A mechanogated nonselective cation channel in proximal tubule that is ATP sensitive

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Hurwitz, Craig G., Vivian Y. Hu, and Alan S. Segal. A mechanogated nonselective cation channel in proximal tubule that is ATP sensitive. Am J Physiol Renal Physiol 283: F93–F104, 2002. First published February 5, 2002; 10.1152/ajprenal.00239.2001.—Ion channels that are gated in response to membrane deformation or “stretch” are empirically designated stretch-activated channels. Here we describe a stretch-activated nonselective cation channel in the basolateral membrane (BLM) of the proximal tubule (PT) that is nucleotide sensitive. Single channels were studied in cell-intact and cell-free patches from the BLM of PT cells that maintain their epithelial polarity. The limiting inward Cs⁺ conductance is ~28 pS, and channel activity persists after excision into a Ca²⁺- and ATP-free bath. The stretch-dose response is sigmoidal, with half-maximal activation of about ~19 mmHg at ~40 mV, and the channel is activated by depolarization. The inward conductance sequence is: NH₄⁺ ~ Cs⁺ ~ Rb⁺ > K⁺ > Na⁺ ~ Li⁺ > Ca²⁺ ~ Ba²⁺ ~ N-methyl-D-glucamine ~ tetraethylammonium. The venom of the common Chilean tarantula, Grammostola spatulata, completely blocks channel activity in cell-attached patches. Hypotonic swelling reversibly activates the channel. Intracellular ATP concentration ([ATP]ᵢ) reversibly blocks the channel (inhibitory constant ~0.48 mM), suggesting that channel function is coupled to the metabolic state of the cell. We conclude that this channel may function as a Ca²⁺ entry pathway and/or be involved in regulation of cell volume. Furthermore, the coupling of SA channel function to cellular metabolism in renal epithelia would provide evidence that this channel may be important when intracellular ATP concentration ([ATP]ᵢ) is depleted, as occurs during increased transepithelial transport or with ischemic injury.

MATERIALS AND METHODS

Solutions and drugs. Experiments were performed using bath solutions that were either nominally Ca²⁺ free or that contained 1.8 mM Ca²⁺. The composition of the standard NaCl bath recording solution was (in mM): 95 NaCl, 2.5 KCl, 1 MgCl₂, 1 EGTA, and 10 HEPES. Unless otherwise noted, patch pipettes were filled with the standard CsCl pipette solution (in mM): 95 CsCl, 2.5 KCl, 1 MgCl₂, 1 EGTA, and 10 HEPES. The rationale for using Cs⁺ in the pipette solution is 1) the SA-nonselective cation (NSC) is the only NSC channel in the BLM that conducts Cs⁺; 2) Cs⁺ tends to block BLM K⁺ channels that might otherwise contaminate recording of the SA-NSC, and 3) the single channel conductance for Cs⁺ is greater than that for Na⁺, providing a better signal-to-noise ratio. After titration to pH 7.5 (model 710A; Orion, Boston, MA), sucrose was added to adjust the osmolality (Vapro model 5520; Wescor, Logan, UT) of the solutions to 200 mosmol/kg H₂O. In bi-ionic experiments, the pipette was

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filled with the chloride or gluconate salt of the test cation so that pipette test cation concentration was equal to bath Na+ concentration. Hypotonic swelling experiments were done at constant ionic strength beginning in an isotonic NaCl bath solution containing (in mM) 58.8 NaCl, 2.5 KCl, 1.8 CaCl2, 1 MgCl2, 10 HEPES, and 76 sucrose. Cells were then exposed to a 38% decrease in osmolality (124 mosmol/kg H2O) by changing to the sucrose-free solution. To test the spider venom from Grammmostola spatulata (Spider Pharm, Yarnell, AZ) as a candidate specific blocker of the channel in cell-attached patches, pipettes were backfilled with a solution containing 10 µl whole venom per milliliter of buffer (the tip was initially venom free). In solutions containing ATP, the nucleotide was added as the magnesium salt. Nucleotides were prepared fresh daily in bath solution. Chemicals used were obtained from Sigma (St. Louis, MO), Calbiochem (La Jolla, CA), or Biomol (Plymouth Meeting, PA).

Cell preparation. Dissociated PT cells were isolated from amphibian kidneys as previously described (35). Briefly, aqueous phase Ambystoma tigrinum kept at 4°C were killed by submergence in 0.2% tricaine methanesulfonate. The kidneys were rapidly removed and placed in ice-cold HEPES-buffered NaCl (in mM: 90 NaCl, 2.5 KCl, 1 MgCl2, 1 EGTA, and 10 HEPES) at pH 7.5. Renal tissue was cut into 1- to 2-mm3 pieces and incubated in collagenase-dispase (0.2 U/ml of collagenase; Boehringer-Mannheim, Indianapolis, IN) on a shaker for 30 min at room temperature. The cells were then mechanically dispersed into suspension by repeated trituration and resuspended in NaCl and stored at 4°C until use. The dissociated PT cells can retain their epithelial polarity for up to 14 days (35). Cells were used for experiments from 1 to 10 days after dissociation.

Electrophysiology. A 5-µl aliquot of Ambystoma cell suspension in NaCl was placed in a recording chamber mounted on an inverted microscope (Olympus IX-70; Optical Analysis, Nashua, NH). PT cells were readily recognized by their characteristic asymmetric bilobated morphology. The standard configurations for the single-channel patch-clamp technique (11) were used to record channel currents from the BLM. Patch pipettes were pulled from Corning 7052 borosilicate glass capillaries (Warner Instrument, Hamden, CT) on a two-step puller (Narishige PP-83), coated with wax to within 200 µm of the tip, and fire polished just before use. The open-tip pipette resistance was 3–8 MΩ when placed in the initial bath solution. A motorized micromanipulator (MP-285; Sutter Instrument, Novato, CA) was used to guide the patch microelectrode to the cell. The success rate of forming gigahm seals on the BLM was ~90%. Data have not been corrected for liquid-junction potentials, which were all <3.25 mV.

Voltage-clamped membrane currents were amplified with an EPC-9 patch-clamp amplifier (HEKA Elektronik) controlled by a Pentium personal computer running PULSE software (HEKA Elektronik) within Windows 98. Currents were low-pass-filtered (at 0.4–1 kHz, LBPF-48-DG; npi electronic) and then simultaneously printed on an analog strip chart recorder (Dash IV; Astro-Med, West Warwick, RI) and digitally sampled at 1–4 ksample/s directly to hard disk. Experiments were carried out at room temperature.

Application of pressure. Negative pressure was applied to the membrane patch through the side port of the pipette holder and measured with an integrated piezoresistive pressure transducer (Fujikura XPFP-03PGVR; Servoflo, Lexington, MA) placed in parallel with the pipette. Steady-state pressure was applied using a pressure transducer tester (DPM-1B; Biotek Instruments, Winooski, VT). For the application of pressure ramps, a syringe pump (YA-12, Yale Ap-
pipette potential, although channel openings will be evident in a minority (10–15%) of patches. Figure 1A shows that a ramp pressure protocol reversibly activates an NSC channel. In this representative excised inside-out membrane patch, rare channel openings are evident at a command potential of −40 mV before application of pressure, and channel activity increases with negative pressure. Conversely, during the ramp back to 0 mmHg, channel activity progressively declines.

Figure 1B shows channel records from an excised membrane patch (held at −40 mV) at a variety of pressure levels that demonstrate a direct relation between channel activity (NP) and pipette pressure. The relationship between applied negative pressure and NP, shown in Fig. 1C indicates that the pressure for half-maximal channel activation (EP50) is −19.3 mmHg at −40 mV. The EP50 tends to decrease (i.e., less negative pressure is required to gate the channel open) with depolarization. This value is fairly consistent but does vary in some cell preparations. This can be manifested as either more activity at 0 mmHg, a lower threshold pressure (e.g., −5 mmHg rather than −12 mmHg), or as a much higher threshold pressure (e.g., −18–25 mmHg), as seen in Fig. 1D.

SA channel inwardly rectifies and is cation nonselective. When BLM membrane patches are excised in the inside-out configuration under symmetrical conditions, measurements of the single channel current demonstrate that the SA-NSC channel is a true weak inward rectifier (Fig. 2, A and B) with a rectification index (g_{in}/g_{out}) of 1.4. Kinetic analyses of one channel patches held at −40 mV and −50 mmHg show that the SA-NSC channel has two open states (6 ms, 75% weight; 25 ms, 25% weight) and three closed states (~0.6 ms, 62% weight; ~16 ms, 22% weight; and ~180 ms, 16% weight), as shown in Fig. 2C. The channel is permeable to a wide variety of cations. Representative channel records from patches excised under bi-ionic conditions (pipette cation concentration equal to bath Na⁺ concentration) are shown in Fig. 3A, and the summarized current-voltage relationships are plotted in Fig. 3B. Note that the reversal potential is about the same for all conditions, so although the conductances differ, the permeabilities of the conducting cations are virtually identical. The single channel conductance for the outward flow of Na⁺ is the same (∼21 pS) regardless of the pipette cation. The inward conductance is divided into groups that can be correlated to the hydrated radius (or the dehydration energy) of the cation. Larger cations (Rb⁺, Ca⁺, and NH₄⁺) with smaller hydrated radii have a higher conductance (gin ∼47 pS) than smaller cations (gin ∼28 pS for Li⁺, Na⁺, and K⁺) with larger hydrated radii. The channel also conducts Ca²⁺, but with different kinetics (more flickery). Both N-methyl-D-glucamine (NMDG⁺) and tetraethylammonium (TEA⁺); large cations usually considered to be impermeant, conduct at ~6 and ~5 pS, respectively (Fig. 3A, inset). The presence or absence of bath Ca²⁺ has negligible effects on channel behavior.

![Figure 1](http://ajprenal.physiology.org/)

**Fig. 1.** Mechanosensitivity of the stretch-activated (SA)-nonselective cation (NSC) channel. A: slow application of negative pressure reversibly activates an NSC channel. In this original recording from an inside-out patch containing a single SA-NSC channel, the pipette contains CsCl, the bath contains Na⁺-gluconate, and the command potential is −40 mV. B: the open probability (Pₒ) of the SA-NSC channels increases with steady-state negative pressure (conditions as in A). C, All channels closed. C: summary of the stretch-dose response of the SA-NSC channel for 5 excised patches (Boltzmann fit). The effective pressure required for half-maximal activation (EP50) is −19.3 mmHg. Symbols represent the mean (●) ± SE (bars). D: a multistep protocol for a patch containing one channel shows that although −20 mmHg is able to maintain the opened channel in the open state, nearly 300 ms at −40 mmHg are required to reopen the channel (conditions as in A).
BLM SA-NSC channel is depolarization activated.

The open probability of the channel is voltage dependent and increases with depolarization (Fig. 4A). The open probability ($NP_o$) was measured from continuous current records at least 30 s long from patches held at constant negative pressure. Because channel activity varies among patches, $NP_o$ was normalized to the maximum $NP_o$ in each patch. Channel activity increases $e$-fold for every 49.5-mV depolarization. The experiment shown in Fig. 4B was designed to examine the instantaneous effects of voltage on channel gating. The pressure system was set to make a step from 0 to $-40 \text{ mmHg}$ and back to 0 mmHg over a duration of 5 ms, and channel recordings were made at $\pm 100 \text{ mV}$. Note that the opening latency time is shorter, and the closing latency time is longer, at $+100 \text{ mV}$ compared with those at $-100 \text{ mV}$. Pooled latency time data from seven experiments are summarized in Fig. 4C. This result suggests that depolarization shortens a closed-state lifetime and prolongs an open-state lifetime, both of which favor a higher open probability.

The multistep pressure protocol in Fig. 4D reinforces this idea. With descending negative pressure steps, $-60 \text{ mmHg}$ opens channels at both $\pm 80 \text{ mV}$, but reducing the pressure to $-40 \text{ mmHg}$ leads to closure of the channels only at $-80 \text{ mV}$. Channels stay open at $+80 \text{ mV}$ even when the pressure is further decreased to $-20 \text{ mmHg}$ but close as expected at 0 mmHg.
ascending negative pressure steps, channels reopen at
−40 mmHg at +80 mV but fail to open at −80 mV until
the pressure increases to −60 mmHg.
This SA channel is Gd₃⁺ insensitive but is blocked
by spider venom. Sensitivity to the lanthanide gado-
linium (Gd³⁺) is a common feature of many mechano-
sensitive channels, especially those that are cation
nonselective (29, 45). The SA-NSC channel in the BLM
appears to be insensitive to Gd³⁺, as detailed in Fig. 5.
Activation of the channel by negative pressure is un-
affected by 100 μM Gd³⁺ in the pipette and/or bath of
excised inside-out (Fig. 5A) or outside-out (Fig. 5B)
patches. The experiment in Fig. 5C shows that addition
of 100 μM Gd³⁺ to an outside-out patch conducting
Na⁺ has no effect on channel activity. Other typical
inhibitors of mechanogated or NSC channels, including

Fig. 3. Ion selectivity of the SA-NSC
channel. A: original current records
showing that the SA channel is nonse-
selective for monovalent cations and also
conducts Ca²⁺. All traces are from ex-
cised inside-out patches in a Na⁺-
gluconate bath at −40 mV with steady-
state negative pressure applied. Inset:
current record from an excised patch in symmetrical tetraethylammonium
(TEA⁺-Cl) at maximum voltage (Vₘ) =
−140 mV with stretch applied (same
scale as A). Similar results from salt
dilution experiments indicate that
the channel has a finite permeability
to TEA⁺ [and N-methyl-D-glucamine
(NMDG⁺)], albeit with a substantially
lower conductance. B: steady-state I-V
relationships for cation from each
group (NH₄⁺, Li⁺, and Ca²⁺).

Fig. 4. Open probability of the SA-
NSC channel is voltage dependent.
A: normalized Nₚₒ (i.e., Nₚₒ/maximum
Nₚₒ) increases with depolarization.
Data are from 4 inside-out membrane
patches with CsCl in the pipette and
an NaCl bath. Solid line is a single
exponential fit with a voltage constant
of 49.5 mV. Symbols represent the
mean (F) ± SE (bars). B: representa-
tive current records at command po-
tentials of Vₘ = 100 mV during a fast
(5-ms) pressure step from 0 to −40
mmHg. Conditions are inside-out BLM
patch with Cs⁻-gluconate in the pi-
pette and Na⁺-gluconate in the bath.
C: pooled latency data from 7 experi-
ments as described in B. Depolariza-
tion exerts a stronger effect on the
lengthening of closing latencies (tₐₒ)
than on the shortening of opening la-
tencies (tₐₑ). In both cases, the change
in latency time is significant. D: cur-
rent records at command potentials of
±80 mV during a multistep pressure
protocol. Conditions as described in B.
See text for details.

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amiloride, flufenamic acid, and niflumic acid, also have no significant effect on the SA-NSC channel (data not shown).

Spider venom from Grammostola has been shown to block SA channels (4). Therefore, cell-attached patches were made using patch pipettes backfilled with solution containing 10 μl whole Grammostola spatulata venom per milliliter of buffer (the tip was initially venom free). Figure 6B represents one of four consecutive patches that showed activity from one to four

![Image of spider venom inhibition](http://ajprenal.physiology.org/)

Fig. 5. SA-NSC channel is insensitive to gadolinium (Gd³⁺). A: Gd³⁺ (100 μM) in both the bath and pipette exerts no inhibitory effect on the stretch response of the SA channel in this inside-out patch. Conditions are Cs⁺-gluconate pipette, Na⁺-gluconate bath, and command voltage of −40 mV. B: same conditions as in A, except patch configuration is outside out, and positive pressure is applied. C: addition of 100 μM Gd³⁺ to an outside-out patch conducting Na⁺ has no effect on channel activity. Channel currents are markedly reduced when bath Na⁺ is replaced by TEA⁺. Conditions are CsCl pipette, test cation bath ± 100 μM Gd³⁺, and command potential of −40 mV. Baseline pressure is +25 mmHg. C1 and C2, all-channels-closed levels that differ due to a liquid junction potential; O, open channel levels.

Fig. 6. Spider venom from Grammostola spatulata completely inhibits SA-NSC channels. Representative channel records from 2 cell-attached patches held at −40 mV with −50 mmHg applied. The bath was Na⁺-gluconate, and the pipette contained CsCl ± 10 μl venom/ml buffer. A: in the absence of pipette venom, channel activity in cell-attached patches persists for the duration of the recording. Channel activity over time (P₀, measured every 10 s) for this patch is summarized in the bar graph. B: in the presence of pipette venom (the tip was initially venom free), an SA-NSC channel with a high open probability over the first 90 s (trace on top) begins to exhibit longer dwells in a closed state (2nd trace) and closes completely after 150 s (traces on bottom and bar graph), presumably coinciding with diffusion of the venom to the membrane patch. Increasing negative pressure fails to reopen the channels. Similar results were observed in 4 consecutive cell-attached experiments with patches containing up to four SA-NSC channels.
channels just after seal formation and subsequently exhibited complete loss of channel openings within a few minutes. Such behavior is unprecedented in the absence of venom (Fig. 6A), as our experience with hundreds of patches has shown that SA channel activity is maintained throughout recordings that may last >1 h. These results strongly suggest that this spider venom contains a specific blocker of our channel.

Cellular swelling reversibly activates the SA-NSC channel. It is reasonable to hypothesize that the SA-NSC channel is involved in cellular volume regulation, and the experiment shown in Fig. 7A is consistent with such a role. After baseline channel activity is established in a cell-attached patch under isotonic conditions, the cell is exposed to hypotonic conditions by removal of sucrose at a constant ionic strength. The response of the channel is biphasic, increasing significantly within a few seconds, reaching a peak (a 70% increase over baseline $NP_o$) within a minute, and then decreasing over the next 2 min. Channel activity decreases or abruptly ceases if cellular swelling is stopped or reversed, respectively. Such behavior suggests that the channel may be involved in the regulatory volume decrease (RVD) response of the cell. A summary of six similar swelling experiments is shown in Fig. 7B.

Sensitivity to adenine nucleotides. Nucleotides have been shown to inhibit some NSC channels (17). The SA channel is sensitive to ADP and ATP, as shown in Fig. 8. In this experiment, a constant negative pressure of $-17$ mmHg activates the channel. Addition of 5 mM UDP to the cytoplasmic face of the inside-out patch does not affect channel activity, but application of 5 mM ADP significantly and reversibly inhibits the channel. Exposure to 5 mM ATP demonstrates a more potent inhibition that also reverses (indeed, greater than that before ATP) after washout. That ADP also blocks the channel indicates that inhibition by adenine nucleotides neither involves a typical protein kinase nor requires hydrolysis by an ATPase. Therefore, chan-

Fig. 7. Hypotonic stress activates the SA channel. A: basolateral single channel currents during 38% reduction in bath tonicity (by removal of sucrose) at constant ionic strength (see MATERIALS AND METHODS) and $-40$ mV command potential. A “priming” pressure of $-20$ mmHg is applied throughout the protocol. Insets show representative current traces from before, during, and after the hypotonic challenge. All channels closed level (c) and open channel levels (o) are indicated. B: summary of 6 experiments as in A. Open probabilities are corrected for the number of channels in each patch.

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nel inhibition most likely occurs when an appropriate adenine nucleotide binds to a cytoplasmic domain of the channel protein. The dose-response curve for ATP is sigmoidal and has an inhibition constant ($K_i$) of 0.48 mM (Fig. 8B). The increase of $P_o$ upon washout suggests that ATP refreshes some channels during the period of inhibition. The degree of inhibition by ATP does not appear to be dependent on the magnitude of negative pressure applied to the patch. The block achieved using 2 mM ATP was compared at two levels of applied negative pressure in the same patch; one at which the baseline $P_o$ was ~0.20 and another at which baseline $P_o$ was ~0.70. There was no significant difference in the potency of block (88 ± 3.8% vs. 87 ± 1.1%, $n = 3$) at the two levels of negative pressure and open probability (Fig. 8C).

**DISCUSSION**

The primary objective of this study was to characterize the electrophysiological properties of an SA-NSC channel found at a very high frequency in the BLM of *Ambystoma* PT cells. Previous work on SA channels in PT has demonstrated regulation by cell volume changes induced by hypotonic shock and uptake of substrate (3, 9, 16, 30). Our data demonstrate that a Ca$^{2+}$-independent, mechanogated NSC channel in the BLM of PT is sensitive to ATP. The likelihood of finding at least one SA-NSC channel in a membrane patch is nearly 80%, suggesting that this integral membrane protein is important to the cellular and molecular physiology of the PT.

**Mechanosensitivity.** Of the cell surface proteins involved in “mechanotransduction” (e.g., integrins and ion channels), mechanosensitive ion channels can theoretically provide a rapid and reversible signal in response to membrane deformation. The application of steady-state negative pressure to membrane patches has been shown to both inhibit (40) and activate (30) mechanosensitive channels. Given the sigmoidal relationship between negative pressure and channel open probability.
probability shown in Fig. 1, the NSC channel described in this study is clearly a SA channel. Across multiple species, stretch sensitivity of basolateral SA channels is similar. For example, the half-maximal activation of our channel (−19.3 mmHg at −40 mV) correlates well with that reported for SA channels in *Xenopus* PT cells (16) and is slightly less stretch sensitive than that described for the BLM SA channels in *Rana temporaria* (12) and *Necturus* (30), where the half-maximal activation occurs around −11 mmHg. An osmolar gradient of 1 mosmol/kgH2O results in a hydrostatic pressure of about 17 mmHg, suggesting these channels operate over a physiologically relevant range. Although there is some variation in the mechanosensitivity of the channel in some of our cell preparations, the majority of patches fall into the range detailed in Fig. 1, B and C. It is unclear what factors are responsible for occasional variations in the mechanosensitivity between preparations.

Kinetic analyses of one-channel patches show that this SA-NSC channel has two open states and three closed states. As reviewed by Sachs and Morris (29) and Sackin (32), mechanosensitive channels have at least one stretch-sensitive closed state with a very long dwell time in the absence of applied pressure. Negative pressure probably lowers the energy barrier separating the closed and open states of the channel. Membrane tension, perhaps by altering the membrane deformation energy, decreases the probability of this closed state. Although it is likely that the longest closed state of the SA-NSC is stretch sensitive, additional experiments are necessary to determine if other states are also stretch sensitive.

**Ion conductance, rectification, and selectivity.** SA channels in amphibian kidney demonstrate a fairly consistent conductance of 25–30 pS (12, 13, 16), consistent with that of the SA channel in the present study. Higher-conductance SA channels have been described in *Necturus* (9) and *R. pipiens* (3). Irrespective of the cation tested (including TEA+), under symmetrical conditions the SA-NSC channel is a weak inward rectifier. In the present study, Mg2+ was always present on the cytosolic side of the patch; therefore, the possibility that the inward rectification is the result of Mg2+ block, as is the case for some K+ channels (20), cannot be excluded.

The inward conductance sequence suggests grouping correlated to the hydrated radius (or the dehydration energy) of the cations. Therefore, the selectivity sequence favors ions like Cs+ and Rb+ because of the relatively low energy required to shed their waters (−71 and −79 kcal/mol, respectively). This implies that conductance increases as atomic radii increase and as hydration energies decrease (*Eisenman sequences* I and II; see Ref. 6). The reduced conductance for Ca2+ may be a reflection of its large hydration energy (−397 kcal/mol).

**Voltage dependence.** Of the SA channels described in renal epithelia, those found in *Necturus* and *Xenopus* are K+ selective and have uniformly demonstrated hyperpolarization activation (16, 33). The SA-NSC channel described here is depolarization activated, which is similar to the SA-NSC channel in *R. temporaria* (12). The observation that depolarization increases NPo even at 0 mmHg suggests that the stretch-sensitive closed state is voltage dependent. Using our high-speed pressure step system to investigate the relationship between voltage and latency times, the depolarization activation is seen from a novel perspective. That is, rather than the steady-state measurement of NPo, we see an instantaneous reflection of the depolarization activation (on the order of ms) in the form of a shortened opening latency and a longer closing latency, both of which favor an increased open probability. This important finding is in sharp contrast to the very slow depolarization activation found in the SA channels of *Xenopus* oocytes (10) and leech central neurons (23), which occurs on the order of seconds to tens of seconds.

Mechanistically, voltage dependence can be the result of voltage-induced changes in 1) channel protein(s), 2) surrounding structures, or 3) membrane deformation. Gil et al. (10) hypothesize that voltage across the patch influences membrane tension and gates SA channels. Because depolarization activation did not occur with soft glass pipettes and was absent in outside-out patches, they concluded that voltage dependence was an artifact resulting from specific interactions between the glass pipette and membrane patch in *Xenopus* oocytes. In contrast, the SA channel described here is activated by depolarization regardless of the excised-patch configuration, similar to that described for mechanogated channels in leech neurons (23). If the same process of voltage-induced membrane deformation were occurring in *Ambystoma* epithelia, *Xenopus* oocytes, and leech neurons, such drastic differences in times to activation would not be expected.

**Blockade.** Since first being described to inhibit the beating of a perfused frog heart (24), lanthanides have become widely used in the study of cellular physiology. Sensitivity to the lanthanide Gd3+ is a common feature of many mechanosensitive channels and is reported in a number of different preparations, including yeast MID1 (15), *Necturus* PT cells (8), renal A6 cells (43), *Xenopus* oocytes (45), and human neurons (28). The SA channel described in this paper is unaffected by 100 μM Gd3+ in the pipette and/or bath of excised inside-out or outside-out patches. However, given the broad range of cross-reactivity, Gd3+ inhibition is currently viewed as a less reliable marker for SA channels (29). Other typical inhibitors of mechanogated or NSC channels, including amiloride, flufenamic acid, and niflumic acid, have no significant effect on this SA-NSC channel. Unfortunately, specific pharmacological inhibitors of SA channels are not readily available, but a natural toxin found in the venom of the common Chilean tarantula, *G. spatulata*, has been shown to block SA channels in GH3 pituitary cells (4). Our results indicate that this venom completely blocks channel activity in cell-attached patches containing SA-NSCs. The block may be because of a 35-amino-acid peptide (GsMTx-4) in the venom recently identified by Suchyna...
and coworkers (39). GsMTx-4 is the first peptide toxin that specifically blocks SA channels. They showed that 5 μM GsMTx-4 completely blocks mechanosensitive cation channels in astrocytes and cardiac myocytes. Based on a molecular weight of 4093.9 and a concentration of 1.95 mM GsMTx-4 in whole venom (39), channels in our cell-attached patches were exposed to ~20 μM GsMTx-4 toxin. To our knowledge, the SA-NSC in the PT is the first epithelial channel blocked by this venom.

Swelling activation. Swelling-activated mechanosensitive channels have been described in many tissues, including the PT (3, 30). The SA channel in the BLM of Ambystoma PT cells is also activated by hypotonic challenge. Unlike activation induced by stretch, swelling activation of the SA-NSC results in a biphasic response. Based on studies implementing simultaneous cell volume and patch recording techniques, it appears that the biphasic response of SA channels during swelling can be directly related to the cell volume changes during RVD (9). Whether or not the SA channel in Ambystoma is actually a component of the RVD remains unknown. From a physiological standpoint, hypotonic swelling likely does not reflect cellular volume changes, as they occur during actual transepithelial transport. That is, hypotonic stress may influence a number of cellular signaling systems (e.g., the cytoskeleton and intracellular messengers), making interpretation of these data more complex (29).

Nucleotide sensitivity and possible physiological role. An important feature of the channel is inhibition by cytoplasmic ATP. That the nucleotide diphosphate ADP also blocks the SA-NSC channel indicates that inhibition by adenine nucleotides probably does not require a typical protein kinase or hydrolysis by an ATPase. Therefore, channel inhibition most likely occurs when an appropriate adenine (but not uridine) nucleotide binds to a cytoplasmic domain of the channel protein. Although ATP (41) or cyclic nucleotides (7) regulate certain members of the NSC channel superfamily, to our knowledge the SA-NSC channel is the only mechanosensitive channel that is also nucleotide sensitive. Reminiscent of ATP-sensitive K⁺ channels (26), the tendency of NP, to increase upon washout of ATP (see Fig. 8) suggests that some degree of channel refreshment by ATP occurs at a second site, most likely resulting from phosphorylation of the channel or an associated protein.

As is the case with many mechanosensitive channels, its precise physiological function remains uncertain. Indeed, only within the past 2 yr have data been presented to support the hypothesis that epithelial SA channels in cell-attached patches underlie the whole cell currents elicited by cellular swelling (44). One mechanism that may be involved in the RVD of epithelial cells exposed to hypotonicity involves Ca²⁺ entry via SA-NSC channels, which in turn activates Ca²⁺-activated K⁺ channels in the same membrane (5). The BLM of PT cells appears to contain at least two K⁺ channels (27), one of which is an ATP-dependent K⁺ (K⁰) channel that is inhibited by Ca²⁺ (21, 22). If the other K⁺ channel is also regulated by Ca²⁺, then Ca²⁺ entry via the SA-NSC channel may contribute to RVD through secondary effects on K⁺ channels also in the BLM (18).

Recently cloned mammalian transient receptor potential (TRP)-like Ca²⁺-permeable NSC channels (OTRPC4, the osmosensitive transient receptor channel 4 (38) and VR-OAC, the vanilloid receptor-related osmotically activated channel (19)) are both activated by hypotonicity and found at high levels in the renal tubules. Interestingly, TRP-like channels have not been reported in patch-clamp experiments in native kidney, raising the possibility that the properties of recombinant channels expressed in vitro differ from those in situ. If so, it is possible that the SA-NSC channel is related to one of these TRP-like channels.

Nucleotide sensitivity is a property that may provide important new insight to the physiological role of this channel because of the possibility that SA channel function is linked to cellular metabolism in the PT. However, care must be exercised regarding interpretation of the physiological significance within the possible range for the changes in ATP and ADP concentrations because we do not yet know what these ranges are, whether the channel responds to the absolute level of these nucleotides or their ratio, and/or if key modifiers are lost after patch excision. On the one hand, the Kᵢ of 0.48 mM for ATP inhibition in excised patches suggests that the channel may not be open given PT [ATP]ᵢ levels in the range of 2–5 mM under healthy conditions (2, 42). Rather, it is possible that the channel becomes important when intracellular ATP is depleted, as occurs during ischemic cell injury. During such a stress, SA-NSC channels would open, providing a cation entry pathway for Ca²⁺ or Na⁺. In the case of the latter, as opposed to Na⁺ entry across the apical membrane, Na⁺ entering across the BLM would decrease net Na⁺ reabsorption and induce further cellular swelling. This raises the possibility that, if the channel conducts Na⁺ in vivo, it may be to promote nonspecific cell death, as has been proposed for liver cells (1).

On the other hand, as has been shown for K⁰ channels, protein-lipid interactions may modify ATP sensitivity, essentially permitting channel function in the presence of what would otherwise be inhibitory concentrations of ATP (36). For this reason, the SA-NSC channel described may play a role during transepithelial transport under healthy conditions. In this case, the SA-NSC channel could become active in response to both cellular swelling (44) and reductions in [ATP]ᵢ, allowing Na⁺ or Ca²⁺ to enter or (less likely) K⁺ to exit. We have previously shown that intracellular Ca²⁺ is an important regulator of the hyperpolarization-activated K⁰ channel in the same membrane (22). With these considerations, it is possible that Ca²⁺ entry or a depolarizing Na⁺ current may be important to optimize the pump-leak coupling that occurs between the Na⁺-K⁺-ATPase and K⁰ channels in the BLM of PT.
We conclude that an ATP-sensitive mechanogated NSC channel is highly expressed in the BLM of PT. This mechanogated channel could be functionally coupled to the metabolic state of the cell and may be important for cellular volume regulation and/or as a Ca\(^{2+}\) entry pathway during transepithelial transport or in the response to ischemic injury.

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