Nonhydrolyzable Analogues of GTP Activate a New Na\(^+\) Current in a Rat Mast Cell Line*

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Whole-cell patch clamp experiments were performed to examine the effects of the nonhydrolyzable GTP analogue, guanosine 5′-3-O-(thio)triphosphate, on membrane currents in rat basophilic leukemia cells. Guanosine 5′-3-O-(thio)triphosphate activated an inward sodium current. This current had a new permeability sequence to monovalent cations and a different pharmacological profile to that of other characterized Na\(^+\) channels. Long hyperpolarizing steps revealed that the current declined during the pulse, and the decline was voltage-dependent. Activation of the current required Mg\(^{2+}\) and ATP. The nonhydrolyzable ATP analogues, adenosine 5′-O-(thio)triphosphate and adenosine 5′-(β,γ-imino)triphosphate, could not substitute for ATP. Soluble second messengers like cAMP, cGMP, inositol phosphates, and Ca\(^{2+}\) did not activate the Na\(^+\) current. These results suggest that nonhydrolyzable GTP analogues activate a Na\(^+\) current in rat basophilic leukemia cells that is new in terms of its selectivity, pharmacology, and activation mechanism. It may be the prototype for a new family of Na\(^+\) channels expressed in certain nonexcitable cells.

Na\(^+\) channels fulfill a variety of physiological roles in different cell types. In electrically excitable cells, voltage-dependent Na\(^+\) channels contribute to the initiation and propagation of the action potential (1). More recently, it has become apparent that Na\(^+\) channels can affect several processes through changes in the cellular Ca\(^{2+}\) buffering capacity. In the heart, activation of Na\(^+\) channels elevates cytosolic Na\(^+\) in a localized region termed the fuzzy space. This reduces Ca\(^{2+}\) extrusion by the Na\(^+\)-Ca\(^{2+}\) exchange, thereby elevating cytosolic Ca\(^{2+}\) and triggering muscular contraction through Ca\(^{2+}\)-induced Ca\(^{2+}\) release (2).

Na\(^+\) channels are not ubiquitously distributed in nonexcitable cells and are absent from several cell types. However, transporting epithelial cells are endowed with several types of Na\(^+\) channel (3). These Na\(^+\) channels share several properties with their voltage-dependent counterparts, including monovalent cation selectivity and single-channel conductances. Na\(^+\) channels in transporting epithelial cells are, therefore, thought to represent the typical Na\(^+\) channel of a nonexcitable cell.

Patch-clamp recordings of rat basophilic leukemia (RBL) cells, an immortalized mast cell line, have not detected the presence of Na\(^+\) currents (4, 5). In the present report, I describe that nonhydrolyzable analogues of GTP do activate a Na\(^+\) current in RBL cells. This current has a selectivity sequence different from that of voltage-dependent and epithelial Na\(^+\) channels, a unique pharmacological profile and a complicated mechanism of activation. The Na\(^+\) current in RBL cells might, therefore, represent a new type of Na\(^+\) channel and could conceivably be the prototype for a new Na\(^+\) channel gene family expressed in certain nonexcitable cells.

**MATERIALS AND METHODS**

Rat basophilic leukemia cells (RBL-1) were purchased from American Type Culture Collection cell lines, Rockville, MD, and were cultured as described previously (5). Patch-clamp experiments were carried out in the tight-seal, whole-cell configuration (6) at room temperature (20–25°C) in a standard saline solution that contained 145 mM NaCl, 2.8 mM KCl, 10 mM Ca\(^{2+}\), 2 mM Mg\(^{2+}\), 10 mM CrCl\(_2\), 11 mM glucose, and 10 mM HEPES-NaOH, pH 7.2, as described previously (7). Ca\(^{2+}\) was present to block the inwardly rectifying K\(^+\) channel (4). Sylgard-coated, fire-polished patch pipettes had resistances of 2–3 megohms after compensation of the EPC-9. Series resistance was between 4 and 10 megohms. Ramps were given every 2 s (−100 to +100 mV in 50 ms), and cells were held at 0 mV between ramps. Currents were filtered at 2.3 KHz and sampled at 10 KHz. The Na\(^+\) currents were analyzed at −80 mV. All currents were leak subtracted by averaging ten ramps prior to onset of the GTP\(_{\beta}S\)-activated Na\(^+\) current and then subtracting the average from all subsequent records. Extracellular solution changes were made by local pressure application from a wide-tipped micropipette placed within 20 μm of the cell.

All chemicals were purchased from Sigma except GTP\(_{\beta}S\), GDP\(_{\beta}S\), GTP, ATP\(_{\beta}S\), and AMP-PCP, which were from Boehringer Mannheim. Whenever these nucleotides were used, the same concentration of MgCl\(_2\) was added to maintain the free Mg\(^{2+}\) levels.

**RESULTS**

GTP\(_{\beta}S\) Activates an Inward Current—Fig. 1 shows the effects of dialyzing individual RBL cells with the nonhydrolyzable GTP analogue, GTP\(_{\beta}S\) (200–500 μM). After a variable latency from break-in (60–600 s), an inward current steadily developed (measured at −80 mV, 134/137 cells) and reached an amplitude of several hundred picoamperes (80–1400 pA). This current grew continuously for several minutes and in some cells reached a plateau about 14 min after its onset (range, 8–20 min, although some recordings were aborted before a plateau had been attained). The current was also inward at a holding potential of 0 mV, and two examples are shown in the lower panels of Fig. 4. Applying 50-ms hyperpolarizing pulses (−100 to +100 mV) revealed that the current was voltage-
FIG. 1. GTP analogues activate an inward current. A, effects of different G protein activators. Each current trace represents a different cell. Either GTPγS (200 μM), Gpp(NH)p (200 μM), GDPβS (500 μM), or AlF₄⁻ (30 μM AlCl₃ and 30 mM NaF) was included in the pipette solution. The cells were held at 0 mV, and voltage ramps that spanned −100 to +100 mV in 50 ms were applied at 0.5 Hz. The current amplitudes were measured at −80 mV. Occasionally, GTPγS activated the store-regulated Ca²⁺ current, and the development of this current preceded that of the much larger inward current. One such example of this is shown for the GTPγS current, where a small inward current was observed prior to development of the larger current. Bi, current response to 50-ms voltage pulses (−100 to +100 mV in 20-mV increments). Bii, ramp I-V curve after activation of the current by GTPγS. The first few ramps after break-in were averaged and then subtracted to give the true GTPγS-activated current. Dotted line, zero current potential. Biii, I-V curves to voltage ramps (○) or voltage pulses (●) are superimposable.
independent, possessed weak inward rectification, and reversed at +70 mV (Fig. 1, Bi and Biii). The inward current before any voltage changes in the figures was not leak current but arose because the GTPyS-activated current was inwardly directed at 0 mV, and the cells were held at this voltage between pulses. An identical I-V relationship was obtained when voltage ramps of 50-ms duration (−100 to +100 mV) were applied (Fig. 1Bii), demonstrating that the ramp protocol was not distorting the I-V relationship over this time scale. In fact, the I-V curves obtained using short duration ramps or brief voltage pulses were superimposable (Fig. 1Biii).

Effects of Other GTP Analogues—The effects of GTPyS were mimicked by Gpp(NH)p, another nonhydrolyzable GTP analogue (200 μM, 5/5 cells; Fig. 1A), but not by GDPβS (500 μM, 5/5 cells; Fig. 1A), GTP itself (1–3 mM, 4/4 cells), ATPγS (2 mM, 5/5 cells), nor by internal solution which did not contain GTPyS (12/12 cells). These results suggest that the current is activated by nonhydrolyzable analogues of GTP. Heterotrimeric G proteins can be activated by AlF4− (8) and regulate a variety of ionic channels, including two types of K+ channel in RBL cells (4). To test for the involvement of such a G protein, both AlCl3 (Al3+, 30 μM), and NaF (F−, 30 mM) were included in the pipette solution. Surprisingly, AlF4− was less effective than GTPyS (Fig. 1A). Two of six cells failed to respond at all, and of the four cells that did respond, the current was smaller than that evoked by GTPyS in adjacent cells taken from the same coverslip (AlF4−, −4.2 ± 1.1 pA/pF, 4 cells; GTPyS, −10.1 ± 2.3, 3 cells, measured at −80 mV). Doubling the Al3+ concentration did not generate bigger currents (3 cells, data not shown).

GTPyS Activates a Na+ Current—The positive reversal potential (Erev) of the current under the present conditions suggests that either Na+ and/or Ca2+ is the charge carrier. Replacing Ca2+ with either Mg2+ (3/3 cells) or Ba2+ (2/2 cells) had no effect on the current, demonstrating that Ca2+ is not the main charge carrier.

Replacing Na+ with either N-methylglucamine (Fig. 2A, NMG, 3/3 cells), Tris (2/2 cells), or tetraethylammonium (3/3 cells) resulted in rapid, complete, and reversible loss of the current. This suggests that the current is carried mainly by Na+. To determine the monovalent cation selectivity sequence of the current, various test cations replaced external Na+ and were applied by local perfusion once the current had started to develop. K+ was not an effective replacement ion and carried only 18.2 ± 3.1% of that of Na+ (Fig. 2B, 6 cells). Neither Cs+ (8.3 ± 2.3%, Fig. 2C, 3 cells), NH4+ (6.25 ± 0.66%, Fig. 2D, 4 cells), nor Rb+ (4.8 ± 4.8%, Fig. 2F, 2 cells) were able to replace Na+ in carrying the current. Li+ is able to fully replace Na+ in carrying the current through Na+ channels (1). However, Li+ was not as good a charge carrier and carried only 37.6 ± 6.3% that of Na+ (Fig. 2E, 5 cells). The charge carrier profile of the current is Na+ > Li+ > K+ > Rb+ > Cs+, NH4+, tetraethylammonium, Tris, and N-methylglucamine.

Although these experiments, based on ion conductance measurements, point to a Na+ -selective current, a more rigorous demonstration requires an estimation of the relative permeabilities of the different cations. This can be achieved by...

FIG. 2. Monovalent cation selectivity of the GTPyS-activated inward current. Test cations were applied as indicated by local perfusion via a pipette placed within 20 μm of the cell. Currents were recorded using voltage ramps and analyzed at −80 mV. In B, C, and F, the discontinuous recordings arose because the ramps were stopped, and step voltage pulses were applied. Each recording represents a different cell. The percentage of current carried by each cation was calculated by averaging the steady-state current in the presence of the test cation (measured at −80 mV in voltage ramps) for three to five recordings and then dividing this by the averaged peak current in the presence of Na+. The Hodgkin and Huxley activation parameters (200 μM) for three to five recordings and then dividing this by the averaged peak current in the presence of Na+.
measuring the shift in \( E_{\text{rev}} \) on replacing external \( \text{Na}^+ \) with the test monovalent cation (1). When \( \text{K}^+ \) replaced \( \text{Na}^+ \), \( E_{\text{rev}} \) was shifted leftwards by 57 \( \pm \) 8.8 mV, and this corresponded to \( P_{\text{K}}/P_{\text{Na}} \) of 0.09 \( \pm \) 0.02. With \( \text{Li}^+ \), this shift was 9 \( \pm \) 1.2 mV, and \( P_{\text{Li}}/P_{\text{Na}} \) was 0.72 \( \pm \) 0.06. Currents in \( \text{Li}^+ \) were smaller than might be expected from the independence principle for an ion having a permeability ratio of 0.72. Interestingly, this is also the case for voltage-gated \( \text{Na}^+ \) channels (9) and has been ascribed to a permeation block by \( \text{Li}^+ \) on the \( \text{Na}^+ \) channel.

When \( \text{Na}^+ \) replaced \( \text{Cs}^+ \) as the main internal cation in the pipette solution (under these conditions, internal and external \( \text{Na}^+ \) concentrations were equal), GTP\( \gamma \)S activated the \( \text{Na}^+ \) current, but now the current reversed at +5 mV, instead of +70 mV (3/3 cells, data not shown). This shift is similar to the theoretical one predicted from the Nernst equation. Therefore, this current is \( \text{Na}^+ \)-selective with a different selectivity series compared with known \( \text{Na}^+ \) channels. This might indicate the presence of a new type of \( \text{Na}^+ \) channel in RBL cells.

**Pharmacology of the \( \text{Na}^+ \) Current**—If the GTP\( \gamma \)S-activated current indeed represents a new \( \text{Na}^+ \) channel, one would expect it to have a very different pharmacological profile from that of other characterized \( \text{Na}^+ \) channels. Tetrodotoxin, a powerful blocker of voltage-dependent \( \text{Na}^+ \) channels, had no effect on the current (20 \( \mu \)M, 3/3 cells; Fig. 3A). The \( \text{K}^+ \) diuretic amiloride, which is a powerful blocker of the renal epithelial \( \text{Na}^+ \) channel with a \( K_i \) in the submicromolar range (3), had virtually no effect at 300 \( \mu \)M (4/4 cells; Fig. 3B).

**Noise Analysis of the \( \text{Na}^+ \) Current**—If the current was flowing through a \( \text{Na}^+ \) channel, one would predict an increase in the variance as the current developed. Fig. 4 shows two examples of how the variance changes as the \( \text{Na}^+ \) current develops at a holding potential of 0 mV (2–1000 Hz bandwidth). The variance clearly increases, indicating a channel mechanism. However, it was not possible to estimate single-channel conductance (see “Discussion”).

**Voltage-dependent Reduction of the Current**—The results of Fig. 1Bii showed that the \( \text{Na}^+ \) current did not decline much during a 50-ms hyperpolarization to −100 mV. However, if the pulse duration was increased to 1 s, then prominent reduction occurred during the pulse. Fig. 5Aii shows such a response on pulsing to −100 mV from a holding potential of 0 mV with \( \text{Cs}^+ \) as the main intracellular cation. This reduction in current during the pulse was independent of the intracellular cation because it was still observed with either \( \text{Na}^+ \)- or Tris\(^+\)-based internal solution (Fig. 5, Aii–Aiii). The time-constant of decline (\( \tau_{\text{decline}} \)) could be fitted with a mono-exponential function and was 158.3 \( \pm \) 18.1 ms for \( \text{Cs}^+ \) and 166.8 \( \pm \) 37.6 ms for Tris\(^+\) at −100 mV (3 and 4 cells, respectively). The decline in current during the pulse was more pronounced at negative potentials (Fig. 5Bi). Fig. 5Bii plots the time-constant of the decline (\( \tau_{\text{decline}} \) versus voltage for Tris\(^+\)-based internal solution. As the potential becomes more negative, the decline becomes more prominent, and this gives rise to a smaller \( \tau \). For membrane potentials more positive than −20 mV, the decline was not observed. Similar results were obtained when \( \text{Cs}^+ \) was the dominant intracellular cation in the pipette (\( \tau_{\text{decline}} \) was 184 \( \pm \) 35.3 ms at −80 mV, 206.7 \( \pm \) 30.2 at −60 mV, and 281.3 \( \pm \) 32 at −40 mV). The ratio of the steady-state current (measured at the end of the pulse) to the peak current (immediately after the membrane is hyperpolarized) provides an indication of the extent of the current decrease during the pulse. A small ratio would reflect substantial reduction. Fig. 5Biii plots this ratio against membrane potential. At −100 mV, the decline is virtually total, but as the holding potential becomes more positive, the decline becomes less. In Fig. 5Biii, data pooled from a number of cells could be fitted with a Boltzmann-type equation of the form

\[
I_{\text{steady-state}}/I_{\text{peak}} = 1/(1 + \exp(V - V_{1/2} + S))
\]

where \( V_{1/2} \) is the voltage when inactivation is one-half of its maximal value, and \( S \) is the slope factor. For the pooled data, \( V_{1/2} \) was −40.1 mV, and the slope was 12.84. This corresponds to a gating valence of almost 2. However, this value provides only an empirical measure of the voltage-dependence of decay. The voltage-dependent decrease during a hyperpolarizing pulse was not affected by changing external \( \text{Ca}^{2+} \) or \( \text{Mg}^{2+} \) (3/3 cells each), suggesting that it might not reflect voltage-dependent...
block by external divalent cations.

Activation of the Na\(^+\) Current—Activation of the Na\(^+\) current had a stringent requirement for ATP (Fig. 6). Omission of ATP from the pipette solution prevented the induction of the current (11/12 cells; Fig. 6A). Neither 2 mM ATP\(_g\)S (4/4 cells), which can be used by a variety of kinases, nor 2 mM AMP-PCP (3/3 cells), another nonhydrolyzable ATP analogue, could substitute for ATP (Fig. 6, B and C).

A variety of soluble second messengers were tested to see if they could activate the Na\(^+\) current in the absence of GTP\(_g\)S. Neither cAMP (100 \(\mu M\), 4/4 cells), cGMP (100 \(\mu M\) 4/4 cells), inositol 1,4,5-trisphosphate (60 \(\mu M\), 7/10 cells), inositol tetrakisphosphate (the 1, 3, 4, 5 and the 2, 3, 4, 6 isomers, 50 \(\mu M\), 3 cells each) or inositol 1,4,5-trisphosphate and inositol tetrakisphosphate.
rakisphosphate together (3/3 cells) were able to activate the current. Cytosolic Ca$^{2+}$ was also not involved, since the current could be activated by GTP$_{\gamma}S$ in the presence of buffered Ca$^{2+}$ (90 nM) or in the presence of either 10 mM EGTA or 10 mM 1,2-bis(\(\alpha\)-aminophenoxy)ethane-\(N,N,N',N'\)-tetraacetic acid, a faster Ca$^{2+}$ chelator. Dialyzing the cells with 1 mM Ca$^{2+}$ alone did not activate the current.

There was a requirement for cytosolic Mg$^{2+}$. In these experiments, no MgCl$_2$ was added to the internal solution. To chelate any contaminating Mg$^{2+}$, 1 mM EDTA was added. ATP was present but as the Na$_2$ATP salt and not as the MgATP one.

With this internal solution, GTP$_{\gamma}S$ failed to activate the Na$^+$ current (4/4 cells, data not shown), whereas it consistently activated the current in paired experiments on the same preparation of cells using the standard internal solution that did contain Mg$^{2+}$.

**DISCUSSION**

In this study, I have described the presence of a Na$^+$ current in RBL cells, an immortalized mast cell line, and have characterized several of its properties. The Na$^+$ current has a different ionic selectivity, pharmacological profile, and mechanism of activation.

![Activation of the Na$^+$ current requires ATP](https://example.com/activation.png)

**FIG. 6.** Activation of the Na$^+$ current requires ATP. A, the Na$^+$ current develops normally when 2 mM ATP is included in the patch pipette solution. If ATP is omitted from the solution, the current does not develop. The two current recordings reflect two different cells recorded under paired conditions. Neither 2 mM ATP$_{\gamma}S$ (B) nor 2 mM AMP-PCP (C) can substitute for ATP. B and C were from the same coverslip, and the same cell that was dialyzed with ATP is shown in both. 2 mM Mg$^{2+}$ was always added together with the nucleotides to maintain free Mg$^{2+}$ levels. Cells were held at 0 mV between voltage ramps, and the currents were analyzed at −80 mV.
activation to that of previously described Na\(^+\) currents and, therefore, likely reflects a new type of Na\(^+\) channel. The RBL cell Na\(^+\) channel may, therefore, be the prototype for an entire new Na\(^+\) channel family present in nonexcitable cells.

**Selectivity of the Na\(^+\) Current**—The current activated by GTP\(_\gamma\)S was highly selective for Na\(^+\) and was lost when external Na\(^+\) was replaced with structurally distinct organic cations or with large group I monovalent cations (Rb\(^+\) and Cs\(^+\)). K\(^+\) carried only a small fraction of the current compared with Na\(^+\). NH\(_3\)^+, which can carry significant current through voltage-dependent Na\(^+\) channels in excitable as well as epithelial cells (1), was not as effective as a charge carrier in comparison with Na\(^+\) and transported only 37.6\% of that of Na\(^+\) (P\(_{Na}\)/P\(_{Na}\) = 0.72).

The charge carrier profile of the current is, therefore, different to that of other specific Na\(^+\) currents. This is likely to reflect a different pore structure and hence a different channel. The ionic selectivity of previously characterized Na\(^+\) channels has been described in terms of Eisenman theory, which considers selectivity as a balance between the fall in energy when the cation binds to the negative site of the channel (determined by the coulombic potential) and the energy required to dehydrate the ion (10). Selectivity of Na\(^+\) channels corresponds to a type XII sequence (1). The RBL cell Na\(^+\) current corresponds to sequence XI, which requires a weaker interaction energy (U) between binding site in the pore and Na\(^+\) ions.

**Pharmacology of the Current**—A further argument in favor of a new Na\(^+\) channel in RBL cells is that both tetrodotoxin, a powerful blocker of voltage-dependent Na\(^+\) channels, and amiloride, which blocks all of the different Na\(^+\) channels in epithelial cells (2) as well as Na\(^+\) currents in other nonexcitable cells like B lymphoid ones (11), had no effect even at high concentrations. A tetrodotoxin-resistant Na\(^+\) channel has been cloned from peripheral neurons (12), but this is voltage-activated and is, therefore, unlikely to be the one observed in RBL cells.

**Voltage-dependent Decrease during a Long Hyperpolarizing Pulse**—The Na\(^+\) current decreased in amplitude during long step hyperpolarizations. The decline was voltage-dependent in that it was more prominent at negative potentials. The decline in current could reflect either inactivation of open channels during the hyperpolarizing pulse or deactivation from the open to the closed state since some channels were already open before the hyperpolarizing pulses were applied. It is rather difficult to distinguish between these two possibilities for a non-voltage-activated channel, and additional experiments are required to resolve this.

The decline during the pulse could occur if intracellular cations bind electrostatically to the inner channel vestibule at negative potentials and induce a voltage-dependent block. An argument against this possibility is that the decline in current was observed regardless of the internal monovalent because it was seen with Cs\(^+\), Na\(^+\), or the larger cations Tris\(^+\) and N-methylglucamine\(^+\). The voltage-dependent decrease could conceivably reflect block by external divalent cations, as is the case with Mg\(^{2+}\) and the N-methyl-D-aspartate receptor channel (13). However, the voltage-dependent decline was still apparent irrespective of the presence of external Ca\(^{2+}\) or Mg\(^{2+}\).

The reduction is unlikely to reflect voltage-dependent block by intracellular Ca\(^{2+}\) because inactivation was still present in the presence of 10 mM 1,2-bis-(o-aminophenoxo)-ethane-N,N,N',N'-tetraacetic acid. Block by cytosolic Mg\(^{2+}\) could not be examined because the current failed to activate in the absence of internal Mg\(^{2+}\). Another possibility for the voltage-dependent decrease is that it represents a process intrinsic to the channel itself.

**Is the Na\(^+\) Current Carried through an Ion Channel Mechanism?**—The activation of the Na\(^+\) current was not associated with any clear single-channel events, raising the question as to whether an ion channel underlied the entry. Several arguments are compatible with the current flowing through a channel rather than a slow carrier/transporter. (a) Step hyperpolarizations, once the current had developed, evoked instantaneous increases in conductance, indicative of transport through a channel. (b) The current showed voltage-dependent block, which can most easily be explained in terms of anionic channel process. (c) Activation of the current was always associated with an increase in the current variance. An increase in variance is diagnostic of a channel. The increase in variance was rather small though, and this could be due to either a low channel conductance or a long-lived open state with little flickering between open and closed states. Alternatively, a small variance increase might reflect low or high open probabilities of the channel. Because variance is largest when open probability is 0.5, one way to estimate conductance from variance measurements is to apply a channel blocker to reduce the current amplitude. Because the typical Na\(^+\) blocker blockers were not effective in reducing the current, it was not possible to use this approach to estimate single-channel conductance.

**Activation of the Na\(^+\) Current**—Voltage-dependent Na\(^+\) channels are activated by membrane depolarization, and Na\(^+\) currents in renal epithelial cells are thought to be directly modulated by heterotrimeric G proteins since Goq,3 activates the Na\(^+\) channels in excised patches (14). The Na\(^+\) current in RBL cells that I have described does not require membrane depolarization to activate, and two results suggest that it might not be activated only by a heterotrimeric G protein. (a) AlF\(_4\)\(^-\), which is routinely used to indiscriminately activate heterotrimeric G proteins (8), was somewhat less effective than GTP\(_\gamma\)S. The concentrations of AlF\(_4\)\(^-\) and F\(^-\) were similar to those widely used to maximally activate heterotrimeric G proteins in RBL cells (4). (b) The activation of the Na\(^+\) current had a stringent requirement for both Mg\(^{2+}\) and ATP. In excised atrial membrane patches, G protein \(\beta_{\gamma}\)-subunits activate the muscarinic-gated potassium channel without any requirement for ATP (15).

This critical requirement for ATP for activation of the RBL cell Na\(^+\) current probably reflects a need for ATP hydrolysis rather than a phosphorylation reaction because neither ATP-S (which can be used by a variety of kinases) nor AMP-PCP (a nonhydrolyzable ATP analogue) could substitute for ATP. One process that requires ATP hydrolysis is the production of second messengers like CAMP. However, several known soluble second messengers like cAMP, cGMP, Na\(^{2+}\), and inositol polyphosphates failed to induce the current themselves. Another process that requires ATP hydrolysis is vesicle transport/fusion (16). Although speculative, it is possible that the Na\(^+\) channels are stored in vesicles within the cytosol and are then inserted into the plasma membrane after stimulation. This would be similar to incorporation of water channels by antidiuretic hormone in the kidney (17). Vesicle fusion is regulated by small G proteins, and it is noteworthy that GTP\(_\gamma\)S is a better activator of the Na\(^+\) current than AlF\(_4\)\(^-\). Although both these agents activate heterotrimeric G proteins, only GTP\(_\gamma\)S directly interferes with small G protein function as well (18). Alternatively, ATP hydrolysis can remodel the cytoskeleton. For example, the intrinsic ATPase activity of myosin can contract actin microfilaments. Because the renal epithelial Na\(^+\) channel is thought to be regulated by actin filaments (19), it is possible that cytoskeletal rearrangements might somehow activate the RBL cell Na\(^+\) current.
A New Family of Na⁺ Channels?—The new monovalent cation selectivity, the unusual pharmacology, the voltage-dependent decline, and the complicated activation mechanism would all be compatible with the notion that the RBL cell Na⁺ current represents a new Na⁺ channel family. Like voltage-dependent Na⁺ channels, the pore-forming α subunits of which are encoded by several distinct genes (20), nonexcitable cells might be able to express Na⁺ channels encoded by different genes.

Possible Role of Na⁺ Current in Pathophysiological States—What role might the Na⁺ current fulfill? Stimulation of either A₃ adenosine or antigen surface receptors, the two main receptors in RBL cells, does not activate the Na⁺ current (7). This would suggest that either an unknown surface receptor activates the current or several stimuli are required to turn it on. One striking observation is that the Na⁺ current is present only in RBL cells, a tumor cell, but evidently does not seem to be present in the parent cell, the mast cell (21). This raises the intriguing possibility that the current might somehow be associated with the malignant state. Future experiments should address whether the Na⁺ current is directly involved in transformation or, perhaps more likely, if it is an indirect consequence of malignancy.

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