We have recently demonstrated that the amiloride-sensitive Na\(^+\) channel in the apical membrane of the renal epithelial cell line, A6, is modulated by the \(\alpha_{1.3}\) subunit of the G\(_4\) protein. We also showed that a 700-kDa protein complex can be purified from the membranes of A6 epithelia which (a) can reconstitute the amiloride-sensitive Na\(^+\) influx in isoposomes and planar bilayer membranes and (b) consists of six major protein bands observed on reducing sodium dodecyl sulfate-polyacrylamide gels with molecular masses ranging from 35 to 320 kDa. The present study was undertaken to determine if the \(\alpha_{1.3}\) subunit is a member of this Na\(^+\) channel complex. G\(_4\) structure and function were identified by Western blotting with specific G\(_1\)-3 subunit antibodies and Na\(^+\) channel antibodies, through ADP-ribosylation with pertussis toxin, and by immunocytochemical localization of the Na\(^+\) channel and G\(_1\)-3 proteins. We demonstrate that two protein substrates are ADP-ribosylated in the 700-kDa complex in the presence of pertussis toxin and are specifically immunoprecipitated with an anti-Na\(^+\) channel polyclonal antibody. One of these substrates, a 41-kDa protein, was identified as the \(\alpha_{1.3}\) subunit of the G\(_4\) protein on Western blots with specific antibodies. Na\(^+\) channel antibodies do not recognize G\(_1\)-3 on Western blots of Golgi membranes which contain \(\alpha_{1.3}\) but not Na\(^+\) channel proteins, nor do they immunoprecipitate from solubilized Golgi membranes; however, \(\alpha_{1.3}\) is coprecipitated as part of the Na\(^+\) channel complex from A6 cell membranes by polyclonal Na\(^+\) channel antibodies. Both \(\alpha_{1.3}\) and the Na\(^+\) channel have been localized in A6 cells by confocal imaging and immunofluorescence with specific antibodies and are found to be in distinct but adjacent domains of the apical cell surface. In functional studies, \(\alpha_{1.3}\), but not \(\alpha_{2.1}\), stimulates Na\(^+\) channel activity. These data are therefore consistent with the localisation of Na\(^+\) channel activity and modulatory \(\alpha_{1.3}\) protein at the apical plasma membrane, which together represent a specific signal transduction pathway for ion channel regulation.

The regulation of Na\(^+\) transport in renal epithelia is in large part a function of the modulation of Na\(^+\) channel activity in the apical membrane. In a number of epithelia, including cultured toad kidney A6 cells, Na\(^+\) channel activity has been shown to be influenced by classical hormone signal transduction pathways at the basolateral membrane involving biochemical signaling through protein kinase activity (1–5). Recent patch-clamp data from our laboratory have demonstrated that the Na\(^+\) channel is activated in excised, pertussis toxin-treated apical membrane patches from A6 cells by addition of the \(\alpha\) subunit of the G\(_3\) protein (6). These studies were further supported by the observation that guanine nucleotides regulate the apical Na\(^+\) channel, thus providing functional evidence for the existence of an endogenous G\(_3\) protein/Na\(^+\) channel complex localized in the apical membrane of the A6 epithelia. This G\(_3\) protein is topographically distinct from receptor-coupled Gs and G\(_2\) proteins traditionally localized in the basolateral membrane which are responsible for the generation of second messengers (7). G\(_1\)-3 is localized predominantly on the Golgi membranes in epithelial cells (7, 8). In this report we demonstrate that the \(\alpha_{1.3}\) subunit is also a structural component of the 700-kDa Na\(^+\) channel complex in the apical membrane of A6 epithelial cells. We show that the Na\(^+\) channel complex contains the \(\alpha_{1.3}\) 41-kDa protein, which is identified using specific \(\alpha_{1.3}\) peptide antibodies and is a substrate for ADP-ribosylation by pertussis toxin. The \(\alpha_{1.3}\) subunit was localized by confocal imaging and was found to be concentrated in a domain in the apical membrane adjacent to the Na\(^+\) channel in A6 cells. The functional significance of this localization is supported by the demonstration that \(\alpha_{1.3}\), but not \(\alpha_{3.2}\), regulates Na\(^+\) channel activity as assessed by the patch-clamp technique. These data indicate that the Na\(^+\) channel complex contains the \(\alpha_{1.3}\) regulatory protein, which is functionally linked to apical Na\(^+\) channel regulation.

**Experimental Procedures**

**Na\(^+\) Channel Purification**—The procedures for the isolation and purification of the amiloride-sensitive Na\(^+\) channel from amphibian A6 cultured cells are detailed in Sariban-Sohraby and Benos (9) and Benos et al. (10) and were used here without modification.

**Cell Culture**—Clonal A6 cells (ATCC no. CCL102) derived from Xenopus laevis renal tubular cells were used between passages 20–36. The procedures for growing the A6 cells on permeable supports (Nucleopore or Millipore HAWP, 0.45-μm filters) are detailed in earlier reports (6, 10). Cells utilized for patch-clamp studies and

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\1 The abbreviations used are: G protein, signal-transducing, nucleotide-binding protein of subunit structure \(\alpha_{i,j,k}\); G\(_4\), or \(\alpha_{1.3}\), the \(\alpha\) subunit family of pertussis toxin-sensitive G proteins responsible for inhibition of adenyl cyclase and modulation of ion channels; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GTP\(^+\)-S, guanosine 5′(3′-O-thio)triphosphate; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; HPLC, high performance liquid chromatography.
immunofluorescence were cultured on glass coverslips in tissue culture medium as previously described (6, 7). Briefly, cells were grown in a Coon's modification of Ham's F-12 and Liebovitz's F-15 media modified to contain 105 mM NACL and 25 mM NaHCO3. The mixture was supplemented with 10% fetal bovine serum (GIBCO). Cells were maintained in atmosphere of 95% air and 5% CO2.

Antibodies—Polyclonal antisera raised against a highly purified preparation of sodium channel from bovine renal papilla were used for immunoprecipitation, Western blotting, and immunolocalization of the Na+ channel (11, 12). Antisera from different rabbits show specificity for the epitelial Na+ channel as assayed by enzyme-linked immunoabsorbent assay, immunoblots, Western blots, and by immunoprecipitation of iodinated channel proteins from a heterogeneous mixture of solubilized kidney membrane proteins. All antisera give the same staining pattern for the Na+ channel by immunofluorescence (see Fig. 1A). Antisera from different rabbits all immunoprecipitate the complex of proteins described previously (~300 to ~30 kDa) (11) (Fig. 4A), but each antisera recognizes only two or three of these proteins by Western blotting. Thus, the antisera used in the present study recognizes only proteins at 320, 150, and 95 kDa by Western blotting (see Fig. 4B).

Polyclonal rabbit antibodies raised against peptides of α1 subunits were the kind gift of Dr. A. Spiegel (National Institutes of Health). An affinity-purified antibody (EC-2), which was raised against a carboxyl-terminal decapeptide of α1, was used to specifically identify α1 on Western blots (13) and for immunolocalization of α1 in LLC-PK1 cells and other cells, as previously described (7, 8).

Patch-Clamp Measurement of Na+ Channel Activity—The patch clamp technique was carried out as we have previously described (6). Data were obtained solely from excised inside-out patches. Holding potentials were between +40 and +80 mV. Pipette-patch solution was (in mM): 115 NaCl, 5 KCl, 0.8 MgSO4, 1.2 CaCl2, and 10 Hepes, pH 7.4. The perfusion solution was a modification of the patch pipette solution where Na+ was replaced in equimolar concentration with K+.

RESULTS

Effect of Amiloride and Pertussis Toxin on Na+ Uptake into A6 Confluent Cells—The uptake of 22Na+ was used to assess the effect of pertussis toxin on amiloride-sensitive Na+ channel activity in confluent monolayers of A6 cells. Utilizing techniques previously developed in our laboratory (5), we observed that the apical membrane obtained from A6 cells grown on filters was solubilized (9), followed by pertussis toxin-induced ADP-ribosylation, which was carried out as previously described (5) in a buffer containing 10 mM Tris base (pH 7.5), 1 mM EDTA, 5% β-mercaptoethanol, 1 mM ATP, and 0.91 mM GTP. The reaction mixture was incubated at 37 °C for 60 min. The reaction was terminated by the addition of 100 mM NaCl and 1 mM NaADP to a final concentration of 10 μM. The solubilized A6 Na+ channel protein complex was incubated for 45 min at 32 °C. The samples were spun for 2 h at 6000 rpm followed by boiling for 3 min in sodium dodecyl sulfate and electrophoresis. Isolated rat liver Golgi membranes were incubated with pertussis toxin for ADP-ribosylation of the Goα3 subunit as described previously (8). Gels were dried and exposed to Kodak X-Omat AR film at ~80 °C for appropriate lengths of time (12 h to 3 days).

Immunofluorescence Localization—Cultures of A6 renal epithelial cells were fixed in 4% paraformaldehyde in PBS for 1 h at 20 °C or 30 min at 4 °C. The cells were then washed in several changes of PBS, permeabilized by exposure to 0.1% Triton X-100 in PBS, and then rinsed in blocking buffer (PBS with 0.5% bovine serum albumin). The cells were incubated in diluted antiserum or control serum for 2 h at 20 °C, washed in several changes of blocking buffer and then incubated with FITC-conjugated goat anti-rabbit IgG for 1 h. Cells on coverslips or on filters were mounted in PBS/glycerol with 1% N-propyl gallate and viewed by conventional epifluorescence or by confocal microscopy on a Zeiss microscope fitted with a Bio-Rad 600 confocal laser imaging system.


**TABLE I**

<table>
<thead>
<tr>
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<th>Minus PTX</th>
<th>Plus PTX</th>
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<tbody>
<tr>
<td>20 nM Na⁺ influx (J\textsubscript{Na⁺})</td>
<td>889 ± 190</td>
<td>501 ± 55\textsuperscript{a}</td>
</tr>
<tr>
<td>Amiloride (10⁻⁶ M)</td>
<td>489 ± 59\textsuperscript{a}</td>
<td>489 ± 52\textsuperscript{a}</td>
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\( p < 0.025 \) versus control for \( n = 30 \) filters.

pertussis toxin completely inhibits amiloride-sensitive Na⁺ channel conductance (6). These studies in intact cells (Table I) and isolated patches (6) raise the possibility that a G\textsubscript{i} regulatory protein is intimately related to the apical membrane Na⁺ channel in A6 cells. The experiments described below were designed to address this question.

Localization of Na⁺ Channel and α\textsubscript{i,3} Subunit in Adjacent Apical Domains of A6 Cells—Conventional epifluorescence and confocal imaging were used to localize the Na⁺ channel on apical microvilli of the A6 cells in a similar distribution to that previously reported (18). Confocal imaging further showed that the Na⁺ channel staining was specifically limited to the protruding portions of the typical finger- or ridge-like microvilli on the apical cell surface (Fig. 1A) and there was no staining on the basal or lateral cell surfaces (data not shown). Immunofluorescence of A6 cells with an antibody specific for the α\textsubscript{i,3} subunit showed punctate staining of this protein throughout the cytoplasm and on membranes of the perinuclear Golgi complex, similar to the localization seen previously in LLC-PK\textsubscript{1} renal epithelial cells and NRK cells (7, 8). In contrast to LLC-PK\textsubscript{1}, and NRK cells, where it was most concentrated on Golgi membranes, α\textsubscript{i,3} was found to be most abundant at the apical pole of the A6 cells. Confocal imaging and comparison of pixel intensities at different levels through the A6 cell layer revealed that α\textsubscript{i,3} is most heavily concentrated in a 3-μm layer associated with the apical cell membrane, just at the base of the microvilli, although the staining did not extend into the projections themselves (Fig. 1, B and C). This localization confirms that the α\textsubscript{i,3} is present at the apical membrane of the A6 cells in a distinct, but adjacent, domain to the Na⁺ channel.

ADP-ribosylation of Solubilized Membrane Proteins from A6 Cells—ADP-ribosylation of solubilized A6 membrane proteins followed by electrophoresis demonstrated three major protein bands at 95, 66, and 41 kDa, respectively (Fig. 2, lane 1). The 66-kDa band is a frequent contaminant of such studies reflecting, at least in part, serum albumin used in toxin activation.\(^2\) Following immunoprecipitation with the Na⁺ channel antibody and electrophoresis, two ADP-ribosylated bands were seen: one at 95 kDa and the other at 41 kDa. Neither protein was immunoprecipitated with preimmune serum (data not shown).

Since amiloride is known to bind to at least one member of the Na⁺ channel complex (e.g. 130/150-kDa protein) (10) it was of interest to see if amiloride binding altered ADP-ribosylation of any of the Na⁺ channel proteins. As seen in Fig. 3, the presence of increasing amounts of amiloride, which functionally block Na⁺ channel activity, had no effect on the ADP-ribosylation of either the 95- or 41-kDa proteins.

Co-purification of α\textsubscript{i,3} in the Na⁺ Channel Complex—The HPLC-purified Na⁺ channel complex containing a number of proteins (Fig. 4A) was immunoblotted with a polyclonal Na⁺ channel antibody and with preimmune serum as a control (Fig. 4B). As previously described, polyclonal Na⁺ channel antibodies variably label only some of the proteins in the complex; this antiserum recognized proteins at 320, 150, and 95 kDa (Fig. 4B, lane 2). Transfers of the purified Na⁺ channel complex were probed with the G\textsubscript{i,3} antibody (Fig. 4C, lane 4), which showed that a 41-kDa protein, corresponding to G\textsubscript{i,3}, was part of this purified complex. In order to further demonstrate that α\textsubscript{i,3} is actually part of the Na⁺ channel complex, this polyclonal Na⁺ channel antibody was used to construct an immunoaffinity column for purification of Na⁺ channel complexes from detergent-solubilized A6 cell homogenates. Affinity-bound proteins, eluted from the Na⁺ channel column with low pH buffer, were electrophoresed and transferred for Western blotting with the G\textsubscript{i,3} antibody (Fig. 4C). The 41-kDa, α\textsubscript{i,3} protein was identified in the eluate from the affinity column (Fig. 4C, lane 6), while there was no protein detected with the control, preimmune serum (Fig. 4C, lane 5). These results suggest that G\textsubscript{i,3} is co-purified as part of a multi-protein Na⁺ channel complex.

In order to confirm that G\textsubscript{i,3}, itself is not recognized by the Na⁺ channel antibody, this antibody was used to Western blot an enriched source of G\textsubscript{i,3}. G\textsubscript{i,3} has previously been localized on Golgi membranes (7, 8). When Golgi membranes isolated from rat liver were used for Western blotting, the antibody to G\textsubscript{i,3} recognized a 41-kDa protein (Fig. 5A, lane 1), but there was no staining of this band, or any other protein band on Golgi membranes, with the Na⁺ channel antibody (Fig. 5A, lane 2). In addition, we confirmed that the Na⁺ channel antibody cannot immunoprecipitate G\textsubscript{i,3} from the Golgi membranes in the absence of other Na⁺ channel proteins. Golgi membranes were ADP-ribosylated with pertussis toxin to label the α\textsubscript{i,3} subunit (Fig. 5B, lane 3), and then solubilized Golgi membrane proteins were used for immunoprecipitation with Na⁺ channel or G\textsubscript{i,3} antibodies. The G\textsubscript{i,3} antibody precipitated some of the ADP-ribosylated α\textsubscript{i,3} subunit (Fig. 5B, lane 4), but the Na⁺ channel antibody was not able to immunoprecipitate the α\textsubscript{i,3} subunit (Fig. 5B, lane 5), providing further evidence that the Na⁺ channel antibody does not directly recognize G\textsubscript{i,3}.

Specificity of α\textsubscript{i,3} as a Functional Stimulator of Na⁺ Channel Activity—The apical membrane localization and co-immunoprecipitation of the α\textsubscript{i,3} subunit with the Na⁺ channel suggested a specific functional role for α\textsubscript{i,3} in Na⁺ channel regulation. Such a role was confirmed by the data presented in Table II. In the presence of pertussis toxin for 1 min there is a decrease in the percent open time of spontaneous Na⁺ channels, which is rapidly reversed by the addition of 20 pm α\textsubscript{i,3}, as we have previously shown (6). In contrast, the addition of a homologous α\textsubscript{i,1} subunit, α\textsubscript{i,2}, had no effect on the recovery of Na⁺ channel activity in the presence of pertussis toxin. As can be seen in Table II, Na⁺ channel activity continued to decay in the presence of pertussis toxin and α\textsubscript{i,2} over 5 min, consistent with our previous data showing that Na⁺ channel activity is near zero after 3 min of pertussis toxin exposure (6). The addition of another homologous G\textsubscript{i,4} subunit, α\textsubscript{i,4} also did not alter single Na⁺ channel conductance in the presence of pertussis toxin (data not shown).

**DISCUSSION**

The transepithelial transport of Na⁺ is a major function of renal epithelial cells. Renal Na⁺ channel activity is known to be under hormonal control (such as vasopressin or aldosterone), and classical second messenger signaling pathways in-

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\(^{2}\) D. A. Ausiello, J. L. Stow, H. F. Cantiello, J. B. de Almeida, and D. J. Benos, unpublished observations.
volving protein kinase A or protein kinase C have been shown to activate or inhibit the amiloride-sensitive Na" channel, respectively, in A6 cells and other renal epithelia (1). However, how these signals are responsible for Na" channel activity at the apical membrane is not known. Recently, utilizing the patch-clamp technique, we have defined the modulation of Na" channel activity in A6 cells by a pathway(s) involving endogenous pertussis toxin-sensitive G proteins (6) and phospholipids (19) located at the apical membrane which are topologically separated from second messenger pathways gen-

**C. Intensity of immunofluorescent staining in A6 cells.**

Fluorescence intensity was measured in a vertical plane through A6 cells in order to compare the amount of Gi-3 staining at different levels.
phosphorylation, although its function is as yet unknown (20). The 130-150-kDa protein binds amiloride, although it is not known if this subunit itself confers the conductive properties involved in Na⁺ channel activity (10). The other proteins in the Na⁺ channel complex are as yet undefined, although it is reasonable to speculate that they may be regulatory proteins forming a complex with the Na⁺ channel itself.

In the present study, immunocytochemistry, utilizing specific antibodies, localized the α3 subunit to a subapical membrane domain at the level of the base of the microvilli in A6 cells. In recent studies we have shown that Go₁₃ is most heavily concentrated on the Golgi membranes of other epithelial cells including LLC-PK₁ and NRK cells (7, 8) where it functions as a regulator of the secretory pathway. Consistent with this general finding, there is also Gα₃ staining on perinuclear Golgi membranes in A6 cells. However, the most intense staining of Gα₃ in the A6 cells is at the apical cell membrane, showing that in some cells, such as A6, Gα₃ is localized in more than one domain. This suggests that in certain cells Gα₃ may perform multiple functions. The subapical membrane domain containing Gα₃ in A6 cells is distinct from, but closely adjacent to, the Na⁺ channel-rich domains on the apical microvilli. Although this localization shows heavy concentrations of Gα₃ in the sub-apical domain, we cannot rule out the possibility that undetectable amounts of Gα₃ in the microvilli themselves are responsible for regulating the Na⁺ channel. However, the localization of Na⁺ channels and Gα₃ in adjacent domains could suggest that Gᵢ protein coupling to the Na⁺ channel may not be direct, but rather its stimulatory capacity is generated by further intermediary signal transducing pathways. These data are consistent with our recent observation that Gᵢ protein modulation of Na⁺ channel activity is the result of activation of phospholipase and lipoxygenase pathways generating phospholipids which are responsible for Na⁺ channel activity (19). Of interest is our recent observation that actin filament network organization is also a regulator of the Na⁺ channel (21). Taken together these data would support the conclusion that an ion channel regulatory complex consisting of a Gᵢ protein, phospholipase, lipoxygenase, and actin-binding proteins could be

![Fig. 2. Pertussis toxin induced ADP-ribosylation of Na⁺ channel complex. Autoradiograph of pertussis toxin induced ADP-ribosylation of solubilized A6 membranes (30 µg of protein) (lane 1) and proteins immunoprecipitated from solubilized membranes with Na⁺ channel antibody (lane 2). Three major protein bands are ribosylated in these membranes; the apparent molecular weights of these proteins are marked according to the molecular weight standards.](image1)

![Fig. 3. Effect of amiloride on ADP-ribosylation. Autoradiograph of pertussis toxin induced 32P ADP-ribosylation of solubilized A6 membrane proteins (30 µg of protein/lane) in the presence of increasing concentrations (0-300 µM) of amiloride. The presence of amiloride has no effect on the level of ribosylation of the labeled proteins.](image2)

![Fig. 4. Western blotting of purified Na⁺ channel complex. A, HPLC-purified Na⁺ channel complex from solubilized A6 membranes was electrophoresed on 8% polyacrylamide gels and silver-stained to show the proteins comprising the complex (220, 150, 95, 70, 55, and 41 kDa) (lane 1). B, purified Na⁺ channel complex proteins (200 ng/lane) were loaded onto polyacrylamide gels, electrophoresed, and then transferred to nitrocellulose for Western blotting. Transfers were probed either with preimmune serum (lane 3) or with the Na⁺ channel antibody (lane 2) followed by alkaline phosphatase conjugates. This Na⁺ channel antibody typically recognizes the 320-, 150-, and 95-kDa bands of the complex by Western blotting (lane 2), although it immunoprecipitates the whole complex (10). C, the HPLC-purified Na⁺ channel complex (200 ng/lane) was Western blotted with the Go₁₃ antibody followed by an alkaline phosphatase conjugate (lane 4). The 41-kDa band of Go₁₃ was detected in the Na⁺ channel complex. Immunoaffinity column-purified Na⁺ channel complex (200 ng/lane) (lanes 5 and 6) was Western blotted with anti-α3 serum (lane 6) or without serum as a control (lane 5), followed by detection with 125I-labeled protein A. There was no specific binding of 125I-protein A in the absence of antiserum (lane 5); the α3, antibody labeled a single band at 41 kDa (lane 6) indicating that α3 is present in the affinity-purified Na⁺ channel complex.](image3)
Na⁺ Channel Co-purifies with Goα₃ Subunit

Fig. 5. Western blotting of isolated rat liver Golgi membranes. A, isolated Golgi membranes from rat liver, used as an enriched source of Goα₃, were electrophoresed on gels (50 µg/lane), transferred, and Western blotted with antibodies specific for α₁₃ (lane 1) and Na⁺ channel (lane 2). Reaction of the Golgi membranes with anti-α₁₃ serum confirmed the presence of the 41-kDa α₁₀₃ subunit (lane 1). There is nonspecific binding of antibody to albumin at a higher molecular weight. Reaction of the Golgi membranes with the Na⁺ channel antibody gave no staining of the 41-kDa band or of any other proteins (lane 2) showing that the Na⁺ channel antibody does not recognize the α₁₀₃ protein. B, isolated Golgi membranes were ADP-ribosylated with pertussis toxin. The major ADP-ribosylated protein in these membranes is the 41-kDa, (lane 3). Solubilized membranes were then used for immunoprecipitation with the Goα₃ antiserum or with Na⁺ channel antiserum. The α₁₀₃ antibody precipitated the 41-kDa α₁₀₃ subunit (lane 4), but the Na⁺ channel antibody did not precipitate the labeled Goα₃ (lane 5).

TABLE II
Effect of purified α₁₀₃ or α₁₀₂ subunits on amiloride-sensitive Na⁺ channel activity measured by the patch-clamp technique

Pertussis toxin (PTX) (100 ng/ml) was added for 1 min, following which open time and mean conductance, γ, were determined (PTX columns). At 1 min, α₁₀₃ or α₁₀₂ was added and the same parameters were again determined at 5 min.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PTX</th>
<th>α₁₀₃ (20 pm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open time (%)</td>
<td>67.5 ± 6.2</td>
<td>16.4 ± 7.0*</td>
<td>55.7 ± 9.8</td>
</tr>
<tr>
<td>γ (pS)</td>
<td>9.6 ± 2.1</td>
<td>9.7 ± 2.1</td>
<td>12.1 ± 4.4</td>
</tr>
<tr>
<td>Control</td>
<td>PTX</td>
<td>α₁₀₂ (20 pm)</td>
<td></td>
</tr>
<tr>
<td>Open time (%)</td>
<td>69.1 ± 10.3</td>
<td>19.0 ± 15.5*</td>
<td>8.1 ± 2.5*</td>
</tr>
<tr>
<td>γ (pS)</td>
<td>10.6 ± 2.1</td>
<td>8.6 ± 2.8</td>
<td>8.2 ± 2.5</td>
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* p < 0.02 versus control, n = 3.

Acknowledgments—We would like to extend our sincere appreciation to Lisa Firicane for excellent secretarial assistance, Deborah Keeton for excellent technical support, and Joanne Doherty for excellent technical and photographic assistance.

REFERENCES
Na+ Channel Co-purifies with Ga1.3 Subunit