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Properties and Rundown of Sodium-activated Potassium Channels in Rat Olfactory Bulb Neurons

Terrance M. Egan, Daniel Dagan,[a] Jürgen Kupper, and Irwin B. Levitan

Graduate Department of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, Massachusetts 02254-9110

We have used single-channel recording techniques to investigate the properties of sodium-activated potassium channels (K_{Na} channels) in cultured rat olfactory bulb neurons, and in large neurons in the mitral cell layer of thin slices of olfactory bulb. Ion channels highly selective for potassium over sodium and chloride, and requiring 10–180 mM internal sodium (Na_i) for their activation, were present in approximately 75% of inside-out membrane patches detached from cultured olfactory bulb neurons. Most of these patches contained several K_{Na} channels. K_{Na} channels were seen in cell-attached patches only when Na_i was raised by including veratridine in the extracellular medium. Preincubation of the cell in TTX or removal of extracellular sodium prevented this effect of veratridine, confirming that the channels observed under these conditions were indeed K_{Na} channels. Lithium did not substitute for Na_i in activating these channels. With 150 mM potassium on both sides of the membrane, K_{Na} channels had a single-channel conductance of 172 pS, and at least two subconducting states were observed in addition to this fully open state. Under these ionic conditions, the channels exhibited linear fully open channel current–voltage curves over the potential range of −100 to 0 mV. At voltages more positive than the potassium equilibrium potential, the single-channel currents exhibited inward rectification as a result of sodium block of outward potassium current. The channels opened in bursts, during which they fluctuated between the fully open and closed states, and the substates. Between bursts they sometimes entered a long-lived inactive state that could last for up to several minutes. In addition, K_{Na} channels in the detached patches exhibited rundown, a progressive irreversible loss in activity, over a time course that varied from less than 1 min to longer than 1 hr. Rundown of K_{Na} channel activity in cell-attached patches (in the presence of veratridine) did not occur, suggesting that some intracellular factor necessary for K_{Na} channel activity is lost when the membrane patch is detached from the cell.

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Correspondence should be addressed to Dr. Irwin B. Levitan, Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254-9110.

[a] Permanent address: Rappaport Family Institute, Technion Faculty of Medicine, Department of Physiology and Biophysics, Haifa 30197, Israel.

Many different types of K^+ channels have been characterized in neurons by electrophysiological techniques, and the recent application of molecular genetic approaches has emphasized the enormous diversity of the K^+ channel family. Within this large family of K^+ channels, one class is modulated by cations. The best known examples of this class are the Ca^{2+}-activated K^+ channels (K_{Ca} channels) (Latorre et al., 1989). Recent evidence has shown that Na^+_i also can activate a K^+ channel. Such K_{Na} channels have been reported in snail neurons (Partridge and Thomas, 1976), vertebrate heart (Kameyama et al., 1984; Luk and Carmeliet, 1990; Wang et al., 1991), cultured quail trigeminal neurons (Bader et al., 1985; Haimann et al., 1990), crayfish neurons (Hartung, 1985), *Drosophila* giant neurons (Saito and Wu, 1991), zebra finch hyperstriatal neurons (Kubota and Saito, 1991), and cultured chick brainstem neurons (Dryer et al., 1989). Voltage-clamp analysis of currents of guinea pig and rat suggests that the K_{Na} channel is also present in mammalian brain (Constanti and Sim, 1987; Schwindt et al., 1989), but thus far no single-channel analysis of K_{Na} channels from mammalian brain has been reported.

We have been interested in the properties of cation-sensitive K^+ channels in mammalian brain and have focused our attention on the rat olfactory bulb. The behavior of the olfactory bulb is determined in part by the electrical activity of the different populations of neurons that comprise it (Shepherd and Greer, 1990). Soon after an odor is detected by the sensory neurons, action potential discharge begins in the principal relay cells of the bulb, the mitral cells (Macrides and Chorover, 1972; Kauer, 1974, 1987, 1991; Hamilton and Kauer, 1985; Meredith, 1986; Døving, 1987; Wellis et al., 1989; Wellis and Scott, 1990). The pattern and intensity of this discharge are determined by the actions of local circuits (Kauer, 1974; Mair, 1982; Scott and Harrison, 1987; Hamilton and Kauer, 1989) and by the inherent ionic conductances of the neurons (Yamamoto et al., 1963; Jahr and Nicoll, 1982; Mori, 1987). A major component of these conductances is likely the result of the activity of K^+ channels.

Most of the information available about the pharmacology and neurophysiology of the olfactory bulb comes from *in vitro* brain slice preparations of turtle and salamander and from *in vivo* studies on rabbit and salamander, although an *in vitro* preparation of rat olfactory bulb has been developed (Frosch and Dichter, 1984; Trombley and Westbrook, 1990). We used two *in vitro* preparations of rat olfactory bulb, thin brain slices and dissociated neurons in primary culture, to study the properties of K^+-selective ion channels. The activity of one type of K^+ channel in these preparations is dependent on $[Na^+]_i$. We studied the behavior of this channel using both the cell-attached con-

figuration of the patch clamp technique while indirectly varying $[Na^+]_i$ (with veratridine), and the cell-detached inside-out and outside-out configurations, which allow more direct control of ion concentration gradients.

A preliminary report of this work has been presented to the Biophysical Society (Egan et al., 1990).

Materials and Methods

Tissue culture. Primary cultures of postnatal rat central neurons were prepared according to the method of Huettner and Baughman (Huettner and Baughman, 1986; Baughman et al., 1991). Long–Evans rat pups, aged 1–3 d, were killed by decapitation. The main and accessory olfactory bulbs were quickly removed from the cranium and cut into thirds with a razor blade. The resulting approximately 1-mm-thick slices were bathed in 3 ml of a physiological saline solution containing 1 mM free Ca^{2+}, papain (Worthington Biochemicals; 150–250 U), cysteine (1 mM), and kynurenic acid (0.1–1 mM) and kept at a temperature of 37°C in a 5% CO_2 incubator. After 30 min, the bath solution was replaced with 3 ml of fresh enzyme solution and left for an additional 30 min. This procedure was repeated again so that the slices were incubated in enzyme for a total of 90 min. The slices were then washed in a solution containing 5% fetal calf serum, bovine serum albumin (1 mg/ml), and trypsin inhibitor (1 mg/ml; Boehringer Mannheim) to stop the enzymatic action of the papain. Cells were dissociated by triturating the slices with fire-polished Pasteur pipettes, and the dispersed neurons and glia were plated onto collagen-coated glass cover slips. The cultures were kept at 37°C in a humid atmosphere containing 5% CO_2. Typically, four pups were used per preparation, yielding about 48 culture plates. The growth medium was Dulbecco's modified Eagle medium containing glutamine (0.5 mM), glucose (20 mM), penicillin (100 U/ml), streptomycin (0.1 mg/ml), 5% fetal calf serum (all from GIBCO Laboratories), and kynurenic acid (0.1–1 mM). Cytosine arabinoside (10–20 μM) was added for 24–48 hr between days 3 and 5 to stop overgrowth of rapidly dividing cells. The medium was exchanged every 5 d. Neurons were viable under these conditions for at least 2 months, and ion channel activity could be recorded from cultures prepared the previous day. We used neurons maintained in culture for 7–21 d for the experiments described in this paper. Except where otherwise noted, chemicals were obtained from Sigma Chemicals.

Brain slices. Thin brain slices were prepared according to the method of Edwards et al. (1989). Long–Evans rats, aged 14–21 d, were killed by decapitation. The cranium was quickly submerged in a cool (4°C) saline solution of the following composition (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 25 glucose, gassed with 95% O_2/5% CO_2. One main olfactory bulb/accessory olfactory bulb complex along with a small portion (3–5 mm) of the attached frontal cortex was dissected from the rest of the brain. The frontal cortex was used as a base to stabilize the sausage-shaped bulbs during slicing. The bulb/frontal cortex was then fastened to the stage of a vibrating microtome (TPI Vibratome 1000) with a drop of cyanoacrylic glue. The Vibratome bath also contained the cool, gassed saline. Depending on how the bulb/cortex complex was positioned on the stage, either horizontal or coronal slices were obtained. The first cut removed the uppermost quarter of the bulb, which was discarded. Then, successive 120–150-μm-thick slices were cut until a total of 5–10 slices was obtained. The slices were transferred to a holding chamber containing the same oxygenated physiological saline solution at room temperature. The slices remained viable under these conditions for at least 8 hr. A single slice was transferred to a tissue bath positioned on the fixed stage of a Zeiss ACM microscope, and cells were visualized with Nomarski optics using a 40× water immersion lens. Total magnification equaled 400×. Mitral and granule cells were identified by their location in the slice. Individual neurons were cleaned with a micropipette by the technique described by Edwards et al. (1989). The pipette solution contained (in mM) 125 NaCl, 2.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, and 10 EDTA; we found that the inclusion of EDTA and the exclusion of divalent cations helped prevent clogging of the pipette tip with debris during the cleaning process.

Electrophysiology. K_{Na} channels were recorded from on-cell, inside-out, and outside-out patches of olfactory bulb neurons using conventional methods (Hamill et al., 1981). Unless otherwise noted, data presented here come from experiments using the inside-out patch clamp configuration on olfactory bulb neuronal cultures. Patch electrodes were pulled from 1.5 mm o.d. borosilicate glass capillary tubes (Jencons H15). The electrodes were lightly fire-polished to a final tip diameter of 1–2 μm and had resistances of 2–10 MΩ when filled with 150 mM K^+ salts. Single-channel activity was recorded with a List EPC-7 or an Axopatch 1-D amplifier using a headstage gain of 50 mV/pA. A head stage gain of 10–20 mV/pA was used for whole-cell voltage clamp. Gigaohm seals were formed by momentarily applying negative pressure to the inside of the patch pipette. When recording from cell-attached and inside-out membrane patches, the pipette contained (in mM) 110 K-gluconate, 40 KCl, 1 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES, and the bath contained 110 Na-gluconate, 40 NaCl, 2 $MgCl_2$, 1 EGTA, and 10 HEPES. For experiments on outside-out membrane patches, the pipette contained (in mM) 110 Na-gluconate, 40 NaCl, 2 $MgCl_2$, 1 EGTA, and 10 HEPES, and the bath contained (in mM) 110 K-gluconate, 40 KCl, 1 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES. All pipette and bath solutions were buffered to a pH of 7.3 with either KOH or NaOH, depending on the experiment. Other bath and pipette solutions are detailed in the text as needed. Quoted potentials are equal to the command voltages minus any offset voltages caused by junction potentials (usually 3–8 mV). Normal cellular convention is used to describe voltage with respect to ground, and inward current through open channels is shown as downward deflections from the closed level baseline. Drugs and solutions of different ionic composition were applied by superfusion. Except where noted, all experiments were performed at room temperature.

Data acquisition and analysis. Single-channel data were stored on a VCR modified for FM recording (Vetter 420 VCR Format Data Recorder). Data were subsequently filtered at 1–2 kHz (−3 dB) using an eight-pole low-pass Bessel filter (Frequency Devices 902LPF), and digitized at 10 kHz using an Everex Step 386/20 computer and a Tecmar Labmaster A/D board. The data were analyzed off line using either a program (SINGIBX) written by Dr. Peter Reinhart, which was based on the Indec Fastlab software library, or by a program (PAT, version 6.1) kindly supplied by John Dempster of the University of Strathclyde. For kinetic analysis of single-channel data, an automated 25–30% crossing threshold routine was used to collect 24,000–30,000 channel transitions. Then, sections of the idealized record were compared by eye to the raw data to insure correct interpretation of the events. Binned data were exported to SIGMAPLOT (Jandel Co.), where curves were fit to amplitude and dwell time histograms using a Levenberg–Marquadt curve-fitting algorithm. Events lasting less than 0.5 msec, which were unlikely to be properly resolved, were excluded by discarding the first bin of the dwell time histograms and therefore did not contribute to the fitted curves.

Channel gating was characterized by bursting behavior in which the channel alternated between the fully open state, the substates and the fully closed state. K_{Na} channels occasionally remained closed for a long time before reopening (a 5–10 min closure was not unusual; see Results). We consider such long closures to reflect an additional state of the channel. This *inactive* state was entered only occasionally, and it contributed little to the total closed time histogram. Thus, closures longer than 1 sec were not included in the kinetic analysis of the data, and closures longer than 2 sec were not included in estimates of P_o. In many experiments, more than one K_{Na} channel was actively gating in the membrane patch, in which case activity was counted as the number of channels (N) multiplied by P_o. Estimates of NP_o included all data, as longer closures of individual channels could not be discerned because of the simultaneous gating of many channels.

Each experiment was repeated at least six times unless otherwise noted. Results are expressed as the sample mean ± SD of n observations.

Results

Our cultures contained predominantly two types of neurons as distinguished by their morphology. The first type was pyramidal shaped and had a single large neurite extending from each of its three corners. The second type was bipolar. The properties of these two neuronal populations have recently been described in detail by Trombley and Westbrook (1990), who suggest that the first type are mitral/tufted cells and the second are granule cells. Most of the experiments described here were performed on the larger pyramidal-shaped neurons, although some experiments used bipolar cells. K_{Na} channel activity was recorded from both cell types, and there was no obvious difference in the behavior of the channel between the two populations.

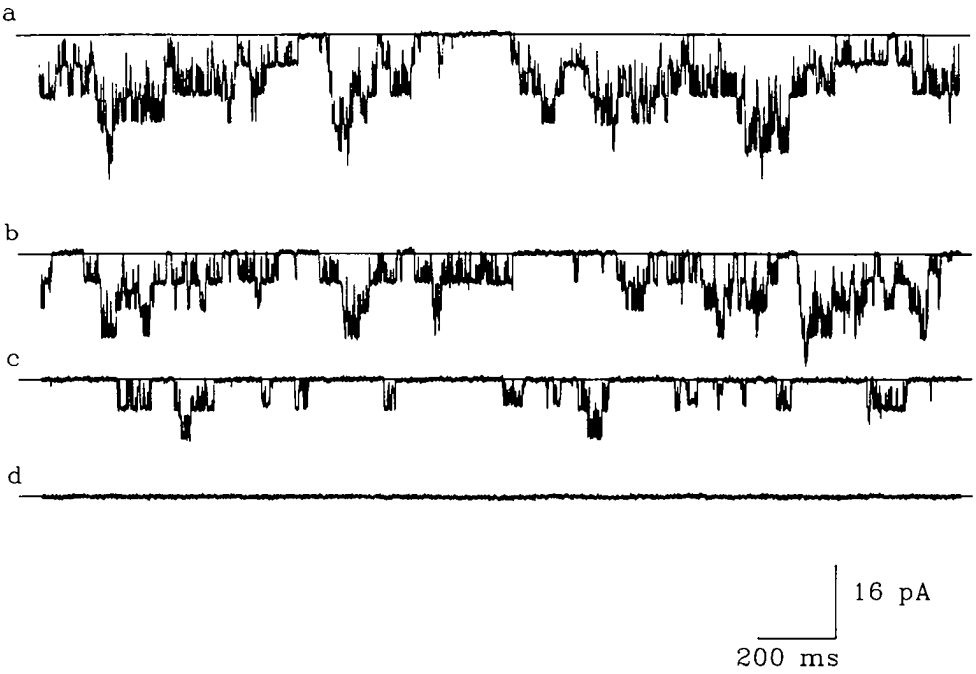

Figure 1. K^+ channels activated by $[Na^+]_i$. The record shows channel activity of an inside-out membrane patch excised from a cultured olfactory bulb neuron and bathed in different $[Na^+]_i$. V_m was −40 mV; $[K^+]_o/[K^+]_i$ equaled 150/0 mM. Under these conditions, inward current through open channels is shown as *downward deflections* from the closed level. The *solid line* indicates the zero current level at which all channels are fully closed. The membrane patch contained at least eight K_{Na} channels. Superimposed openings were common when $[Na^+]_i$ equaled 60 mM (*a*). The channels were open less often when $[Na^+]_i$ equaled 40 mM (*b*) and 20 mM (*c*). No channels were open in the absence of $[Na^+]_i$ (*d*). Channel activity returned to near control levels upon reintroduction of $[Na^+]_i$ (not shown).

Several different classes of K^+-selective channels were recorded in inside-out and outside-out patches of cultured olfactory bulb neurons. Two of these channel classes were distinguished by their selective activation by different cations. The first class required Ca^{2+}_i and, on the basis of data obtained in the presence of low $[Na^+]_i$/high $[Ca^{2+}]_i$ solutions, was determined to be a member of the "maxi-K" K_{Ca} channel family (Egan et al., 1990). The second class was active when $[Na^+]_i$ was greater than about 10–20 mM and thus was classified as a K_{Na} channel (Fig. 1). In a survey of 120 inside-out patches in which $[Na^+]_i$ and $[Ca^{2+}]_i$ exceeded 20 mM and 10 μM, respectively, 43% (52 of 120) of the patches had only K_{Na} channels, 15% (18 of 120) had only K_{Ca} channels, and 32% (38 of 120) had both K_{Na} and K_{Ca} channels. Only a few patches (12 of 120, 10%) had neither channel type. When a patch contained both types of cation-activated channels, K_{Na} channels were isolated by chelating Ca^{2+}_i with EGTA ($[Ca^{2+}]_i$ < 10 nM). Usually, membrane patches contained several active K_{Na} channels, although single-channel patches were observed. For example, in a survey of 90 patches containing K_{Na} channels (150 mM $[Na^+]_i$; V_m = −40 mV), seven had single channels. We concluded that we had a single K_{Na} channel in a patch only when no single-channel current superpositions were seen when the P_o was very high (≥0.8).

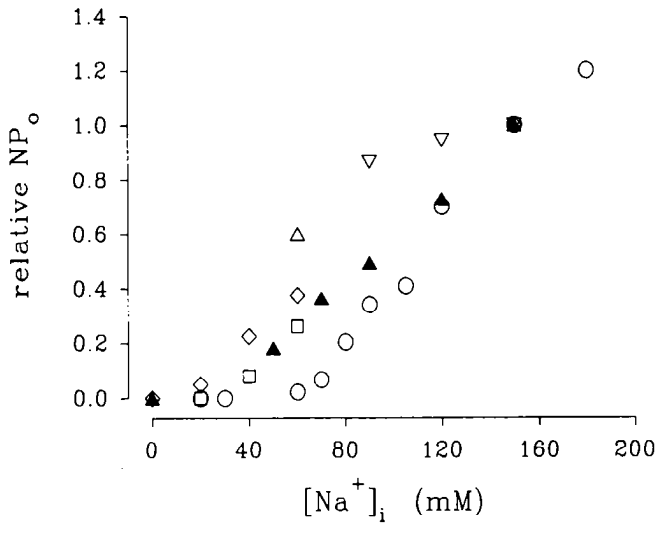

Figure 2. $[Na^+]_i$ increases K_{Na} channel activity in a dose-dependent manner. NP_o was measured at many different $[Na^+]_i$ levels for six multichannel inside-out membrane patches (*separate symbols* for each patch). The graph shows relative NP_o plotted against $[Na^+]_i$, where the relative NP_o equaled the NP_o measured at a given $[Na^+]_i$ divided by the NP_o measured at 150 mM $[Na^+]_i$. V_m equaled −40 mV.

Some K^+ channels are $[Na^+]_i$ dependent

We did not observe channel activity in on-cell patches of olfactory bulb neurons held at the resting V_m under normal conditions. However, a common event upon excision of an inside-out membrane patch into a 150 mM Na^+/low Ca^{2+} solution was the immediate appearance of channel gating activity. The amount of channel activity was determined by $[Na^+]_i$. The patch of Figure 1 contained at least eight K_{Na} channels, all of which were actively gating at 150 mM $[Na^+]_i$, and the current trace never had a period of zero channel activity with this high $[Na^+]_i$ (not shown). When $[Na^+]_i$ was decreased to 60 mM by replacement with equimolar K^+_i, the P_o fell so that discreet openings and closings from a stable zero current baseline were seen (Fig. 1*a*). Channel gating decreased further in a graded manner as $[Na^+]_i$ was lowered from 60 to 20 mM (Fig. 1*a–c*), and no channel openings were apparent in the absence of Na^+_i (Fig. 1*d*). Channel gating recovered when Na^+_i was again introduced (not shown). Li^+_i (60–150 mM), an effective substitute for Na^+_i in many physiological processes, did not open K_{Na} channels when it replaced Na^+ as the major ion bathing the internal surface of inside-out membrane patches. As stated above, many patches contained both K_{Na} and K_{Ca} channels, which differed in mean conductance, gating behavior, and the presence of substates (see below). However, in patches containing only K_{Na} channels, changes in $[Ca^{2+}]_i$ did not affect K_{Na} channel activity. K^+_i (up to 150 mM)

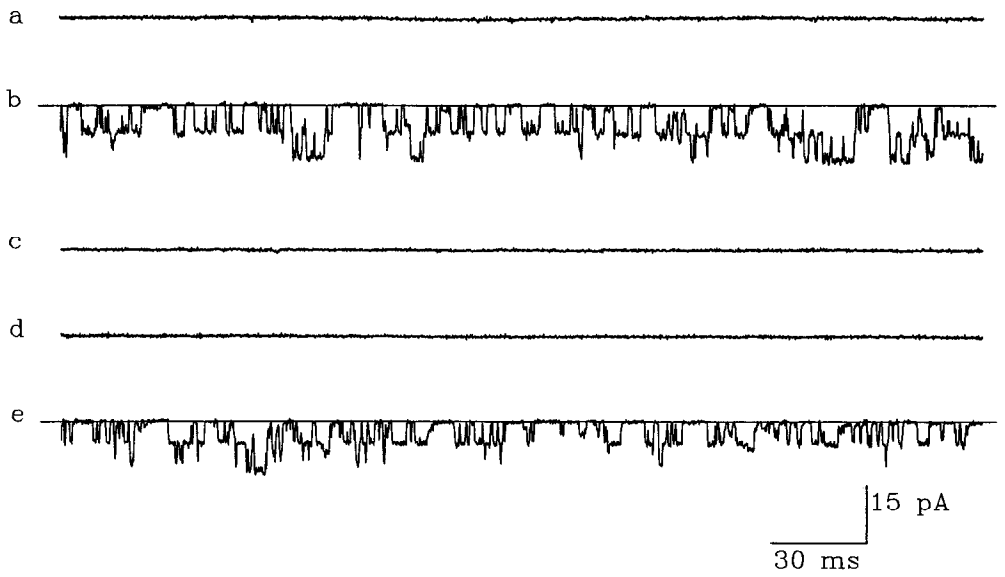

Figure 3. K_{Na} channels recorded from on-cell patches: records from a cell-attached patch of cultured olfactory bulb neuronal membrane. The recording pipette contained 150 mM [K^+], and the applied pipette voltage was 40 mV. *a,* The cell was bathed initially in a solution containing 150 mM Na^+. Under these conditions, no channel activity was observed. *b,* Addition of 100 μM veratridine to the bath solution resulted in the onset of channel gating. At least two channels were present in the membrane patch. *c,* A few minutes after 150 mM $[K^+]_o$ replaced 150 mM $[Na^+]_o$, channel gating ceased even in the continued presence of veratridine. Channel gating recovered after changing back to a 150 mM $[Na^+]_o$ plus veratridine solution (not shown). *d,* Addition of TTX (100 nM) also prevented channel gating in the presence of 150 mM $[Na^+]_i$, presumably by preventing Na^+ influx through voltage-activated Na^+ channels kept open by veratridine (100 μM). *e,* Patch excision into a 150 mM $[Na^+]_i$ solution restores channel activity even when TTX is present.

and Mg^{2+}_i (up to 10 mM) also were ineffective in opening these channels.

The initial $[Na^+]_i$ sensitivity of our sample group of K_{Na} channels varied from cell to cell as determined by measuring the P_o (or the NP_o) at a V_m of −40 to −60 mV immediately after excising into 150 mM $[Na^+]_i$. The variability is connected, we believe, to the phenomenon of rundown (see below). Many K_{Na} channels had very high P_o values in 150 mM $[Na^+]_i$ (P_o near 0.8, measured at −40 mV), and the activities of these channels were not further increased by raising $[Na^+]_i$. However, channels that were less sensitive to 150 mM $[Na^+]_i$ ($P_o < 0.8$) did respond to increases in $[Na^+]_i$. To determine the average $[Na^+]_i$ versus NP_o relationship, we constructed $[Na^+]_i$ versus NP_o curves for channel activity in six multiple-channel patches (Fig. 2). Dose–response curves of activity from multiple-channel patches showed that $[Na^+]_i$ continued to increase NP_o even at very high $[Na^+]_i$. For example, in one patch (open circles, Fig. 2) activity was dose dependent over the entire range of $[Na^+]_i$ tested, and even at the highest $[Na^+]_i$ (180 mM), the curve did not plateau. The absence of saturation of the $[Na^+]_i$ versus NP_o curve in this range of $[Na^+]_i$ has also been reported for K_{Na} channels in multiple-channel patches of chick cultured brainstem neurons (Dryer et al., 1989).

As stated above, no channel activity was normally seen in recordings from on-cell membrane patches (Fig. 3*a*). However, it was possible to activate K_{Na} channels in cell-attached patches when $[Na^+]_i$ was raised with veratridine. This drug prevents inactivation of voltage-dependent Na^+ channels, thereby allowing Na^+ to leak into the cell. Superfusion of veratridine (1–100 μM) resulted in the appearance of channels resembling K_{Na} channels with respect to the presence of multiple substates (see below) and an absolute dependence on $[Na^+]_i$. K_{Na} channels began gating 1–5 min after superfusion of veratridine (Fig. 3*b*). Interestingly, the K_{Na} single-channel current amplitude increased during the first few minutes of the effect of veratridine. This increase in current amplitude most likely resulted from an increased driving force for current flow through these K^+-selective channels (see below), suggesting that veratridine hyperpolarized these cells by indirectly opening K_{Na} channels. K_{Na} channels recorded from on-cell patches under these conditions routinely continued to gate in a regular manner for as long as the patch was held (up to 90 min). Further, in the continued presence of veratridine, $[Na^+]_i$ was manipulated by changing $[Na^+]_o$ or by preventing Na^+ influx through voltage-gated Na^+ channels with TTX (0.1–10 μM). Channel activity ceased when $[Na^+]_o$ was decreased from 150 to 0 mM (Fig. 3*c*), or when TTX (100 nM) was added in the presence of normal $[Na^+]_o$ (Fig. 3*d*). TTX had no direct effect on K_{Na} channels (Fig. 3*e*; see also below), and in any event in these experiments it was applied to the outside of the patch electrode. Likewise, channel activity gradually disappeared after washout of veratridine if the external solution contained at least 5 mM $[K^+]_o$, but not in the absence of K^+_o, suggesting that an active Na^+/K^+ pump is necessary to lower $[Na^+]_i$ following washout of veratridine.

Conductance and ion selectivity

Inside-out and outside-out membrane patches were used to determine the conductance and selectivity of the fully open channel. Using symmetrical 150 mM $[K^+]$ conditions in an inside-out patch where $[Na^+]_i$ was 60 mM, the *I–V* curve was linear and the slope conductance measured 161 pS over the potential range of −10 to −100 mV (Fig. 4). E_{rev} was 0 mV, equal to the predicted value of the K^+ equilibrium potential, even in the presence of these high levels of $[Na^+]_i$, suggesting that the channel was more permeable to K^+ than to Na^+. In other experiments using symmetrical 150 mM $[K^+]$ and 60–120 mM $[Na^+]_i$, the

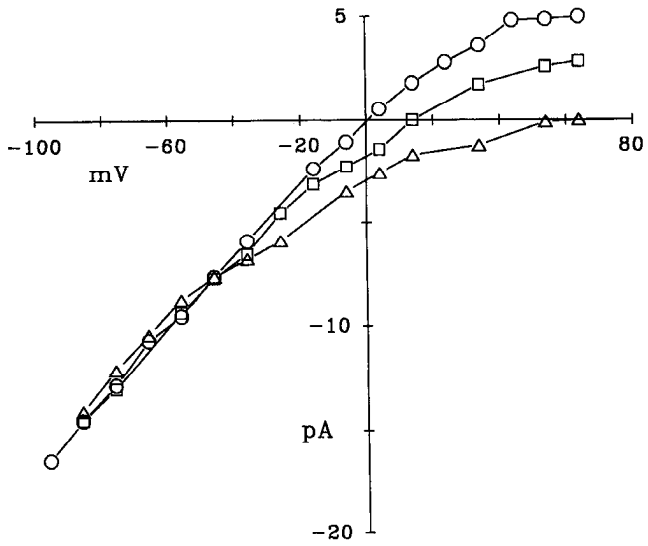

Figure 4. K_{Na} channels are K^+ selective: *I–V* curves for K_{Na} channels of an inside-out membrane patch bathed in different $[K^+]_i$ and $[Na^+]_i$. In all three cases, $[K^+]_o$ equaled 150 mM. *Top curve* (○), $[K^+]_i$ equaled 150 mM and $[Na^+]_i$ equaled 60 mM. We did not correct for the osmotic imbalance caused by Na^+_i. *Middle curve* (□), $[K^+]_i$ equaled 60 mM and $[Na^+]_i$ equaled 90 mM. *Lower curve* (△), $[K^+]_i$ equaled 0 mM and $[Na^+]_i$ equaled 150 mM. Slope conductance, measured from the linear portion of the *I–V* curve at negative V_m under symmetrical 150 mM $[K^+]$ conditions (○), was 161 pS.

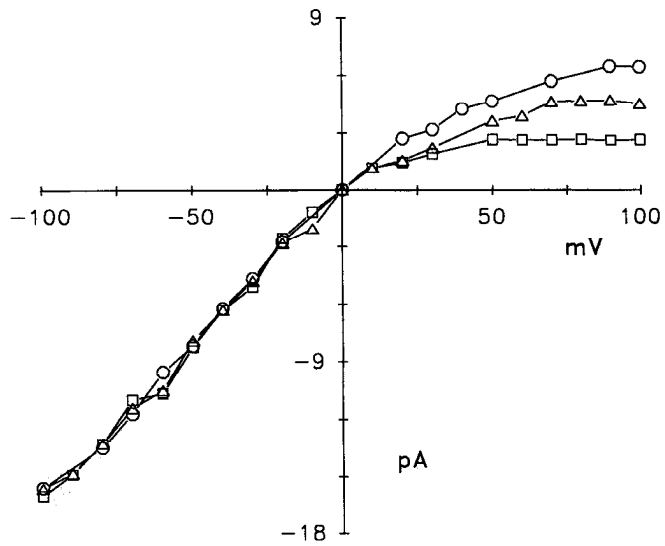

Figure 5. $[Na^+]_i$ increases inward rectification. K_{Na} single-channel current was measured in inside-out membrane patches. $[K^+]_o$ and $[K^+]_i$ equaled 150 mM and $[Na^+]_i$ equaled 30 (○), 60 (△), and 120 mM (□). At negative voltages, *I–V* curves were linear and had slope conductances of about 145 pS. At positive potentials, $[Na^+]_i$ blocked the outward flow of K^+.

average slope conductance at negative V_m was 172 ± 8 pS (n = 8) and the average E_{rev} was 0 ± 2 mV (n = 14). At positive V_m, the channel showed inward rectification.

Selectivity was further measured by varying $[Na^+]_i$ and $[K^+]_i$ (Fig. 4). No outward current could be measured when Na^+_i completely replaced K^+_i. When $[K^+]_o/[K^+]_i$ equaled 150/60 and $[Na^+]_i$ equaled 90 mM, the average E_{rev} was 18.9 ± 2.9 mV, which is close to the value of 23 mV predicted by the Nernst equation for a K^+-selective pore, and the average slope conductance was 149.3 ± 9.2 pS (n = 4). The *I–V* curves were unaffected by changes in $[Cl^-]_i$ and $[Cl^-]_o$.

The degree of inward rectification seen at positive V_m depended on $[Na^+]_i$ (Fig. 5). This was shown by varying $[Na^+]_i$ between 30 and 120 mM while keeping $[K^+]_i$ and $[K^+]_o$ constant at 150 mM each. Inward rectification increased as $[Na^+]_i$ increased, suggesting that $[Na^+]_i$ can block outward flow of K^+ through the open channel. No increase in open-channel noise was evident in the limited bandwidth used in these experiments, and Na^+ may therefore be a very fast blocker of K_{Na} channels. Similar evidence of fast Na^+ block of K_{Na} channels of heart has recently been presented by Wang et al. (1991). Although, as stated above, Li^+_i failed to activate olfactory bulb K_{Na} channels, it shared with Na^+ the ability to block K_{Na} channels (data not shown). Ca^{2+} had no effect on the current–voltage relationship.

K_{Na} channels are voltage dependent

P_o was measured at different V_m to determine the effect of voltage on channel activity. The experimental protocol was as follows. Inside-out membrane patches were held at different V_m levels in a staggered order (e.g., −80, +80, −40, +40 mV) to avoid confusing a change in the P_o caused by rundown (P_o vs. time; see below) with a change resulting from the effect of voltage (P_o vs. V_m). $[K^+]_o$ and $[K^+]_i$ equaled 150 mM. P_o, measured at −80 mV, was kept low (less than 0.6) by changing $[Na^+]_i$ (30–120 mM). Under these conditions, the P_o varied as a function of V_m, increasing with depolarization and decreasing with hyperpolarization. This effect of voltage was most pronounced at low initial P_o (measured at −80 mV). For example, in one experiment changing V_m from −80 mV to −40 mV resulted in a threefold change in the P_o (change from 0.06 to 0.18). However, at higher initial P_o values, changing V_m had less of an effect, and at very high initial P_o values (near 0.8 at −80 mV), no voltage dependence was observed.

Gating kinetics

Gating behavior was characterized by long periods of bursting activity in which the channel rapidly alternated between a fully open state, multiple subconductance states (see below), and a fully closed state (Fig. 6*a*). Between groups of bursts, the channel occasionally entered an inactive state where it remained closed for several hundreds of milliseconds to minutes, after which it returned to its bursting mode for several minutes before falling quiet again. This pattern of occasional long quiet periods separating groups of bursts has also been reported for the K_{Na} channel gating of mammalian heart (Luk and Carmeliet, 1990). As mentioned above, most membrane patches contained many K_{Na} channels. Also, most K_{Na} channels showed a gradual decrease in the P_o with time after excision (i.e., rundown; see below). These two facts confounded analysis of K_{Na} channel transition rate constants. However, in a few patches, single K_{Na} channels gated for the entire period beginning immediately after excision into 150 mM $[Na^+]$ and lasting until the seal deteriorated (see, e.g., Fig. 13); such patches were used for kinetic analysis.

Kinetic rate constants were determined from data collected during extended periods of stable P_o. Two minutes of data were used to construct dwell time histograms of all the open times and for closed times shorter than 1 sec. Open and closed time histograms for two K_{Na} channels with the P_o equal to 0.55 and 0.85 are shown in Figures 6*b* and 7. The open time histograms for the two channels were almost identical; each was fit well by

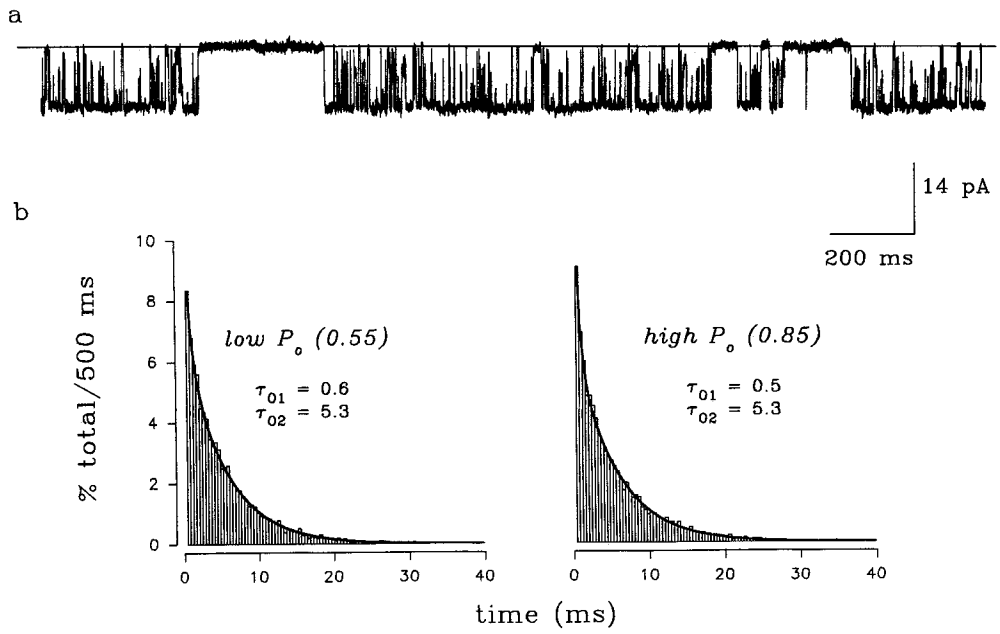

Figure 6. Open time histograms. *a,* A K_{Na} channel showed bursts of openings and closures punctuated by longer closures. This channel occasionally remained closed for up to 1 min (not shown). The *solid line* indicates the zero current level. V_m was −70 mV. $[K^+]_o/[K^+]_i$ equaled 150/0 mM, and $[Na^+]_i$ equaled 150 mM. *b,* Open time histograms of data of two different channels differing in P_o. Open times were measured for a 2 min stretch of data. The data of each histogram were fit well by the sum of two exponential functions. *Left histogram,* Data from experiment pictured in *a.* The P_o equaled 0.55. τ_{O1} was 0.6 msec and τ_{O2} was 5.3 msec. *Right histogram,* V_m was −80 mV. $[K^+]_o/[K^+]_i$ equaled 150/0 mM and $[Na^+]_i$ equaled 150 mM. The P_o equaled 0.85. τ_{O1} and τ_{O2} equaled 0.5 and 5.3 msec, respectively.

the sum of two exponentials with τ_{O1} equal to about 0.6 msec and τ_{O2} equal to about 5.3 msec (Fig. 6*b*). The closed time histograms differed (Fig. 7). For both channels, about 95% of the closures lasted less than 15 msec, and the closed time histograms of data in this range were roughly equivalent for the two experiments; each was described by the sum of two exponential functions (Fig. 7, upper panels). The biggest difference in gating patterns involved longer closures. This is shown in the lower panels of Figure 7, where closures in the 15–1000 msec range are tabulated. These data from the less active channel were fit by two additional exponential functions where τ_{C3} and τ_{C4} equaled 10.5 and 142 msec, respectively (Fig. 7, bottom left panel). We did not measure τ_{C3} and τ_{C4} for the more active channel because, although we collected about 25,000 total transitions, longer closures occurred infrequently, and we therefore could not reliably assign rate constants to these data. Even for the less active channel, τ_{C3} and τ_{C4} were derived from a relatively small number of events and should therefore be considered to estimate the true values of these parameters. Table 1 shows the kinetic time constants for six different channels. These data

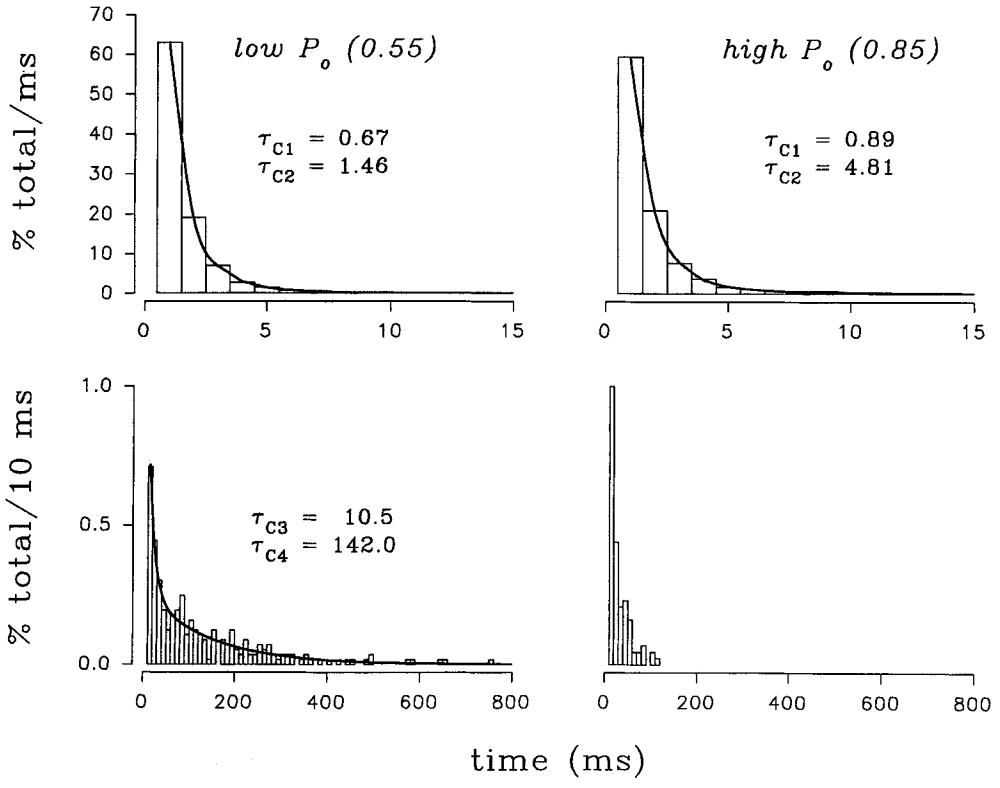

Figure 7. Closed time histograms: data from the same experiments as in Figure 6*b.* Two closed time histograms were generated for each experiment. Curves are the best fit to the data determined by an iterative curve-fitting program. *Upper panels* tabulate closures in the 0.5–15 msec range. *Top left panel,* The P_o equaled 0.55. τ_{C1} and τ_{C2} equaled 0.7 and 1.5 msec, respectively. *Top right panel,* The P_o equaled 0.85. τ_{C1} and τ_{C2} equaled 0.9 and 4.8 msec, respectively. *Lower panels* contain data in the 15–1000 msec range. *Bottom left panel,* same experiment as *top left panel.* τ_{C3} equaled 10.5 msec and τ_{C4} equaled 142 msec. *Bottom right panel,* Same experiment as *top right panel.* The histogram shows that few closures occur with durations in this time range in channels of high P_o.

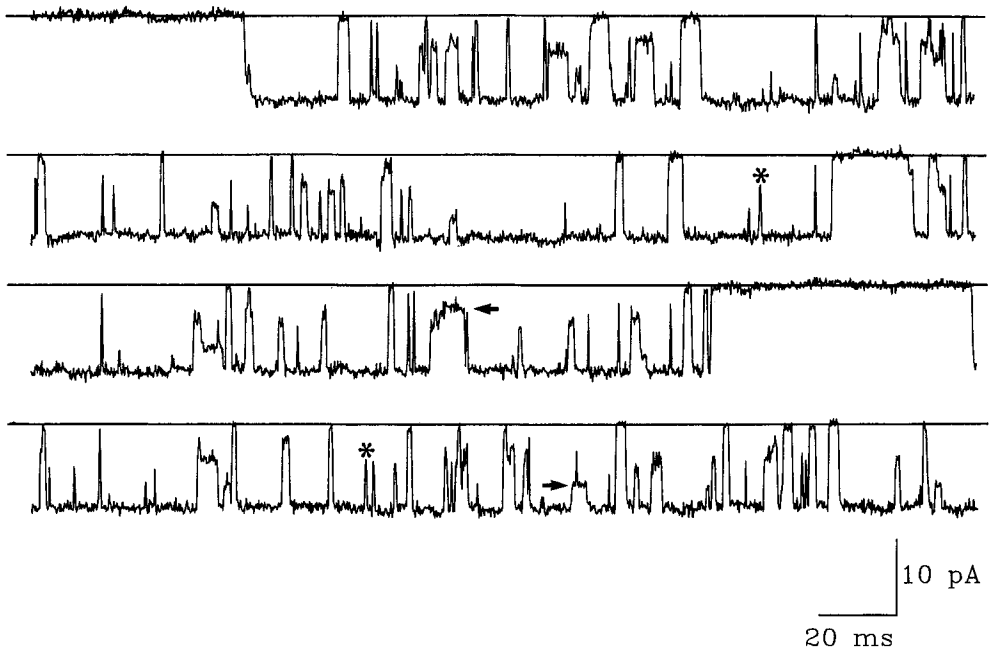

Figure 8. K_{Na} channel current substates. Data are taken from an inside-out patch containing only a single K_{Na} channel. V_m was −70 mV, $[K^+]_o/[K^+]_i$ equaled 150/0, and $[Na^+]_i$ was 150 mM. Inward currents typical of the open channel under these conditions are seen as downward deflections from the closed level (*solid line*). The *arrows* depict different substate levels, and the *asterisks* apparent substates that are not well resolved at our filter cutoff frequency (see Results).

emphasize that the open times are remarkably constant from one channel to another regardless of the P_o, and only the closed times differ.

Substates

A distinguishing characteristic of the K_{Na} channel was the presence of substates (Fig. 8; see also Figs. 10–12). These substates were seen as partial openings or partial closures during the burst interval. Within a burst a substate was entered from either the fully open or fully closed states, or from a different substate level. However, partial openings from the interburst fully closed state were rarely seen. Figure 9*a* shows the all-points current amplitude histogram taken from an inside-out membrane patch containing a single K_{Na} channel exposed to 150 mM $[Na^+]_i$ and held at −70 mV. The three data points at the beginning and end of each opening and closing were excluded from the raw data to prevent transition slurring of the binned data. $[K^+]_o/[K^+]_i$ equaled 150/0, and the single-channel conductance measured under these conditions was 175 pS. The histogram showed two clear peaks at about 0 and 12.25 pA, representing the fully closed and fully open states, respectively, each of which was fit well by a single Gaussian function. Between these two clear peaks were additional current amplitude points caused by the occurrence of multiple substates.

To estimate the number and relative size of the substates, idealized current amplitude data, corresponding to substate levels determined from the threshold crossing routine described in Materials and Methods, were tabulated and binned into a histogram (Fig. 9*b*). This histogram excluded data belonging to the fully opened or fully closed states, and included most of the substate amplitude data. Substate current amplitude histograms were not fit well by a single Gaussian function, suggesting the presence of at least two substates. Fitting the data with two Gaussian functions gave substate current amplitudes that measured about 41% and 57% of the fully open channel amplitude. Visual inspection of the raw data confirms the presence of multiple substates (arrows, Fig. 8). In five experiments analyzed in this manner, substates averaged 39 ± 6% and 62 ± 12% of the fully open channel amplitude. However, from the raw data we were able to identify more than two substates (see Fig. 8). In addition, the 2 kHz cutoff frequency used in these experiments may have resulted in our failure to record accurately the full amplitude of some substate levels (asterisks, Fig. 8). Because of this uncertainty, and because the channel spent relatively little

Table 1. Transition rate constants for six K_{Na} channels

Experiment	V_m	P_o	τ_{O_1}	τ_{O_2}	τ_{C_1}	τ_{C_2}	τ_{C_3}	τ_{C_4}
1	−40	0.48	1.21 (19)	5.13 (81)	0.89	1.42	20.03	70.86
2	−70	0.55	0.64 (44)	5.27 (56)	0.67	1.46	10.04	142.00
3	−60	0.59	0.97 (10)	5.80 (90)	0.79	3.35	28.12	45.50
4	−60	0.81	0.85 (38)	6.82 (62)	0.70	3.77	—	—
5	−80	0.85	0.51 (40)	5.33 (60)	0.90	4.82	—	—
6	−80	0.89	1.23 (31)	7.08 (69)	0.89	4.21	—	—

V_m values are given in mV; τ values are given in msec. The numbers in parentheses are the percent weight of each τ_O. Because τ_{C_1} and τ_{C_2}, and τ_{C_3} and τ_{C_4} are estimated from different histograms, we could not accurately measure the percentage of total closed times described by each τ. Also, because τ_{C_3} and τ_{C_4} are derived from a small number of closures, curve fits estimate the real values of τ_{C_3} and τ_{C_4}.

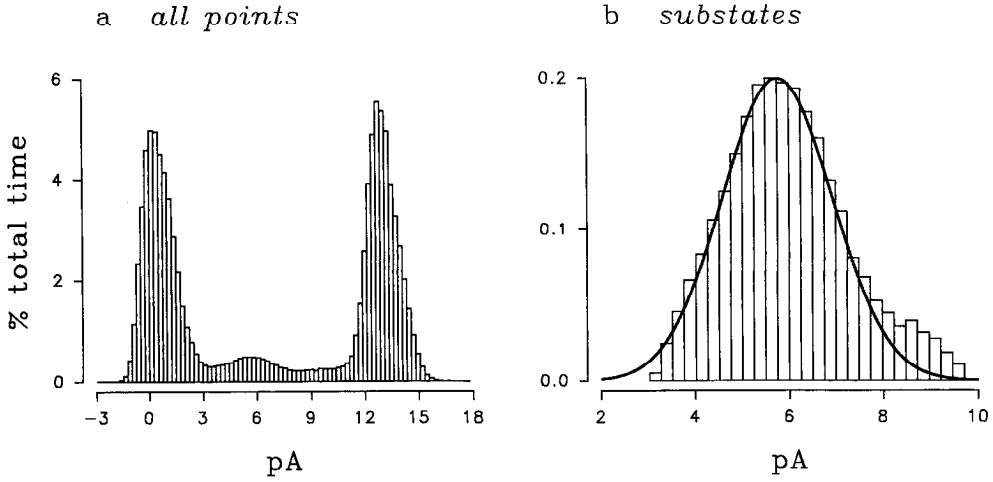

Figure 9. Amplitude histograms. Current amplitude histograms were constructed for data from the experiment pictured in Figure 8. *a,* Binned data of about 24,000 transitions. The all-points histogram showed two prominent peaks centered at 0 and 12.25 pA, and a small broad peak at about 5 pA. *b,* The histogram of the substate current amplitudes was obtained from idealized current records to avoid complications due to baseline and open channel noise. It was not fit well by a single Gaussian function (*smooth line*), suggesting that at least two substate levels were present.

time in the multiple substate levels, we did not attempt to characterize the absolute number of substates.

The effect of $[Na^+]_i$ on substate levels was tested for a K_{Na} channel in an inside-out patch held at −60 mV. $[K^+]_i$ and $[K^+]_o$ were kept constant at 150 mM each, and $[Na^+]_i$ was changed to 60, 90, 120, and 150 mM. $[Na^+]_i$ had no effect on either the relative amplitude or the relative occurrence of the two most prominent substate levels.

Pharmacology

K^+ channels can be classified according to susceptibility to specific blockers. We tested some of these blockers on K_{Na} channels. *d*-Tubocurarine (*d*-TC) reduces the P_o of K_{Ca} channels (Smart, 1987). We found that this drug also decreases the P_o of K_{Na} channels, but at 10× higher concentrations than those needed for K_{Ca} channels. Internal application of *d*-TC (10–100 μM) reduced the P_o of K_{Na} channels by changing the mean open and closed times without changing the single-channel conductance. Figure 10 shows an example of the effect of 50 μM *d*-TC applied to the internal membrane surface of an inside-out patch containing a single K_{Na} channel. In the absence of drug, the channel was open about 50% of the time, and the mean open and closed times were 4.8 and 5.8 msec, respectively. In the presence of *d*-TC, the P_o fell to 0.06, the mean open time decreased to 1.9 msec, and the mean closed time increased to 26.3 msec. Application of *d*-TC to the external surface of the membrane had no effect in the concentration range tested here.

The piperidine R56865 has been reported to block the K_{Na} channel of heart muscle (Luk and Carmeliet, 1990). In olfactory bulb neurons, R56865 (2.5 μM; $n = 3$) acted like *d*-TC to reduce the P_o of K_{Na} channels when applied to the internal membrane surface (Fig. 11), as did the related compound R58735 (sabeluzole; 2.5 μM; $n = 2$). Similar concentrations of both piperidines also decreased the P_o of K_{Ca} channels in cultured olfactory bulb neurons, although another unidentified type of K^+ channel was not affected (T. M. Egan and J. Kupper, unpublished observations). R56865 and R58735 therefore should not be considered selective blockers of K_{Na} channels. Neither the voltage-activated

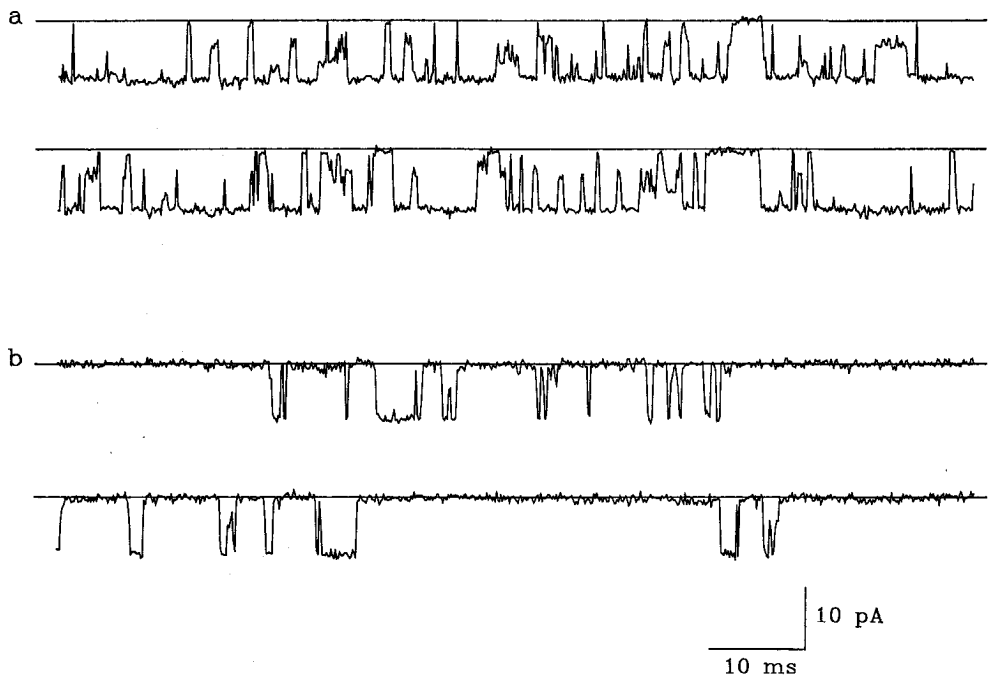

Figure 10. *d*-TC blocks K_{Na} channels: data from an inside-out membrane patch containing a single K_{Na} channel. V_m equaled −40 mV and $[Na^+]_i$ was 150 mM. *Solid lines* indicate closed levels. *a,* In the absence of drug, the channel was open about 50% of the time. *b,* After 50 μM *d*-TC was added to the cytoplasmic surface, the channel was open only 6% of the time. The channel activity returned to control levels shortly after removing *d*-TC (not shown).

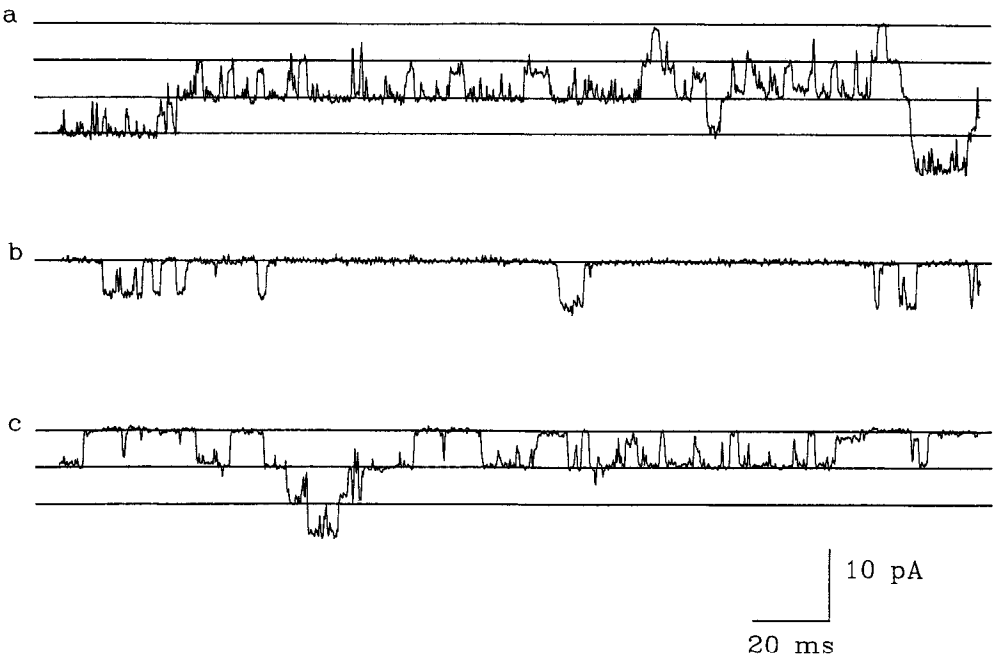

Figure 11. The piperidine R56865 blocks K_{Na} channels: data from a patch containing at least four K_{Na} channels. V_m equaled −43 mV and $[Na^+]_i$ was 150 mM. The *solid lines* indicate closed levels for the individual channels. *a*, In the absence of drug, superimposed channel openings are common. *b*, In the presence of 2.5 μM R56865, the channels open less often. *c*, Superimposed channel openings occur again after washout of the drug. Note, however, that the overall channel activity is lower than control because of channel rundown (see Results for details).

Na^+ channel blocker TTX (1–10 μM; see Fig. 3*e*), the K_{Ca} channel blocker charybdotoxin (0.01–10 μM), nor strophanthidin (1 mM; $n = 2$), which inhibits the Na^+/K^+ pump, blocked K_{Na} channels when applied to either the internal (using inside-out membrane patches) or external (using outside-out membrane patches) membrane surfaces.

P_o *decreases with time in detached patches*

In a typical experiment using the inside-out patch configuration and an $[Na^+]_i$ equal to 150 mM, the P_o (or the NP_o) decreased over a period of time lasting from a few tens of seconds to up to 5 hr. An example of rapid rundown of K_{Na} channel activity, with complete cessation of activity by 2 min after patch excision, is shown in Figure 12. Plots of NP_o as a function of time after patch excision for several patches (Fig. 13) illustrate the variability in time course of rundown observed, even in a single culture dish on 1 d. Variability was greater among groups of neurons cultured from different litters of rats on different days than from neurons of the same culture date. The length of time in culture (7–28 d) did not influence the extent or time course of rundown (although we did not extensively test cells younger than 6 d in culture). Often, the P_o would decrease until virtually no channel gating was recorded. In these patches, channel activity could be recovered temporarily by increasing $[Na^+]_i$; how-

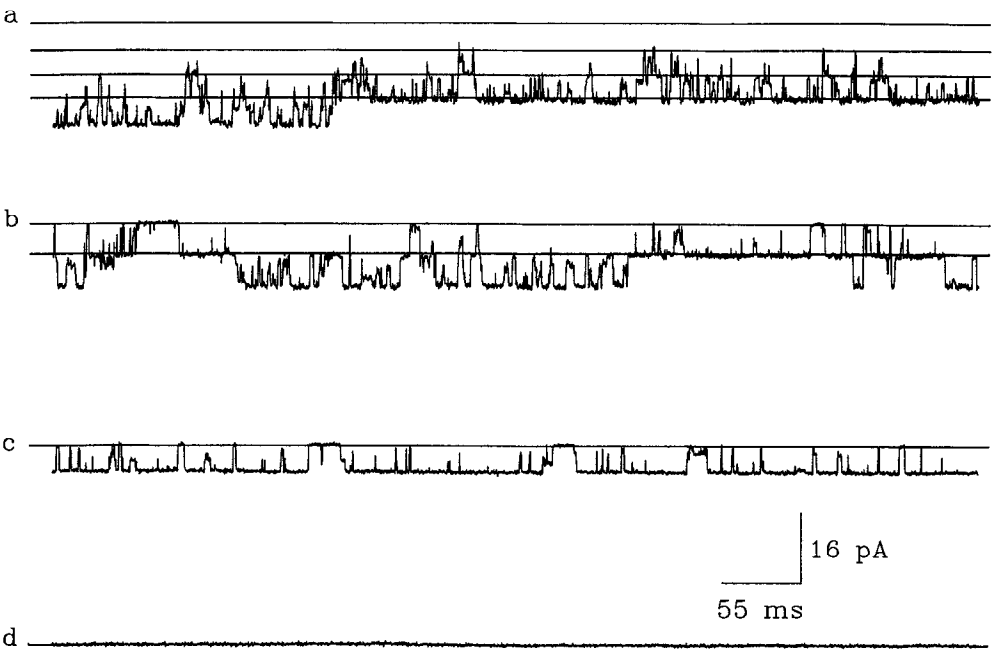

Figure 12. K_{Na} channel rundown: Data from a four channel patch showing a rapid decrease in channel activity following membrane patch excision. $[Na^+]_i$ equaled 150 mM, $[K^+]_o/[K^+]_i$ equaled 150/0, and V_m was −40 mV. Inward current through the open channel was shown as *downward deflections* from the baseline. *Solid lines* indicate closed levels. Channel activity was high immediately after patch excision (*a*), but was lower at 30 sec (*b*) and 60 sec (*c*) post-excision. No openings were seen after about 90 sec (*d*).

ever, channel activity again quickly fell to zero as the channels became insensitive to the higher $[Na^+]_i$.

Rundown was not activity dependent; that is, it did not depend on the channel opening a given number of times. This was tested in the following way. Experiments were performed on neurons of the same culture date to eliminate some of the variability seen among cultures as detailed above. Control patches were excised into a 150 mM $[Na^+]_i$ solution and NP_o was measured to determine the time course of rundown. In this set of control experiments, rundown occurred rapidly so that NP_o fell from values near 0.8 to almost 0 after about 10 min postexcision. Then, patches taken from other neurons of the same culture date were excised immediately into a 0 mM $[Na^+]_i$ solution to prevent channel activity. After 30 min, $[Na^+]_i$ was increased to 150 mM to induce channel openings. If rundown was activity dependent, then we would have expected that introduction of $[Na^+]_i$ would result in about the same average NP_o seen in the control patches immediately following excision into 150 mM $[Na^+]_i$. This was not the case. Channel activity was abnormally low or absent in the test patches after introduction of 150 mM $[Na^+]_i$ following a 30 min preincubation in 0 mM $[Na^+]_i$, suggesting that rundown is not influenced by prior channel activity (or lack thereof).

We do not know what causes rundown. One possibility is that a cytoplasmic intermediate needed for prolonged channel activity is lost upon membrane excision. This is supported by our finding that channels in on-cell patches (in the presence of veratridine) do not show the rundown phenomenon. If some factor essential for gating is lost upon excision of the patch, then one would expect that gating would be modulated by adding an exogenous factor. We tested several possible modulators for their ability to influence channel gating behavior. Channel activity recorded from inside-out membrane patches was not affected by application of cAMP, cGMP, ATP (all at 1 mM), cAMP-dependent protein kinase with 1 mM ATP (n = 2), or protein kinase C (n = 2) to the internal membrane surface. Furthermore, none of these paradigms prevented channel rundown. Changing the temperature from about 25°C to 37°C did not affect the time course of rundown in inside-out patches. Forskolin (50 μM), a membrane-permeable activator of adenyl cyclase, did not affect K_{Na} channel activity recorded from on-cell patches in the presence of veratridine (n = 3). Likewise, a 1–10 hr preincubation in pertussis toxin (200–400 ng/ml) did not affect the ability of veratridine to activate K_{Na} channels. Forskolin or pertussis toxin pretreatment also did not prevent channel rundown upon patch excision.

K_{Na} channels in thin slices of olfactory bulb

K_{Na} channels were recorded from visually identified neurons in the mitral cell body layer of thin slices of olfactory bulb. K_{Na} channels identical to those of cultured olfactory bulb neurons were activated in inside-out patches by 150 mM $[Na^+]_i$. Channel activity decreased to zero when $[Na^+]_i$ was replaced with $[K^+]_i$ but was not affected by changes in $[Ca^{2+}]_i$. The channel behavior resembled that of K_{Na} channels of cultured olfactory bulb neurons with respect to the presence of multiple substates and the occurrence of rundown. d-TC (60 μM) applied to the internal surface of the membrane blocked the channel. K_{Na} channels could also be recorded in on-cell patches from mitral neurons after addition of veratridine (10–60 μM) to the bath solution (n = 3). Thus far, in six attempts we have not observed K_{Na} channels in granule cell neurons in thin slices.

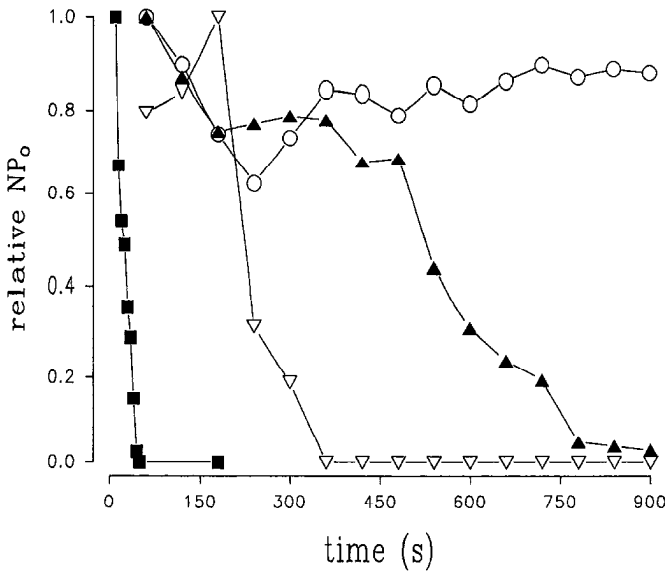

Figure 13. Variable rates of channel rundown. NP_o values for four different patches (*different symbols*) as a function of time after patch excision to the inside-out configuration. NP_o values were calculated for 5 sec (■) or 60 sec (▽, ▲, ○) intervals and were normalized to the maximum NP_o level for each patch. $[Na^+]_i$ equaled 150 mM, $[K^+]_o/[K^+]_i$ equaled 150/0, and V_m was −40 mV for all patches. Three of these patches (▽, ▲, ○) were taken from neurons in a single culture dish on the same day; the other (■) is the patch whose channel activity is illustrated in Figure 12. The kinetic analysis in Figures 6 and 7 was done on patches exhibiting stable kinetic behavior, such as the one depicted with ○ here.

Whole-cell currents

Current flow through K_{Na} channels may contribute to a brief outward current that follows the voltage-activated Na^+ current in some preparations (Bader et al., 1985; Hartung, 1985; Dryer et al., 1989; but see also Dryer, 1991). Whole-cell voltage clamp of cultured rat olfactory bulb neurons failed to demonstrate any transient outward current resembling the K_{Na} whole-cell current of other tissues except when very high concentrations (≥50 mM) of Na^+ were included in the pipette solution. When the pipette contained 30 mM Na^+, the $[Na^+]_i$ used to evoke whole-cell K_{Na} current in experiments on other tissues (see Discussion), a step depolarization to −30 mV from a resting V_m of −80 mV resulted in an inward Na^+ current only. Only when the pipette contained 50 mM or more Na^+ was the inward Na^+ current followed by a transient outward current that lasted about 5–10 msec. Both inward and outward currents were absent after superfusion of TTX (100 nM), and both currents returned after washout of TTX.

Discussion

K_{Na} channels have previously been recorded from mammalian heart (Kameyama et al., 1984; Luk and Carmeliet, 1990; Wang et al., 1991) and from nonmammalian nervous tissue (see Martin and Dryer, 1989; Kubota and Saito, 1991; Saito and Wu, 1991). In mammalian brain, voltage-clamp currents that are thought to be K_{Na} currents have been reported (Constanti and Sim, 1987; Schwindt et al., 1989). We report here recordings of single K_{Na} channel activity from mammalian CNS. Such single-channel measurements eliminate the possibility of voltage-clamp artifacts (see Dryer, 1991) and provide unequivocal evidence

for a population of K^+-selective ion channels that are activated by Na_i. These channels are present in neurons from a variety of regions of the rat brain, although their density appears to be the highest in olfactory bulb (T. M. Egan, D. Dagan, J. Kupper, and I. B. Levitan, unpublished observations). The K_{Na} channel was the predominant K^+-selective channel recorded from inside-out patches of olfactory bulb neurons when $[Na^+]_i$ was greater than about 10–20 mM and V_m was hyperpolarized. It was easily differentiated from other K^+ channels by its conductance and gating behavior and by the presence of several distinct substates. Most patches contained several K_{Na} channels, and it was not unusual to have four or more actively gating K_{Na} channels in a patch of membrane enclosed by the 1–2 μm diameter tip of the recording electrode. If, as the numbers imply, the density of K_{Na} channels is high in these neurons, then several important questions about some of the unusual properties of this channel come to mind.

Regulation of gating by $[Na^+]_i$

K_{Na} channels opened when $[Na^+]_i$ exceeded some minimal concentration, above which the P_o depended on $[Na^+]_i$. One important question is why the channel is relatively insensitive to its major activator, $[Na^+]_i$. Relatively little $Ca^{2+}{}_i$ (tens or hundreds of nanomolar) is needed to activate K_{Ca} channels. However, we needed at least 10–20 mM $Na^+{}_i$ to cause a noticeable activation of K_{Na} channels in experiments on inside-out membrane patches, and even higher concentrations to activate the channel half-maximally. These data agree with measurements of the $[Na^+]_i$ sensitivity of K_{Na} channels of other tissues. However, it seems unlikely that such high $[Na^+]_i$ is ever reached in healthy, intact neurons, even at the peak of the action potential. Thus, it seems possible that the true $[Na^+]_i$ sensitivity of the channel is higher. Several observations suggest that this is indeed the case. First, K_{Na} channels had a wide range of sensitivity to $[Na^+]_i$. For example, while 20 mM $Na^+{}_i$ often induced K_{Na} channel activity (Fig. 1), in some experiments $[Na^+]_i$ had to exceed 60 mM before significant activation occurred. Further, the P_o measured immediately upon excision into 150 mM $[Na^+]_i$ also varied greatly for our sample of the K_{Na} channel population, ranging from 0.2 to about 0.8. While the activities of channels of high initial P_o values were not increased by further raising $[Na^+]_i$ (up to 180 mM), K_{Na} channels that were fairly insensitive to 150 mM $[Na^+]_i$ (P_o values < 0.8) did respond to additional $[Na^+]_i$. Also, in patches containing many K_{Na} channels, NP_o continued to increase even at the highest $[Na^+]_i$ tested (180 mM). This variability makes it difficult to pinpoint the true $[Na^+]_i$ sensitivity of the channel. We believe that the variable $[Na^+]_i$ sensitivity may be related to the phenomenon of rundown.

Rundown was a prominent feature of many if not all K_{Na} channels in off-cell patches. Channel activity, which was normally high immediately following excision, decreased with time, such that the P_o (or the NP_o) sometimes fell to almost zero in just a few minutes (Fig. 12). While the very rapid rundown depicted in Figure 12 was observed infrequently, channel activity did decrease during most experiments (Fig. 13). Therefore, because it took 15–30 min to construct the $[Na^+]_i$ versus P_o curves, rundown probably resulted in an underestimate of the true $[Na^+]_i$ sensitivity. How much we have underestimated the $[Na^+]_i$ sensitivity is unknown. We noticed that channel activity was always very high in the first few seconds following patch excision, after which it would quickly settle to a new level from which it would fall more slowly with time. This very rapid decrease in channel activity immediately following excision was difficult to quantify, and may result from the same phenomenon responsible for the slower phase of rundown that we observed routinely. Thus, a degree of rundown may have occurred even in those channels that showed a relatively high initial $[Na^+]_i$ sensitivity, and even here $[Na^+]_i$ versus P_o curves may have underestimated the true $[Na^+]_i$ sensitivity.

Finally, $[Na^+]_i$ versus P_o was measured routinely at a V_m of −40 to −60 mV. Other experiments showed that K_{Na} channels are voltage dependent, and opened more often at depolarized V_m at any one $[Na^+]_i$. We measured $[Na^+]_i$ sensitivity at negative V_m because we found it difficult to maintain gigaohm seals when patches were held at positive V_m for more than a few minutes. Also, single-channel currents were more difficult to measure at positive V_m because of the Na^+ block of K_{Na} channels that gave rise to inward rectification. We expect that K_{Na} channels would appear to be more sensitive to $[Na^+]_i$ if this parameter could be measured accurately at positive V_m. This may be important, because $[Na^+]_i$ would be expected to be highest at the peak of the action potential, a time at which the cell would also be very depolarized.

What is the function of K_{Na} in olfactory bulb neurons?

Several possible functions have been postulated for K_{Na} channels of other tissues. These include a role in repolarizing the cell following an action potential (Bader et al., 1985; Hartung, 1985), contributing to the generation of the resting V_m (Dryer et al., 1989), protecting the cell from $[Na^+]_i$ or $[Ca^{2+}]$ overload (Kameyama et al., 1984; Luk and Carmeliet, 1990), and reducing excitability during maintained subthreshold excitation (Schwindt et al., 1989). The channel may also play a role in neurotransmitter modulation of firing patterns (Constanti and Sim, 1987; Foehring et al., 1989). So far, experiments designed to test two of these ideas have not defined a role for the K_{Na} channel in olfactory bulb neurons. First, it seems unlikely that the channel normally contributes to the resting V_m because we never saw an active channel in on-cell patches (except in the presence of veratridine). Second, whole-cell voltage clamp of cultured olfactory bulb neurons failed to implicate K_{Na} currents in repolarization following inward Na^+ currents. In other tissues, step depolarizations from appropriate resting V_m produce large voltage-dependent inward Na^+ currents that are immediately followed by transient outward K^+ currents (Bader et al., 1985; Hartung, 1985; Dryer et al., 1989). It has been suggested that this outward current results from current flow through K_{Na} channels. However, recent evidence has shown that these transient outward currents may result from an artifact of the experimental procedure and not from activation of K_{Na} channels (Dryer, 1991). Using the same conditions that produced outward currents in other tissues (i.e., 30 mM $[Na^+]_i$), we did not record a transient outward current following induction of inward Na^+ current. As our data suggest that the K_{Na} channels may be controlled by an intracellular intermediate, which is lost in off-cell patches, one explanation for our failure to record whole-cell K_{Na} currents may be that the concentration of this same intracellular intermediate decreases as a consequence of the perfusion of the inside of the neurons by the contents of the whole-cell patch electrode.

The idea that the K_{Na} channel is affected by neurotransmitters is intriguing. Most of the incoming synaptic input to the mitral cells occurs on the distal dendrites. If the K_{Na} channel is present

in the dendrites, and if the channel is, as suggested, more sensitive to $[Na^+]_i$ than we have so far been able to measure, then it is possible that $[Na^+]_i$ may rise in response to an excitatory neurotransmitter to a level high enough to activate K_{Na} channels. Likewise, if, as suggested by the presence of rundown, the channel activity is controlled by some diffusible substance, then it is feasible that a neurotransmitter that influences the postsynaptic concentration of this substance would affect K_{Na} channel gating.

What do we know about rundown?

A defining characteristic of the K_{Na} channel of rat olfactory bulb neurons was the gradual decrease in channel activity recorded from off-cell patches. Rundown does not appear to be a general feature of K_{Na} channels of most other tissues, although it is seen in channels in inside-out patches of cultured chick olfactory bulb neurons (S. E. Dryer, personal communication) and in *Xenopus* oocytes (J. Kupper and I. B. Levitan, unpublished observations). We do not know at present what causes the activity to decrease with time. One obvious explanation is that some diffusible intracellular substance that is required for the maintenance of channel gating is lost upon excision of the patch. This hypothesis is strengthened by the observation that on-cell recordings of K_{Na} channels in veratridine-treated cells did not show rundown. The outcome of losing the diffusible substance apparently is a shift in the sensitivity of the channel to $[Na^+]_i$, as channel activity is momentarily recovered after rundown by addition of high $[Na^+]_i$, showing that the channel is still able to gate but requires a higher $[Na^+]_i$ to do so. If the channel requires some chemical to continue gating, then it is expected that the channel gating would be affected in off-cell patches by adding this chemical to the solution bathing the internal surface of the membrane. Several preliminary attempts at adding back putative channel modulators have so far failed to affect channel gating or rundown, and the diffusible intracellular substance remains to be identified. By understanding what causes rundown we expect to gain insight into how the cell modulates the activity of these channels.

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