

Gating of Na Channels in the Rat Cortical Collecting Tubule: Effects of Voltage and Membrane Stretch

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ABSTRACT The gating kinetics of apical membrane Na channels in the rat cortical collecting tubule were assessed in cell-attached and inside-out excised patches from split-open tubules using the patch-clamp technique. In patches containing a single channel the open probability (P_o) was variable, ranging from 0.05 to 0.9. The average P_o was 0.5. However, the individual values were not distributed normally, but were mainly ≤ 0.25 or ≥ 0.75 . Mean open times and mean closed times were correlated directly and inversely, respectively, with P_o . In patches where a sufficient number of events could be recorded, two time constants were required to describe the open-time and closed-time distributions. In most patches in which basal P_o was < 0.3 the channels could be activated by hyperpolarization of the apical membrane. In five such patches containing a single channel hyperpolarization by 40 mV increased P_o by 10-fold, from 0.055 ± 0.023 to 0.58 ± 0.07 . This change reflected an increase in the mean open time of the channels from 52 ± 17 to 494 ± 175 ms and a decrease in the mean closed time from $1,940 \pm 350$ to 336 ± 100 ms. These responses, however, could not be described by a simple voltage dependence of the opening and closing rates. In many cases significant delays in both the activation by hyperpolarization and deactivation by depolarization were observed. These delays ranged from several seconds to several tens of seconds. Similar effects of voltage were seen in cell-attached and excised patches, arguing against a voltage-dependent chemical modification of the channel, such as a phosphorylation. Rather, the channels appeared to switch between gating modes. These switches could be spontaneous but were strongly influenced by changes in membrane voltage. Voltage dependence of channel gating was also observed under whole-cell clamp conditions. To see if mechanical perturbations could also influence channel kinetics or gating mode, negative pressures of 10–60 mm Hg were applied to the patch pipette. In most cases (15 out of 22), this maneuver had no significant effect on channel behavior. In 6 out of 22 patches, however, there was a rapid and reversible increase in P_o when the pressure was applied. In one patch, there was a reversible decrease. While no consistent effects of pressure could be documented, membrane deformation could contribute to the variation in P_o under some conditions.

INTRODUCTION

Epithelial Na channels in such tissues as the rat cortical collecting tubule (CCT), toad urinary bladder and in amphibian cells (A6) in culture are characterized by a high selectivity for Na over K, single-channel conductance of 4–5 pS at room temperature (8–9 pS at 37°C) and long mean open and closed times (Eaton and Hamilton, 1988; Garty and Benos, 1988; Smith and Benos, 1991; Palmer, 1992). The open probability of these channels is, however, quite variable. In the rat

CCT under apparently identical physiological conditions P_o can range from very low levels (< 0.05) to very high values (> 0.95) (Palmer and Frindt, 1988; Pácha et al., 1993). In some cases, two channels in the same patch can apparently have different values of P_o (Palmer and Frindt, 1988; Marunaka et al., 1992). Variables that have been proposed to affect P_o include intracellular pH (Palmer and Frindt, 1987), intracellular Ca (Silver et al., 1993), activation of PKC (Ling and Eaton, 1989), activation of G-proteins (Ohara et al., 1993), insulin, (Marunaka et al., 1992) and aldosterone (Kemendy et al., 1992).

Another factor that can influence P_o is the membrane voltage. In contrast to the effects of voltage on Na channels from excitable cells, hyperpolarization of

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the cell membrane activates epithelial Na channels while depolarization deactivates them (Palmer and Frindt, 1988; Frindt et al., 1993). The response to voltage was weak compared with classical voltage-gated channels, with P_o increasing by $\sim 2\%$ per mV (Palmer and Frindt, 1988). The voltage dependence of the channels may be important in mediating feedback control of channel activity, especially during changes in the rate of Na entry into the cells (Frindt et al., 1993). These effects have not been studied in detail.

Recently epithelial Na channels have been cloned from the rat colon (Canessa et al., 1993; Lingueglia et al., 1993; Canessa et al., 1994). These clones called α , β , and γ rENaC's, show no significant homology to other known vertebrate ion channels. They do share, however, homology of both nucleotide and protein sequences as well as similarity of apparent membrane topology with several genes from the nematode *Caenorhabditis elegans* which are expressed in sensory neurons and are thought to be involved in the transduction of mechanical stimuli (Driscoll and Chalfie, 1991; Chalfie et al., 1993; Huang and Chalfie, 1994). This raises the possibility that the epithelial Na channels are related to mechanosensory channels. The sensitivity of the Na channels to mechanical perturbations of the membrane, e.g., membrane stretch, has not yet been examined.

In this paper we describe the kinetic properties of the epithelial Na channel from the rat renal cortical collecting tubule, and the effects of changes in membrane voltage and transmembrane pressures on channel gating.

MATERIALS AND METHODS

Biological Preparations

Sprague-Dawley rats of either sex (100–150 g) raised free of viral infections (Charles River Laboratories, Kingston, NY) were fed a low Na diet for at least one week to enhance Na channel activity. Animals were killed by cervical dislocation, the kidneys removed, and the CCT dissected free and opened manually to expose the luminal surface. The split tubules were attached to a small plastic rectangle coated with Cell-Tak (Collaborative Research, Bedford, MA) and placed in a perfusion chamber mounted on an inverted microscope. The chamber was continuously perfused with solution which was prewarmed to 37°C by a miniature water-jacketed glass coil (Radnotti Glass Technology, Monrovia, CA). Principal cells of the tubule were identified visually as described previously (Pácha et al., 1991). To apply pressure across the patch, suction was applied to the back of the pipette through the pipette holder using a micrometer syringe. The pressure was monitored using a pressure transducer (model DPM1-B; Bio-Tek).

Solutions

Tubules were superfused with a solution consisting of (millimolar): 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 2 glucose, and 10 *N*-2-

hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) adjusted to pH 7.4 with NaOH. Modifications of the solution are described in the text. The patch-clamp pipettes were filled with solutions containing (millimolar): 140 LiCl, 3 MgCl₂, and 10 Hepes, adjusted to pH 7.5 with LiOH. Li was used as the primary cation in the pipette because the single-channel conductance is higher to Li than to Na, giving a better signal-to-noise ratio (Silver et al., 1993).

For whole-cell recordings the tubules were bathed with a solution in which NaCl was replaced by Na methane sulfonate to reduce membrane Cl⁻ conductance. The pipette solution contained (millimolar): 7 KCl, 123 aspartic acid, 20 CsOH, 20 TEAOH, 5 EGTA, 10 Hepes, 3 MgATP, and 0.3 NaGTP with the pH adjusted to 7.4 with KOH.

Electrical

Basic patch-clamp methods were as described previously (Frindt et al., 1993; Silver et al., 1993). Recording of currents and analysis of data were carried out with an Atari 1040 ST computer equipped with interface and data acquisition software (Instrutech, Mineola, NY). Current records were stored on video tape using a pulse-code modulator (Instrutech). Computation of the mean number of open channels (NP_o), open probability (P_o) and mean open and closed times was carried out using the TAC program (Instrutech). In patches containing a single channel the open- and closed-time distributions were plotted and analyzed according to the method of Sigworth and Sine (Sigworth and Sine, 1987). Fitting of the distributions was carried out using the maximum likelihood method with the TAC program.

RESULTS

Kinetics of Na Channels in Single-Channel Patches

It is rare to find single Na channels in patches from the rat CCT. When the rat is on a low-Na diet, most patches contain many channels, with the average around five (Pácha et al., 1993). When the animal is on a normal diet, there is almost no Na channel activity at all. Intermediate conditions have been difficult to establish. Nevertheless over a period of about four years a reasonable number of patches which did contain just one active channel were observed. In such patches, only two current levels, corresponding to the open and closed state of the channels, were observed over a period of 3 min or more. These channels had conductances (12–13 pS) similar to those reported previously for the same conditions (Frindt et al., 1993). This value is higher than the 5 pS value associated with this channel (Palmer, 1992) because Li rather than Na was the major permeant ion and the measurements were made at 37°C rather than room temperature. The kinetics of these channels are described in Figs. 1–3. A striking finding was the variability in the mean open and closed times and the open probability within this group.

Fig. 1 *A* shows an example of a channel with a low P_o . The activity of the channel was recorded for 11.5 min. The P_o , which averaged 0.093, varied somewhat over this time period but there was no systematic change

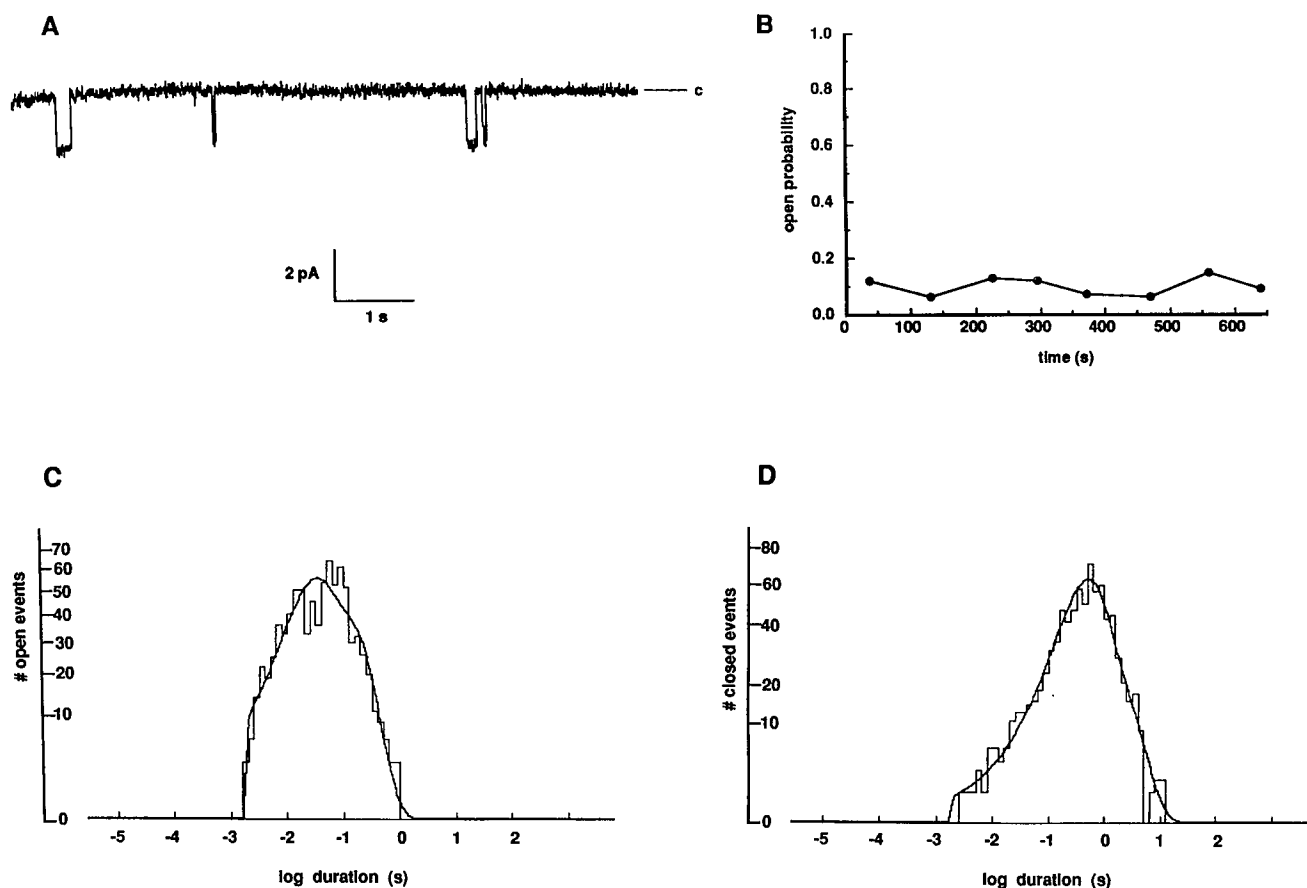


FIGURE 1. Kinetics of a channel with low P_o (0.093). (A) Portion of the original recording which was 695 s long. The pipette potential was +20 mV. (B) Plot of P_o as a function of time. (C) Open-time histogram. The kinetics are plotted according to the method of Sigworth and Sine (1987). The solid line represents a fit to two exponentials with time constants 123 ms (51% of total events) and 24 ms (49%). (D) Closed-time histogram. The solid line represents a fit to two exponentials with time constants 1,580 (27%) and 454 ms (73%).

(Fig. 1 B). It was necessary to establish that the P_o was constant to use the entire record for assessing the kinetic properties of the channel. The time interval was selected arbitrarily. The mean open time was 77 ms, and the mean closed time was 760 ms. Open and closed-time distributions are shown in Fig. 1, C and D. The open-time histogram could be fitted by two exponentials, as described in the figure legend. The closed-time histogram could be described fairly well with a single exponential, although a better fit was obtained with two exponentials. Five out of six of the channels within this group with $P_o < 0.22$ required two exponentials to fit the open-time distribution. In all of these cases the closed times could be reasonably well described with a single exponential.

Fig. 2 A shows a record from a patch with a channel of intermediate P_o . The total recording time was 7 min and P_o was stable with an average value of 0.55 (Fig. 2 B). The mean open time was 350 ms. As in the case above, the open-time distribution indicated two time constants (Fig. 2 C). The mean closed time was 286 ms

and was fairly well described by a single exponential (Fig. 2 D). There were only three channels in the group with intermediate P_o values between 0.25 and 0.75. All indicated two open times. One of the three also required two time constants to fit the closed-time distributions. In this patch the pipette potential was zero, compared with +20 mV for Figs. 1 and 3. No comparable record at +20 mV was available for this group.

Fig. 3 A shows a record from a patch with a single channel with a high P_o . This recording lasted for 10 min. The average P_o was 0.87 and again was relatively constant over the recording period (Fig. 3 B). The mean open time was 1.62 s. The open times were best described by two exponentials, although most (88%) of the events were within the distribution of longer openings (mean open time 1.81 s). The mean closed time was 237 ms. In this case the closed time histogram indicated the presence of two closed time constants of 340 and 41 ms.

The relationship between P_o and open and closed times is shown in Fig. 4. In this and subsequent figures

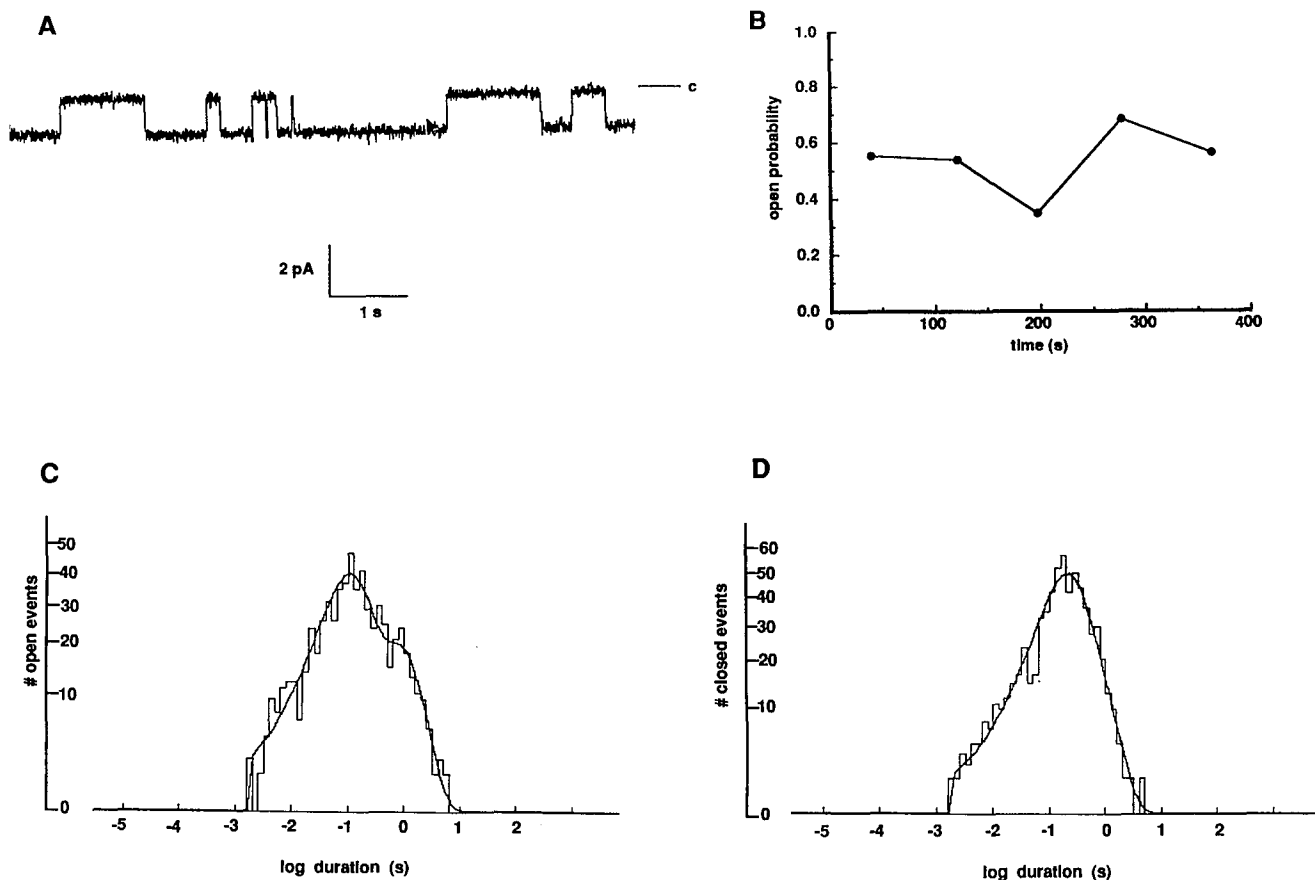


FIGURE 2. Kinetics of a channel with intermediate P_o (0.55). (A) Portion of the original recording that was 399 s long. The pipette potential was 0 mV. (B) Plot of P_o as a function of time. (C) Open-time histogram. The kinetics are plotted according to the method of Sigworth and Sine (1987). The solid line represents a fit to two exponentials with time constants 761 (37% of total events) and 95 ms (63%). (D) Closed-time histogram. The solid line represents a fit to two exponentials with time constants 761 ms (37%) and 171 ms (63%).

only the mean open and closed times are shown. Recordings of < 5 min under the same conditions were not long enough to accurately resolve multiple time constants as shown in Figs. 1–3. The mean open and closed times are weighted averages of two or more populations of open or closed events. There is a clear increase in mean open times and a decrease in mean closed times as P_o increases. Three patches appeared to fall somewhat outside this relationship. These are shown as circled symbols in the figure, and had more rapid, flickery kinetics. Both the mean open times (25–60 ms) and mean closed times (120–190 ms) were short. All three of these patches were obtained within a three day period on the same batch of rats. The channels had a normal i - V relationship characteristic of the Na channels, with a slope conductance for inward current of 12 pS. We do not know why they behaved differently.

In previous studies using mainly multichannel patches the mean values of P_o were given as 0.4–0.5 (Palmer and Frindt, 1988; Pácha et al., 1993). Curiously,

there were very few single channels which had a P_o in this range. It is possible that the criterion of having a single channel selects for conditions with extreme values of P_o . Another possibility is that multichannel patches contain two populations of channels with high and low P_o which tend to average. Indeed the average P_o of the 19 single-channel patches was 0.50 ± 0.08 . This suggests the presence of at least two gating “modes” for the channels, one with high P_o and one with low P_o . Multichannel patches may then have a mix of these modes.

Effects of Voltage on Channel Gating

Further evidence for gating modes, and for inter conversion between them, was obtained from observations of the effects of voltage on channel gating. We have previously reported that hyperpolarization can activate the epithelial Na channel (Palmer, 1988; Frindt, 1993). In the present study we examined this effect in more detail using patches in which the initial P_o was low, such

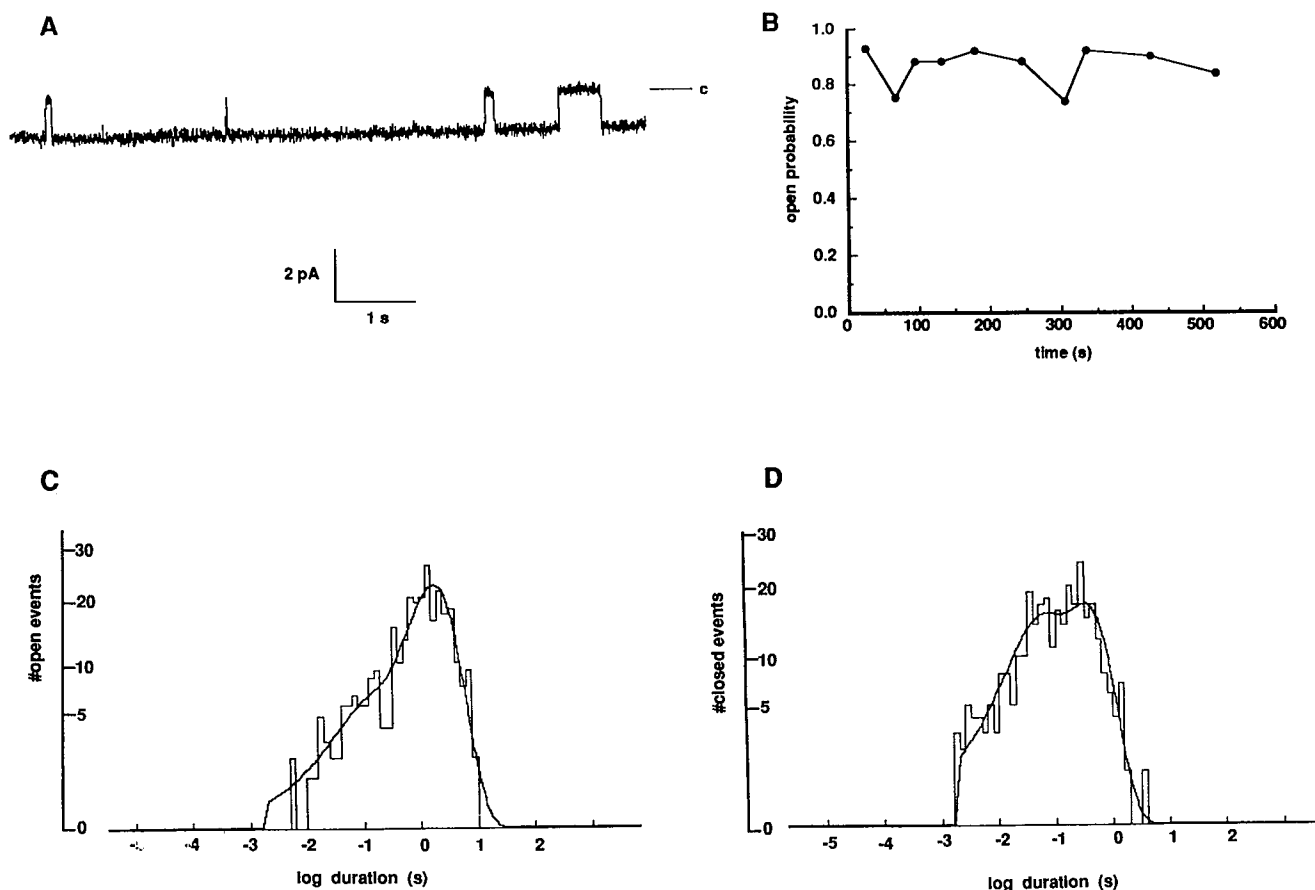


FIGURE 3. Kinetics of a channel with high P_o (0.87). (A) Portion of the original recording which was 578 s long. The pipette potential was +20 mV. (B) Plot of P_o as a function of time. (C) Open-time histogram. The kinetics are plotted according to the method of Sigworth and Sine (1987). The solid line represents a fit to two exponentials with time constants 1810 ms (88% of total events) and 82 ms (12%). (D) Closed-time histogram. The solid line represents a fit to two exponentials with time constants 340 (64%) and 41 ms (36%).

as that shown in Fig. 1. Most (>80%) channels in this group could be activated by voltage, although the extent of the hyperpolarization required for activation was variable. The time course of the effect of voltage for five such patches is shown in Fig. 5. Three of these had channels with a low P_o at the resting potential of the cell ($V_{\text{pipette}} = 0$) and activated after a hyperpolarization of 40 mV. The other two had channels which maintained a low P_o for more than 1 min at $V_{\text{pipette}} = 40$ mV, but activated with a further 40 mV hyperpolarization. There were no consistent differences in the two data sets which are therefore combined. NP_o increased within 30 s and continued to rise, on the average, for 3 min. We did not study more prolonged hyperpolarizations. Upon returning the patches to the initial level NP_o decreased but remained above the original level for 1–2 min.

The kinetics of the channels before and after voltage activation are shown in Fig. 6. This summarizes experiments similar to those shown in Fig. 5, but where there was a single active channel in the patch. The initial volt-

age was 0 to 40 mV, and the patch was hyperpolarized by 40 to 80 mV. The increase in P_o is shown in A. B and C show the increase in mean open times and the decrease in mean closed times, respectively. In these cases the recording time at any one voltage was not sufficient to resolve multiple open or closed states. Clearly, however, changes in both opening and closing rates contribute to the increased P_o .

As indicated in Fig. 5, the effects of voltage on channel behavior often involved substantial delays. An example of an individual record illustrating delayed activation is shown in Fig. 7. Here the pipette voltage was initially 0 mV and NP_o was quite low. Hyperpolarization of the patch by 80 mV (changing V_{pipette} to +80 mV) had little immediate effect on P_o but after ~ 16 s the channel suddenly activated. The increased NP_o was sustained for at least 20 s but was reversed by returning V_{pipette} to 0. A time course of NP_o for this patch is shown in Fig. 8. In this case the channel was activated three times in succession. In each case there was a significant delay between the hyperpolarization and the activation.

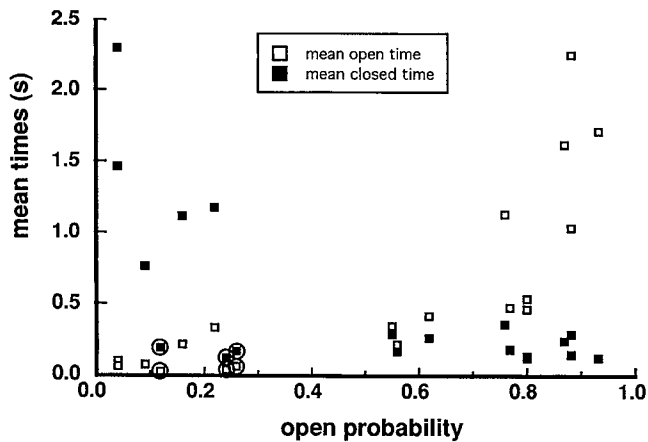


FIGURE 4. Plot of mean open and closed times for various values of P_o . Each pair of points represents a different patch. Three patches in which both the mean open and closed times were unusually short are circled.

In other cases long delays in the deactivation of the channels by depolarization were observed. One striking example is shown in Fig. 9. Here again the channel activity was low but was increased by hyperpolarization. In this case the activation was sustained for at least two minutes despite the return of the voltage to its original value. P_o eventually returned to low levels after a further depolarization of the membrane.

The delays in activation and deactivation are summarized in Fig. 10 for a series of patches in which the original P_o was < 0.25 . This required a somewhat arbitrary definition of activation. We considered the channels to be activated if the relative value NP_o increased by a factor of two and if the absolute value of NP_o increased by at least 0.01. The second condition was necessary for channels with very low P_o values where relatively small increases in activity could double the NP_o . The distributions show that $\sim 1/3$ of the patches activated quickly, i.e., within 5 s. A better resolution was not possible because measurement of NP_o took a finite amount of

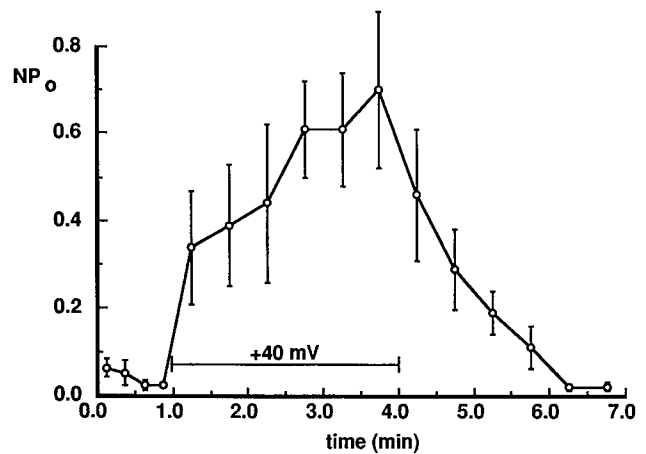


FIGURE 5. Activation of channels by hyperpolarization. Channel activity was measured for 1 min at $V_{\text{pipette}} = 0$ (three patches) or $+40$ mV (two patches), during which time NP_o remained low. The voltage was then increased by 40 mV for 3 min and decreased to the initial level for an additional 3 min. NP_o was monitored continuously. Values were averaged over 15 (initial period) or 30 s (final two periods) intervals, and are reported as means \pm SEM for the five patches.

time. In the other patches activation and deactivation involved delays of up to 30 s.

The possibility that the delayed activation of the channels by hyperpolarization might be due to a voltage-dependent biochemical reaction was tested using excised, inside-out patches. An example is shown in Fig. 11. The P_o was initially stable at a low value. Hyperpolarization of the excised patch increased P_o after a delay that was similar to that observed in cell-attached patches. Similar results were seen in two other inside-out patches. This does not rule out a voltage-dependent chemical reaction, but does imply that the factors involved in such an effect must be retained with the patch after excision.

The complicated, history-dependent effects of voltage on the kinetics of the channel make it difficult to define a precise relationship between open probability

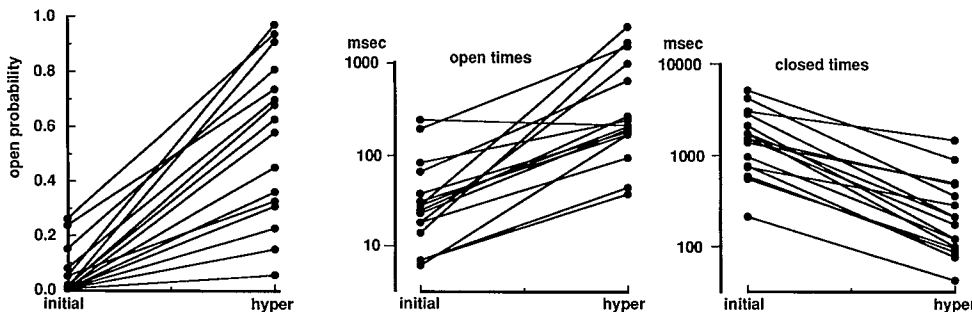


FIGURE 6. Effect of voltage activation on P_o (left), mean open time (center), and mean closed time (right). All the data were from cell-attached patches selected to have a single channel and an initial P_o of < 0.3 . Straight lines connect measurements from the same patch before and after hyperpolarization. The initial V_{pipette} ranged from 0 to $+100$ mV. The increase in V_{pipette} ranged from 40 to 100 mV.

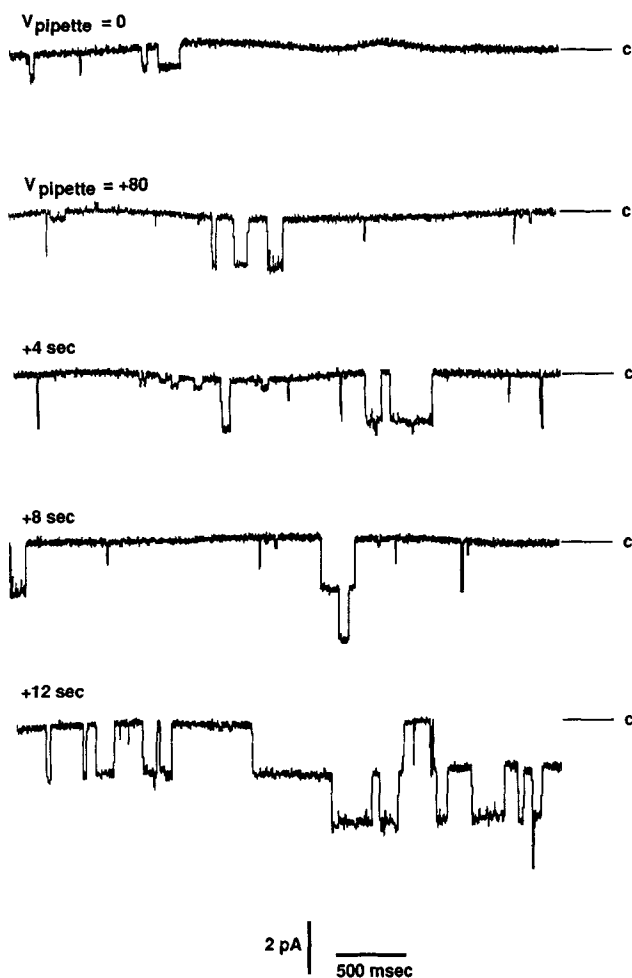


FIGURE 7. Delayed activation by hyperpolarization. The patch was initially held at $V_{\text{pipette}} = 0$, and NP_o was low. After hyperpolarization by 80 mV the channel activity remained low for several seconds before activation occurred. Times on the left represent the time after the voltage jump. The channels responsible for the smaller transitions observed after hyperpolarization have not been identified.

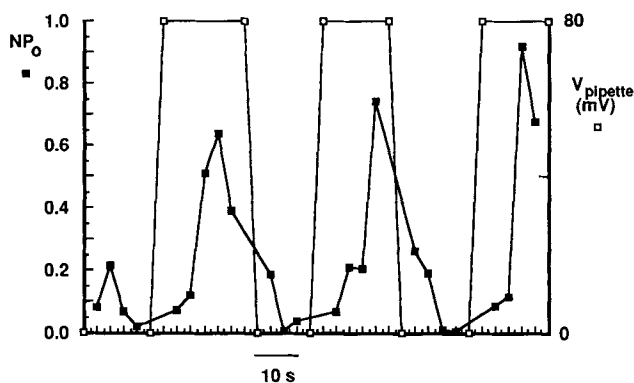


FIGURE 8. Time course of NP_o for the patch shown in Fig. 7. V_{pipette} was switched between 0 and +80 mV three successive times.

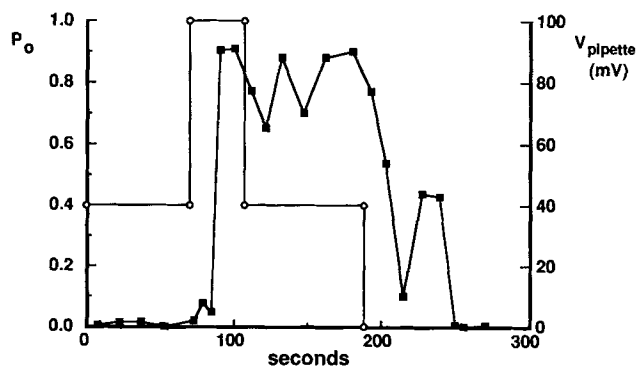


FIGURE 9. Time course of P_o for a patch containing a single channel in which deactivation by depolarization was delayed. V_{pipette} was originally +40 mV, and P_o was low. Hyperpolarization to $V_{\text{pipette}} = +100$ mV activated the channel after a delay of ~ 20 s. P_o remained high for ~ 1 min after returning the potential to its original level. Further depolarization to $V_{\text{pipette}} = 0$ mV resulted in the eventual deactivation of the channel.

and voltage at the single-channel level. Therefore, the effects of voltage on Na channels were further assessed using the whole-cell clamp configuration of the patch-clamp technique. Previous studies showed that principal cells in the rat CCT are not electrically coupled, so that individual cells within an intact epithelium can be clamped (Frindt et al., 1990). Cells were held at 0 mV and hyperpolarized to various voltages up to 100 mV for 10 s. The protocols were then repeated in the presence of 10 μM amiloride. A typical response is shown in Fig. 12 A. "Instantaneous" currents were measured after the decay of the capacitive transient, within a few milliseconds of the voltage change. The inward current then increased rapidly, over the course of several hundred milliseconds, and then more slowly during the next 5–10 s. Neither the rapid nor the slow rise were seen in the presence of amiloride. The average initial and steady state levels of amiloride-sensitive current are plotted vs voltage in Fig. 12 B. The difference in the two currents presumably represents the voltage-dependent activation of the channels. There was an 80% increase in current at -100 mV. Substantial, although somewhat smaller increases were seen in the physiological voltage range ~ -60 to -80 mV.

Effects of Stretch on Channel Gating

It has been suggested that the epithelial Na channel is related through evolution to mechanosensitive channels found in nematodes. We therefore examined whether mechanical perturbation could alter the gating kinetics or gating mode of the channels in the CCT. To test this possibility we used the protocol of applying suction to the patch-clamp pipette during recording from a cell-attached patch (Sachs, 1992). To optimize

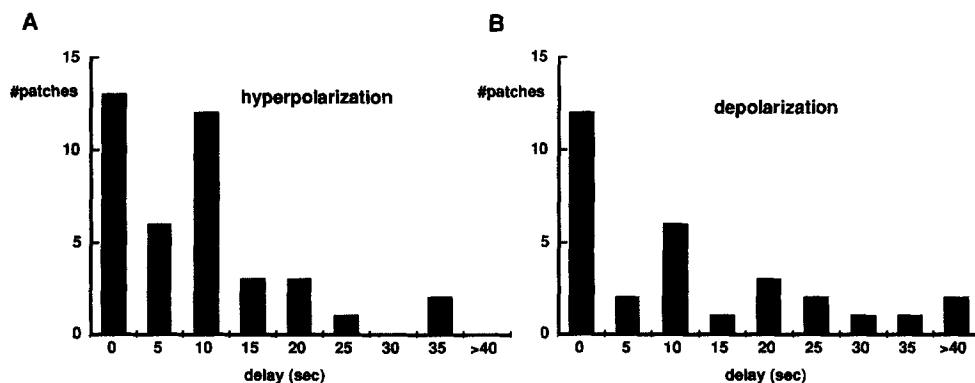


FIGURE 10. Histograms of the delay times for activation by hyperpolarization (A) and deactivation by depolarization (B).

the chances of seeing an effect of mechanical perturbation, patches with a low basal P_o were selected. In most cases negative pressures of up to 80 mm Hg had no obvious effect on the channels (Fig. 13 A). However, in six cases there was a substantial, reversible activation of the channels (Fig. 13 B). One of these positive results is illustrated in Fig. 14. In this case the negative pressure was increased in three steps up to 24 mm Hg. The first step to 8 mm Hg had no significant effect on channel gating. The second step to 16 mm Hg elicited only a brief burst of channel openings. Increasing the pressure to 24 mm Hg brought about a prompt, sustained activation of the channels. The increase in P_o was reversed after releasing the pressure. This deactivation was not immediate, being complete 20 s after the pressure changed. In the six responsive patches P_o was increased by application of between 10 and 30 mm Hg. The mean value of NP_o in these patches increased from 0.048 ± 0.04 to 0.22 ± 0.09 . In one other patch (not shown), P_o was reduced by negative pressure and increased again when the pressure was released.

DISCUSSION

Gating Modes

The main purpose of this study was to examine the variations in open probability of the epithelial Na channel

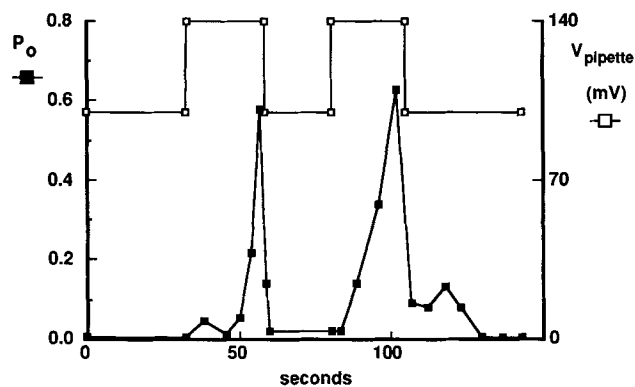


FIGURE 11. Delayed activation in an excised, inside-out patch. The patch was originally held at $V_{\text{pipette}} = +100$ mV. P_o increased upon hyperpolarization to $V_{\text{pipette}} = +140$ mV after a delay of ~ 30 s.

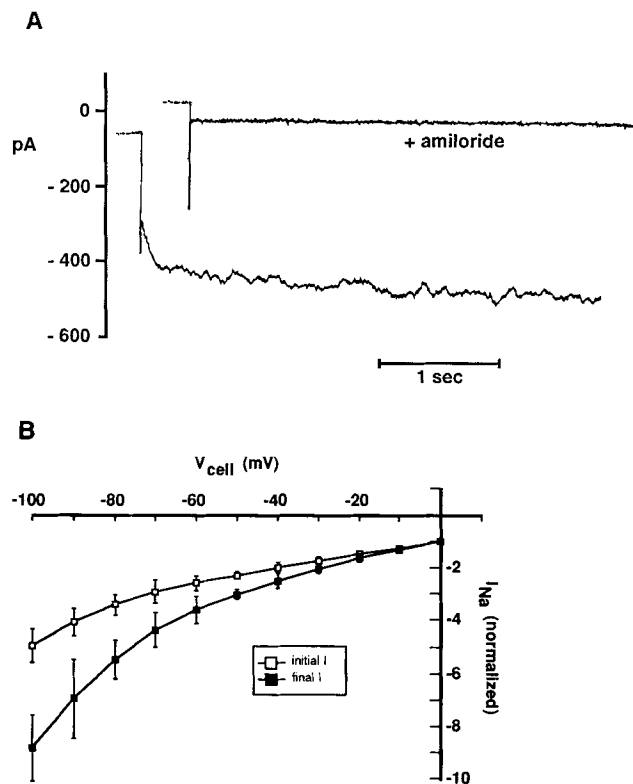


FIGURE 12. Effect of voltage under whole-cell clamp conditions. Cell voltages were initially held at 0 mV and then switched to negative values between -10 and -100 mV. (A) The response of an individual cell to a hyperpolarization to -100 mV. Traces from the same cell in the absence (top) and presence (bottom) of $10 \mu\text{M}$ amiloride are shown. Negative values represent inward current. (B) The amiloride-sensitive current as a function of voltage. Currents were measured just after the decay of the capacitive transient (initial values) and at the end of the voltage pulse (steady state values). All currents were normalized to the value at 0 mV, and are presented as means \pm SEM of four cells.

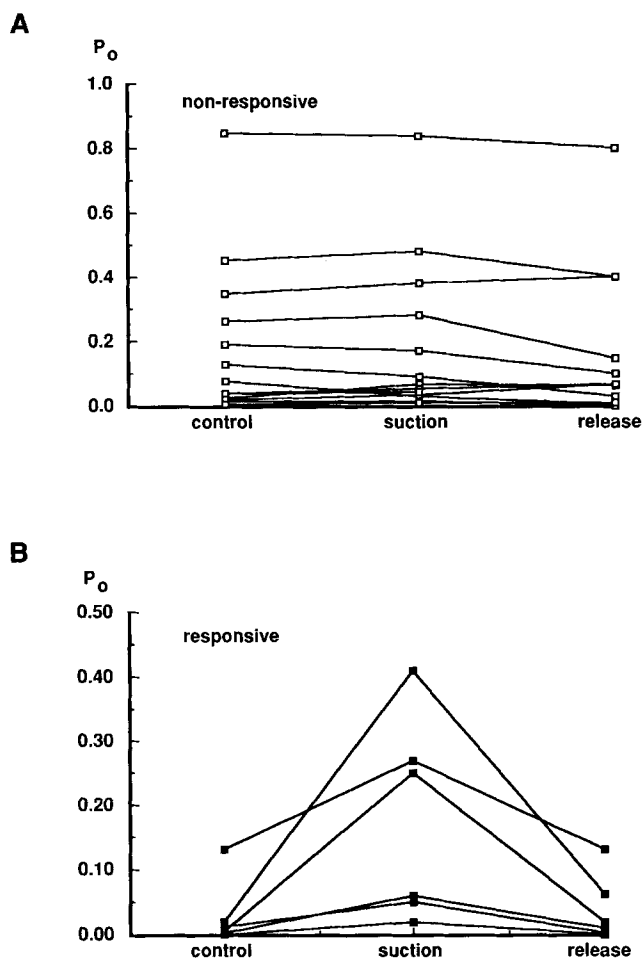


FIGURE 13. Effects of negative pressure on channel gating. (A) 15 patches in which there was no obvious effect of negative pressure. The applied suction ranged from 20 to 50 mm Hg. (B) Six patches in which suction of 10–30 mm Hg appeared to activate the channels reversibly.

and to identify some of the mechanisms underlying this variability. The extent of the variation is illustrated in Fig. 4. Basal values of P_o in single-channel patches ranged from < 0.05 to greater than 0.9. This variability confirms previous studies showing similar ranges of apparent P_o in multichannel patches from the rat CCT (Palmer and Frindt, 1988; Pácha et al., 1993). A large variability of P_o has also been reported for Na channels in A6 cells, a culture model derived from the amphibian distal tubule (Ling and Eaton, 1989; Ohara et al., 1993). What was somewhat surprising was the clustering of these P_o values toward the low and high end of the spectrum.

The records with high and low P_o appear to represent the same basic channel type. All of the channels reported in this study had similar single-channel conductances of 12–13 pS, and strongly positive reversal potentials. More importantly, at least some of the channels

with low P_o could be converted to a high P_o by membrane hyperpolarization or stretch. This suggests that the kinetic variations represent different gating modes, rather than different channel molecules. However, the data do not rule out a contribution of structural heterogeneity of channels to the variation in kinetic properties.

A gating mode is defined here as a set of kinetic states (open and closed states) governing channel gating which can undergo a shift to a second set of kinetic states, or a second set of rate constants for transitions between states. This shift occurs on a time scale which is slow relative to the lifetimes of open and closed states within a mode. Other ion channels have been shown to undergo such changes in gating mode. Voltage-dependent Ca channels, for example, can undergo changes in gating patterns which are influenced by dihydropyridines (Hess et al., 1984). Voltage-dependent Na channels can exhibit spontaneous shifts in their P_o -V relationship even after reconstitution in planar lipid bilayers (O'Connell, 1992).

Previous studies had indicated that the basic Na channel kinetics in the absence of amiloride could be described in terms of a single open state and a single closed state (Palmer and Frindt, 1986; Eaton and Marunaka, 1990). In the case of the CCT, this conclusion was inferred primarily from recordings from multi-channel patches. The relatively long single-channel recordings shown here reveal a more complex kinetic pattern. The presence of two time constants in the open-time and closed-time histograms suggest the existence of at least two open and two closed states. It is not yet clear, however, if the channel has access to all of these states at the same time. It is possible, for instance, that the different open and closed time constants arise from relatively short, reversible shifts in gating mode. This would predict that the distribution of open and closed times of sufficiently short records should be describable with single exponentials. Unfortunately, recordings of < 5 min do not contain enough events to rigorously test this prediction.

Voltage Activation

The gating of the channels is influenced by the membrane voltage. Hyperpolarization increased P_o as described previously (Palmer and Frindt, 1988; Frindt et al., 1993). A more complete examination of the effect of voltage revealed that the effect on P_o involved considerable delays. These delays are not accounted for by a simple two-state model of channel gating in which the rates of opening and closing involve movement of charge through the electric field of the membrane and are therefore directly influenced by voltage. In this case the effect of changing the transmembrane voltage on the rates of channel opening and closure would be instantaneous, and the rate of change in P_o would be de-

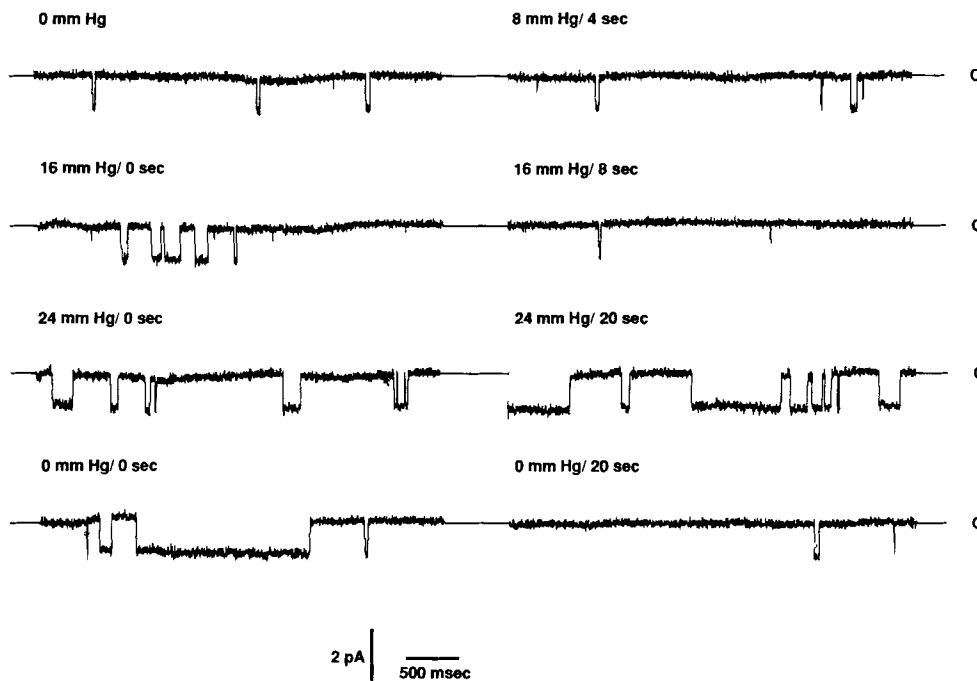


FIGURE 14. Original tracings from a patch which responded to negative pressure. V_{pipette} was held constant at +20 mV. Activity in the absence of suction is shown above on the left. Application of 8 mm Hg did not activate the channel (*above right*). Increasing the negative pressure to 16 mm Hg elicited a brief burst of channel openings (*second line*). Increasing the negative pressure to 24 mm Hg resulted in a sustained increase in P_o (*third line*). After release of the suction P_o remained high for ~ 10 s and then decreased (*bottom line*). Times refer to duration of the applied suction at the beginning of the individual trace.

terminated by the sum of the new opening and closing rates. The observations are consistent with the idea that the channels have two or more gating modes, with hyperpolarization favoring the high P_o mode and depolarization favoring the low P_o mode. This mechanism can account for the long delays and the hysteresis effects seen when the voltage was changed.

The observation of shifts in channel kinetics in excised patches suggests that the channels themselves, or a closely associated membrane component, is responding to the transmembrane voltage. This does not rule out the possibility that other events, such as biochemical modification of the channel, might also influence the gating mode. In A6 cells, for example, aldosterone was shown to shift channels from a low P_o to a high P_o state (Kemendy et al., 1992). In contrast, the P_o of channels in the rat CCT was not influenced by mineralocorticoid status (Pácha et al., 1993). Increases in cytoplasmic Ca^{2+} (Silver et al., 1993) and activators of protein kinase C (Ling and Eaton, 1989) reduce channel activity, possibly by favoring the low P_o state. Insulin, on the other hand, increased P_o of Na channels (Marunaka et al., 1992). Activation of G-proteins can also have a strong effect on channel gating. Both P_o and mean open time of Na channels in A6 cells were increased by GDP and decreased by GTP in excised patches (Ohara et al., 1993).

The voltage dependence of the channels could also be observed under whole-cell conditions. This indicates that the sensitivity of the channels to voltage is not an artifact of the mechanical isolation of the channels within the patch. These data showed that switching the

voltage from 0 to -100 mV could activate the channels by 80%. Assuming that this activation involved only an increase in open probability, without a concomitant insertion of new channels into the membrane, it can be concluded that the average P_o with a holding potential of zero was at most 0.56. Since the voltage activation showed no signs of saturation as the voltage was increased to -100 mV, the actual initial P_o was probably considerably lower.

Mechanical Activation

Recent cloning of the epithelial Na channel from the rat colon (Canessa et al., 1993; Lingueglia et al., 1993; Canessa et al., 1994) indicated homology between the subunits of this channel and certain genes identified in the nematode *C. elegans* involved in touch-avoidance responses (Driscoll and Chalfie, 1991; Chalfie et al., 1993; Huang and Chalfie, 1994). The homology, together with the similarity of the predicted membrane topology of the two families, suggests that the *C. elegans* genes may encode a mechanosensitive ion channel. This raises the question of whether the epithelial Na channels may also be influenced by mechanical perturbations of the membrane. We were unable to elicit a consistent response of the channels in the CCT to changes in transmembrane pressure, although in a minority of cases channel activation was observed. The variability in the response could reflect differences in the mechanical deformations of the apical membrane within the tip of the pipette. Alternatively, the Na channels may not respond to pressure in the same way as do the well

characterized stretch-sensitive channels (Sachs, 1992; Sackin, 1994). It is possible that the protocol used to test for mechanosensitivity was not the most appropriate one for these channels. It has been shown, for example, that cell swelling can activate Na channels in A6 cells (Wills et al., 1991). Whether this reflects a direct

effect on the channels is not yet clear. Substantial delays have been observed between the change in cell volume and the increase in channel activity. As shown here, however, this does not necessarily preclude a direct influence on the gating mode.

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