Steroids and Exogenous γ-ENaC Subunit Modulate Cation Channels Formed by α-ENaC in Human B Lymphocytes*

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Previous studies using whole-cell recording methods suggest that human B lymphocytes express an amiloride-sensitive, sodium-permeable channel. The present studies aim to determine whether this channel has biophysical properties and a molecular structure related to the α, β, and γ subunits of the epithelial sodium channel (ENaC). Reverse transcriptase polymerase chain reaction and Northern blots showed that human B lymphocytes express messages for both α- and β- but not γ-ENaC. Western blots showed that both α- and β- but not γ-ENaC proteins are expressed and strongly reduced by antisense oligonucleotides. Patch clamp experiments demonstrated that lymphocyte sodium channels are not active in cell-attached patches. However, membrane stretch can activate a 21-pS nonselective cation channel. The frequency of observance of this channel was significantly reduced by antisense oligonucleotide against α-ENaC but not by antisense oligonucleotide against β-ENaC, indicating that only the α subunit of ENaC is necessary to form stretch-activated cation channels. Aldosterone (1.5 μM) reduced the frequency of observance of 21-pS α-ENaC channels and simultaneously induced the appearance of spontaneously active 10-pS channels. Antisense oligonucleotide experiments showed that this 10-pS channel is formed from α- and β-ENaC. After expression of exogenous γ-ENaC, aldosterone again reduced the frequency of observance of the 21-pS α-ENaC channel but induced the appearance of a 5-pS channel, presumably a αβγ-ENaC channel. In the absence of aldosterone, the α subunit forms an α-cryptic channel that is activated by stretch, and in the presence of aldosterone, β and α subunits together form an active channel that is modulated by aldosterone.

The superfamily of proteins to which the epithelial sodium channel (ENaC)1 belongs generally mediates cation transport across cell membranes. ENaC, itself, is usually associated with sodium transport across the apical membrane of a variety of epithelia including the colon, lung, and kidney. Since 1994, when ENaC was initially cloned from rat colon (1), the biophysical properties and molecular structure of ENaC have been studied extensively. Several lines of evidence suggest that ENaC, including human ENaC (hENaC), is typically composed of three subunits, α, β, and γ, and that all three subunits are required to form a functional αγγ-ENaC channel complex (1–6). In heterologous expression systems, maximal expression of the hENaC channel requires co-expression of all three subunits (7, 8), and in oocytes, expression of α-ENaC cRNA alone produces little expression of any amiloride-sensitive currents. However, in other cell types, expression of the exogenous α-ENaC subunit alone can form a stretch-activated nonselective cation channel (9), and a similar nonselective cation channel has also been described in native lung epithelial alveolar type II cells (10–12). This lung cation channel appears to be formed from α-ENaC alone and is equally permeable to Na+ and K+, is sensitive to steroid hormones, and has a higher unit conductance (21 pS) than that of αβγ-ENaC (4–5 pS) (13, 14). These data suggest that the α-ENaC subunit alone under the correct conditions is able to form a channel. But the biophysical properties of this channel are different from those of the ENaC channel complex formed by all three subunits, α-, β-, and γ-ENaC.

Results from whole-cell recordings show that an amiloride-sensitive conductance is expressed in human B lymphocytes (15–18). However, whether this channel is related to ENaC remains to be determined by single-channel and biochemical experiments. Therefore, in the present study, cell-attached recordings were performed in human B lymphocytes to characterize the amiloride-sensitive, Na+-permeable channel at a single-channel level. Reverse transcriptase (RT)-PCR, Northern blotting, and Western blotting experiments were also carried out to determine whether human B lymphocytes express ENaC subunits. Thereafter, antisense oligonucleotide methods were used to determine which ENaC subunit was involved in forming the lymphocyte Na+-permeable channel. In addition, we determined how steroid hormones regulate the expression of this channel. We found that human B lymphocytes express a stretch-activated channel apparently formed from α-ENaC alone. When the cells were exposed to aldosterone, the frequency of observance of this channel decreased, and the frequency of observance of a 10-pS channel increased. The 10-pS channel was apparently composed of α and β subunits. When exogenous γ-ENaC subunit was transfected into lymphocytes, aldosterone induced a 5-pS channel which appeared to be com-
posed of α, β, and γ subunits and was indistinguishable from the channels in sodium-transporting epithelial tissues.

MATERIALS AND METHODS

Cell Culture—Human B lymphocytes (the Daudi cell line) were purchased from the American Type Culture Collection. The cells were continuously cultured in RPMI 1640 medium (Invitrogen) with 10% fetal bovine serum (Invitrogen) by incubator in which the temperature was set at 37 °C with a constant CO₂ of 5%.

Patch Clamp Techniques—Immediately before use, human B lymphocytes were thoroughly washed with NaCl bath solution (see “Chemicals and Solutions” below) and transferred into the patch recording chamber mounted on the stage of a Leitz inverted microscope. Whole-cell and cell-attached configurations were established with polished micropipettes with a tip resistance of 2.5–5 megohms. Only patches with a seal resistance above 10 gigohms were used for the experiments. In whole-cell recordings, a voltage-ramp protocol from −120 mV to +60 mV at a holding potential of −60 mV was used to quickly obtain the current-voltage relationship. After the characterization of macroscopic currents by whole-cell recordings, single-channel analysis was also done in the cell-attached mode. For cell-attached experiments, the fraction of patches with channel activity was used to estimate the channel density as described previously (13, 14).

RT-PCR—RT-PCR of total RNA obtained from human B lymphocytes was carried out with subunit-specific primers designed according to published sequences for human ENaC. PCR reactions were run in the linear range of amplification with glyceraldehyde-3-phosphate dehydrogenase as the standard for comparison and to establish equal starting amounts of message. These primers amplified a single fragment of the expected size for each RNA (346 bp for the α subunit of human ENaC (α-ENaC), 520 bp for β-ENaC, or 624 bp for γ-ENaC). The PCR products were not seen in control reactions without RNA. The primers specific for each subunit did amplify plasmid DNA for the target subunit but did not amplify plasmid DNA for any other subunits. The sequences of the amplified fragments were confirmed by DNA sequencing.

Preparation of Antisense and Sense Oligonucleotides to α, β, and γ-ENaC—Oligonucleotides either complementary (for antisense) or identical (for sense) to α-(first 22), β- (first 23), and γ-ENaC (first 23) sequences beginning with the initiation codon AUG were synthesized as described in the “Chemical Methods” section. These phosphothiorate oligonucleotides by the Emory University Microchemical Facility. The sequences were: α-sense, 5′-TAC GTC TCA TGG AGG GGA A-3′; α-antisense, 5′-TCC CCC ATG AGA CTA GGT A-3′; β-sense, 5′-CAG GTG CCA TCA TGA TGC A-3′; and β-antisense, 5′-CTT CAC GTG CAT ATG GCC ACC TG-3′. A BLAST search of the GenBank data base revealed no similarity of these oligonucleotide sequences with sequences of other Na⁺ channels and only limited similarity of these oligonucleotides between each ENaC subunit. The cells were pretreated with serum-free medium containing either 10 μM antisense or sense oligonucleotides to either the α- or β-ENaC subunit for 24 h. In some experiments, the culture medium contained 10% fetal bovine serum plus aldosterone to determine the effect of aldosterone. In those experiments, the oligonucleotides were repeatedly added into the culture medium every 6 h to ensure that they remained present at a significant concentration.

Transfection of Human B Lymphocytes with γ-ENaC—The rat γ-ENaC in the pSport vector, provided by Dr. Bernard C. Rossier (University of Lausanne, Switzerland), was subcloned into pcDNA3.1 (+) vector (Invitrogen) and verified by restriction digestion and DNA sequence analysis. The correct sequence was then subcloned into pcDNA3.1 (+) vector using EcoRI and HindIII restriction sites. The cells were transiently transfected with this plasmid DNA of rat γ-ENaC by using LipofectAMINE™ plus reagent (Invitrogen) according to the manufacturer’s specifications. The day before transfection, the cells were split into 25-cm² flasks with a resulting density of 10⁶ cells in each flask on the next day. γ-ENaC DNA was diluted with serum-free medium (1 μg of DNA/50 μl of medium), mixed with Plus™ reagent, and incubated at room temperature for 15 min. LipofectAMINE™ reagent was then diluted with serum-free medium (1 μl of LipofectAMINE™/25 μl of medium), mixed with the DNA/Plus™ reagent, and incubated at room temperature for 15 min. The transfection solution containing DNA, Plus™ reagent, and LipofectAMINE™ reagent was then added to the cells. After incubation of the cells at 37 °C for 6 h, the transfection solution was replaced with regular medium, and the cells were incubated for 24–48 h before patch clamp experiments.

Chemicals and Solutions—Chemicals were obtained from Sigma and Research Biochemicals International. The NaCl bath solution for cell-attached experiments contained (in mM): 145 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, and 10 HEPES, at a pH of 7.4. The KCl bath solution contained (in mM): 145 KCl, 5 NaCl, 1 CaCl₂, 1 MgCl₂, and 10 HEPES, at a pH of 7.4. The pipette solution for whole-cell recordings contained: 140 mM KCl, 5 mM NaCl, 50 mM free Ca²⁺ (after titration with 2 mM EGTA), 1 mM MgCl₂, 2 mM K₂-ATP, and 10 mM HEPES, at a pH of 7.2. For cell-attached recordings, NaCl bath solution without or with 100 μM quinine (a K⁺ channel blocker) was used for filling patch pipettes.

RESULTS

There Is No Spontaneously Active Na⁺-permeable Channel Activity in Human B Lymphocytes—Previous studies from whole-cell experiments suggest that human B lymphocytes, especially from patients with Liddle’s syndrome, appear to express an amiloride-sensitive, sodium-permeable conductance (17, 18). However, the amiloride-sensitive whole-cell current exhibited substantial fluctuations and was observed only when the cell membrane was strongly hyperpolarized. However, in single-channel recordings, there has been no evidence for channels consistent with these properties; therefore, the exact nature of the amiloride-sensitive, rapidly fluctuating whole-cell currents observed in human B lymphocytes remains unclear. The apparent increase in whole-cell current in Liddle’s syndrome and the amiloride sensitivity of the current have suggested to some investigators that the lymphocyte current might be related to epithelial sodium channels (ENaC); however, the observation that the biophysical characteristics are so different from the properties of ENaC in epithelial cells or when ENaC subunits are expressed in oocytes has cast doubt on this hypothesis.

In the present study, both whole-cell and single-channel recordings were performed in human B lymphocytes to detect any possible ENaC activity. Consistent with our previous report (19), no outward K⁺ currents (but not any inward currents) were recorded as shown by a representative whole-cell recording from a total of 118 experiments (Fig. 1A). Although flickery inward currents were occasionally observed when the cell membrane was hyperpolarized to −140 mV or below, the flickery inward currents were never observed at any membrane potential above −140 mV. Consistent with the results from whole-cell experiments, single-channel recordings also demonstrated that no inward channel events were observed in 98 of 106 cell-attached patches. A representative cell-attached recording is shown in Fig. 1B. Only eight cell-attached patches had inward single-channel events. However, the channel events appeared only immediately after patch formation and disappeared within 30 s. Although the patch membrane was stretched when forming a patch, we hypothesized that these channel events, which were observed in less than 10% of cell-attached patches, might be activated by stretch.

Membrane Stretch Activates a 21-pS Nonselective Cation Channel—To test the above hypothesis that human B lymphocytes express a stretch-activated channel, a negative pressure was applied to the patch pipette in the cell-attached mode, which was usually established immediately after releasing the gentle positive pressure (1–2 cm H₂O) applied to the patch pipette before the pipette was lowered into the bath. In some cases, a very gentle negative pressure (<2 cm H₂O) was required to form a good seal, which was immediately released after forming the cell-attached mode, and these patches were allowed a few minutes for the patch membrane to recover from the stretch that was applied to form the seal. We found that
There is no spontaneous channel activity under resting conditions in human B lymphocytes. A, a representative whole-cell current trace induced by a voltage-ramp protocol in which the bath solution contained either 145 mM Na\(^+\) (upper trace) or 145 mM Na\(^+\) plus 100 \(\mu\)M quinine for eliminating K\(^+\) currents (lower trace). The pipette solution contained 144 mM K\(^+\), B, single-channel recordings under applied pipette potentials in a representative cell-attached patch. The bath solution contained 145 mM Na\(^+\), whereas the pipette solution contained 145 mM Na\(^+\) and 100 \(\mu\)M quinine (for eliminating K\(^+\) channel activity).

Membrane stretch activates a 20-pS nonselective cation channel. A, representative single-channel recordings in cell-attached patches in which patch pipettes contained 145 mM NaCl (upper trace), 145 mM NMDG-Cl (middle trace), or 145 mM KCl (lower trace), respectively. B, I-V relationship where patch pipettes contained 145 mM NaCl (open circles), 145 mM NMDG-Cl (open squares), or 145 mM KCl (open triangles), respectively. C, blockade of the stretch-activated channel activity by 10 \(\mu\)M amiloride in the patch pipette. Solid bars indicate the state in which channels are closed. Downward channel events represent the channel-open state, indicating Na\(^+\) influx. Solid bars show the period of negative pressure applied to the patch pipette. The single-channel current traces shown in A and C (also in the other figures, excluding some especially indicated) were recorded at the resting membrane potential (\(-V_{\text{pipette}} = 0\) mV).

A Cation Channel Formed by \(\alpha\)-ENaC in Human B Lymphocytes

Fig. 1. Membrane stretch activates a 20-pS nonselective cation channel. A, representative single-channel recordings in cell-attached patches in which patch pipettes contained 145 mM NaCl (upper trace), 145 mM NMDG-Cl (middle trace), or 145 mM KCl (lower trace), respectively. B, I-V relationship where patch pipettes contained 145 mM NaCl (open circles), 145 mM NMDG-Cl (open squares), or 145 mM KCl (open triangles), respectively. C, blockade of the stretch-activated channel activity by 10 \(\mu\)M amiloride in the patch pipette. Solid bars indicate the state in which channels are closed. Downward channel events represent the channel-open state, indicating Na\(^+\) influx. Solid bars show the period of negative pressure applied to the patch pipette. The single-channel current traces shown in A and C (also in the other figures, excluding some especially indicated) were recorded at the resting membrane potential (\(-V_{\text{pipette}} = 0\) mV).

Fig. 2. Membrane stretch activates a 20-pS nonselective cation channel. A, representative single-channel recordings in cell-attached patches in which patch pipettes contained 145 mM NaCl (upper trace), 145 mM NMDG-Cl (middle trace), or 145 mM KCl (lower trace), respectively. B, I-V relationship where patch pipettes contained 145 mM NaCl (open circles), 145 mM NMDG-Cl (open squares), or 145 mM KCl (open triangles), respectively. C, blockade of the stretch-activated channel activity by 10 \(\mu\)M amiloride in the patch pipette. Solid bars indicate the state in which channels are closed. Downward channel events represent the channel-open state, indicating Na\(^+\) influx. Solid bars show the period of negative pressure applied to the patch pipette. The single-channel current traces shown in A and C (also in the other figures, excluding some especially indicated) were recorded at the resting membrane potential (\(-V_{\text{pipette}} = 0\) mV).

Application of negative pressure to the patch pipette induced significant inward channel events in cell-attached patches when the patch pipette contained 145 mM Na\(^+\). To determine ion selectivity, ion substitution experiments were performed. The replacement of Na\(^+\) with NMDG\(^+\) in the pipette eliminated the channel events, indicating that the channel is a cation channel (Fig. 2A). However, the channel events remained when Na\(^+\) in the patch pipette was replaced with K\(^+\), and the replacement did not shift the reversal potential, suggesting that this cation channel is nonselective for Na\(^+\) over K\(^+\) (\(P_{\text{Na}}/P_{\text{K}} = 1/1\)). Similar to stretch-activated channels observed in other tissues, the channel had a linear I-V relationship with a unitary conductance of ~21 pS (Fig. 2B). However, the channel activity was blocked with 10 \(\mu\)M amiloride (Fig. 2C).

Human B Lymphocytes Express the Messages for the \(\alpha\) and \(\beta\) Subunits of ENaC—Previous studies have demonstrated that expression of exogenous \(\alpha\)-ENaC results in a stretch-activated cation channel (9). Nonetheless, we hypothesized that \(\alpha\)-ENaC may be involved in forming the stretch-activated cation channel observed in human B lymphocytes. To test whether human B lymphocytes express \(\alpha\), \(\beta\), and \(\gamma\)-ENaC subunits, RT-PCR experiments were performed by using subunit-specific primers, which amplify ENaC subunits from human kidney. Similar to observation by others (17), the data demonstrated that \(\alpha\) (346 bp) and \(\beta\) (520 bp), but not \(\gamma\) (624 bp), were amplified in human B lymphocytes (Fig. 3A), indicating that messages for \(\alpha\)- and \(\beta\)-ENaC subunits was present in human B lymphocytes. To further confirm the results from RT-PCR experiments, Northern blotting experiments were performed using subunit-specific probes for ENaC subunits. Consistent with the results from RT-PCR experiments, the data demonstrated that \(\alpha\)- and \(\beta\)-ENaC subunits were detected as multiple bands, but \(\gamma\) is undetectable (Fig. 3B).

Human B Lymphocytes Express \(\alpha\)- and \(\beta\)-ENaC Subunit Protein, and Antisense Oligonucleotides Can Knock Down the Expression of the Subunits—Fig. 4 shows that lymphocytes contain detectable amounts of \(\alpha\)- and \(\beta\)-ENaC subunit protein. There is, however, no detectable \(\gamma\)-ENaC. Fig. 4 also shows that
Antisense Oligonucleotide against \( \alpha \)-ENaC Eliminates the Stretch-activated Cation Channel —In lung alveolar type II cells, 21-pS nonselective cation channels are formed from \( \alpha \) subunit protein alone or in combination with some subunit other than \( \beta \) and \( \gamma \) (13, 14). To further determine whether the 21-pS stretch-activated cation channel in lymphocytes is formed by \( \alpha \)-ENaC alone or \( \alpha \)-ENaC in combination with \( \beta \)-ENaC, human B lymphocytes were treated with antisense or sense oligonucleotides to either \( \alpha \)- or \( \beta \)-ENaC sequences (see “Materials and Methods”). The experiments were performed in three sets; each set contained five groups in which the cells were under control conditions or treated with antisense or sense oligonucleotides to either \( \alpha \)- or \( \beta \)-ENaC, respectively. Under control conditions, the 21-pS stretch-activated channel was recorded in ~75% of cell-attached patches (\( n = 23 \)). After treatment with antisense oligonucleotide to \( \alpha \)-ENaC, only 33% of cell-attached patches (\( n = 2 \)) contained the stretch-activated channel. In contrast, 69% of cell-attached patches (\( n = 13 \)) contained the stretch-activated channel when the cells were treated with sense oligonucleotide to \( \alpha \)-ENaC. The cation selectivity of the 21-pS channel in these experiments was also determined by ion substitution experiments as described in the legend to Fig. 2, showing that \( P_{\text{Na}}/P_{\text{K}} \) is 1/1. Compared with either control experiments or sense treatment, antisense oligonucleotide to \( \alpha \)-ENaC significantly reduced the frequency of observance of the stretch-activated channel (\( p < 0.01 \)). In other words, the channel density in the cell membrane was decreased after treatment with antisense oligonucleotide to \( \alpha \)-ENaC, indicating that \( \alpha \)-ENaC is necessary to form the stretch-activated channel. However, the frequency of observance of the stretch-activated channel was unaffected by treatments with either antisense (\( n = 16 \)) or sense oligonucleotide (\( n = 14 \)) to \( \beta \)-ENaC. Representative cell-attached recordings from each group experiment are shown in Fig. 5A. The summary percentages of each group from three sets of experiments are also shown in Fig. 5B. These data together suggest that \( \alpha \)-ENaC (but not \( \beta \)-ENaC) is necessary to form the 21-pS stretch-activated cation channel in human B lymphocytes.

Aldosterone Activates a 10-pS Sodium-permeable Channel, Which Can Be Blocked by Antisense Oligonucleotides to Both \( \alpha \)- and \( \beta \)-ENaC —The above experiments suggest that \( \alpha \)-ENaC (but not \( \beta \)-ENaC) is necessary to form the 21-pS stretch-activated cation channel in human B lymphocytes despite the fact that RT-PCR and Northern blotting experiments suggest that \( \beta \)-ENaC message is present in human B lymphocytes. However, in epithelial cells, \( \beta \)-ENaC appears to act as an auxiliary subunit to regulate ENaC activity and trafficking to the plasma membrane (20–22). Although the data from other investigators have shown that aldosterone has no chronic effect on the expression of \( \beta \)-ENaC in rat kidney (22, 23), our results have shown that aldosterone stimulates expression of \( \beta \)-ENaC in A6 cells (24). Therefore, it is possible that the \( \alpha \)-ENaC channel in human B lymphocytes is regulated by aldosterone via expression of the \( \beta \) subunit. Human B lymphocytes were treated with 1.5 \( \mu \)M aldosterone for 6 h before performing cell-attached recordings. As we described in Figs. 1 and 2, under control conditions in the absence of aldosterone, there was no apparent spontaneous 21-pS \( \alpha \)-ENaC-associated channel activity in Daudi B cells, even though nonselective channel activity was observed after application of stretch in 17 of 23 patches (74%). Interestingly, in 20 patches on aldosterone-treated lymphocytes, the stretch-activated 21-pS \( \alpha \)-ENaC channel was not observed; however, in 12 of the 20 patches (60%), aldosterone induced a 10-pS channel that spontaneously opened and closed without requiring stretch activation. This channel activity was also blocked by 5 \( \mu \)M amiloride. Therefore, we hypothesized that this 10-pS channel might be related to the stretch-activated \( \alpha \)-ENaC channel. To test this hypothesis, human B lymphocytes were treated with antisense and sense oligonucleotides to \( \alpha \)-ENaC to determine whether the treatments affect
the aldosterone-induced channel activity. Under control conditions, the 10-pS channel induced by aldosterone was observed in ~60% of cell-attached patches (n = 20). Treatment with antisense oligonucleotide to α-ENaC significantly reduced the frequency of observance of the 10-pS channel from 50% (sense control, n = 15) to 14% (antisense, n = 15) (p < 0.05), indicating that α-ENaC is not only necessary to form the stretch-activated 21-pS α-ENaC channel but is also necessary to form the aldosterone-induced 10-pS channel. Interestingly, the frequency of observance of the 10-pS channel in cell-attached patches was also significantly reduced after treatment with antisense oligonucleotide to β-ENaC from 55% (sense control, n = 13) to 20% (antisense, n = 20), suggesting that β-ENaC is also necessary to form the aldosterone-induced, 10-pS channel.

Representative cell-attached recordings of each type of experiment are shown in Fig. 6A. Fig. 6B shows that the 10-pS channel had a linear I-V relationship. To determine cation selectivity of the 10-pS channel, ion substitution experiments were performed. The replacement of Na+ with NMDG+ in the pipette eliminated the channel events, indicating that the channel is a cation channel. The replacement of Na+ with K+ in the pipette shifted the reversal potential from 61 mV to 34 mV. Using the Goldman-Hodgkin-Katz equation, the calculated sodium-to-potassium permeability ratio PNa/PK was 3.2/1. After treatment with antisense oligonucleotides to β-ENaC, the frequency of observance of the channels was reduced; however, the selectivity and conductance of the channels that remained were unaffected; i.e. they were still 10 pS with a selectivity of about 3 to 1. This implied to us that even though α-ENaC protein was still present, the cells did not express at measurable levels the 21-pS α-only channels (although we could not rule out the possibility of low level expression or the possibility that we would have to stretch the membranes to observe the channel). This selectivity is similar to that for a 10-pS amiloride-sensitive channel originally described in a renal cell line, A6 (25). The summary percentages of each group from three sets of experiments are shown in Fig. 6C.

Aldosterone Induces a 5-pS Amiloride-sensitive Sodium Channel after Expression of Exogenous γ-ENaC—Epithelial cells that express all three ENaC subunits, α, β, and γ, usually express a 5-pS highly selective amiloride-sensitive channel. In contrast, B lymphocytes that do not normally express the γ subunit, as one might expect, do not express such channels. However, because lymphocytes express the α subunit and, in the presence of aldosterone, the β subunit, we thought that if we expressed the exogenous γ subunit in lymphocytes, we might observe the 5-pS αβγ-ENaC channel. Therefore, we transfected human B lymphocytes with the γ subunit of rat ENaC. Unfortunately, expression of exogenous γ-ENaC in the absence of aldosterone failed to induce any spontaneous ENaC-like channel activity. However, in the presence of 1.5 μM aldosterone, exogenous γ-ENaC induced spontaneous inward single-channel events (Fig. 7A). The I-V relationship inwardly rectified and had a unitary conductance of 5 pS when the cell membrane was hyperpolarized (Fig. 7B). To determine ion selectivity, ion substitution experiments were performed. The replacement of Na+ with NMDG+ in the pipette eliminated the channel events, indicating that the channel is a cation channel. The replacement of Na+ with K+ in the pipette shifted the reversal potential to the left. This shift implies that the 5-pS channel is selective for Na+ and K+. The mean open time was reduced by 1 μM amiloride (Fig. 7C) from 512 ± 126 ms to 76 ± 14 ms (p < 0.01) (Fig. 7D).

DISCUSSION

It has been known for a number of years that under whole-cell recording conditions, human B lymphocytes have an amiloride-sensitive Na+ current (15–18). However, the molecular basis for the current remains controversial because the whole-cell currents rapidly fluctuate in a manner unlike ENaC currents in other cells, and they are detectable only when the lymphocyte membrane is strongly hyperpolarized. To address this issue, in the present study we performed both whole-cell and cell-attached experiments. In whole-cell recordings, as we expected, we did not observe any inward currents in cells that were not strongly hyperpolarized (Fig. 1A). We did observe inward currents when the cells were repeatedly stimulated with a hyperpolarizing stimulus of ~140 mV or below (data not shown). Similar to the current observed by others (18), this hyperpolarization-associated current contains large rapidly fluctuating currents. Therefore, it is likely that the open state of the “cryptic” Na+-permeable channel in human B lymphocytes is not very stable but is activated by hyperpolarization. Although it can also be activated by physical stretch, the ratio of “cytoplasmic” (patch pipette) osmolarity versus “extracellu-
lar" (bath) osmolarity may affect the channel as well and may explain the discrepancy between our work and some previous reports.

Consistent with our whole-cell data, single-channel recordings showed that there was no spontaneous Na\(^+\)/H\(^+\)-permeable channel activity in cell-attached patches (Fig. 1B). Although a nonselective cation channel was observed after stretching the patch membrane, the channel was equally permeable to Na\(^+\)/H\(^+\) or K\(^+\)/H\(^+\) with a unitary conductance of 21 pS (Fig. 2). Despite the fact that a nonselective cation channel rather than the more typical highly selective 5-pS ENaC channel was observed, we speculated that one or more ENaC subunits might form the molecular basis for the nonselective cation channel. Consistent with another report (17), our results from RT-PCR, Northern blotting, and Western blotting experiments suggest that human B lymphocytes do contain the messages and protein for \(\alpha\)- and \(\beta\)-ENaC subunits (Fig. 3 and 4). Using antisense oligonucleotide methods, we found that only \(\alpha\)-ENaC but not \(\beta\)-ENaC is necessary to form the stretch-activated 21-pS Na\(^+\)/H\(^+\)-permeable channel (Fig. 5). Further investigation will be required to determine why the \(\beta\)-ENaC subunit is not involved. Recent studies have demonstrated that ENaC is regulated by Nedd4, a ubiquitin protein ligase (26–30). In A6 cells, a proteosome inhibitor strongly increases the cellular amount of \(\beta\)-ENaC but
only causes a slight increase in α-ENaC protein (31). These results suggest the possibility that degradation of the plasma membrane β-ENaC might be much faster than that of α-ENaC in human B lymphocytes; thus, leading to a situation in which, although there is message and protein for both α- and β-ENaC, only α-ENaC is present in the membrane at a high enough concentration to be the predominant channel-forming subunit. More experiments will be required to show how β-ENaC is regulated and trafficked in human B lymphocytes.

One interesting finding of the present study was that aldosterone can induce 10-pS channels in human B lymphocytes. Unlike the 21-pS, presumably an α-only channel present in the absence of aldosterone, the aldosterone-induced channel spontaneously opens and closes at the resting membrane potential in cell-attached patches and was blocked by antisysteol ouabainloctides to either α- or β-ENaC (Fig. 6). These results suggest that the aldosterone-induced channel consists of, at least, α and β subunits. Another steroid, dexamethasone, is known to increase expression of β- and γ-ENaC protein levels in lung epithelial cells (32). Our earlier studies also suggest that aldosterone increases the mRNA level of β-ENaC and the protein level of γ-ENaC in A6 distal nephron cells (24). The concentration of aldosterone we used was high enough that it could have activated both mineralocorticoid and glucocorticoid receptors. Regardless of the steroid receptor, we suggest that steroids like aldosterone may promote loss of the stretch-activated 21-pS α-ENaC channel and appearance of the 10-pS αβ-ENaC channel by increasing the membrane expression of β-ENaC protein.

Our speculations about the nature of the channels were confirmed by the expression of exogenous γ-ENaC, which led to the formation of a “traditional” ENaC channel, at least in the presence of aldosterone. Interestingly, in the absence of aldosterone, only a 21-pS stretch-activated channel composed of α-ENaC alone was observed regardless of whether γ-ENaC was expressed.

In conclusion, the single-channel properties and molecular structure of an amiloride-sensitive channel in human B lymphocytes are different from those of amiloride-sensitive channels in many epithelial cells. First, the channel is not spontaneously active. Second, it can be directly activated by membrane stretch unlike ENaC, which requires special conditions for stretch activation (33). Third, it has distinct biological characteristics: a 21-pS unit conductance rather than membrane stretch unlike ENaC, which requires special conditions for stretch activation (34). Finally, it has diagnostic significance for maintaining lymphocytes in an inactive state. When lymphocytes are exposed to hypertonicity (to induce stretch) or steroid hormones, the channels can be activated. This characteristic may have pathophysiological significance for activating lymphocytes in either inflammatory or immunosuppressive states. Alternatively, steroids are often elevated (naturally or pharmacologically) under states when lymphocytes are activated, suggesting the possibility that the transition to the alternative channel presumably formed from α and β subunits of ENaC may be important in this process.

Originally, amiloride-sensitive channels in lymphocytes were examined as a possible indicator of excessive ENaC activity that might be predictive of abnormalities in total body sodium balance and hypertension (15–19). With the failure to easily observe amiloride-sensitive channels at a single-channel level in lymphocytes, some of the impetus for this approach was lost. However, the current work demonstrates the conditions under which channels associated with at least the α and β subunits of ENaC can be observed in lymphocytes. Therefore, the possibility of using lymphocytes as a diagnostic tool to examine functional mutations in α or β subunits that could lead to hypertension can be reconsidered.

REFERENCES

1. Canessa, C. M., Schild, L., Buell, G., Thoresen, B., Gauthier, I., Horisberger, J. D., and Rossier, B. C. (1994) Nature 367, 463–467
2. Pyke, G. K., Quinn, A., and Canessa, C. M. (1998) Biochem. Pharmacol. 56, 138–151
3. Horisberger, J. D. (1998) Clin. Sci. 95, 445–449
4. Linguegella, E., Voiley, N., Waldmann, R., Ladunzinski, M., and Barthy, P. (1993) FEBS Lett. 318, 35–99
5. Pyke, G. K., and Canessa, C. M. (1998) J. Gen. Physiol. 112, 423–432
6. Firsov, D., Gauthier, I., Merillat, A. M., Rossier, B. C., and Schild, L. (1998) EMBO J. 17, 344–352
7. McDonald, F. J., Price, M. P., Snyder, P. M., and Welsh, M. J. (1995) Am. J. Physiol. 268, C1157–C1163
8. McDonald, F. J., Snyder, P. M., McCray, P. B., Jr., and Welsh, M. J. (1994) Am. J. Physiol. 266, L724–L734
9. Kizer, N., Guo, X. L., and Hruska, K. (1997) Prog. Natl. Acad. Sci. U. S. A. 94, 1013–1018
10. Jain, L., Chen, J. X., Brown, L. A., and Eaton, D. C. (1998) Am. J. Physiol. 274, L475–L484
11. Marunaka, Y., Niisato, N., O’Broovich, H., and Eaton, D. C. (1999) J. Physiol. (Lond.) 515, 669–683
12. Yue, G., Russell, W. J., Benos, D. J., Jackson, R. M., Olman, M. A., and Matalon, S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8418–8422
13. Jain, L., Chen, J. X., Malik, B., Al-Khalili, O., and Eaton, D. C. (1999) Am. J. Physiol. 276, L1046–L1051
14. Jain, L., Chen, J. X., Ramosevae, S., Brown, L. A., and Eaton, D. C. (2001) Am. J. Physiol. 280, L646–L658
15. Achard, J. M., Buhen, J. K., Benos, D. J., and Warnock, D. G. (1996) Am. J. Physiol. 270, C224–C234
16. Bradford, A. L., Ismailov, I., Achard, J. M., Warnock, D. G., Buhen, J. K., and Benos, D. J. (1995) Am. J. Physiol. 269, C601–C611
17. Buhen, J. K., Watson, B., Khan, M. A., Langlois, A. L., Fuller, C. M., Berdiev, B., Tousson, A., and Benos, D. J. (2001) J. Biol. Chem. 276, 8557–8566
18. Buhen, J. K., and Warnock, D. G. (1995) Am. J. Physiol. 269, C1157–C1183
19. Zhou, Z. H., Unalp, M. T., Li, L., and Ma, H. (2000) J. Membr. Biol. 188, 97–105
20. McDonald, F. J., Yang, B., Hrstka, R. F., Drummond, H. A., Tarr, D. E., McCray, P. B., Jr., Stokes, J. B., Welsh, M. J., and Williamson, R. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1727–1731
21. Cui, Y., Su, Y. R., Rutkowski, M., Reif, M., Menon, A. G., and Pun, R. Y. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9962–9966
22. Awaysa, M. S., Tousson, A., and Benos, D. J. (1997) Am. J. Physiol. 273, C1889–C1899
23. Maslaliman, S., Kim, G. H., Mitchell, C., Wade, J. B., and Knepper, M. A. (1999) J. Clin. Investig. 104, 19–23
24. Malik, B., Schlenger, L., Al-Khalili, O., Yue, G., and Eaton, D. C. (2002) FASEB J. 16, A477 (abstr.)
25. Hamilton, K. L., and Eaton, D. C. (1986) Membr. Biochem. 6, 149–171
26. Debonville, C., Flores, S. Y., Kamynina, E., Plant, P. J., Taine, C., Thomas, M. A., Munster, C., Chrabai, A., Pratt, J. H., Horisberger, J. D., Pearce, D., Loffing, J., and Staub, O. (2001) EMBO J. 20, 7052–7059
27. Kamynina, E., Taine, C., and Staub, O. (2001) Am. J. Physiol. 281, F469–F477
28. Kamynina, E., Debonville, C., Beno, M., Vandewalle, A., and Staub, O. (2001) FASEB J. 15, 294–211
29. Staub, O., Dho, S., Henry, F., Correa, J., Ishikawa, T., McClade, G., and Rotin, D. (1996) EMBO J. 15, 2371–2380
30. Staub, O., Breiel, H., Plant, P., Ishikawa, T., Kanels, V., Saleki, R., Horisberger, J. D., Schild, L., and Rotin, D. (2000) Kidney Int. 57, 809–815
31. Malik, B., Schlenger, L., Al-Khalili, O., Bao, H. F., Yue, G., Price, S. R., Mitch, W. E., and Eaton, D. C. (2001) J. Biol. Chem. 276, 12903–12910
32. Lazarak, A., Samanta, A., Venetsanou, K., Barphy, P., and Matalon, S. (2000) J. Membr. Biol. 179, 275–2770
33. Ma, H. P., Lu, L., Zhou, Z. H., Eaton, D. C., and Warnock, D. G. (2002) Am. J. Physiol. 282, F501–F505

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