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Effects of Internal pH on the Nonselective Cation Channel from the Mouse Collecting Tubule

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Abstract. We investigated the effects of internal pH on Ca-activated, nucleotide-inhibited nonselective cation channels in the basolateral membranes of mouse collecting tubules, using the inside-out variant of the patch clamp technique. pH modulated the channel open probability (P_o), giving a bell-shaped curve peaking at pH 6.8/7.0: P_o at pH 6.0 was 11 ± 6% of P_o at pH 7.2 and 32 ± 7% at pH 8.0. The open and closed time distributions, best fitted to the sum of two exponentials, were differently sensitive to acid and alkaline conditions. Low pH reduced the short and long open times to 38 and 24% of their pH 7.2 values, while high pH produced a 4-fold increase in the long closed time. As previously reported, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS) induced a quasi-permanent opening of the channel. The inhibition of the channel produced by high pH disappeared in the presence of SITS, while the inhibition produced by low pH was unaffected. These results suggest that the pH dependence of the channel is due to two separate mechanisms. pH was without effect on the ATP-evoked inhibition of the channel, while high pH profoundly reduced the steepness of the AMP inhibition curve, without altering the half-maximal inhibitory AMP concentration.

Key words: Cation channel — pH — Nucleotides

Introduction

Most parts of the renal tubule contain a calcium-activated nonselective cation channel in the basolateral membranes [2, 30]. This channel has a linear current-voltage relationship with a unit conductance of about 20–30 pS, it does not discriminate between Na^+ and K^+, and is barely permeant to calcium. It is activated by internal calcium, and inhibited by a range of nucleotides acting at the internal surface of the channel [21]. These properties are characteristic of the Ca-NS^+ channels (according to the terminology of Reale et al. [23]), originally described in cultured cardiac ventricular cells by Colquhoun et al. [4] and, subsequently found in a wide range of tissues and cultured cells [26, 29, for reviews].

The calcium concentrations required to open the channel vary from one part of the renal tubule to another and are 1 to 100 μmol/l. The variability of the calcium response compares well with that in other tissues [26, 29], and suggests that the calcium sensitivity of the channel is controlled by other, as yet unknown, factors. Maruyama and Petersen [16] showed that the calcium sensitivity decreased rapidly after excision of membrane patches, possibly because some intracellular factor had been washed out. More recently, Razani-Boroujerdi and Partridge [22] reported that cAMP-dependent protein kinase reduced Ca-NS^+ channel activity in inside-out patches from embryonic chick sensory neurons. Thus, the low calcium sensitivity of the Ca-NS^+ channel, encountered in several types of cells, and especially in the collecting duct, is not incompatible with calcium being the predominant physiological modulator of the channel, at least under ad hoc conditions. Another possibility is that there are further regulatory processes involved in the control of the Ca-NS^+ channel, which are not presently understood.

This second possibility is supported by the fact that 1 mmol/l ATP almost completely abolishes the activity of maximally activated (1 mmol/l internal calcium) Ca-NS^+ channels [2, 21, 27], suggesting that this channel should be closed under all physiological conditions. Nevertheless, Thorn and Petersen [31] pointed out that there was a second ATP-binding site, which helped

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maintain channel activity and high calcium sensitivity. Another type of regulation by nucleotides was recently demonstrated by Reale et al. [23]. They showed that a fraction of Ca-NS$^+$ channels in an insulinoma cell line are activated by cGMP or cAMP at low concentrations (0.1–10 μmol/l), while they are inhibited by high concentrations (100 μmol/l) of the same nucleotides.

These collective results paint a complex picture of Ca-NS$^+$ channel regulation, in which the influences of calcium and ATP, the major regulators of channel activity, may be modulated or counterbalanced by other factors. The present study investigates the effects of an additional regulator of the Ca-NS$^+$ channel, intracellular pH. The open-state probability of the Ca-NS$^+$ channel was found to be a bell-shaped function intracellular pH with a peak at pH 6.8–7.0. There was also evidence that the inhibition of the channel by AMP—but not of ATP—was profoundly modified at high pH.

Materials and Methods

Tubule Isolation

Cortical collecting ducts (CCD) were isolated from the kidneys of male mice (15–20 g) by incubation in a Ringer solution (pH 7.4) containing 1 mmol/l $CaCl_2$ and collagenase (Worthington, CLS II, 300 U/ml) as described previously [2]. The procedure was designed to allow access to the basolateral plasma membrane by removing the basement membrane covering the tubules.

Recording Methods

Single-channel currents were recorded from CCD basolateral membranes using the inside-out configuration of the patch clamp technique [12] at room temperature. Patch pipettes were made from borosilicate hematocrits (Assistent, Hecht, Paris, France), pulled in two stages with a M 720 vertical puller (David Kopf Inst, Tuyunga, CA), coated with a silicon elastomer (Sylgard, Dow Corning, Seruppe, Belgium) and heat-polished. Currents were measured with an LM-EPC7 patch clamp amplifier (List, Electronics, Darmstadt, Germany), displayed on an oscilloscope (Tektronix, Beaserton, OR) and stored on videotape after analog/digital conversion (Biologic, Claix, France). The bath reference was 0.5 mol/l KCl in a 4% agar bridge connected to an Ag/AgCl pellet. The applied membrane potentials correspond to the potential of the inner side of the membrane patch with respect to the ground; cation currents from the inner to the outer side of the membrane are given a positive sign and are shown upwards in the illustrations.

Data Analysis

Signals were filtered at 500 Hz with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA) and digitized at 1 kHz using a Labmaster interface and Fetchex Software (Axon Instruments, Foster City, CA). The open probability (P_o) was calculated from $P_o = I/n \times i$, where n, the maximal number of nonselective cation channels per patch was estimated from the number of current levels detected over the whole record. The time-averaged current (I) passing through the n channels in the patch and the unit current (i) were determined on amplitude histograms, using software designed by T. Van Den Abbeele (Lab. de Physiologie de l'Oreille Interne, Faculté de Médecine Lariboisière, Paris, France).

For kinetic studies, stretches of data (1–5 min) were filtered at 1 kHz and digitized at 2 kHz. Open and closed lifetime distributions were determined using idealized records obtained with a half-amplitude threshold algorithm and fitted by a least-squares method (Biopatch, Biologic, Claix, France) to the equation: $a_1\ \tau_1^{-1}\ e^{-t/\tau_1} + a_2\ \tau_2^{-1}\ e^{-t/\tau_2}$ where τ_1 and τ_2 are the short and long time constants, and a_1 and a_2 the areas of each component. The number of events ranged from 700 to 6600 and only 3/22 stretches of data had less than 1000 events (700–900).

The paired or unpaired Student t-test were used to compare means.

Solutions

At the beginning of each experiment, the CCD tubule was bathed in NaCl Ringer containing (mmol/l): 140 NaCl; 4.8 KCl; 1.2 $MgCl_2$, 1 $CaCl_2$, 10 N-[2-Hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid] (HEPES) adjusted to pH 7.4 with NaOH. The pipette solution had a similar ionic composition except that it was calcium-free and contained 2 mmol/l ethylene glycol-bis (β-aminoethylether) N,N,N′,N′ tetraacetic acid (EGTA); pH was adjusted to 7.2. On formation of an excised patch, the inner surface of the membrane patch was superfused with a stream of solution from one of a series of outlet pipes.

The solution at pH 7.2 was the control for all tests on the effects of pH. Buffers were HEPES (pH 6.8–8.0) or 2-[N-morpholino] ethanesulfonic acid (MES, pH 6.0–6.8). The pH and the concentrations of free Ca^{2+} and Mg^{2+} were kept constant when testing the effects of ATP, by taking into account appropriate constants [9, 14]. These experiments were done in the presence of excess Mg (5 mmol/l) to minimize changes in the concentrations of free ATP with pH. All reagent were obtained from Sigma (St-Quentin-Fallavier, France), except 4-acetamido-4′-iso thiocyanato stilbene-2,2′-disulfonic acid (SITS) which was from Aldrich (St-Quentin-Fallavier, France).

Results

Single-channel recordings were obtained from inside-out patches, excised from the basolateral membranes of CCD tubules, bathed with NaCl-Ringer. The bath solution contained 1 mmol/l $CaCl_2$ to maintain maximal activity of the Ca-NS$^+$ channels [2, 30]. Under these conditions, the Ca-NS$^+$ channel was detected in almost every patch and was easily distinguished from less frequent, smaller, channels (10 pS).

Effects of Internal pH

Gray and Argent [11] reported that increasing the internal pH from 7.4 to 7.9 strongly reduced the open state probability of the Ca-NS$^+$ channel on pancreatic duct cells. Tests on the effects of pH on the CCD Ca-NS$^+$ channel over a wider pH range, revealed that low pH (<6.8) also reduced P_o. Fig. 1*A* shows excerpts of single-channel recordings, obtained at various pHs from one

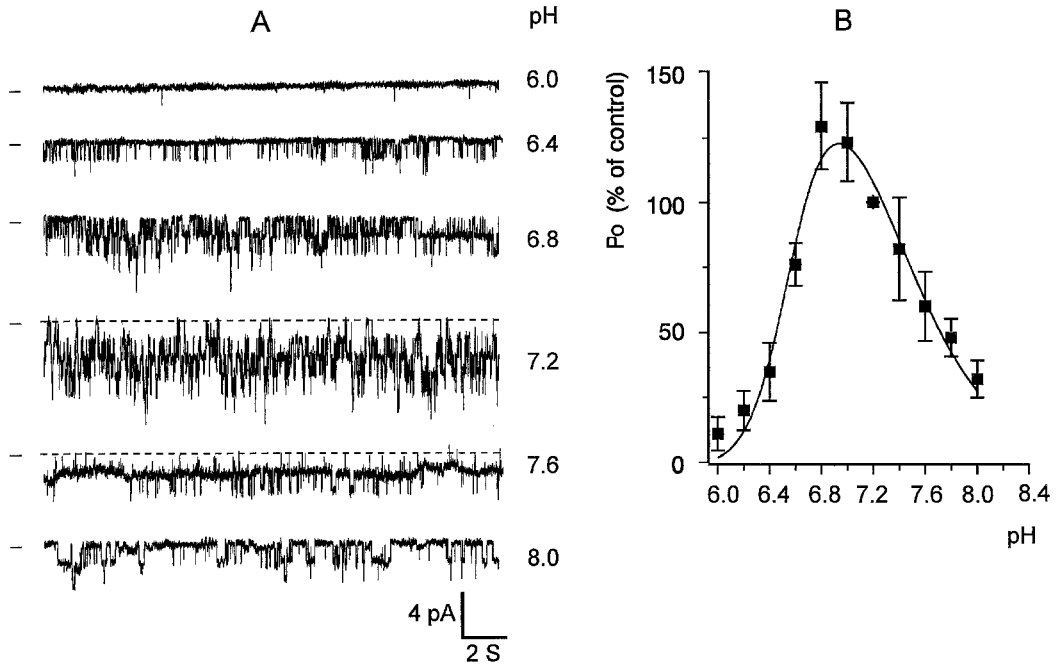

Fig. 1. Effects of acid and alkaline conditions on channel activity. (*A*) Single-channel current recordings from one inside-out patch containing five channels at several pHs from 6.0 to 8.0. All solutions were NaCl-Ringer buffered with 10 mmol/l HEPES (pH 7.2–7.6) or MES (6.0–6.8). The pipette solution was at pH 7.4. The closed channel current is indicated by a mark to the left of the traces. −50 mV membrane potential. (*B*) Dose-response curve for the effect of internal pH on single-channel open probability. The mean open probability (P_o expressed as percentage of P_o at pH 7.2) is plotted against internal pH. Filled circles and bars indicate mean values ± SEM from 7–18 patches (except at pH 6.2, $n = 4$). The curve was fitted to the equation $P_o = P_o\text{max}/(1 + 10^{(pK_1 - pH)n_1}) + P_o\text{max}/(1 + 10^{(pK_2 + pH)n_2}) - P_o\text{max}$, according to a Marquardt least squares method (Fig. P, Biosoft, Cambridge, UK). pK: half-maximal inhibitory pHs; *n*: Hill coefficients. Best fits were obtained for $pK_1 = 6.6$, $pK_2 = 7.4$, $n_1 = 2.6$, $n_2 = 1.2$ and $P_o\text{max} = 187\%$.

inside-out patch containing 5 Ca-NS$^+$ channels: P_o was maximal at pH 7.2 ($P_o = 0.37$); it decreased at high pH as observed previously [11]. Low pH also reduced P_o, giving a bell-shaped appearance to the plot of P_o against pH. Similar curves were obtained in all cases when P_o was measured over a broad pH range, but the curve shape and pH at which P_o was maximal were somewhat variable. The mean P_o, expressed in % of P_o at pH 7.2, is plotted against the internal pH in Fig. 1*B*. P_o was maximal at pH 6.8 ($P_o = 129.6 \pm 16.5\%$ of control, $n = 18$) and 7.0 ($P_o = 123.2 \pm 15.1\%$ of control, $n = 8$) and decreased on both sides of this value with unequal slopes. Compared to P_o value at pH 7.2, the P_o at pH 6.0 was 11 ± 6% ($n = 12$) and 32 ± 7% at pH 8.0 ($n = 14$). The experimental points were fitted to a combination of allosteric-type equations to obtain a quantitative description of the curve: the acid branch had a pK of 6.6 and a Hill coefficient of 2.6; the alkaline branch had a pK of 7.4 and a Hill coefficient of 1.2. Since the solutions at pH 6.0–6.6 and at pH 6.8–8.0 contained different buffers (MES and HEPES), we checked that the P_o, at an intermediate pH, 6.8, was similar with both buffers (HEPES: $P_o = 129.2 \pm 16.5\%$ of control, $n = 18$; MES: $P_o = 125.6 \pm 24.9\%$ of control, $n = 6$; NS).

The effects of pH on channel activity were independent of the applied voltage. The P_o at pH 6.0, was 4 ± 2% ($n = 4$) of control with a membrane potential of 60 mV and 14 ± 10 ($n = 4$) % of control (P_o at P 7.2) with a −60 mV membrane potential (NS); at pH 8.0. They were 37 ± 16% of control at +60 mV and 30 ± 11% of control ($n = 4$) at +60 and −60 mV membrane potential (NS).

In contrast to its effects on the open probability, the internal pH had no effect on the unit conductance of the channel: the unit current amplitudes, measured at −60 mV membrane potential, at pH 6.6 ($i = 1.51 \pm 0.06$ pA; $n = 8$) and 8.0 ($i = 1.49 \pm 0.06$ pA, $n = 10$) were identical to those measured at pH 7.2 ($i = 1.50 \pm 0.05$ pA, $n = 10$).

Channel Kinetics

The distribution of channel open and closed times was examined using patches containing only one channel level to gain some insight into the mechanisms responsible for the pH effects. The open and closed time distributions were best fitted by the sum of two exponentials, as in other tissues [5, 11, 32]. An example of channel lifetime distribution is shown in Fig. 2. At pH 7.2, with 1 mmol/l $CaCl_2$ in the bath, and 10^{-9} mol/l $CaCl_2$ in the pipette, the mean open time constants were 7.4 ± 2.3 msec and 103.5 ± 43.0 msec ($n = 7$). The corresponding areas under each component of the curve (*see* Materials

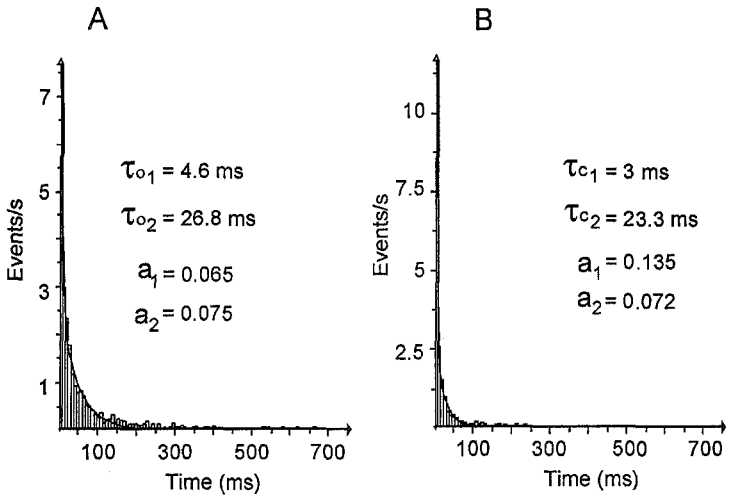

Fig. 2. Distribution of channel lifetimes. Open-time (*A*) and closed-time (*B*) histograms for a nonselective cation channel in an inside-out patch at −60 mV membrane potential. The curves are best fits of the sum of two exponentials to the dwell-time histogram data by a least-squares method. Number of events: 1728.

and Methods), a_1 and a_2 were 0.042 ± 0.020 and 0.057 ± 0.019, respectively (n = 7). The mean closed time constants were 2.2 ± 0.7 msec and 26.2 ± 6.2 msec (n = 7) with a_1 = 0.030 ± 0.007 and a_2 = 0.021 ± 0.007.

The main effect of low pH (6.4–6.6) was to reduce the short and long open times (Fig. 3*B*) to 2.8 ± 0.5 msec (n = 8) and 24.5 ± 3.8 msec (n = 7), respectively (statistically different from same values at pH 7.2, $P < 0.05$). Low pH had no effect on closed times. A high pH (pH 8.0) did not significantly reduce the open times (n = 7) but, it did increase the length of long closed times from 26.2 ± 6.2 msec to 103.5 ± 42.8 msec (n = 7) (Fig. 3*B*; statistically different from the value of the same parameter at pH 7.2, $P < 0.05$). The small increase in short closed times at pH 8.0 was not significant (n = 7). Fig. 3*A* shows long-duration traces (about 100 sec) from one inside-out patch containing only one channel: the contrasting effects of acid and alkaline conditions are apparent.

Modulation of pH Effects by SITS

In agreement with previous studies [5, 10], SITS (0.1 mmol/l), locked Ca-NS$^+$ channels in the open state when applied to the internal surface of membrane patches, increasing P_o from 0.45 ± 0.14 to 0.87 ± 0.06 (n = 6). Kinetic analysis of two patches containing only one channel suggested that SITS acted primarily on the closed time distribution, reducing the short closed time from about 3 msec to less than 1 msec and the long closed time from 52 msec to 13 msec, while the open time distribution was essentially unaltered. As previously reported [10], the effect of SITS was not readily reversible. Although SITS stabilized the channel in its open state, it did not prevent the gradual decline of channel activity (channel "rundown") which sometimes occurs in CCD preparations (*data not* shown).

The influence of SITS on effects of low and high pH on Ca-NS$^+$ channel was also checked (Fig. 4*A*). This inside-out patch contained only one Ca-NS$^+$ channel, plus another type of channel with a much smaller unit conductance. The traces in the left-hand column in Fig. 4*a* show channel activity before addition of SITS under control, acid and alkaline conditions. In agreement with results of previous section, although solutions at pH 8.0 (middle trace) and pH 6.4 (bottom trace) blocked the channel to the same extent, they did not influence the same kinetic parameters. The channel bathed with a pH 8.0 solution showed few sparse, rather long openings while it showed more frequent shorter openings at pH 6.4. The inside-out patch was then perfused with solutions adjusted to pH 7.2 containing 0.1 mmol/l SITS for at least 2 min and then challenged, in the continued presence of SITS, with solutions at pH 6.4 or pH 8.0 (right-hand column). The addition of SITS to a Ringer at pH 7.2 induced a large increase in P_o. (from 0.29 to 0.99, right-hand column, upper trace). At pH 8.0, with presence of SITS, the Ca-NS$^+$ channel was no longer inhibited by high pH (right-hand column, middle trace) but it was still inhibited by low pH (right-hand column, lower trace). Similar results were obtained with 6 different patches (Fig. 4*B*). The inhibitions induced by low pH (6.4 or 6.0) were similar in the presence (82.4 ± 11.0% inhibition) or absence of SITS (61.6 ± 17.4%, n = 6), while the inhibition induced by high pH (pH 8.0) under control condition (74.6 ± 9.1%, n = 6) was almost completely lost in the presence of SITS (11.8 ± 4.9% inhibition, n = 6, $P < 0.01$).

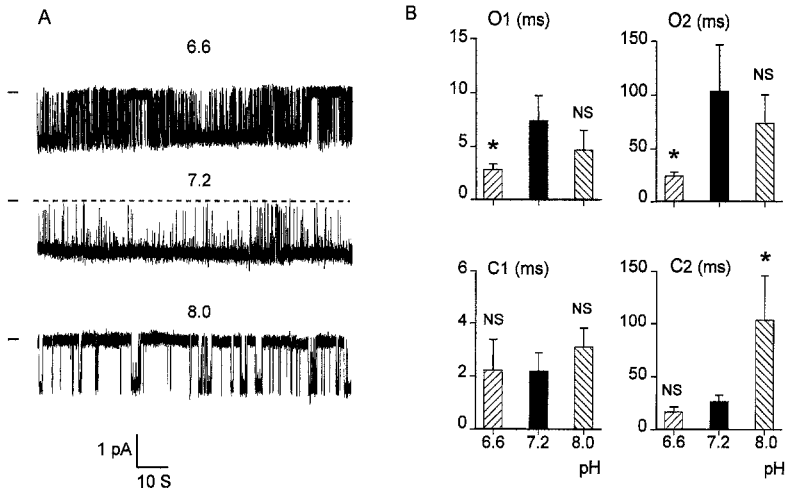

Fig. 3. Changes in the kinetic parameters of the channel induced by low and high pH. A/Single-channel current recording from one inside-out patch containing only one Ca-NS$^+$ channel, held at –60 mV. These traces are excerpts from longer recordings which were used to calculate kinetic parameters. In this particular example, the following values (in msec) were obtained at pH 6.6, 7.2 and 8.0 for the short open times: 10, 18.8, 11.3; the long open times: 54.5, 444.1, 226.4; the short closed times: 3.4, 3.5, 15.5; the long closed times: 43.0, 39.7, 208.1. The mark to the left of the traces indicates the closed current level. B/Kinetic parameters of the channel as a function of pH. O_1, O_2, C_1, C_2 are the short and long open times, and the short and long closed times, respectively. Values are mean from 7–8 patches. Statistically different from values at pH 7.2: $*P < 0.05$).

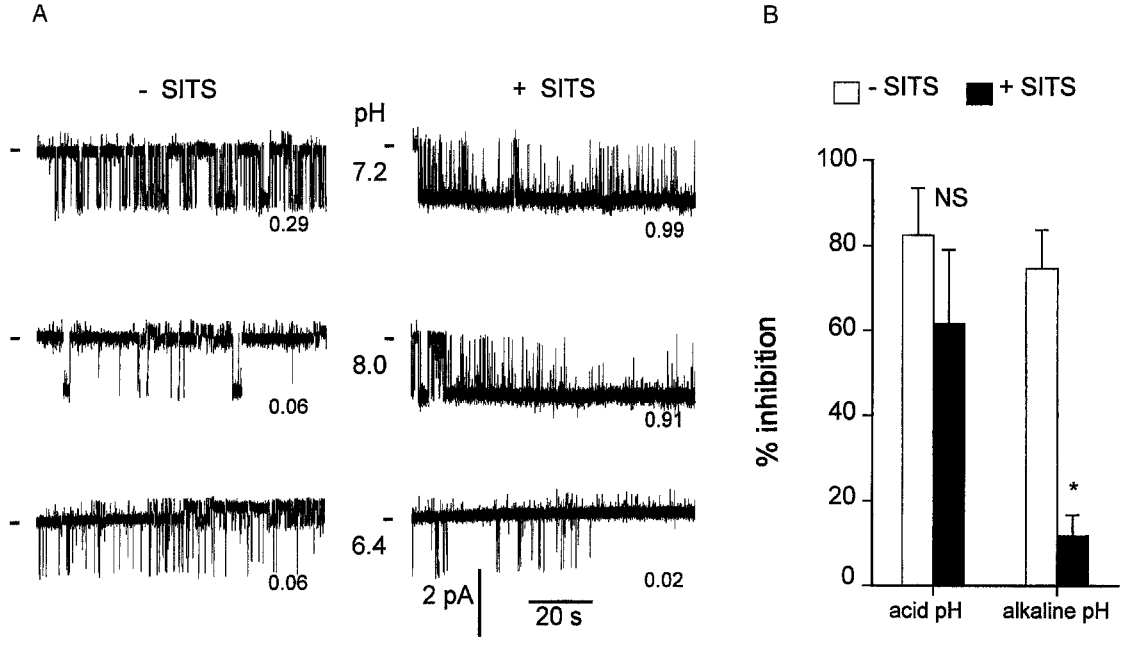

Fig. 4. (*A*) Effects of SITS applied to the internal side of an inside-out patch containing one channel. The left-hand panel shows the activity of the channel at pH 7.2 (upper trace), 8.0 (middle trace) and 6.4 (lower trace) in the absence of SITS. The acid and alkaline solutions induced a near complete inhibition. The same patch was then exposed to 0.1 mmol/l SITS for several minutes and challenged with acid or alkaline solutions in the continued presence of SITS (right-hand side panel). SITS, at pH 7.2, induced a quasi-permanent opening of the channel (upper trace). In the presence of SITS, the channel inhibition evoked by high pH almost disappeared (middle trace), while the effect of low pH was unchanged (lower trace). Numbers below the traces give P_o values. The marks on the left of the recordings point to the current level when the Ca-NS$^+$ channel is closed. There was another type of channel with a smaller conductance in this patch (smaller current deflections better seen in the left-hand panel). (*B*) Channel inhibition evoked by acid and alkaline conditions in the presence or absence of SITS (1 mmol/l). The mean inhibition evoked by pH in the absence (open bars) or in the presence (solid bars) of SITS is expressed as a percentage of (P_o at test pH – P_o at pH 7.2)/P_o at pH 7.2. The inhibition induced by low pH was unchanged in the presence of SITS, while that induced by high pH was profoundly impaired by SITS. $*P < 0.05$.

Interactions Between the Inhibition Evoked by Nucleotides and Internal pH

It has been reported that internal pH may have an effect on the ATP-induced inhibition of the ATP-sensitive K$^+$ channel [7, 8, 13]. We postulated that pH also modified inhibition of the Ca-NS$^+$ channel by nucleotides. The inhibitory effects of AMP on the Ca-NS$^+$ channel P_o at pH 6.6 and 8.0, were first determined to establish dose-response curves at both pH (Fig. 5). The traces in Fig. 5*A* were obtained from one inside-out patch containing two channels. The inhibition by AMP was almost complete at 10^{-5}mol/l AMP. The same inhibition occured at pH 7.2 (*see* the traces in Fig. 5*B*, which were obtained from another inside-out patch) but 10^{-5}mol/l AMP was less inhibitory at pH 8.0 (about 70% inhibition) than at

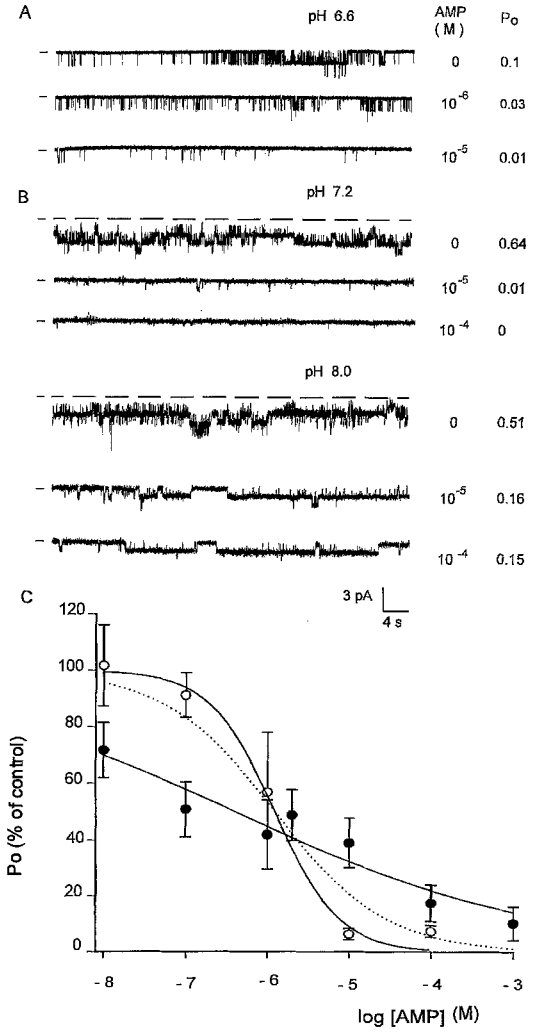

Fig. 5. Inhibition of the nonselective cation channel by AMP in acid and alkaline conditions. (*A*) Single-channel current recordings were obtained from one inside-out patch bathed with NaCl-Ringer solutions (pipette: pH 7.4) at pH 6.6 in the presence of 10^{-6} or 10^{-5} mol/l AMP. (*B*) Single-channel current recording from another inside-out patch bathed NaCl-Ringer at pH 7.2 or 8.0 in the presence of 10^{-5} or 10^{-4} mol/l AMP. Inhibition was almost complete at 10^{-5}mmol/l AMP at pH 7.2, while about 30% channel activity was preserved at pH 8.0 in the presence of the same concentration of AMP. The marks to the left of the traces indicate the number of open channels, the upper mark indicating the closed current level. Filtered 500 Hz. –50 mV membrane potential. (*c*) Modulation of inhibition by AMP of the nonselective cation channel measured at pH 6.6 (open circles) and 8.0 (filled circles) are expressed as percentages of P_o in the absence of AMP and plotted against the logarithm of AMP concentration. Mean values from 4–9 patches (except for 10^{-3} mol/l AMP where $n = 3$) were fitted to the equation $P_o = 100/(1+(c/c50)^n)$ where $c50$ and n are the half-maximal inhibitory concentration and the "Hill" coefficient respectively. pH 6.6:c50 = 1.2×10^{-6}mol/l, $n = 1.09$; pH 8.0:c50 = 4.1×10^{-7}mol/l, $n = 0.23$. The broken line is the dose-response curve for AMP at pH 7.2 (Chraïbi et al., 1994).

pH 6.6 or 7.2. 10^{-4}mol/l AMP did not completely inhibit the channel at this pH.

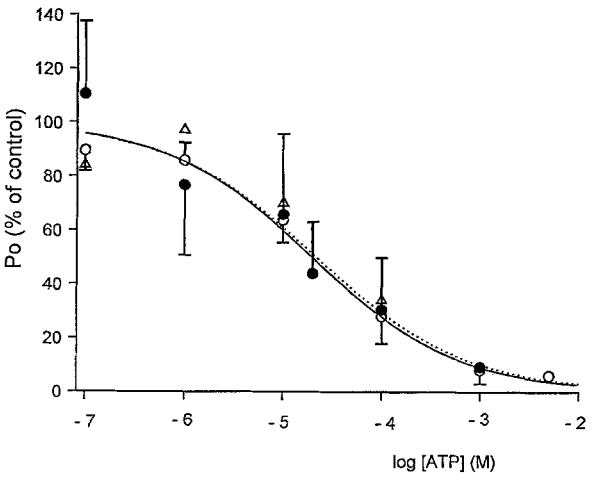

Fig. 6. Influence of internal pH on the dose-dependent inhibition of the nonselective cation channel by ATP. The open probabilities (P_o) at pH 7.2 (open circles) and 8.0 (filled circles) are plotted against the logarithm of ATP concentration. Mean values from 4–10 patches (except for 2×10^{-5}mol/l ATP at pH 6.6 and 5×10^{-3}mol/l ATP at pH 7.2 where $n = 2$) were fitted to the same equations as in Fig. 6. c50 = 2×10^{-5}mol/l (pH 7.2 and 8.0); $n = 0.57$ (pH 7.2) or 0.59 (pH 8.0). Means for two measurements at pH 6.6 (ATP concentrations: 10^{-7}, 10^{-5} and 10^{-4}mol/l) are shown as open triangles.

The dose-response curves in Fig. 5*C* were constructed from the results obtained in 5–8 patches. P_o are expressed as a percentage of the control P_o at the same pH (i.e., 6.6 or 8.0) to facilitate the comparison between the 2 curves. A third curve established at pH 7.2 in a previous study [2] is shown as a broken line for comparison. The half-maximal inhibitory concentrations at pH 7.2 (1.2×10^{-6}mol/l; [2]), at pH 6.6 (1.2×10^{-6}mol/l) and 8.0 (4.1×10^{-7}mol/l) were comparable. The main difference was in the slopes of the curves; the Hill coefficient was 1.1 at pH 6.6 and 0.23 at pH 8.0, with an intermediate value of 0.63 at pH 7.2 [2]. Fitting the experimental points collected at pH 8.0 to an allosteric type of equation has probably no substantial meaning under these circumstances but was nevertheless done to quantify the decrease in steepness of the curve.

The same type of experiments was then used to investigate whether the ATP-evoked inhibition of the Ca-NS^+ channel was also modulated by internal pH (Fig. 6). Dose-response curves for ATP inhibition were constructed at pH 7.2 and 8.0 using solutions containing 5 mM $MgCl_2$ to maintain the free ATP concentration approximately equal at both pH. The curves mostly overlapped with identical half-maximal inhibition concentrations (2×10^{-5}mol/l ATP) and comparable Hill coefficients ($n = 0.59$ at pH 8.00, $n = 0.57$ at pH 7.2) (Fig. 6). Some experiments were also done at pH 6.6 ($n = 2$). The results (open triangles) suggest that the low pH had no definite effect on the ATP-induced inhibition of the Ca-NS^+ channel.

Discussion

Two Mechanisms for the Modulation of the Ca-NS^+ Channel by pH

The initial observation by Gray and Argent [11] that high pH reduced the activity of the Ca-NS^+ channel on pancreatic duct cells, is confirmed and extended in the present study. The internal pH (pH_i) modulated Ca-NS^+ channel activity to give a bell-shaped function peaking at pH 6.8/7.0. Although protein enzyme activities often display a bell-shaped curve as a function of pH, there are very few reports of such pH profiles for ion channels. The only example so far reported seems to be the macroscopic conductance of Cl^- channels from the torpedo electric organ [18]. We know of no other example of channel inhibition at high pH.

In contrast, several channels are known to have reduced activity at low pH. It was first observed in pancreatic β cells for a maxi K^+ channel [5], and has been confirmed afterwards in other cell types [3, 17] and for other channels, including the amiloride-sensitive Na^+ channel [19] and several calcium-insensitive K^+ channels in rat CCD [25, 28, 33] in thick ascending limb [1] and in a proximal tubule cell line [20]. The half-maximal inhibitory pH (pK_i) is generally around 7.3/7.5. The inhibition caused by low pH is also a component of the effects of pH on the Ca-NS^+ channel, but, here, the inhibition curve is shifted towards a lower pH, since the pK_i was 6.5, well within the range of pH effects for another calcium-dependent channel, the maxi K^+ channel [5].

The pH dependence of the Ca-NS^+ channel is probably due to a combination of two separate effects of internal pH. The evidence for this is that the slopes for the acidic and alkaline branches of the P_o/pH curve were unequal, and the acid and alkaline conditions did not influence the same kinetic parameters. The low pH reduced the open times (by 70–80%), while the high pH increased the long closed time. Lastly, SITS removed the channel inhibition induced by high pH, but did not alter the effect of low pH.

Our results are then compatible with a scheme in which two distinct mechanisms contribute to pH effects. However, we have yet to determine whether the effects are mediated by specific sites, i.e., a proton-binding site and a high pH-sensitive site or, whether they are due to interactions with binding site for calcium and nucleotides. In analogy to the Ca- and voltage-activated maxi K^+ channel [3, 5], protons might inhibit the Ca-NS^+ channel by binding to its calcium site. But this is unlikely, because the low pH reduces the open times, while internal calcium seems to control channel activity by modulating closed times [22].

Effects of SITS

Previous reports have shown that SITS, applied at the internal surface of the membrane patch, irreversibly stabilizes the Ca-NS^+ channel in the open state [6, 10], without preventing its closure in response to AMP [6] or lowering the Ca concentration [10]. It is then interesting that SITS prevent inhibition of the channel by high pH. We have no ready explanation for SITS effects. The SITS may interact with an high pH-sensitive site on the channel. Alternatively, the effect of SITS on high pH-induced inhibition might result from the fact that both phenomena modulate the closed time distribution. However, calcium control over channel activity, although it is also exerted via closed time parameters [22], is not impaired by SITS [10].

Interactions Between AMP and High pH

Our search for Ca-NS^+ channel modulators, included possible interactions between pH and nucleotide-evoked inhibition because of results for K_{ATP} channels. Low pH decreases the sensitivity of this channel to ATP by a factor of two [13] to ten [8], without modifying the steepness of the dose-response curve. The results for the Ca-NS^+ channel are quite different. The ATP-evoked inhibition is not altered by acid or alkaline conditions, and the changes in the AMP inhibition curve, caused by high pH, are very different from those obtained for ATP for K_{ATP} channels at low pH. There was a large decrease in the steepness of the dose-response curve when the pH was increased from 6.6 to 8.0, but no significant change in the half-maximal inhibitory concentration. These results seem to indicate that high pH does not act like a competitive antagonist for AMP, but that the alkaline conditions reduce the effectiveness of AMP binding to its site. Nevertheless, the effect of low concentrations of AMP is enhanced at high pH.

The fact that the high pH does not alter inhibition of the channel by ATP as it does for AMP is puzzling, and suggests the possibility of several binding sites for nucleotides on the Ca-NS^+ channel.

Physiological Relevance

The Ca-NS^+ channel is usually not recorded from CCD cells in the basal state and, unlike its homologs in exocrine acini [15, 24, 31], has no identified hormonal activators. However, provided that channels are open under conditions yet to be determined, pH would be a physiological modulator of the channel, since channel activity is very sensitive to internal pH at values close to the normal intracellular pH (7.2/7.3). P_o decreases by 20% per 0.2 unit pH between 7.0 and 8.0. The secretory K^+

channel [33] and the epithelial Na^+ channel [19] are located at the apical membrane of collecting tubule, and they are both sensitive to pH in the opposite way to that the Ca-NS^+ channel over the same pH range. This suggests that intracellular pH could be an important modulator of ion transport in the collecting tubule [25].

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