Potassium Current Activated by Intracellular Sodium in Quail Trigeminal Ganglion Neurons

C. HAIMANN, L. BERNHEIM, D. BERTRAND, and C. R. BADER

From the Department of Physiology, Centre Médical Universitaire, the Division of Clinical Neurophysiology, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland; and the Department of Pharmacology Center for Peripheral Neuropathies, CNR Center of Cytopharmacology, 20129 Milan, Italy

ABSTRACT   Whole-cell voltage clamp and single-channel recordings were performed on cultured trigeminal ganglion neurons from quail embryos in order to study a sodium-activated potassium current (K_{Na}). When K_{Na} was activated by a step depolarization in voltage clamp, there was a proportionality between K_{Na} and I_{Na} at all voltages between the threshold of I_{Na} and E_{Na}. Single-channel recordings indicated that K_{Na} could be activated already by 12 mM intracellular sodium and was almost fully activated at 50 mM sodium. 100 mM lithium, 100 mM choline, or 5 μM calcium did not activate K_{Na}. The relationship between the probability for the channel to be open (P_o) vs. the sodium concentration and the relationship of K_{Na} open time-distributions vs. the sodium concentration suggest that two to three sodium ions bind cooperatively before K_{Na} channels open. K_{Na} channels were sensitive to depolarization; at 12 mM sodium, a 42-mV depolarization caused an e-fold increase in P_o. Under physiological conditions, the conductance of the K_{Na} channel was 50 pS. This conductance increased to 174 pS when the intra- and extracellular potassium concentrations were 75 and 150 mM, respectively.

INTRODUCTION

Several potassium currents play an important role in the control of neuronal excitability: they influence the resting membrane potential, control the firing pattern during a depolarization, and are able to put an end to a burst of action potentials. These various tasks are achieved by potassium currents with distinct properties. Some currents are voltage-gated like the delayed rectifier (Hodgkin and Huxley, 1952), the A current (Connor and Stevens, 1971), the slowly inactivating I_D current (Storm, 1988), or the inward rectifier (Katz, 1943; Sakmann and Trube, 1984). Other potassium currents are ligand-gated. Among these, are the various calcium-activated potassium channels that are activated as a consequence of the ionic fluxes that occur during action potentials. In this paper we describe another current of

this type, a potassium current activated by intracellular sodium. Such a current was initially described in heart cells (Kameyama et al., 1984). Later on, a sodium-activated potassium current (K_{Na}) has been found in invertebrate neurons (Hartung, 1985), in chick ganglionic neurons (Bader et al., 1985), in chick brainstem neurons (Dryer et al., 1989), and in cat cortical cells (Schwindt et al., 1989).

In this paper K_{Na} was investigated in cultured quail trigeminal ganglion neurons by both the whole-cell and single-channel recording techniques. The physiological role of K_{Na} has not yet been assessed but data from whole-cell recording suggest that this current might be activated by the inward sodium current underlying spike generation and thereby participates in the repolarization phase of the action potential. Single-channel recording indicates that this current is substantially activated at physiological intracellular sodium concentrations, suggesting a possible contribution of K_{Na} to the resting potential of neurons.

MATERIALS AND METHODS

Quail eggs from a commercial source were incubated 7–8 d at 38°C. Trigeminal ganglia were dissected and cultured using the method described by Kato and Rey (1982) for ciliary ganglia. Cultures were kept at 37°C in an humidified incubator gassed with a 10% CO_2/air mixture. Experiments could be performed as early as 4 h after plating. At this time neurons have almost no neurites and are sufficiently well attached to the bottom of the petri dish to be superfused (Bader et al., 1987). The experiments reported here were done either on freshly plated neurons (3–6 h) or on neurons in culture for 2–3 d. The culture medium was alpha-minimum essential medium (α-MEM, Gibco Laboratories, Grand Island, NY), supplemented with 10% heat inactivated horse serum, MEM vitamins, penicillin (100 U/ml), and streptomycin (0.1 mg/ml).

Whole-Cell Recording

For whole-cell recording, a petri dish was mounted on the stage of an inverted microscope. The superfusion solutions were gassed with oxygen, pumped into the dish through fine polyethylene tubing, and removed by suction through a second tube. The perfusion rate was 0.75–1.5 ml/min, depending on the experiment, and the changes in bathing solutions took ~1 min. Experiments were carried out at room temperature (22°C). Between recordings, cells were superfused with a solution containing (in millimolar unless specified otherwise): NaCl (150), KCl (5.4), $CaCl_2$ (3), HEPES (5), $MgCl_2$ (0.8), glutamine (2), glucose (16.7), bovine serum albumin (20 μg/ml), MEM vitamins, penicillin (100 U/ml), and streptomycin (0.1 mg/ml). During intracellular recording, a solution containing 3 mM cobalt chloride instead of calcium was superfused and is referred to as the "control solution." Sodium and potassium blocking agents, tetrodotoxin (TTX, Calbiochem-Behring Corp., San Diego, CA) tetraethylammonium (TEA, bromide salt, Fluka, Buchs, Switzerland), and 4-aminopyridine (4AP, Fluka) were simply added to this solution. The pH was adjusted to 7.4 with NaOH.

Recordings were made with patch electrodes and the whole-cell recording method (Hamill et al., 1981). Current injection and voltage recording were done with the same electrode using a chopping technique (Brenneke and Lindeman, 1974). The chopping frequency was 27.7 kHz. Voltage and current records were digitized (12 bit resolution) and stored on disks using a 16-bit microcomputer (PC-AT, IBM Corp., Danbury, CT). The current was filtered at 3 kHz (Krohn-Hite 3202) before digitization. Data could be replayed on- and off-line and

plotted on a digital plotter (model 7550A, Hewlett-Packard Co., Palo Alto, CA). Acquisition, analysis and graphical representation was performed with the program DATAC (Bertrand and Bader, 1986).

Patch electrodes (1-μm tip opening) were prepared from borosilicate tubing (No. 7740, Corning Glass Works, Corning, NY) with a microprocessor-controlled puller (BB-CH, Mecanex S.A., Nyon, Switzerland); the fire-polishing which occurred during the symmetrical pulling procedure (Bertrand et al., 1983) was sufficient to give good seals between the electrode and the neuronal membrane. With a few exceptions mentioned in the text the electrodes were filled with (in millimolar): potassium acetate (130), KCl (10), NaCl (10), EGTA (5), HEPES (5), and glucose (5); the pH was adjusted to 7.4 with NaOH. The electrode resistance in the superfusion solution was <5 MΩ and the membrane-electrode resistance before breaking the patch exceeded 5 GΩ, as measured by the amplitude of the voltage deflection induced by a 25-pA current pulse applied through the constant current injection circuit of our head stage (Colburn and Schwartz, 1972).

Recording from single cells was possible for 20 min or more. In most cells, however, a given experimental protocol could be completed in <10 min. In one series of experiments, a second patch electrode was used to monitor the voltage at a site distant from the voltage clamping electrode. The second electrode was connected to an amplifier of the same type as that used for the voltage clamping electrode. The performance of the single-electrode voltage clamp was slightly affected by the presence of the second electrode. Optimal functioning of the clamp was achieved by adjusting the capacity compensation of the second amplifier. The adjustment was made so as to obtain the best rise time on a 90-mV voltage step in voltage clamp and care was taken to avoid oscillations of the second amplifier. The voltage clamp amplifier was then further adjusted with the gain and the capacity compensation to obtain optimal rise time without oscillation in the initial part of the voltage step.

Single-Channel Recording

For single-channel recordings, trigeminal neurons cultured between 4 h and 2 d were used. Experiments were performed at room temperature. Thick-wall, borosilicate glass electrodes (10 MΩ) were used. Inside-out patches were obtained by realizing first a cell-attached patch. Once the seal between the electrode and the membrane was formed, a calcium-free solution containing 20 mM EGTA was superfused and the pipette pulled away from the cell. The tip of the pipette was then exposed to air for ~500 ms and dipped again in the superfusion solution (Hamill et al., 1981). The resistance of the seals between the electrode and the cell membrane exceeded 5 GΩ. Before and after the electrophysiological recordings neurons were continuously superfused with (in millimolar unless specified otherwise): NaCl (150), KCl (5.4), CaCl$_2$ (0.5), MgCl$_2$ (5), HEPES (5), glutamine (2), glucose (16.7), bovine serum albumin (20 μg/ml), MEM vitamins, penicillin (100 U/ml), streptomycin (0.1 mg/ml). The pipette or extracellular solutions and the bath or intracellular solutions (pH 7.3–7.4) used in individual experiments are described in the figure legends.

Single-channel currents were recorded with a patch-clamp amplifier (EPC-7, List Electronic, Darmstadt, FRG) and filtered with a low-pass filter at 2 kHz (3202; Krohn-Hite Co., Avon, MA) before digitization. Voltage and current records were digitized at 3.3 or 10 kHz (Fig. 5) at 12-bit resolution. Single-channel current amplitudes were measured either directly on the screen or from current amplitude histograms.

For both whole-cell and single-channel recordings, the grounding of the bath was ensured through a silver/silver chloride electrode dipped in a polyethylene tubing filled with a saline/agarose gel. In the various superfusion solutions, the liquid junction potential with the electrode in the bath did not vary by >2 mV.

RESULTS

K_{Na} in Whole-Cell Recordings

We reported previously that a current which disappears in the presence of TTX during a step depolarization to -10 mV from a steady holding level of -100 mV consists of an early inward current followed by an outward current which decays within a few milliseconds (Bader et al., 1985). >80% of the neurons had this outward current suppressible by TTX. Taking into account the well-established specificity of TTX as a sodium channel blocker (Narahashi et al., 1964; Ritchie and Rogart, 1977), it did not seem reasonable to believe that TTX was directly affecting an outward current channel. A more likely explanation was that the disappearance of this current was related to the suppression of the inward sodium current by TTX. Indeed, replacing extracellular sodium ions with choline (three neurons) or Tris (two neurons) gave the same results as added TTX (Fig. 1). It can be seen in Fig. 1 that the progressive decrease of the sodium current during superfusion with the sodium-free solution was accompanied by a parallel reduction in the outward current. The effects of sodium substitution were fully reversible. Therefore, it appeared that trigeminal ganglion neurons possess an outward current that can be activated by sodium influx.

The effect of sodium on this outward current is not mediated by a secondary rise in the intracellular calcium concentration. The presence of EGTA (5 or 20 mM on occasions) in the intracellular perfusate should have kept the intracellular calcium concentration constant and therefore have prevented the activation of calcium-dependent potassium channels. Sodium entry in a neuron, by inducing a metabolic load, might decrease the adenosine triphosphate (ATP) concentration and thereby activate ATP-dependent potassium channels (Noma, 1983). This does not seem very likely either, since the outward current described here can also be observed in the presence of 5 mM ATP in the intracellular fluid.

Potassium ions carry the outward current. The nature of the ion carrying the outward current was determined by examining the relaxation currents and the reversal potential of the TTX-sensitive outward current (Fig. 2). The differences between the relaxation currents recorded at a given voltage in the absence and presence of TTX were measured at the time indicated by the arrow in Fig. 2 *A* and were plotted as a function of the final voltage in Fig. 2 *B*. The data can be fitted adequately with a Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin and Katz, 1949):

$$I_K = Pz^2(F^2 \ V/RT)([K]_i - [K]_o \exp[-zVF/RT])/(1 - \exp[-zVF/RT]) \qquad (1)$$

A similar observation was made in four neurons. When the extracellular potassium concentration was increased, the behavior of the relaxation currents was again that predicted for a current carried by potassium ions (three neurons, data not shown), suggesting that potassium ions are the major charge carriers for the current, which we call K_{Na}.

K_{Na} can be activated steadily by an increase in the intracellular sodium concentration. This was found by recording from a cell with a patch pipette containing 50 mM sodium and by measuring the current before and after application of 4AP and TEA. The difference in current intensity in the absence and in the presence of these two

drugs reveals the size of the potassium current activated by the elevated intracellular sodium concentration.

A few precautions had to be taken in the design of this experiment. First, it was necessary to demonstrate that the current suppressed by 4AP and TEA was indeed a potassium current. TEA and 4AP have been shown to reduce a neuronal current carried by hydrogen ions in an invertebrate preparation (Byerly et al., 1984). Secondly, the voltage range over which the sodium-activated current is measured must not overlap the voltage range over which voltage-dependent potassium currents are activated. Thirdly, in these experiments the presence of a steady high intracellular sodium concentration may lead to indirect changes in the concentration of intracellular calcium or ATP; any of these changes might in turn activate potassium currents (Meech, 1978; Noma, 1983). In an attempt to minimize these changes, we used both EGTA and ATP at a concentration of 5 mM in the patch pipette.
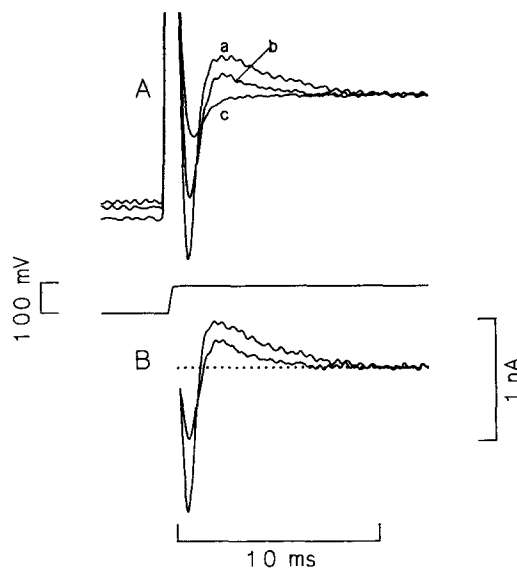

FIGURE 1. Inward and outward currents suppressible by low extracellular sodium. A neuron was superfused with the control solution described in the Methods. (*A*) The voltage was held at −100 mV and stepped every 5 s to −10 mV for 10 ms and the current was recorded (trace labeled *a*). A solution of the same composition but with choline replacing sodium ions was then superfused and the current was recorded during a same voltage step. The traces labeled *b* and *c* were recorded 1 and 2 min after the beginning of the superfusion with the sodium-free solution. (*B*) The difference between traces *a* and *c* in *A* was plotted together with the difference between traces *b* and *c*.

The results of three different experiments are illustrated in Fig. 2 *C*. In one case, the current-voltage relationship was determined while the intracellular medium contained 50 mM choline and it can be seen in Fig. 2 *C* (△) that no current was suppressed by 4AP and TEA. In the two other experiments illustrated in Fig. 2 *C*, the sodium concentration inside the pipette was 50 mM and in both cases 4AP and TEA suppressed a current (○ and +). The data can be fitted by a Goldman-Hodgkin-Katz equation (see Eq. 1) computed with the actual intra- and extracellular potassium concentrations present in these two experimental conditions. The results suggest that sodium ions, present at a concentration of 50 mM in the patch pipette, activate a steady current suppressible by a mixture of 4AP and TEA, and that this current is a potassium current since its polarity and apparent reversal potential behave as expected for a current carried by potassium ions.
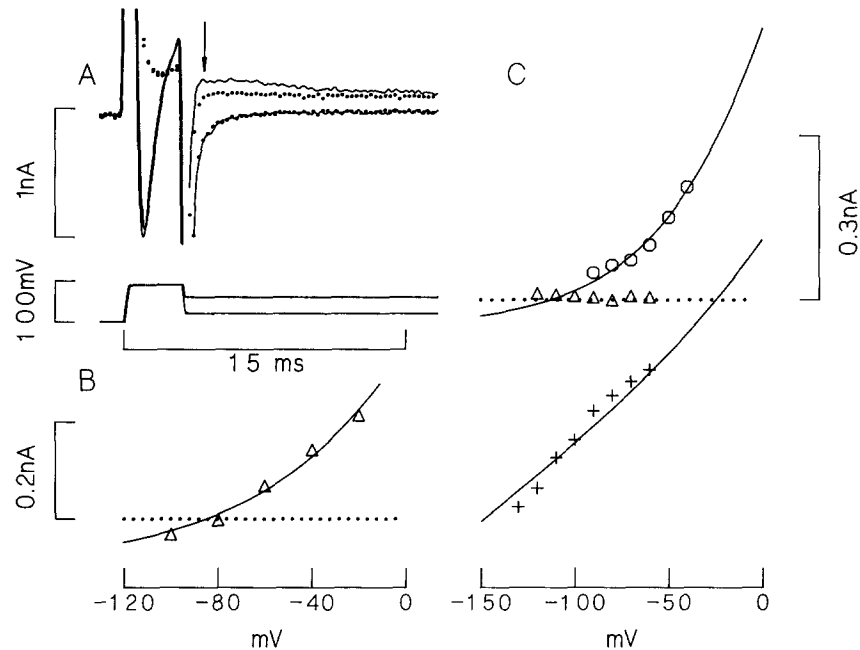
FIGURE 2. Reversal potential of the outward current activated by sodium. (A) The voltage of a neuron was held at −100 and stepped to −10 mV for 3 ms, to activate the outward current (*continuous line*). The voltage was then stepped to another value (−40 and −80 mV are illustrated), and the resulting relaxation currents were recorded. TTX was then added to the superfusion solution and the same protocol was repeated (*dotted line*). Note that at −80 mV, a voltage near E_K, the dots superimpose on the continuous line. (B) The difference in the relaxation currents measured in A for a given voltage in the absence and in the presence of TTX were measured at the time indicated by the arrow. The values were plotted as a function of the final voltage. The continuous line is Eq. 1 fitted to the data using the potassium concentrations in the superfusion solution (5.4 mM) and in the patch pipette solution (140 mM). (C) Steady activation of a potassium current by intracellular sodium. Results from three neurons. The voltage of a neuron was held at −100 mV and stepped for 30 ms to several voltages between −130 and −40 mV. This experimental procedure was done first in the absence and then in the presence of 20 mM TEA and 4 mM 4AP. The difference between the currents recorded at a given voltage in these two conditions was measured and plotted as a function of the voltage. (△) Measurements obtained when recording from a neuron with a patch pipette containing (in millimolar): choline chloride (50), EGTA (5), potassium acetate (79), KCl (20), KOH (13), HEPES (5), and glucose (5). The superfusion solution was a modified control solution (110 mM NaCl and 40 mM KCl). The crosses represent measurements obtained in conditions similar to that just described except that the intracellular solution contained 50 mM sodium (40 mM NaCl and 5 mM Na$_2$ATP) instead of choline (E_K was at −24 mV). Eq. 1 was fitted to the data (*continuous line*). (○) Measurements obtained when the superfusion solution contained 1.2 mM potassium and the intracellular potassium concentration was set to 90 mM (E_K was −110 mV and the intracellular sodium concentration was 50 mM).

Could K_{Na} be a voltage clamp artifact? An outward potassium current similar to that illustrated in Fig. 1 might be generated as a consequence of inadequate voltage clamping. If the potential of part of the membrane was not adequately controlled, an action potential (partial or complete) might be triggered in that part of the membrane during a depolarizing step and this action potential could, in turn, activate additional voltage-gated potassium currents. Obviously, such unclamped sodium action potentials would disappear in the presence of tetrodotoxin or in the absence of sodium. Therefore, a difference between outward currents recorded in the presence and absence of sodium could simply be due to additional voltage-gated potassium currents activated by the unclamped action potential.

To evaluate whether isopotentiality of freshly plated neurons was satisfied under our voltage clamp conditions, we recorded the potential at a second site, distant from the voltage clamp electrode by ~10 μm (cell diameter ~20 μm). One electrode was used for the single-electrode voltage clamp with the chopping technique, per-
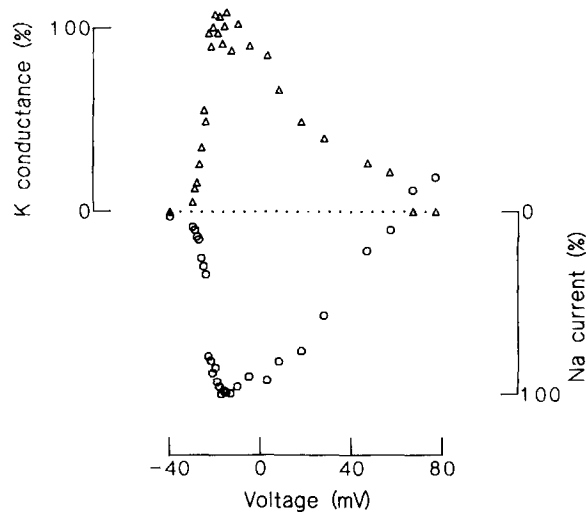

FIGURE 3. Correlation between the size of the sodium current and the conductance of K_{Na}. Trigeminal neuron, 4 h in culture. Peak amplitudes of the sodium current (O) were plotted as a function of the voltage during a depolarizing step from −100 mV. The peak inward current suppressed by TTX at each voltage was normalized to the maximum value of the current. K_{Na} conductance (Δ) was calculated from the maximum difference in outward current between pairs of current recordings obtained at the same voltage in the absence and in the presence of TTX.

mitting a direct reading of the voltage at the tip of this electrode. The second electrode was used exclusively to record voltage. In three neurons, the potential recorded by the two electrodes during a depolarizing voltage step exactly superimposed. However, we also found neurons in which the voltages recorded by the two electrodes were not the same. In six neurons, a brief voltage deflection of 5–15 mV was recorded at the distant electrode at the beginning of the step to −10 mV. Later during the step, there was a sustained small hyperpolarization (2–3 mV) with respect to the voltage recorded by the voltage clamping electrode. Thus, in some cases the soma appears to be isopotential under voltage clamp while in other cases it is not. To conclude, experiments with two electrodes suggest that spherical somata without processes are not always isopotential under our single-electrode voltage clamp conditions.

A simpler way of assessing the quality of the voltage clamp consists of examining how the clamp controls the inward sodium current in a range of membrane poten-

tials between the threshold for the activation of the sodium current and the voltage at which this current reaches its maximum amplitude. It is in this voltage range where the fast regenerative process underlying an action potential develops that an escape from adequate clamping is likely to occur, giving rise to a sharp discontinuity in the current-voltage relationship. In Fig. 3, an example of an adequate voltage clamp in a trigeminal neuron, the amplitude of the sodium current (○) increases progressively with depolarizations above −30 mV. The K_{Na} conductance was computed from the maximum difference in outward current between pairs of recordings obtained at the same voltage in the absence and in the presence of TTX. Fig. 3 shows that the size of the K_{Na} conductance (△) parallels the inward sodium current. In particular, it can be seen that K_{Na} drops progressively as the membrane potential approaches the sodium equilibrium potential, as one would expect from a conductance linked to the magnitude of the sodium current. This experiment speaks against K_{Na} being an artifact, at least in neurons adequately clamped as judged by the criterion illustrated in Fig. 3. In a total of 30 cells the peak value of K_{Na} conductance during a step depolarization to −10 mV was 8.7 ± 3.5 nS (mean ± SD).

Single-Channel Recording of K_{Na}

An inside-out patch of membrane was held at a potential of −50 mV (cytoplasmic face of the membrane negative) and several solutions were applied to the cytoplasmic side of the membrane. In the presence of 100 mM choline there was no channel activity (Fig. 4 A) but during superfusion of 100 mM sodium (Fig. 4 B), two channels became active and this activity persisted during the several-minute exposure to sodium. Lithium (100 mM) failed to evoke single-channel activity (Fig. 4 C), but activity was restored as soon as sodium was applied again (Fig. 4 D). Note that the lithium solution also contained a calcium buffered solution (5 μM) and that the sodium solution contained EGTA and no calcium. Thus, the channel activity observed in Fig. 4 cannot be due to calcium-activated channels. At −50 mV, a potential which corresponds to the resting membrane potential for cultured trigeminal neurons, the channel activity occurred in bursts with a rapid flickering between open and closed states. Relatively long periods without activity separated the bursts. In a series of 65 inside-out patches, 38.5% had sodium-activated channels. Of these, 66% had more than one channel. The number of channels, as determined from the number of increasing current steps, remained constant for the duration of the experiment. In addition, membrane patches often contained other channels with conductances smaller than that of the sodium-activated channel but these channels will not be considered here.

Fig. 5 A illustrates, on a faster time scale, examples of the elementary current in a patch containing only one channel. It appears that the channel may enter at least two open states in addition to the main conducting state. The current amplitude of the substates represents ~29% and 76% of the maximum amplitude of the elementary current. Substates were usually preceded and followed by the main conducting state and were only occasionally seen in isolation. The amplitude distribution of the single-channel current in Fig. 5 B indicates the presence of three current peaks (3.6, 9.4, and 12.4 pA). The leftmost peak (0 pA) represents the amplitude distribution of the noise. There are no additional current peaks to the right of the largest

current level, indicating that the various current peaks correspond probably to substates of a same channel and not to several types of channels present in the patch.

The amplitude distribution was fitted with a sum of four Gaussian functions (Fig. 5 B). The width of the distribution of the two sublevels is larger than that of the distribution of the noise or of the main level. This could reflect rapid and unresolved flickering between substates. Another possibility is that there are additional substates. The area under each Gaussian function was divided by the total area of the histogram to evaluate the probability for the K_{Na} channel to be in one of the
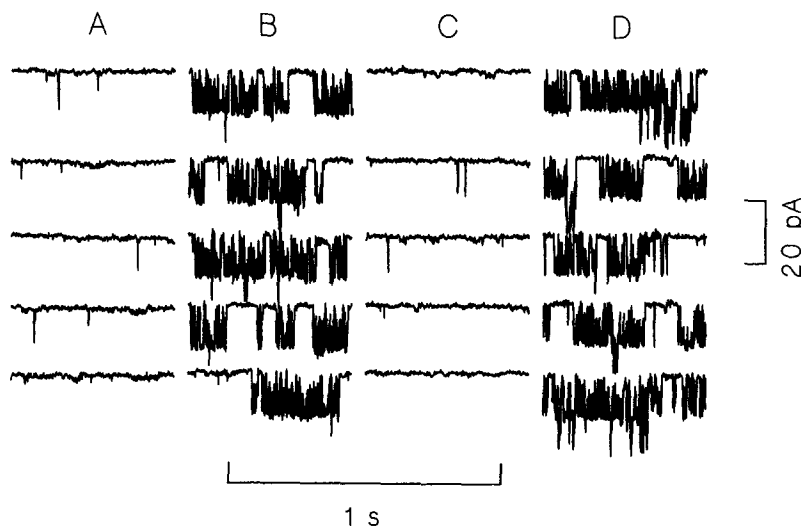

FIGURE 4. Effect of sodium, choline and lithium on K_{Na}. An inside-out membrane patch was excised from a trigeminal neuron and was maintained at a potential of -50 mV. Downward deflections represent openings of the channel. (A) Five consecutive 600-ms traces taken every 600 ms while the intracellular face of the patch was exposed to a sodium-free solution of the following composition (in millimolar): choline chloride (100), KF (75), HEPES (5), and EGTA (20), pH 7.3. (B) Five consecutive traces from the same patch as in A exposed to a solution in which 100 mM NaCl replaced choline chloride. (C) The patch was then exposed to a solution in which sodium ions were replaced by lithium and the calcium concentration was set to 5 μM (19.66 mM $CaCl_2$ were added to the solution containing 20 mM EGTA). (D) The same solution as in B was superfused again. The solution inside the pipette was (in millimolar unless specified otherwise): NaCl (5), KCl (140), KF (10), HEPES (5), EGTA (5), and tetrodotoxin (1 μM).

open states. These probabilities were 0.44 (12.4 pA), 0.19 (9.4 pA), and 0.13 (3.6 pA). The overall probability for the channel to be in an open configuration was 0.76 at this intracellular sodium concentration (100 mM) and voltage (-50 mV). Substates were also present when the membrane was depolarized, but a detailed analysis on their voltage dependence was not made.

Conductance and ionic selectivity of K_{Na} channels. Fig. 6 A shows the current–voltage relationship measured in five different patches superfused at the cytoplasmic face of the membrane with a solution containing 100 mM sodium and 75 mM

potassium. The solution in the pipette contained 150 mM potassium. The current–voltage relationship is well described by a Goldman-Hodgkin-Katz equation (Eq. 1). The adequacy of the fit indicates that potassium ions are the major charge carriers and that there is no rectification of the channel over the voltage range tested. Between −70 and 0 mV, the elementary conductance of the sodium-activated potassium channel was 174 ± 24 pS (mean ± SD of five patches).

A selective permeability for potassium ions was confirmed by experiments in which inside-out patches were exposed to solutions with different ratios of intra- to
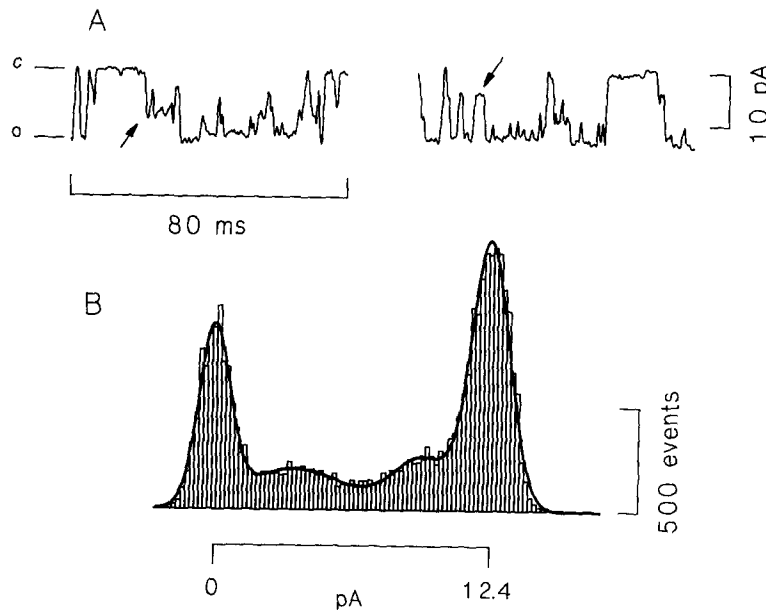

FIGURE 5.   Substates for K_{Na} channels. An inside-out patch of membrane was excised from a trigeminal neuron. The patch was maintained at a potential of −50 mV during exposure to 100 mM sodium. (A) Two traces are represented and the letters c and o indicate the level of the current when the channel is closed or open in the maximally conducting state, respectively. The arrows indicate substate levels. (B) Amplitude histogram of a 12-s recording of the channel activity (60 current traces each lasting 200 ms were sampled every 600 ms; sampling frequency 10 kHz, filter 2 kHz). The histogram was fitted with a sum of four Gaussian functions, centered at 0, 3.6, 9.4, and 12.4 pA. The 0 pA level corresponds to the peak of the noise. The solution inside the pipette was as in Fig. 4. The superfusion solution was (in millimolar): NaCl (100), KF (75), HEPES (5), and EGTA (20).

extracellular concentrations of potassium. Single-channel currents reversed near the predicted equilibrium potential for potassium (Fig. 6 B) and modifications of the intracellular sodium or chloride concentrations had no influence on the reversal potential of K_{Na}.

A change in chord conductance was seen when the potassium concentration in the pipette, i.e., the extracellular potassium concentration (K_o), was modified. Potassium was replaced by sodium and the concentrations of all other ions were kept
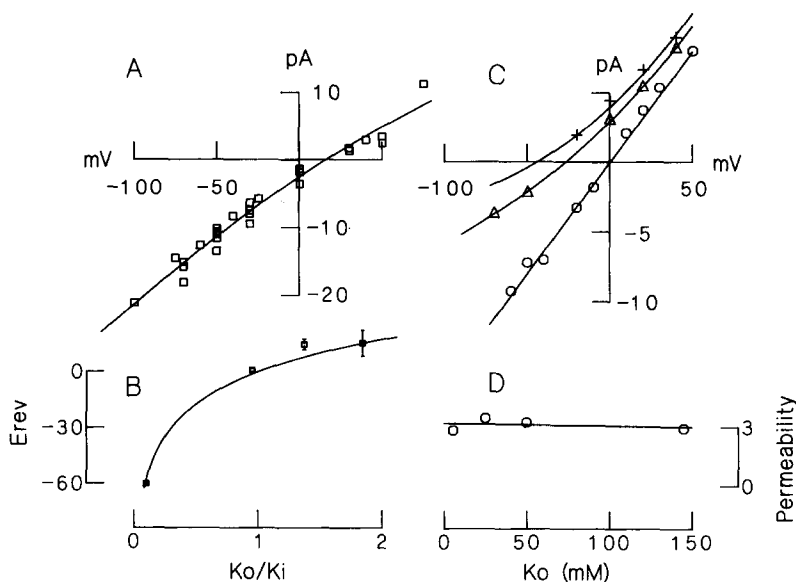
FIGURE 6. Reversal potential of K_{Na}. (A) Current–voltage relationship measured in five inside-out patches exposed to 100 mM sodium. The voltage was changed stepwise and the amplitude of the current measured as a function of voltage. The line through the data is Eq. 1. Between -70 and 0 mV the conductance is 174 pS. The superfusion solution and the solution in the pipette were the same as in Fig. 5. (B) The reversal potential of K_{Na} in several inside-out patches were plotted as a function of the ratio between the extra- and intracellular potassium concentration. The continuous line is the Nernst equation. The potassium concentration ratios were (number of patches tested are in parenthesis): 0.1 (1), 1 (8), 1.4 (5), and 1.8 (5). The bars are standard deviations. (C) Effect of the extracellular potassium concentration on the current–voltage relationship of K_{Na} in an outside-out patch. The patch pipette contained (in millimolar): KF (130), KCl (20), EGTA (20), Mg (1), ATP (5), glucose (5), HEPES (5), and NaCl (58), which was added to activate K_{Na}. The outside-out patch was exposed to 150 mM K (O), 50 mM K ($\triangle$), and 25 mM K (+). The extracellular solution with 150 mM potassium contained (in millimolar unless specified otherwise): $CoCl_2$ (3), TTX (1 μM), HEPES (5), $MgCl_2$ (0.8), glutamine (2), glucose (16.7), bovine serum albumin (20 μg/ml), MEM vitamins, penicillin (100 U/ml), streptomycin (0.1 mg/ml), KCl (140), KF (10), and NaCl (40). Other potassium concentrations were obtained by replacing KCl by equimolar amounts of NaCl. The lines are Eq. 1. (D) The permeability, computed by fitting Eq. 1 to the data in C, was plotted as a function of the extracellular potassium concentration (regression line: $y = -0.0017x + 3.17$). Permeability is expressed as 10^{-13} cm^3/s.

constant. In this condition, the conductance decreased from 174 ± 24 pS (n = 5) in 150 mM K_o to 139 ± 10 pS (n = 8) in 75 mM K_o (the intracellular potassium concentration was 75 mM in both experiments). To examine whether or not the decrease in conductance might be due to the reduction in the concentration of potassium ions in the extracellular medium, we applied several extracellular potassium concentrations on an outside-out patch, in which single-channel activity was induced by 50 mM sodium in the patch pipette. The K_{Na} channel was distinguished

from other channels which could have been present in the patch by its elevated elementary conductance and by the typical pattern of openings in bursts at −50 mV. Fig. 6 C shows that the current intensity in different extracellular potassium concentrations fits predictions of the Goldman-Hodgkin-Katz equation assuming a constant permeability (Fig. 6 D). Thus, we conclude that the reduction in conductance observed when the extracellular potassium concentration is reduced can be
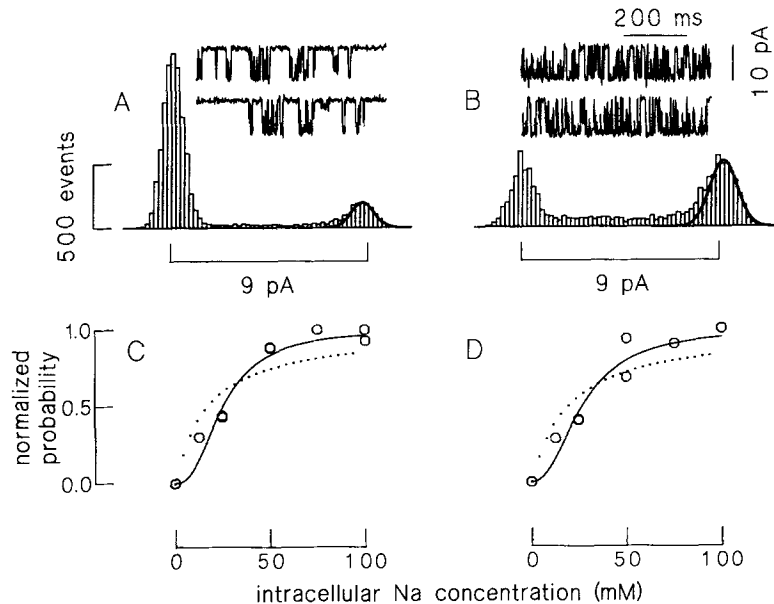

FIGURE 7.   Effect of different intracellular sodium concentrations on the activity of K_{Na} in an inside-out patch of membrane. (A and B) Channel activity and amplitude histograms of the activity recorded in the presence of 12.5 and 50 mM intracellular sodium, respectively. Consecutive 600-ms traces are plotted to illustrate the behavior of K_{Na} in both situations. The holding potential was −50 mV. The pipette solution was as described in Fig. 4. The superfusion solution contained (in millimolar): KF (75), EGTA (20), HEPES (5), and either NaCl (12.5) and choline (87.5) or NaCl (50) and choline (50). (C) The probability for the K_{Na} channel to be in the maximally conducting state was normalized at various sodium concentrations to the maximum probability for each of the two patches (see Table I). At each concentration, the probability was computed by dividing the area of the Gaussian curves (continuous lines in A and B) by the total area of the corresponding histogram. (D) Same as in C except that the probability to be in any conducting state was considered. The lines are Eq. 2 fitted to both sets of data. Dotted lines are Eq. 2 with $n = 1$ (i.e., a Michaelis-Menten equation) fitted to the data. Concentrations of sodium <100 mM were obtained by substituting NaCl by equimolar amounts of choline chloride.

explained by the constant-field theory. In the presence of physiological intra- and extracellular potassium concentrations the conductance of K_{Na} was 50 pS at +10 mV.

K_{Na} sensitivity to intracellular sodium. The results presented so far indicate that K_{Na} channels are activated when high concentrations of sodium are present at the

cytoplasmic face of the membrane. Does K_{Na} activation also occur in the presence of lower, probably more physiological sodium concentrations? To answer this question the dose–response relationship between K_{Na} activity and sodium concentration was determined in inside-out patches. Fig. 7, A and B illustrates two sets of recordings obtained in the same patch exposed to 12.5 or to 50 mM sodium, at a membrane potential of −50 mV. K_{Na} activity was still present with a sodium concentration of 12.5 mM. When the intracellular sodium concentration was increased, the probability for the channel to be open increased. In both amplitude histograms, the area under the rightmost peak relative to the area of the entire histogram represents the fraction of the time the channel was in the maximally conducting state. Increasing the sodium concentration from 12.5 to 50 mM increased this fraction from 0.13 to 0.39 in this patch.

In Fig. 7 C the normalized probability for K_{Na} to be in the maximally conducting state in two patches is plotted as a function of sodium concentration (see Table I, for the actual values). The range of sodium concentrations examined was 0–100 mM and the potential was kept at −50 mV. It can be seen that there is a steep

TABLE I

Probability for K_{Na} Channels To Be Open in Different Sodium Concentrations

Sodium (mM)	12.5	25	50	75	100
Patch A main state	ND	0.10	0.21	0.24	0.22
Total	ND	0.25	0.38	0.50	0.55
Patch B main state	0.13	0.19	0.39	ND	0.44
Total	0.22	0.31	0.71	ND	0.76

Two inside-out patches (A and B) were exposed to several intracellular sodium concentrations. The probability to be in the main open state was obtained by dividing the area of the main current level peak in the amplitude histogram by the total area of the histogram (see Fig. 7, A and B). The total probability was computed by dividing the total area minus the noise peak by the total area of a histogram. Underlined numbers were used for the normalization procedure that yielded Fig. 7, C and D. ND, not determined.

dependence on sodium concentration up to 50 mM and that beyond this concentration saturation occurs. The dose–response relationship could be fitted with a Hill equation (Hill, 1910):

$$Y = K_h [X]^n / (1 + K_h [X]^n) \tag{2}$$

where the Hill coefficient (n) was 2.39 and the value of K_h 4.5 × 10^{-4}. A Hill equation with n equal to 1 did not fit the data as well (*dotted line*, Fig. 7 C). The same procedure was repeated with the overall probability for K_{Na} to be open (all states considered). This probability was calculated by measuring the area under the noise and the total area of the amplitude histograms, and plotted as a function of the sodium concentration in Fig. 7 D. The fit with Eq. 2 gave a value of 2.16 for n and 8.2 × 10^{-4} for K_h. Apparently all substates are similarly affected by the intracellular sodium concentration.

To examine the effect of intracellular sodium concentration on the open time of K_{Na}, open time distributions (all substates considered) were determined in record-

ings taken in the presence of 12, 50, and 100 mM sodium. The open time distributions were fitted with a single exponential or a sum of two or three exponentials with a simplex algorithm (see Bertrand and Bader, 1986). At all concentrations, a sum of two exponentials gave a better fit than a single exponential, based on the calculated goodness of fit. Three exponentials did not improve the fit with respect to two. We found that different intracellular sodium concentrations did not modify the open time distributions, which were made up of a short and of a long open time population. For the three pairs of exponentials the ratios of the amplitude coefficients (shorter open time population/longer open time population) were similar (4.2 ± 0.6, mean ± SD) as were the time constants (0.93 ± 0.05 and 9 ± 1.8 ms), indicating that the relative distribution of short and long open times remained the
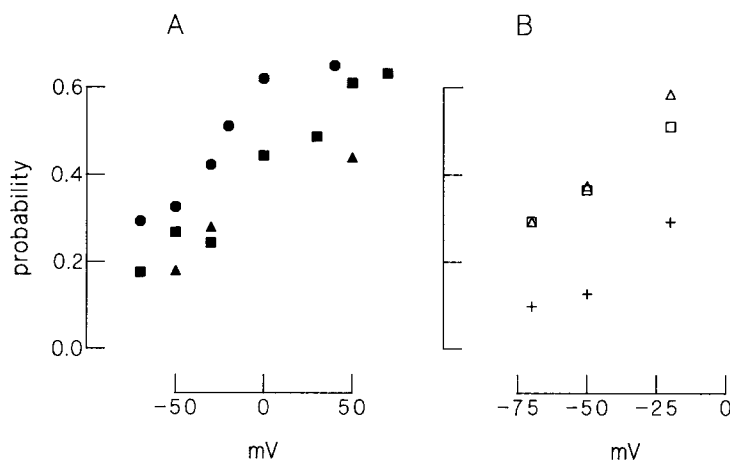

FIGURE 8.   Effect of membrane potential on K_{Na}. (A) The probability for the K_{Na} channel to be in the maximally conducting state was plotted as a function of the holding voltage in three inside-out patches. The potassium concentration in the pipette was 150 mM (O and □) and 85 mM (△). The superfusion solution contained 100 mM Na and 75 mM K (O); 75 mM Na, 25 mM choline, and 75 mM K (□); 75 mM Na, 70 mM sucrose, and 62 mM K (△). (B) Effect of the potential examined at different intracellular sodium concentrations. Same patch as illustrated by the circles in A. The sodium concentrations were 12.5 mM (+), 50 mM (△) and 100 mM (□).

same in the presence of the three sodium concentrations tested. Thus, intracellular sodium promotes the opening of the K_{Na} channel but has little influence on the duration of individual openings.

Effects of membrane potential on K_{Na}. Whole-cell recordings indicated that K_{Na} is activated very rapidly during a depolarizing step, suggesting a role for this current in the repolarization phase of the action potential (Bader et al., 1985). Rapid activation of K_{Na} could be due to an accumulation of sodium underneath the membrane leading rapidly to a high concentration. Another factor that could also influence K_{Na} activation could be the depolarization itself. The relationship between membrane voltage and the probability for K_{Na} to be in the main open state was investigated first at a sodium concentration that induced the maximal response when the patch was

held at -50 mV. Fig. 8 A illustrates the results obtained in three patches exposed to a high concentration of sodium. During depolarization there was approximately a threefold increase of the probability over a voltage range between -70 and $+70$ mV. In the absence of intracellular sodium, membrane depolarization alone did not activate K_{Na}, indicating that the gating was primarily sodium-dependent. The effect of membrane potential at different sodium concentrations is shown in Fig. 8 B. In the same patch, a depolarization by 50 mV (from -70 to -20 mV) increased the probability nearly three times at 12 mM intracellular sodium and approximately twice at 50 and 100 mM sodium.

DISCUSSION

Sodium-activated potassium currents have now been reported in several preparations, guinea-pig heart myocytes (Kameyama et al., 1984), invertebrate neurons (Hartung, 1985), avian ganglionic (Bader et al., 1985) and brainstem neurons (Dryer et al., 1989), and cat cortical neurons (Schwindt et al., 1989). The present study indicates that intracellular sodium concentrations in the physiological range can activate K_{Na} single-channel activity in trigeminal neurons and that the opening of the channel, as we shall discuss, seems linked to the cooperative binding of two or three sodium ions. The channel does not rectify and the conductance increase observed in high extracellular potassium can be explained by the constant field theory. The probability for K_{Na} channels to be open increases at depolarized voltages.

While single-channel recording clearly demonstrated the existence of K_{Na}, in whole-cell recordings we were concerned that what we thought to be a transient activation of a sodium-activated current was the consequence of an imperfect space clamp. However, the close correlation between the magnitude of the K_{Na} conductance and the amplitude of the sodium current over a wide range of voltages, from the threshold of the sodium current to its reversal potential is evidence against this possibility. In addition, it is important to note that the drop of the K_{Na} conductance observed at voltages more depolarized than -20 mV (Fig. 3) occurred in spite of the increasing driving force for potassium with depolarization. Therefore, the size of K_{Na} was correlated primarily with the size of the sodium current, as one would expect from a current activated by the influx of sodium into the cell and K_{Na} is not an artifact.

This conclusion agrees with results obtained by Dryer et al. (1989) in brainstem neurons. They found that lithium did not activate K_{Na} in single-channel or in whole-cell recording despite the fact that lithium was as effective as sodium in carrying the inward current during depolarizing steps in brainstem neurons. Unfortunately, experiments with lithium are not as clear in preparations other than brainstem neurons. In whole-cell recordings from invertebrate neurons, Hartung (1985) reported that K_{Na} was activated by lithium. We found with single-channel recording that in trigeminal neurons lithium did not activate K_{Na}. In whole-cell recordings, however, our results were inconclusive. First, whereas in several neurons no K_{Na} was visible in the presence of lithium, in others K_{Na} was still present even after a long exposure to lithium. Secondly, a decrease in the amplitude of K_{Na} during exposure to lithium was observed in some neurons but this decrease could not be reversed by superfusion with sodium. Finally, it is important to emphasize that the failure to detect K_{Na}

in whole-cell recording does not necessarily imply that this current is absent or has been suppressed: K_{Na} can be masked by a large or long-lasting sodium current (see Bader et al., 1990, Fig. 6.7). Since the visualization of K_{Na} in whole-cell recording depends upon the relative contribution of this current with respect to that of the sodium current, the disappearance of K_{Na} during superfusion with lithium must be considered with some caution, unless K_{Na} reappears when sodium ions are again superfused.

Single-Channel Properties of K_{Na} in Different Preparations

There are certain differences among the sodium-activated potassium channels described so far. In heart myocytes, where the channel was first described, the elementary conductance is high (207 pS with K_i 49 mM and K_o 150 mM), there is marked inward rectification of the channel below the potassium equilibrium potential and no substates were reported. In neurons the conductance was found to be 50 pS in the presence of a physiological transmembrane potassium gradient (Dryer et al., 1989; the present study). When high potassium concentrations were present on both sides of the membrane the conductance was higher. In trigeminal neurons, we found a conductance of 174 pS (K_i 75, K_o 150 mM) for the main conducting state. This increased conductance could be explained by the constant field theory. In brainstem neurons, the conductance was 105 pS (K_i 75 mM, K_o 150 mM) and no substates were described. In both neuronal preparations there is essentially no inward rectification of the channel and this in spite of the presence of intracellular magnesium (10 mM, Dryer et al., 1989 and 1 mM, this study, Fig. 6 C), which, like calcium (Mazzanti and DiFrancesco, 1989), was found to induce inward rectification in heart potassium channels (Matsuda, 1988). Thus, the K_{Na} channel family seems to include several subtypes.

In both heart cells and trigeminal neurons, a power relationship exists between the fraction of time the channel is open and the intracellular sodium concentration. In trigeminal neurons, the Hill coefficient is greater than 2 and it is therefore possible that two or three sodium ions bind to the channel before it opens. Another explanation for the power relationship would be that the channel is opened by the binding of one sodium ion, and that the open time depends on the intracellular sodium concentration. We found that the open time distribution was described by two time constants and that with different intracellular sodium concentrations the relationship between brief and longer mean open times remained nearly the same. At 12 mM sodium, there were 1.94 long-lasting openings for each short opening. Corresponding values at 50 and 100 mM sodium were 2.23 and 2.45, respectively. This means that an eightfold increase in Na_i affected the long open time distribution by a factor of less than 1.3. For comparison, a fivefold increase in Ca_i on large K_{Ca} channels of rat muscle increased six times the number of long open times (Barrett et al., 1982). Therefore, our results suggest that the power relationship between intracellular sodium concentration and the percentage of time the channel is open is a consequence of the cooperative binding of two to three sodium ions which precedes the channel opening. In this respect, the behavior of the K_{Na} channel differs from that of big K_{Ca} channels. Another difference between sodium- and calcium-activated

potassium channels is their sensitivity to voltage. The percentage of time spent in the open state by calcium-activated channels increased e-fold for a depolarization of 15 mV (Barrett et al., 1982) or 25 mV (Gorman and Thomas, 1980). For K_{Na} channels an e-fold increase required a depolarization of at least 42 mV in the presence of 12 mM intracellular sodium. At higher sodium concentrations (50 and 100 mM) a depolarization of 108 mV was needed for an e-fold increase.

Functional Role of K_{Na}

The high sodium concentration needed to activate K_{Na} in heart myocytes led Kameyama et al. (1984) to conclude that this type of channel might become active exclusively under pathological conditions. In neurons, however, the results of Dryer et al. (1989) and our data suggest that the channel may be active already in physiological conditions. Intracellular sodium concentrations in neurons measured by various means can vary between 5 mM (rat sympathetic neurons; Galvan et al., 1984) and 4–50 mM (frog motoneurons; Grafe et al., 1982; Bührle and Sonnhof, 1983). Our finding that the percentage of time K_{Na} was open at 12 mM sodium was 20% suggests that K_{Na} channel openings at a low frequency may contribute to the resting potential of neurons.

It is more difficult to evaluate the activation of K_{Na} by sodium ions entering the neuron during a single action potential (Hartung, 1985; Dryer et al., 1989). Sodium ions are expected to diffuse rapidly away from their entry site and a significant local increase in sodium concentration can only occur if a diffusion barrier exists combined with inhomogeneities in the distribution of channels. For example, if K_{Na} channels were colocalized with sodium channels in patches of membrane containing high densities of sodium channels, the presence of a diffusion barrier underneath the membrane would create the condition for a local buildup in sodium concentration which could trigger the activation of K_{Na}. At the node of Ranvier of myelinated axons, where sodium channels are highly concentrated, the intracellular concentration of sodium changes after a few action potentials (Bergman, 1970). An increase in sodium concentration of up to 2–5 mM can be detected in the soma of frog motoneurons during synaptic stimulation at 20 Hz for 10–20 s (Grafe et al., 1982).

The functional role of K_{Na} is not yet known and it will be difficult to establish until a specific blocking agent is found. So far, no pharmacological studies on this channel are available; the fact that 4AP (Dryer et al., 1989) or a combination of 4AP and TEA (Bader et al., 1985) block K_{Na} simply indicates that this channel has some similarities with voltage-gated potassium channels. Results in whole-cell recording suggests that K_{Na} might accelerate the repolarizing phase of the action potential. The time-course of K_{Na}, which is similar to that of the A-current (Connor and Stevens, 1971), may confer to K_{Na} a role of slowing down the discharge frequency of action potentials during a depolarization. More generally, it can be said that whenever the intracellular sodium increases, K_{Na} will promote a hyperpolarization of the neuron. This may contribute to reactivate those currents that were inactivated by depolarization (sodium current, A-current and low-threshold calcium current [Carbone and Lux, 1984; Nowycky et al., 1985]). We report here that in the presence of sodium, the probability for K_{Na} channels to be in the open state increases with depolariza-

tion. This property is consistent with the observation by Hartung (1985) that the potassium current induced by intracellular injection of sodium was only revealed during depolarizing voltage steps. Thus, during an action potential, sodium influx and depolarization may combine to promote the activation of K_{Na}.

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