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A CATION CHANNEL IN THE THICK ASCENDING LIMB OF HENLE'S LOOP OF THE MOUSE KIDNEY: INHIBITION BY ADENINE NUCLEOTIDES

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SUMMARY

1. Patch-clamp single-channel current recordings were used to study the inhibition of Ca^{2+}-activated non-selective cation channels by internal nucleotides in patches excised from basolateral membranes of the thick ascending limb of Henle's loop of the mouse kidney.

2. The application of ATP, ADP or AMP to the cytoplasmic face of excised inside-out membrane patches reduced the open-state probability of the channels (P_o) in a dose-dependent way without effect upon the unitary current amplitude. Dose–response curves gave half-maximal inhibitory concentrations of 20, 21 and 2·5 μM for ATP, ADP and AMP, respectively, while the Hill coefficient was close to one in all three cases.

3. Cyclic AMP partially inhibited channel activity ($P_o = 35 \pm 17$% of control) only at high, unphysiological concentrations (10^{-3} M) while adenosine (10^{-3} M) had very little effect ($P_o = 83 \pm 7$% of control).

4. Replacement of adenine with other purines (guanine, hypoxanthine) or pyrimidine (uridine) bases very largely reduced inhibitory activity. Cyclic GMP had no effect.

5. Non-hydrolysable analogues of ATP, AMP-PNP (10^{-3} M) and ATP-γ-S (5×10^{-4} M), were effective inhibitors of the channel ($P_o = 24 \pm 7$ and 9 ± 4% of control, respectively).

INTRODUCTION

We have recently shown that a Ca^{2+}-activated non-specific cation channel (Ca^{2+}-NSC channel) is present in the basolateral membrane of the thick ascending limb of Henle's loop in the mouse (Teulon, Paulais & Bouthier, 1987). This finding was unexpected, since no non-selective basolateral conductance had been revealed by microelectrode studies in this or any other segment of the nephron. Consequently, due to lack of electrophysiological information, the physiological significance of the Ca^{2+}-NSC channel in the overall function of Henle's loop is, as yet, uncertain. While this channel was described for the first time in renal cell membranes, it shows striking similarities to a cation channel found in the plasma membrane of several other tissues, including cultured cardiac ventricular cells (Colquhoun, Neher, Reuter & Stevens, 1981), neuroblastoma (Yellen, 1982), pancreatic acinar cells (Maruyama &

Petersen, 1982*a*, 1984), lacrimal gland cells (Marty, Tan & Trautmann, 1984), cultured Schwann cells (Bevan, Gray & Ritchie, 1984), and cultured insulin-secreting cells (Sturgess, Hales & Ashford, 1986, 1987). Partridge & Swandulla (1988) have reviewed this area.

To date, the Ca^{2+}-NSC channel has only been detected in the thick ascending limb of Henle's loop, but other types of cation channel seem to occur in different parts of the renal tubule. A non-selective ion channel, permeable to both cations and anions (Gögelein & Greger, 1987), has been reported in the basolateral membrane of the rabbit proximal tubule. A non-selective cation channel with a conductance of 27 pS has been found in primary cultures of rat inner medullary collecting ducts. The latter, which is equally permeable to Na^+ and K^+, is sensitive to external amiloride and not clearly dependent on internal calcium (Light, McCann, Keller & Stanton, 1988). Interestingly, Light & Stanton (1988) found it was inhibited by internal cyclic GMP.

In the present study we demonstrate that the renal Ca^{2+}-NSC channel is sensitive to adenine nucleotides applied to the cytoplasmic side of isolated membrane patches but is not inhibited by cyclic GMP. In this and other respects, the Ca^{2+}-NSC channel in the thick ascending limb of Henle's loop differs from the cation channel present in renal medullary collecting ducts.

Considering that Sturgess *et al.* (1986, 1987) have previously shown that a Ca^{2+}-NSC channel is sensitive to adenine derivatives in an insulin-secreting cell line, it is probable that sensitivity to adenine nucleotides is a general property of this type of cation channel. Some of these results were previously communicated in abstract form to the American Society of Nephrology (Teulon, Paulais, Bouthier & Anagnostopoulos, 1988).

METHODS

Tissue preparation

Tissues were prepared essentially as previously described (Teulon *et al.* 1987). Male mice (15–20 g) were killed by stunning and cervical dislocation. Cortical thick ascending limbs of Henle's loop (cTALH) were isolated from the kidneys by incubation in a Ringer solution (pH 7·4, 1 mM-$CaCl_2$, see below for composition) which contained collagenase (Worthington CLSPA or CLS II) at 200–400 U/ml followed by manual microdissection at 4 °C. The process was designed to allow access to the basolateral membrane by removal of the basement membrane which covers the tubules (Teulon *et al.* 1987). Incubation time and temperature (30–60 min, 30–37 °C) had important effects on the yield of successful seals and were adjusted appropriately.

Measurements

A fragment of cTALH tubule was placed in a chamber on the stage of an inverted microscope (Carl Zeiss, D-7082 Oberkochen, FRG). Single-channel currents were recorded from excised inside-out patches of basolateral membranes of cTALH using the patch-clamp techniques described by Hamill, Marty, Neher, Sakmann & Sigworth (1981). Currents were measured with an LM-EPC 7 patch-clamp amplifier (List Electronics, Darmstadt, FRG) and stored on FM magnetic tape (Euromag 1, Enertec, Villacoublay, France). Records were subsequently replayed onto an oscilloscope (Tektronix, Beaserton, OR, USA) through a VBF/8 filter (Kemo Ltd, Beckenham, UK) at 400–1000 Hz. Pipettes were made from microhaematocrit capillary tubes (CHR Badram, Bizkerod, Denmark) and coated with Sylgard (Dow Corning, Seneffe, Belgium). The sign of the membrane potential (V_c) refers to the bath side with respect to the pipette interior. Positive single-channel currents correspond to a cation flux from the bath *into* the pipette.

The open-state probability (P_o) of the channel was obtained by measurement of the total time

that the individual channels spent in the open state during a given recording period divided by the total time of the recording, taking into account the maximum number of open channels observed in the patch in nucleotide-free solution. Current records were analysed either with an IBM-compatible microcomputer or manually. In the first case, data recorded over 20–120 s were pre-filtered at 1 kHz and digitized at 2 kHz using an analog–digital interface (Data Translation, Inc., Marlborough, MA, USA). The total time that one to three channels were open simultaneously was determined sequentially with an automated single-channel analysis program (IPROC, see Sachs, Neil & Barkakati, 1982). In other instances, especially when four and more levels were present, the analysis was carried out manually by playing back data through the Kemo filter into a high-speed ultraviolet chart-recorder (OM4501, Schlumberger, Paris, France) equipped with a 1·8 kHz galvanometer, running at a speed of 10–25 cm/s. Dose–response curves for the inhibitory action of nucleotides were fitted to the equation $y = 100/(1+(x/c)^b)$ where c is the half-maximal inhibitory concentration and b is the Hill coefficient, according to a Marquardt least-squares method (Enzfitter, Elsevier-Biosoft, Cambridge, UK). Experimental values are given as mean ± S.E.M.; n denotes the number of results. Statistical comparisons were made using the non-parametric Mann–Whitney U test.

Media

At the beginning of each experiment, the cTALH fragment was immersed in a Ringer solution containing (mM): NaCl, 140; KCl, 4·8; $MgCl_2$ 1·2; $CaCl_2$ 1; HEPES, 10; adjusted to pH 7·4 with NaOH. The same solution was present in the recording pipette where pH was adjusted to 7·2, and the calcium concentration usually reduced to 10^{-5} M. A 'low-Na^+' solution (42 mM-NaCl, complemented with sucrose) was used on some occasions. On formation of an inside-out patch, the recording pipette was perfused with a stream of solution from one of a series of piped outlets (Yellen, 1982). The control solution was a Ringer solution at pH 7·2; test solutions were identical except that they contained various amounts of different nucleotides. Since, as we previously reported, activation of the Ca^{2+}-NSC channel by calcium reaches a plateau at about 10^{-4} M-calcium (Teulon *et al.* 1987), a high concentration of 'internal' calcium (10^{-3} M) was used throughout this study in order to maximally activate the channel and thus facilitate the observation of inhibition by nucleotides. The concentrations of free Ca^{2+} and Mg^{2+} were kept constant at 1 and 1·2 mM, respectively, by taking into account published stability constants for H^+, Mg^{2+}, Ca^{2+} and nucleotides (Martell & Smith, 1974; Fabiato, 1981). For instance, to compensate for chelation of calcium and magnesium by ATP, the concentrations of these ions were increased to (mM): $CaCl_2$, 1·3; $MgCl_2$, 1·9, for 1 mM-ATP; and $CaCl_2$, 1·1; $MgCl_2$, 1·5, for 0·5 mM-ATP.

Adenosine 5′-triphosphate (ATP, disodium salt, vanadium free), adenosine 5′-diphosphate (ADP, sodium salt), adenosine 5′-monophosphate (AMP, sodium salt), cyclic adenosine 5′-monophosphate (cyclic AMP, sodium salt), guanosine 5′-triphosphate (GTP, sodium salt), cyclic guanosine 5′-monophosphate (cyclic GMP, sodium salt), inosine 5′-triphosphate (ITP, sodium salt), uridine 5′-triphosphate (UTP, trisodium salt), adenosine 5′-adenylyl imido diphosphate (AMP-PNP, lithium salt), β-γ-methylenadenosine-5′-triphosphate (AMP-PCP, sodium salt), adenosine (hemisulphate salt), were all obtained from Sigma (St Louis, MO, USA). Adenosine 5′-*o*-(3-thiotriphosphate) (ATP-γ-S, lithium salt) was obtained from Boehringer (Mannheim, FRG).

RESULTS

Single-channel recordings were obtained from inside-out patches of basolateral membranes of cTALH fragments, bathed on both sides by Na^+-rich solutions with 1 mM-free Ca^{2+} bathing the internal face of the excised membranes. In such conditions, the 25–30 pS Ca^{2+}-NSC channel was the only observed ionic channel, although a smaller cation-selective conductance of 8–10 pS was occasionally found (openings of this type may be seen in insets *b* and *c*, Fig. 1); in the control state, the open-state probability (P_o) of the Ca^{2+}-NSC channel had a mean value of 0·64 ± 0·03 ($n = 63$).

Figure 1 shows a typical experiment. Up to nine single-channel current levels were

observed in control solution with an average P_o of 0·90. When 5×10^{-4} M-ATP was applied to the cytoplasmic side of the membrane patch, channel activity was strongly inhibited ($P_o = 0{\cdot}02$) such that only brief openings of two single-channel current levels were seen. Channel activity recovered almost completely ($P_o = 0{\cdot}70$)

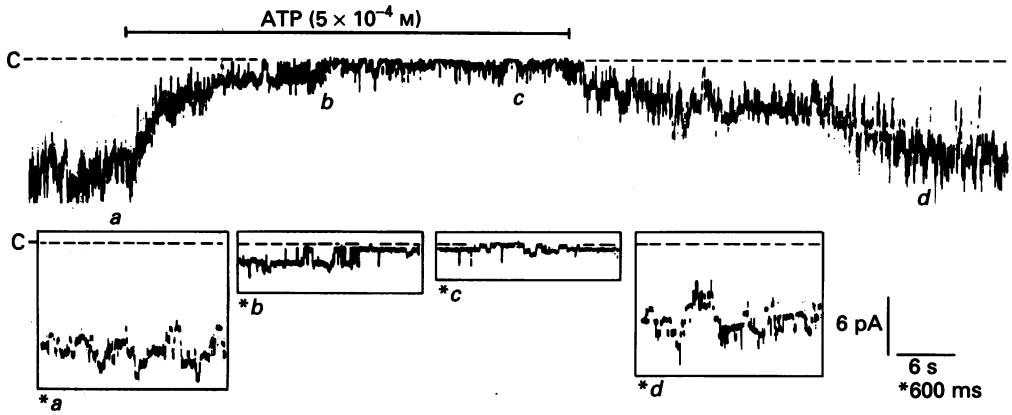

Fig. 1. The effect of internal ATP upon Ca^{2+}-NSC channel activity. The upper trace represents a continuous recording from an excised inside-out patch. The pipette contained a Ringer solution and the bath a low-Na^+ solution (42 mM-NaCl). In this and subsequent figures, the dashed lines, indicated by the letter C, represent the patch current level recorded when all channels were closed; downward deflections of the current trace represent currents flowing from the extracellular side of membrane to the intracellular side. The bar indicates the period for which the membrane patch was exposed to a low-Na^+ solution containing 5×10^{-4} M-ATP. $V_c = -60$ mV membrane potential; traces *a–d* represent records with a faster time base taken from the indicated sections of the upper trace; filtering, 400 Hz.

when ATP was washed away from the membrane. It can be seen in Fig. 1, and more clearly in Fig. 2*A*, that ATP reduced the open probability of the Ca^{2+}-NSC channel without affecting the unitary channel current amplitude.

A graded inhibition of channel activity was observed with increasing concentrations of ATP. This is illustrated in Fig. 2*A* where the application of 10^{-5} M-ATP to the cytoplasmic side of this membrane patch reduced P_o to 75% of control; 10^{-4} M-ATP further reduced P_o to 4% of control and no activity was observed with 5×10^{-4} (not shown) or 10^{-3} M-ATP. In most cases, however, some channel activity was still seen at high ATP concentrations, especially when several channels were active in the same patch: ranges of P_o values were 1–13 and 0–12% of control at 5×10^{-4} ($n = 10$) and 10^{-3} ($n = 7$) M-ATP, respectively. The dose–response curve is shown in Fig. 2*B*. The calculated half-maximal inhibitory concentration of ATP was 20 μM and the Hill coefficient had a value of 0·85.

ADP and AMP applied to the internal surfaces of excised membrane patches were also found to inhibit the activity of the Ca^{2+}-NSC channel. Figure 3*A* illustrates the effects of various concentrations of ADP on an inside-out membrane patch. It can be seen that channel activity is greatly reduced at 5×10^{-5} M-ADP ($P_o = 6\%$ of control) and almost totally blocked at 5×10^{-4} M. However, it should be emphasized that in

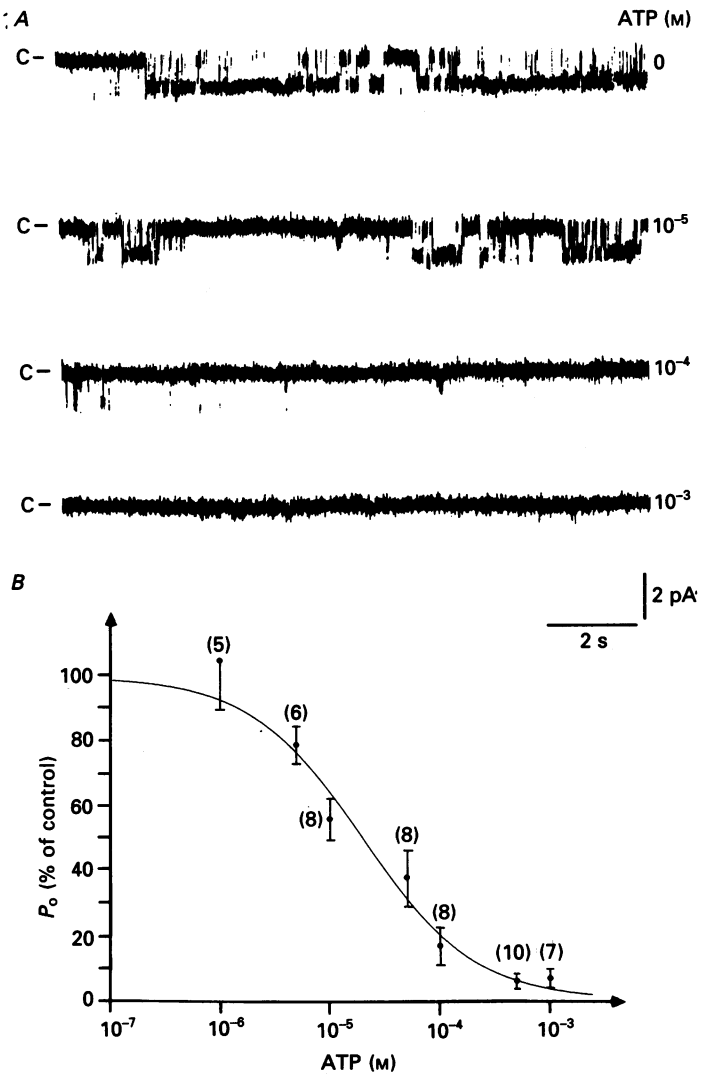

Fig. 2. Dose-dependent inhibition of Ca^{2+}-NSC channel evoked by internal ATP. *A*, single-channel recordings from one inside-out membrane patch bathed with Ringer solution on both sides of the membrane. $V_c = -60$ mV membrane potential; filtering, 600 Hz. *B*, dose–response curve for the inhibitory effect of ATP on single-channel open probability of the Ca^{2+}-NSC channel. The open probability (expressed as percentage of the control state) is plotted against the logarithm of ATP concentration. Filled circles and bars indicate mean values ± S.E.M. from five to ten patches as denoted by numbers in parentheses.

most cases, as previously noted for ATP, some channel openings could still be detected at 10^{-3} M-ADP (P_o ranged from 0 to 16% of control, $n = 7$). A half-maximal inhibitory concentration of 21 μM and a Hill coefficient of 0·78 were calculated for ADP (Fig. 3*B*). AMP was found to be the most potent inhibitor of three nucleotides. The recording in Fig. 4 shows a total inhibition of channel activity at a concentration

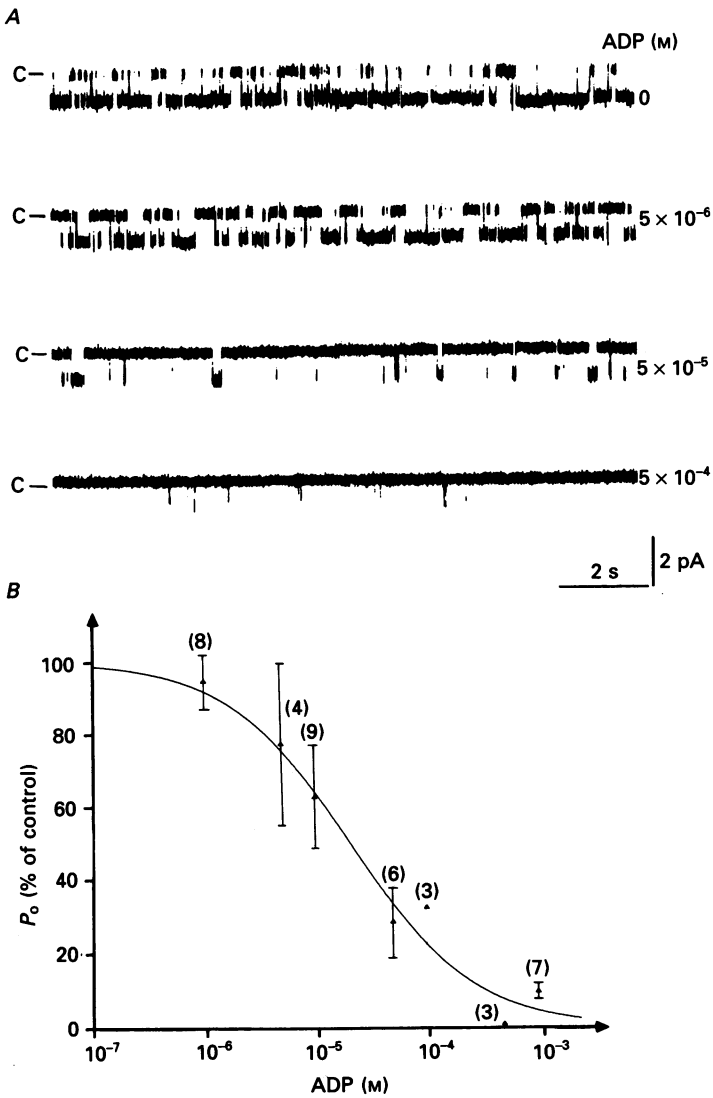

Fig. 3. ADP-evoked inhibition of the Ca^{2+}-NSC channel. *A*, single-channel current recordings from one inside-out membrane patch bathed with Ringer solution on both sides of the membrane. $V_c = -60$ mV membrane potential; filtering, 500 Hz. *B*, dose–response curve for the inhibition of the Ca^{2+}-NSC channel evoked by ADP. The open probability (% of control) is plotted against the logarithm of ADP concentration. Filled triangles and bars are mean values ± S.E.M. from three to nine patches.

of 5×10^{-5} M-AMP. The half-maximal inhibitory concentration of AMP was 2·5 μM and the Hill coefficient was 0·81.

Excised membrane patches were voltage-clamped at a variety of both positive (outward-going membrane currents) and negative (inward-going membrane currents) membrane potentials during this study. We observed no transmembrane potential dependence of nucleotide-induced Ca^{2+}-NSC channel inhibition. In the three

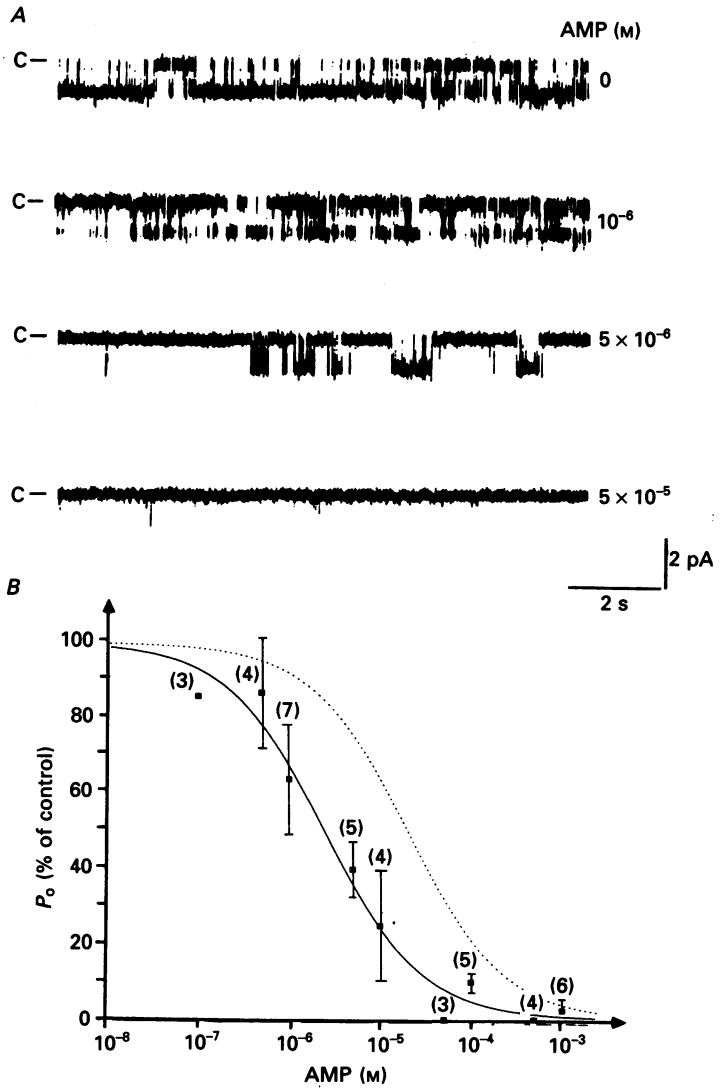

Fig. 4. AMP-evoked inhibition of the Ca^{2+}-NSC channel. *A*, single-channel current recordings from one inside-out membrane patch bathed with Ringer solution on both sides of the membrane (records taken from the same patch as in Fig. 3*A*). $V_c = -60$ mV membrane potential; filtering, 600 Hz. *B*, dose–response curve for the inhibition of the Ca^{2+}-NSC channel evoked by AMP. The open probability (% of control) is plotted against the logarithm of AMP concentration. Filled squares and bars are mean values ± S.E.M. from three to seven patches. The dotted line is the dose–response curve for ATP taken from Fig. 2*B* and shown for comparison.

membrane patches where this point was specifically tested, no difference in the inhibition evoked by internal ATP was detected when the membranes were first voltage-clamped at a positive membrane potential and then at a negative membrane potential.

In contrast to adenine nucleotides, the nucleoside adenosine and nucleotides cyclic AMP, GTP, ITP, UTP and cyclic GMP had weaker inhibitory effects. The application of 10^{-3} M-adenosine to the internal face of the membrane reduced P_o to 83 ± 7% of control ($n = 9$). A part of a continuous record in Fig. 5 shows the effect

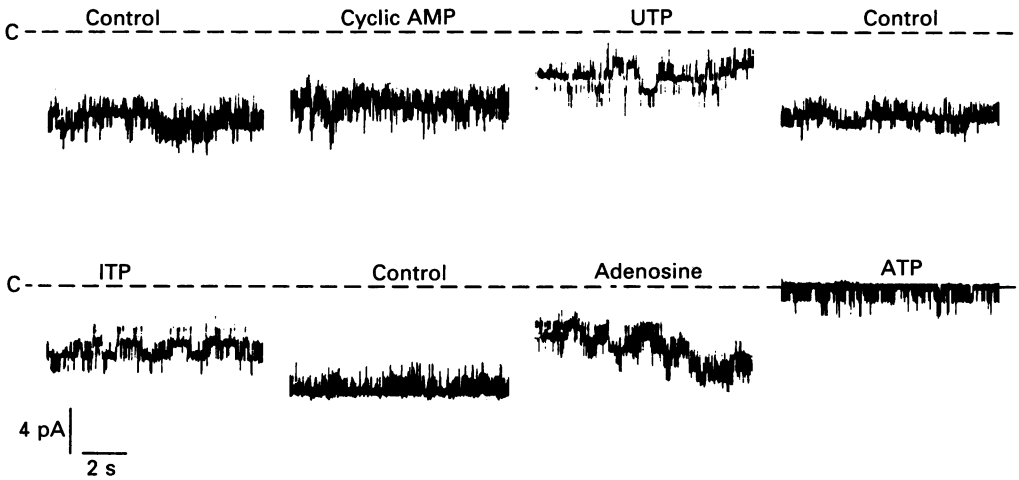

Fig. 5. The effects of cyclic AMP, UTP, ITP, adenosine and ATP upon the activity of the Ca^{2+}-NSC channel. These traces were taken sequentially from a continuous recording from one inside-out patch bathed with Ringer solution on both sides. $V_c = -40$ mV membrane potential; filtering, 300 Hz. Cyclic AMP (at a concentration of 10^{-4} M), UTP, ITP, adenosine and ATP (all at concentrations of 10^{-3} M) were applied to the internal surface of the membrane patch as indicated above the respective traces.

of 10^{-3} M-adenosine ($P_o = 65$% of control in this particular recording). Cyclic AMP at 10^{-3} M (not shown) elicited a clear inhibition of channel activity ($P_o = 35 \pm 17$% of control, $n = 4$), not significantly different from that of ATP at the same concentration. Lower concentrations of cyclic AMP had smaller effects: $P_o = 79$% of control ($n = 3$) at 10^{-4} M (see Fig. 5) and $P_o = 83 \pm 6$% of control ($n = 4$) at 10^{-5} M (not shown). These inhibitory effects of adenosine (10^{-3} M) and cyclic AMP (10^{-4} and 10^{-5} M) were statistically different from those induced by ATP at concentrations ranging upward from 10^{-5} M (for adenosine, $P < 0{\cdot}01$) and upward from 5×10^{-5} M (for cyclic AMP, $P < 0{\cdot}01$). As for the non-adenosine-based nucleotides GTP (not shown), ITP and UTP (Fig. 5), when applied to the inner face of the membrane patch at a concentration of 10^{-3} M, all three elicited reductions of channel activity: P_o fell to 68 ± 3 ($n = 5$), 61 ($n = 3$) and 69 ($n = 3$)% of control, respectively. Cyclic GMP had little effect (not shown), on the other hand, P_o being 93 ($n = 2$), 93 ± 8 ($n = 4$) and 106 ± 4% ($n = 4$) of control, at concentrations of 10^{-3}, 10^{-4} and 10^{-5} M, respectively. Inhibitory effects of non-adenosine-based nucleotides were statistically significantly less than those of ATP at concentrations ranging upward from 10^{-4} M in the case of GTP ($P < 0{\cdot}01$), UTP ($P < 0{\cdot}01$) and ITP ($P < 0{\cdot}05$) and upward from 10^{-5} M in the case of cyclic GMP ($P < 0{\cdot}05$).

Non-hydrolysable ATP analogues also reversibly blocked the activity of the Ca^{2+}-

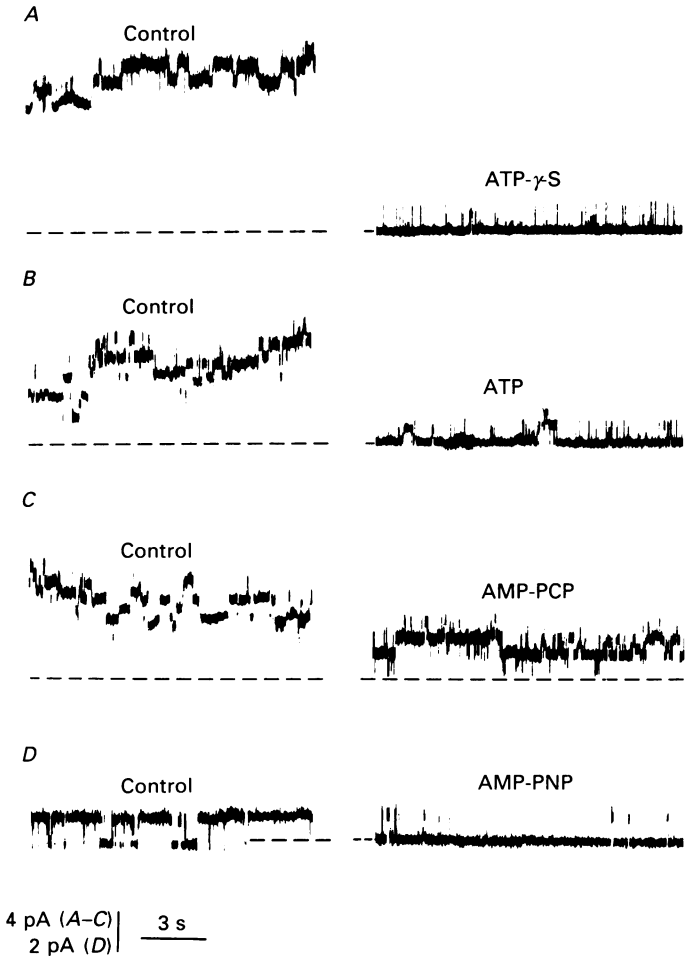

Fig. 6. The effects of ATP, ATP-γ-S, AMP-PCP and AMP-PNP upon the activity of the Ca^{2+}-NSC channel. *A–C* are records obtained from one membrane patch held at a potential of +50 mV; *D* is a record obtained from a separate preparation held at a potential of +60 mV. The patch was successively bathed in control Ringer solution and then in the same solution which contained 5×10^{-4} M-ATP-γ-S (*A*), 10^{-4} M-ATP (*B*), 10^{-3} M-AMP-PCP (*C*) and 10^{-3} M-AMP-PNP (*D*).

NSC channel. 10^{-3} M-AMP-PCP reduced P_o to 34% of control (three preparations, not significantly different from that of 10^{-3} M-ATP), while 10^{-3} M-AMP-PNP (six preparations, less potent than 10^{-3} M-ATP at the 0·05 level) and 5×10^{-4} M-ATP-γ-S (four preparations, not significantly different from that of ATP at the same concentration), provoked a stronger inhibition of the Ca^{2+}-NSC channel (24 ± 7 and 9 ± 4% of control, respectively). Figure 6*A–C* shows parts of a continuous record demonstrating the effects of ATP-γ-S ($P_o = 10$% of control), ATP ($P_o = 8$% of control) and AMP-PCP ($P_o = 28$% of control); Fig. 6*D* shows a record from another patch with inhibition evoked by AMP-PNP ($P_o = 4$% of control); all inhibitory effects were reversible.

DISCUSSION

This study shows that adenine nucleotides reversibly reduce the open-state probability of the renal Ca^{2+}-NSC channel when presented to the intracellular face of the membrane patch. The sensitivity of the Ca^{2+}-NSC channel to inhibition by adenine derivatives, as deduced from the half-maximal inhibitory concentrations for ATP, ADP and AMP (20, 21 and 2·5 μM, respectively), follows the sequence AMP > ADP = ATP $\gg$ adenosine. Since 10^{-3} M-adenosine lowered P_o by only 17%, this result clearly indicates that the channel is mainly sensitive to nucleotides. The intermediate effectiveness of cyclic AMP (much less powerful than AMP or ATP but stronger than adenosine) might support the idea that a full inhibitory action on the Ca^{2+}-NSC channel requires the presence of a plain phosphate group. It should be emphasized that cyclic AMP was effective only at very high concentrations, thus a physiological role for cyclic AMP as a direct inhibitor of the Ca^{2+}-NSC channel is unlikely.

The triphosphate nucleotides ITP, GTP and UTP were able to elicit inhibition of the Ca^{2+}-NSC channel, but based on their effectiveness at 10^{-3} M they appeared to be at least ten times less potent than ATP. These results suggest that the binding site(s) associated with channel protein can discriminate between the nucleotide bases and exhibit(s) a marked preference for the adenine base. The Hill coefficients for inhibition by ATP, ADP and AMP, close to one in all three cases, suggest that each nucleotide binds to only one site on or close to the channel protein.

We tested the effects of several non-hydrolysable ATP analogues and found that ATP-γ-S, AMP-PNP and to a lesser extent AMP-PCP were fully effective as inhibitors of the Ca^{2+}-NSC channel. The similar time courses of channel inhibition by ATP and ATP-γ-S, as well as the full reversibility of ATP-γ-S effects, make it unlikely that ATP-γ-S acted by phosphorylating the channel protein. Moreover, AMP-PNP and AMP-PCP, unlike ATP-γ-S, cannot be hydrolysed (Yount, 1975) and, although less effective than ATP, they still elicited a clear inhibition of the Ca^{2+}-NSC channel. It is thus probable that the metabolism of nucleotides is not involved in the observed inhibition of the Ca^{2+}-NSC channel. The potency variations among ATP analogues would then be better explained by stereochemical differences.

Sturgess *et al.* (1986, 1987) have studied the nucleotide-evoked inhibition of the Ca^{2+}-NSC channel in an insulin-secreting cell line (CRI-G1), where they found that adenine derivatives reduce the open-state probability of the Ca^{2+}-NSC channel according to the sequence AMP > ADP > ATP > adenosine (Sturgess *et al.* 1986). A half-maximal inhibitory concentration of 8 μM, a figure close to our 20 μM, was calculated from the dose–response curve for ATP, together with a Hill coefficient of 1·65 (Sturgess *et al.* 1987). They also reported that the Ca^{2+}-NSC channel was fully inhibited by the non-hydrolysable ATP analogue AMP-PNP (Sturgess *et al.* 1987) and that the inhibitory action is specific for adenine base, since the guanosine monophosphate was ineffective (Sturgess *et al.* 1986). Our results are at variance on minor points only. In our hands, adenosine was hardly effective in blocking the Ca^{2+}-NSC channel, and we found that ATP was as potent an inhibitor as ADP. The relative agreement between the reports of Sturgess *et al.* (1986, 1987) and this study suggests that adenine nucleotide sensitivity is probably a general property of the Ca^{2+}-NSC

channel. Thus, as noted by Sturgess *et al.* (1987), the ATP-sensitive K^+ channel (K^+-ATP channel) is not the only channel type to display an adenine nucleotide sensitivity. First demonstrated in cardiac muscle cells (Noma, 1983), it displays quite a different sensitivity to intracellular nucleotides, being most sensitive to ATP, much less sensitive to ADP and insensitive to AMP (see recent reviews: Petersen & Findlay, 1987; Stanfield, 1987; Ashcroft, 1988).

Since the Ca^{2+}-NSC channel activity recorded in excised inside-out patches depends not only on Ca^{2+} but also, as our results show, on nucleotides, these two factors could maintain the channel closed in resting, non-stimulated conditions. The resting intracellular Ca^{2+} concentration of renal cells is about 10^{-7} M (Murphy, Chamberlin & Mandel, 1986), while the Ca^{2+} threshold for opening the channel is tenfold higher (10^{-6} M) in excised membrane patches. However, we have observed that renal Ca^{2+}-NSC channels may be activated by a lower concentration of internal calcium (5×10^{-7} M), during the first 2–3 min following excision (Teulon *et al.* 1987). The Ca^{2+} threshold might therefore be lower in intact cells than in excised membrane patches, and the Ca^{2+}-NSC channels in intact cells may well be activated by a moderate elevation of cytosolic calcium, for example, due to hormonal stimulation. This has yet to be demonstrated in renal cells, but Maruyama & Petersen (1982*b*) have shown that hormonal activation of mouse pancreatic acini by cholecystokinin, thought to occur via a rise in intracellular calcium, results in opening of the Ca^{2+}-NSC channel.

On the other hand, ATP content of renal cells is generally estimated to be about 2 mM (Soltoff, 1986). It is clear that this ATP concentration is sufficient to maintain the Ca^{2+}-NSC channel closed in the presence of physiological cellular Ca^{2+} concentrations. Moreover, ADP and AMP, as highly effective inhibitors of the Ca^{2+}-NSC channel, might also contribute to keeping the channel closed. Nucleotide inhibition would therefore have to be suppressed or bypassed for the Ca^{2+}-NSC channel to be activated through calcium elevation. The following two possibilities may be considered. The sensitivity of the Ca^{2+}-NSC channel to intracellular nucleotides could be higher in excised patches than in intact cells, as was found by Kakei, Noma & Shibasaki (1985) for the K^+-ATP channel of cardiac muscle. Alternatively, the sensitivity of the Ca^{2+}-NSC channel to calcium and/or nucleotides could be modified by some external (hormonal) activating agent. These possibilities will have to be tested in the near future.

Although the physiological significance of the Ca^{2+}-NSC channel in cTALH, a site of NaCl reabsorption, is speculative, it could play a role in the modulation of the NaCl reabsorptive flux through the epithelium. In a simplified scheme (see Greger & Velazquez, 1987), transcellular NaCl transport across the cTALH is achieved by the following transport processes: (1) an apical Na^+–K^+–$2Cl^-$ co-transport system brings Na^+ and Cl^- ions into the cell (in some species, including mouse, synchronous apical Na^+–H^+ and Cl^-–HCO_3^--exchangers are also present), (2) K^+ channels recycle K^+ ions across the apical membrane, (3) Na^+ ions are removed from the cell by the basolateral $Na^+ + K^+$ pump, (4) a basolateral Cl^- conductance allows peritubular Cl^- diffusion. Activation of the Ca^{2+}-NSC channel, which probably results in membrane depolarization (see Partridge & Swandulla, 1988), Na^+ influx (Marty *et al.* 1984; Maruyama & Petersen, 1984) and K^+ efflux, is potentially capable of affecting almost

every cellular step of NaCl transport: membrane depolarization would increase the electromotive driving force for recycling K^+ ions but reduce the electromotive driving force for absorbing Cl^- ions; Na^+ influx (through possible elevation of cellular Na^+ concentration) could result in reduced activity of the Na^+–K^+–$2Cl^-$ and Na^+–H^+-coupled transport systems, but at the same time, could lead to activation of the $Na^+ + K^+$ pump. Simultaneous rate changes for several ion transport systems would have conflicting effects on overall NaCl reabsorption. Inasmuch as we do not know which of these systems actually limits the reabsorptive capacity of the epithelium, we cannot resolve whether the possible events following Ca^{2+}-NSC channel activation would eventually result in up or down regulation of NaCl reabsorption in the thick ascending limb of Henle's loop. A full characterization of the properties of the Ca^{2+}-NSC channel, including its activators and inhibitors, will be necessary before the role of this channel in the overall function of Henle's loop can be assessed experimentally.

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