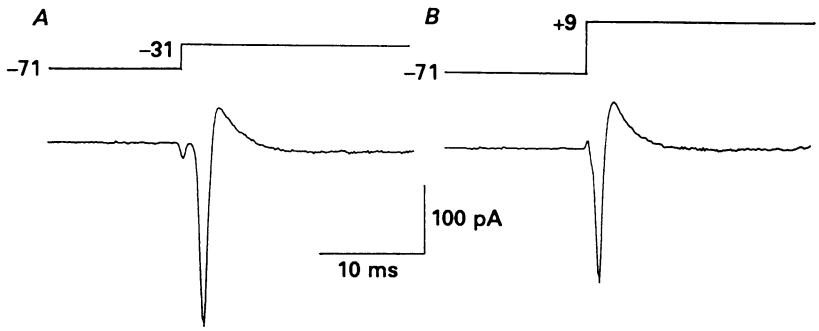

Fig. 4. Dependence of TTX-sensitive outward current on previous inward current. *A*: current produced by step from −71 to −31 mV. *B*: step to +9 mV produced inward current 24% smaller than in *A*, because of reduced driving force for inward Na^+ flux. Outward current was larger than in *A* because of increased driving force for outward K^+ flux, but underlying peak conductance was smaller by 27% (see text).

with increasing repolarizations, and then reversed polarity. The reversal potential was near the calculated K^+ equilibrium potential (−79 mV), suggesting that the current was carried mainly, and perhaps exclusively, by K^+ ions. The calculated equilibrium potentials for Cl^- and Na^+ were −18 and +39 mV respectively.

Experiments like that shown in Fig. 4 were performed as an additional test of the

Na^+ dependence of the transient outward current. The figure shows the TTX-sensitive inward and outward currents produced in the same cell by two different depolarizing pulses from a holding potential of −71 mV, the first to −31 mV and the second to +9 mV. The inward current in the second trial was smaller than that in the first because of the reduced driving force for Na^+ following the larger depolarizing step. The outward current following the first step had a peak value of about 50 pA.

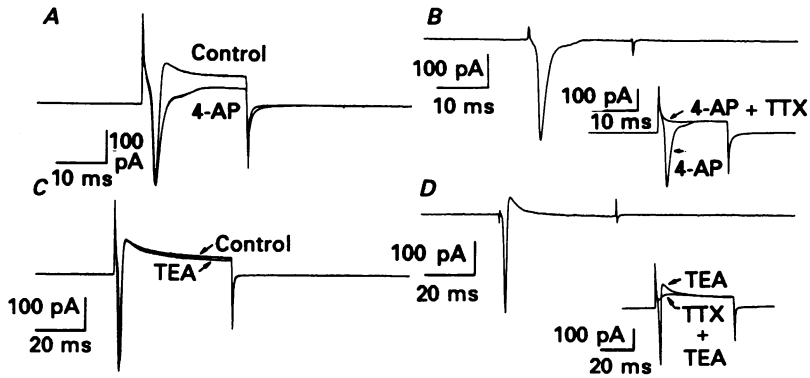

Fig. 5. Effects of 4-AP and TEA on outward K^+ current. *A*: currents evoked by 40 mV depolarizing pulse from −71 mV holding potential. Transient outward current and part of late outward current were abolished by 4-AP (4 mM). *B*: TTX-sensitive current in 4-AP, with only inward current. *C*: currents evoked by depolarizing pulse were reduced only slightly by TEA (2 mM). *D*: TTX-sensitive current in TEA, with both inward and outward components.

With a reversal potential of −79 mV, this corresponded to a peak conductance of 1·04 nS. The second step produced a larger outward current (67 pA), but this was because of the increased driving force; the peak conductance change was only 0·76 nS, a reduction of 27% from that produced by the first trial. This corresponds reasonably well with the reduction in amplitude of the inward current (24%), providing quantitative support for the idea that the channels subserving the outward current were activated by Na^+ entry. In other experiments (not shown), Na^+ currents were partially inactivated by stepping from less negative holding potentials. This procedure again produced a corresponding reduction in the peak conductance underlying the subsequent outward current.

A number of compounds are known to block various K^+ currents in invertebrate and vertebrate neurones. It was of interest to see if any of these were capable of blocking the transient outward current seen in the present experiments which, for brevity, will now be referred to as $I_{K(Na)}$. The effect of 4-AP on total membrane current is shown in Fig. 5*A*. Before addition of the drug (Control), a voltage step to −31 mV from a holding potential of −71 mV produced the familiar inward and outward current pattern. After the addition of 4-AP (4 mM), the transient outward current and a fraction of the later sustained current were absent. The specific effect on the TTX-sensitive outward current (i.e. on $I_{K(Na)}$) is shown in Fig. 5*B*, where the current in 4-AP after addition of TTX has been subtracted from the total current in 4-AP alone (see inset). $I_{K(Na)}$ was totally absent in the presence of 4-AP.

In contrast to 4-AP, TEA (2 mM) had no effect on the early transient outward current and attenuated the sustained outward current only slightly, as indicated by the total current records in Fig. 5*C*. The lack of an effect on $I_{K(Na)}$ is shown specifically by the subtracted record in Fig. 5*D*, in which the TTX-sensitive outward current recorded in the presence of TEA had its usual time course and amplitude.

Single-channel currents

Records were made from 'inside-out' membrane patches to see if single-channel currents with characteristics equivalent to the macroscopic currents discussed so far could be detected; i.e. currents through Na^+-activated K^+ channels. As described in Methods, patches were pulled from cells bathed in Ca^{2+}-free bathing solution with the Na^+ replaced by Li^+ or glucosamine. Consequently, the inside-out patch saw neither Ca^{2+} nor Na^+ at its cytoplasmic face. The pipette solution contained a high concentration of K^+, so the K^+ concentration gradient through the patch was the reverse of that normally seen by the membrane. Thus, with no applied potential, K^+ currents flowed outward from the pipette, from the external to the internal membrane surface.

A continuous record from such an inside-out patch is shown in Fig. 6*A*. This particular patch exhibited only modest channel activity in the Na^+-free bathing solution. Replacement of Li^+ by Na^+ in the bath perfusate was followed by a marked increase in activity that was maintained as long as Na^+ was present on the cytoplasmic face, and declined when the original bathing solution was restored. As with the macroscopic currents, the channel currents reversed at potentials close to E_K (not shown), which in these experiments was +85 mV. More modest channel activity was produced by lower Na^+ concentrations in the bathing solution. The records in Fig. 6*B* were obtained from a patch in a solution containing 100 mM-glucosamine as the major cation, with 50 mM-Li^+. When the Li^+ was replaced by Na^+, channel activity appeared. Reversal was again near E_K, and the channel conductance was about 50 pS ($[K^+]_o$ = 150 mM, $[K^+]_i$ = 5 mM).

Reversal of currents through the Na^+-activated channels is illustrated in Fig. 7. In this experiment the K^+ concentration in the bath was increased to 75 mM and the channels were activated with a Na^+ concentration of 50 mM. As shown in the voltage–current relation, current through the channels reversed at about +17 mV (E_K). The voltage–current relation suggested a slight inward rectification and the channel conductance at the reversal potential under these ionic conditions ($[K^+]_i$ = 150 mM, $[K^+]_o$ = 75 mM) was 105 pS.

It seemed clear from the magnitude and time course of the macroscopic currents that these K^+ channels, activated by Na^+ influx during the rising phase of an action potential, must make a substantial contribution to action potential repolarization. The question then arose whether the channels were likely to contribute to resting K^+ permeability as well, activated by the resting cytoplasmic Na^+ concentration, which can be expected to be in the range of 10–30 mM (see Discussion). With this question in mind, the relation between channel activity and Na^+ concentration at the cytoplasmic face of the patch was examined. One such experiment is illustrated in Fig. 8. In this experiment there were probably no more than two Na^+-activated channels in the patch, and channel openings were sparse in the absence of Na^+ in the

bathing solution. Increasing the Na^+ concentration, first to 37·5 mM and then to 75 and 150 mM, resulted in progressively increasing activity. At the lowest concentration, the channels opened irregularly in bursts; as the Na^+ concentration was increased the bursts became more frequent until they were virtually continuous.

In some experiments there were sufficient numbers of active channels in a patch to produce a continuous current across the membrane at all but the lowest Na^+

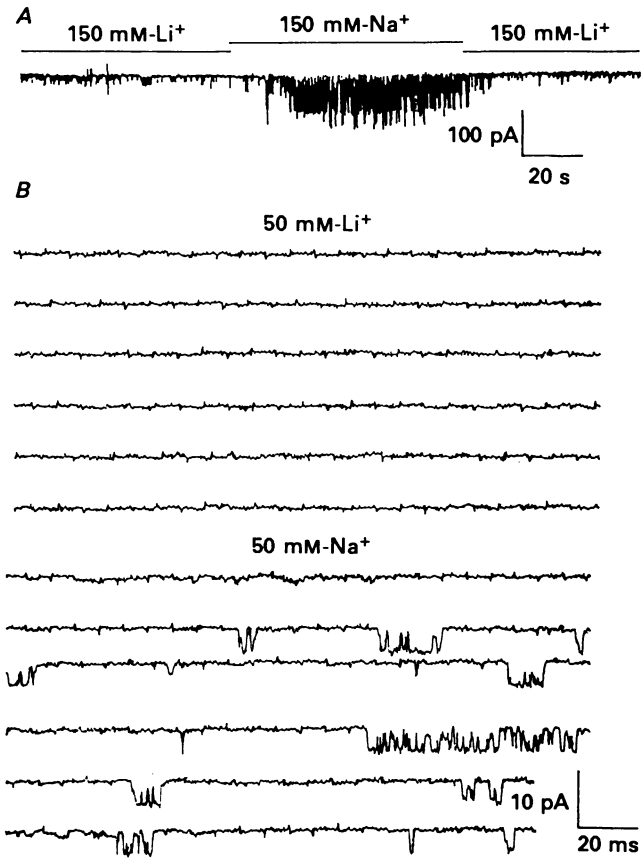

Fig. 6. Single-channel currents from inside-out patches. *A*: patch formed in 150 mM-Li^+ solution showed a marked, reversible increase in channel activity when bathing solution was changed to 150 mM-Na^+. *B*: single-channel activity in 100 mM-glucosamine, 50 mM-Li^+ solution (upper traces) and after replacement with 100 mM-glucosamine, 50 mM-Na^+ (lower traces).

concentrations. A record from one such experiment is shown in Fig. 9*A*. There was very modest channel activity in Na^+-free solution, with single-channel currents of the order of 4·5 pA. Changing briefly to a solution containing 150 mM-Na^+ produced a large fluctuating current with a mean amplitude of more than 40 pA. The results of this and two other experiments are plotted in Fig. 9*B*. The mean current varied from one patch to the next, presumably reflecting variations in the number of active

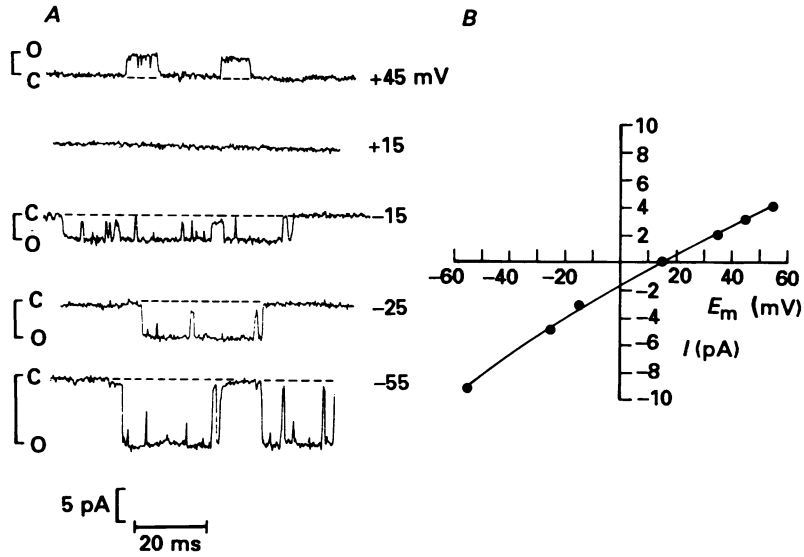

Fig. 7. Reversal of single-channel currents in 50 mM-Na^+ solution, containing 75 mM-K^+. *A*: currents at indicated holding potentials (O and C indicate open and closed channel currents). *B*: voltage–current relation with current reversal near E_K (+17 mV).

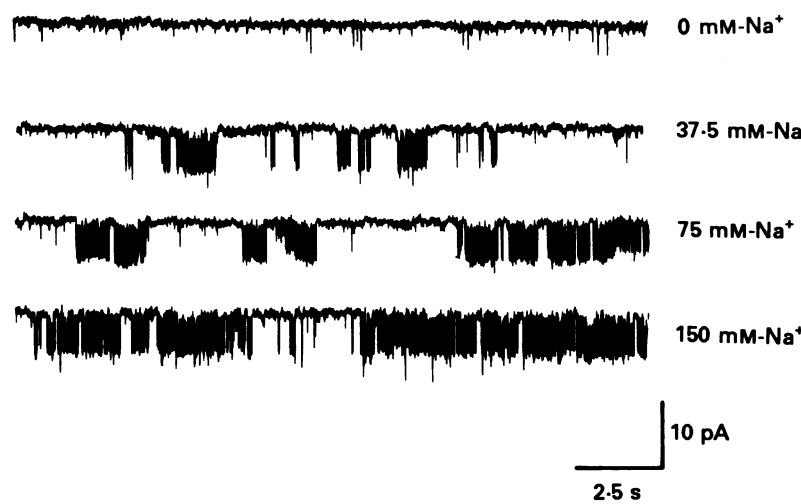

Fig. 8. Na^+ dependence of single-channel activity in inside-out patch, produced by replacing Li^+ by the indicated concentrations of Na^+.

channels present. In all three experiments the mean membrane current increased almost linearly with Na^+ concentration, with little or no sign of saturation, even at 150 mM.

DISCUSSION

The results presented here provide strong evidence for a Na^+-activated K^+ current ($I_{K(Na)}$) in brain stem neurones cultured from chick embryos. The currents appear to be identical to those found by Bader *et al.* (1985) in cultured chick's trigeminal

ganglion cells. They are different, however, from the Na^+-activated K^+ currents reported by Hartung (1985) in crayfish motoneurones, in that the K^+ currents in the crayfish cells were activated by Li^+ as well as by Na^+. This difference suggests that $I_{K(Na)}$ might be subserved by at least two classes of channel, distinguishable on the basis of their sensitivity to Li^+. If so, such heterogeneity would be similar to that

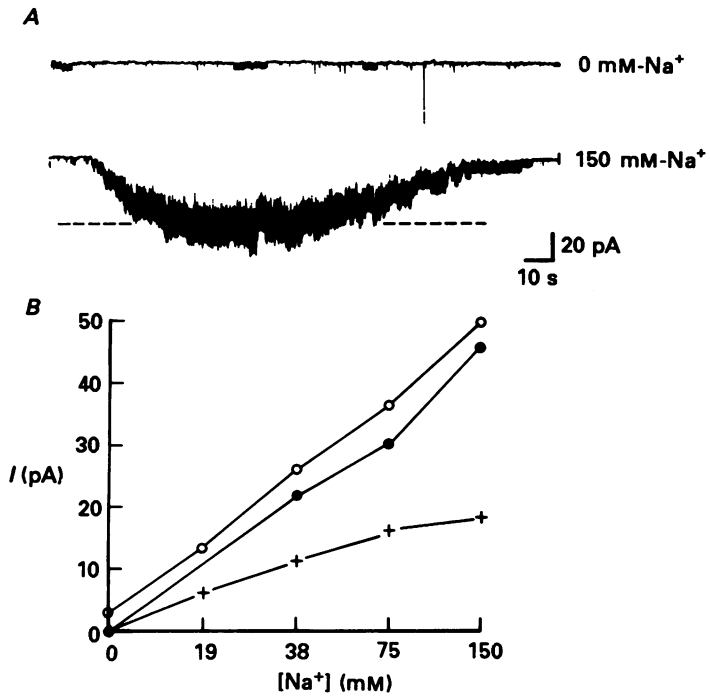

Fig. 9. Dependence of membrane current on Na^+ concentration in multichannel, inside-out patch. *A*: upper trace, channel activity in 150 mM-Li^+ solution. Lower trace, brief (1 min) change to 150 mM-Na^+ produced marked channel activity, with mean current (dashed line) of 46 pA. Activity returned towards previous level as Na^+ was washed out. *B*: relation between mean current and Na^+ concentration in three such patches.

observed in Na^+-dependent transport systems. Li^+ is ineffective in activating some of these (Yamamura & Snyder, 1973; Skou, 1975), but is effective in others (Lopilato, Tschuyia & Wilson, 1978; Beck & Rosen, 1979).

Recently, Constanti & Simm (1987) and Schwindt, Spain, Foehring, Chubb & Crill (1988) have described a slow K^+ current in central nervous system cortical neurones that is blocked by TTX and by exposure to Na^+-free solutions. It would seem from the slow time course of the current that it is different from that reported here. However, the difference may be related to Na^+, not K^+ kinetics: cortical neurones also display a non-inactivating TTX-sensitive Na^+ current (Stafstrom, Schwindt, Chubb & Crill, 1985; Constanti & Simm, 1987), which would allow a steady influx of Na^+ and, consequently, prolonged activation of $I_{K(Na)}$.

The present results provide information about the channels underlying $I_{K(Na)}$. We have not yet studied their kinetics, beyond the simple observation that they appear to open in bursts. With approximately normal, but reversed, K^+ concentrations,

their conductance was of the order of 50 pS, which is in the middle of the range of K^+ channel conductances (for a summary see Hille, 1984). With 75 mM-K^+ on the cytoplasmic face of the patch (rather than 5 mM) the channel conductance was about 100 pS. This effect of concentration provides some information about the nature of ion movement through the channel. It can be mimicked by any number of kinetic models, where K^+ ions 'hop' over a succession of energy barriers within the channel (see Hille, 1975), but is inconsistent with free diffusion of K^+ ions along a constant voltage gradient (a 'constant-field' model). The constant-field model predicts that the increase in K^+ concentration should have increased channel conductance about 6-fold, rather than by the observed factor of two. The conductance at the higher K^+ concentration was still considerably lower than that of Na^+-activated K^+ channels in mammalian cardiac myocytes, reported by Kameyama *et al.* (1984). Those had a channel conductance of about 200 pS at 30–35 °C, with $[K^+]_o = 150$ mM and $[K^+]_i = 49$ mM. In addition, the myocyte channels exhibited considerably more inward rectification than the channels reported here (Fig. 7).

Although $I_{K(Na)}$ was present in the absence of extracellular Ca^{2+}, it is nevertheless possible that the current was activated by displacement of Ca^{2+} from internal stores following Na^+ entry. This seems unlikely, however, as in cell-free patches single channels were activated by Na^+ with neither membrane face exposed to Ca^{2+}.

It seems reasonably clear that $I_{K(Na)}$ must contribute substantially to repolarization of the action potential. When the current was eliminated by Li^+ substitution (Fig. 2) or by 4-AP (Fig. 5*A*), the net inward current was prolonged, indicating overlap of the inward and outward TTX-sensitive currents. Moreover, the magnitude of $I_{K(Na)}$ at its peak was at least as great as that of the late outward current through the delayed rectifier channels (Figs 1, 2 and 4). Allowing for overlap between the inward and outward currents, it can be calculated (for example from Fig. 1) that about 60% of the inward charge movement through the Na^+ channels is restored by $I_{K(Na)}$.

The single-channel records in Figs 8 and 9 indicate that K^+ channel activation increases progressively with Na^+ concentrations ranging from 20 mM or less up to at least 150 mM. Assuming that E_{Na} is of the order of 50 mV, then intracellular Na^+ concentration will normally be somewhere in the range of 10–30 mM. This suggests that $I_{K(Na)}$ must make a finite contribution to the resting K^+ conductance. In addition, the observed range of sensitivities poses a quantitative dilemma: although concentrations in the millimolar range are required to activate the channels, the macroscopic experiments have shown that the entry of an insignificant amount of Na^+ results in a substantial outward current. For example, in Fig. 1 the net inward Na^+ movement (allowing for overlap between inward and outward currents) was about 500 fC, or 5×10^{-18} mol. When dispersed in a cell of radius 20 μm, this would produce a Na^+ concentration increase of less than 2×10^{-10} M. The mobility of Na^+ ions in free solution, compared with their rate of entry through the membrane, makes it unlikely that their bulk concentration would be transiently much higher near the membrane, but even if they were sequestered briefly in a rind as thin as 20 nm, the resulting increase in local concentration would still only amount to about 5×10^{-5} M.

One possible solution to the apparent quantitative disparity is that the voltage-sensitive Na^+ channels and the channels subserving $I_{K(Na)}$ are co-localized in the

membrane so that incoming Na^+ ions can act immediately on the Na^+-activated K^+ channels. An analogous theoretical problem arises with Ca^{2+}-activated K^+ channels, where such co-localization has already been demonstrated in vestibular hair cells (Roberts & Hudspeth, 1987). On the other hand, no such problem arises with the voltage-sensitive K^+ channels subserving the late current and, accordingly, co-localization with voltage-sensitive Na^+ channels appears not to occur, at least in skeletal muscle (Almers, Stanfield & Stühmer, 1983). Evidence available to date, then, suggests that ion-activated channels are co-localized with appropriate voltage-sensitive channels to form a functional unit. How two channels that are presumably separately coded protein entities are inserted into the plasma membrane with the required proximity presents an interesting problem in molecular biology.

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