G protein activation inhibits gating charge movement in rat sympathetic neurons

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Hernández-Ochoa EO, García-Ferreiro RE, García DE. G protein activation inhibits gating charge movement in rat sympathetic neurons. Am J Physiol Cell Physiol 292: C2226–C2238, 2007. First published February 21, 2007; doi:10.1152/ajpcell.00540.2006.—G protein-coupled receptors (GPCRs) control neuronal functions via ion channel modulation. For voltage-gated ion channels, gating charge movement precedes and underlies channel opening. Therefore, we sought to investigate the effects of G protein activation on gating charge movement. Nonlinear capacitive currents were recorded using the whole cell patch-clamp technique in cultured rat sympathetic neurons. Our results show that gating charge movement depends on voltage with average Boltzmann parameters: maximum charge per unit of linear capacitance (Qmax) = 6.1 ± 0.6 nC/μF, midpoint (Vh) = −29.2 ± 0.5 mV, and measure of steepness (k) = 8.4 ± 0.4 mV. Intracellular dialysis with GTPγS produces a nonreversible ~34% decrease in Qmax, a ~10 mV shift in Vh, and a ~63% increase in k with respect to the control. Norepinephrine induces a ~7 mV shift in Vh and ~40% increase in k. Overexpression of G protein β1γδ subunits produces a ~13% decrease in Qmax, a ~9 mV shift in Vh, and a ~28% increase in k. We correlate charge movement modulation with the modulated behavior of voltage-gated channels. Concurrently, G protein activation by transmitters and GTPγS also inhibit both Na+ and N-type Ca2+ channels. These results reveal an inhibition of gating charge movement by G protein activation that parallels the inhibition of both Na+ and N-type Ca2+ currents. We propose that gating charge movement decrement may precede or accompany some forms of GPCR-mediated channel current inhibition or downregulation. This may be a common step in the GPCR-mediated inhibition of distinct populations of voltage-gated ion channels.

IN VOLTAGE-GATED ION CHANNELS, the probability of opening is modified by the membrane potential. This is achieved through voltage sensors that detect the voltage and transfer energy to the pore to control gate (12, 13, 35). Membrane depolarization elicits the movement of voltage sensors located within the channels; their movement constitutes the “gating charge movement” (12, 20, 30).

The activity of almost all types of voltage-gated ion channels is modulated by at least one kind of extra- or intracellular signaling process. A broad array of neuromodulators—monoamines, peptides, glutamate, and acetylcholine—have been shown to alter the function of voltage-gated channels in neurons. The modulation of voltage-gated ion channels by signaling pathways activated by G protein-coupled receptors (GPCRs) constitutes a widespread mechanism for regulating neuronal excitability, neurotransmitter release, and plasticity (15, 18). The mechanisms that underlie the ion channel modulation mediated by GPCR-dependent signaling pathways alter channel function by modifying their opening probability, kinetics, and voltage dependence without altering, however, ionic selectivity and unitary conductance (15, 17, 23, 32, 37, 39). In almost all cases, this has been established by solely examining the properties of ionic current flowing through ion channels. Only in a few cases has voltage-gated ion channel modulation been examined at the level of gating charge movement (7, 36). This study was undertaken to investigate whether G protein-dependent signaling pathways modulate gating charge movement in a native neuronal system. We analyzed total gating charge movement in rat superior cervical ganglion (SCG) neurons. Our results reveal that G protein activation induces a mixed pattern of inhibitory modulation on gating charge movement. Part of the inhibitory pattern is voltage dependent, whereas the other is voltage independent. We establish a correlation between gating charge movement modulation and the modulated behavior of two populations of voltage-gated ion channels present in these neurons. We report that TTX-sensitive Na+, similar to N-type Ca2+-channel currents, is inhibited by GPCR-dependent signaling pathways. Our results show an inhibition of gating charge movement by G protein activation in a manner that closely parallels the inhibition of Na+ and N-type Ca2+ channels. We propose that gating charge movement trapping or its decrement may precede or accompany some forms of ion channel inhibition and/or ion channel downregulation. This process might be a common step in the GPCR-mediated modulation of distinct voltage-gated ion channels.

MATERIALS AND METHODS

Cell culture. SCG neurons were enzymatically dissociated from 5-wk-old male Wistar rats as described previously (26). Rats were placed in a container and exposed to CO2 in a rising concentration and were then euthanized by rapid decapitation, performed according to authorized procedures of our institution. Animals were used in accordance with the procedures approved by the Institutional Animal Care and Use Committee at the University of Maryland. After dissection, ganglia were desheathed, cut into eight to ten small nearly identical pieces, and transferred to a modified Hank's solution containing 20 U/ml papain. After 20 min at 37°C, the papain solution was replaced with a solution containing 1 mg/ml collagenase type I and 10 mg/ml dispase. Ganglia were incubated for 40 min in this solution and...
mechanically triturated every 20 min. The preparation was then centrifuged and resuspended twice in Leibovitz’s L-15 medium and once in Dulbecco’s modified Eagle’s medium, both supplemented with 5% (vol/vol) heat-inactivated fetal bovine serum and 1% penicillin-streptomycin. Neurons were then plated on polystyrene culture dishes coated with poly-L-lysine and stored in a humidified atmosphere of 95% air-5% CO2 at 37°C. Neurons were studied 8–18 h after plating.

Intracellular microinjection. For some experiments, after a 4-h wait for attachment to the substrate, the neurons were intracellularly microinjected using an Eppendorf 5242 pressure microinjector and a 5171 micromanipulator system (Eppendorf, Madison, WI) as described previously (25). The injection solution contained cDNA construct coding for a green fluorescent protein (GFP) mutant fused to the G protein β1-subunit (Gβ1-GFP, 100 ng/µl) and the G protein γ7-subunit (Gγ7, 100 ng/µl) expression plasmids mixed with 1 mg/ml of 10,000 kDa dextran-fluorescein used as an injection marker. The employment of the tagged subunits facilitated the identification of cells that positively express Gβγ subunits. Injection pressures of 10–20 kPa for 0.5–0.8 s resulted in no obvious increase in cell volume. After 12–16 h, successfully injected neurons were identified by their characteristic greenish-blue GFP fluorescence using an inverted microscope equipped with epifluorescence and fluorescent optics.

Solutions. Neurons were constantly and locally perfused during recording (1–2 ml/min) with appropriate solutions designed to isolate total nonlinear capacitive currents, Ba2+ currents (flowing through N-type Ca2+ channels), Na+ currents, and K+ currents. For current measurements through Ca2+ channels, the bath solution contained (in mM) 162.5 tetraethylammonium (TEA) chloride (TEACl), 2–5 BaCl2, Na2GTP, and 0.1 leupeptin, pH adjusted to 7.4 with CsOH. The standard pipette solution (internal solution) contained (in mM) 103 TEACl, 60 NaCl, 10 HEPES, 1 MgCl2, and 0.1 CdCl2, pH 7.4 with TEAOH. The Ca2+ current of rat SCG neurons is set at ~85–90% in N-type channels and the remainder in L-type channels, with no detectable P- or Q-type channels component (26, 46, 49). Therefore, the N-type Ca2+ current was defined as the component of the current sensitive to 100 µM CdCl2 in the presence of 5 µM nifedipine. For Na+ current measurements shown in Figs. 2 and 6, the bath solution contained (in mM) 103 TEACl, 60 NaCl, 10 HEPES, 8 glucose, 2.9 MgCl2, and 0.1 CdCl2, pH adjusted to 7.4 with TEAOH. For Na+ currents measurements shown in Fig. 3, the bath solution contained (in mM) 153 NaCl, 10 TEACl, 10 HEPES, 8 glucose, 2.9 MgCl2, and 0.1 CdCl2, pH adjusted to 7.4 with NaOH. For K+ currents measurements, the bath solution contained (in mM) 163 NaCl, 10 HEPES, 8 glucose, 5 KCl, 2.9 MgCl2, 0.0002 TTX, and 0.1 CdCl2, pH adjusted to 7.4 with NaOH. The external solution for nonlinear capacitive currents was designed to eliminate voltage-gated ionic currents and consisted of (in mM) 161.5 TEACl, 10 HEPES, 8 glucose, 2.8 MgCl2, 1 CsCl, 0.0001 TTX, 0.1 CdCl2, 0.1 LaCl3, pH adjusted to 7.4 with TEAOH.

The solution composition was the result of trial and error tests in preliminary experiments and left the charge movement apparently unaltered and caused minimal changes (~4 mV shift) in surface potential. Where noted, 100 µM norepinephrine (NE) was added to the external solution. Nifedipine-containing external solution was prepared daily from 25 mM absolute ethanol stock solution and protected from light whenever possible. For most of the experiments of this study, we evaluated short-term actions of transmitters on membrane currents. Neurons were constantly and locally perfused during measuring membrane currents. The bath solution contained (in mM) 103 TEACl, 60 NaCl, 10 HEPES, 8 glucose, 2.9 MgCl2, and 0.1 CdCl2, pH adjusted to 7.4 with TEAOH. For Na+ currents measurements shown in Fig. 3, the bath solution contained (in mM) 153 NaCl, 10 TEACl, 10 HEPES, 8 glucose, 2.9 MgCl2, and 0.1 CdCl2, pH adjusted to 7.4 with NaOH. The external solution for nonlinear capacitive currents was designed to eliminate voltage-gated ionic currents and consisted of (in mM) 161.5 TEACl, 10 HEPES, 8 glucose, 2.8 MgCl2, 1 CsCl, 0.0001 TTX, 0.1 CdCl2, 0.1 LaCl3, pH adjusted to 7.4 with TEAOH.

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single-channel amplitude $i$. Variance-mean plots were calculated from data beginning 2 ms after the start of the voltage pulse to the maximum of the mean current amplitude. For nonstationary noise analysis experiments, after the establishment of the whole cell configuration, the neuron under evaluation was lifted off the bottom of the dish and positioned in front of a local perfusion system.

Because the magnitude of the $\text{Ba}^{2+}$, $\text{Na}^+$, $\text{K}^+$, and nonlinear capacitive currents were dependent on cell size, aggregate current data and the amount of gating charge movement are presented as current or charge density normalized to cell capacitance. To avoid one source of systematic bias, experimental and control measurements were alternated whenever possible, and concurrent controls were always performed. All recordings were obtained at room temperature (19–22°C). Where appropriate, data are expressed as means ± SE, with $n$ representing the number of observations. Statistical significance was determined by using an unpaired Student’s t-test. Data were considered significant when $P < 0.05$.

RESULTS

Nonlinear capacitive currents in rat sympathetic neurons. Nonlinear capacitive currents can be recorded from the cell body of SCG neurons in the presence of internal and external solutions designed to eliminate ionic currents. Figure 1A shows that depolarizations to potentials more positive than $-60$ mV elicit transient-outward and -inward currents at the onset and at the end of depolarization, respectively. Their amplitude increases with pulse amplitude (Fig. 1A). As expected for nonlinear capacitive currents, charge saturates at large depolarizations. Fig. 1D shows the charge-voltage ($Q$-$V$) relationships for $Q_{on}$ and $Q_{off}$. HP, holding potential; SHP, subholding potential.

$$Q = Q_{max}/[1 + \exp((-V + V_h)/k)]$$  
(2)

where $Q_{max}$ is the maximum charge per unit of linear capacitance, $V_h$ is the midpoint, and $k$ a measure of steepness. Nonlinear charge movement depended on voltage with average Boltzmann parameters: $Q_{max} = 6.1 \pm 0.6 \text{nC/\mu F}$, $V_h = -29.2 \pm 0.5 \text{mV}$, and $k = 8.4 \pm 0.4 \text{mV}$ ($n = 14$). Both the amounts of charge that were moved following the onset ($Q_{on}$) and the end ($Q_{off}$) of the voltage-clamp step attained a maximal value of around $+40$ mV (Fig. 1D).

There is a general agreement between $Q_{on}$ and $Q_{off}$ for all voltages, except that the amplitude of $Q_{off}$ was less than $Q_{on}$ for...
depolarizations above \(-20\) mV (Fig. 1D). The ratio of \(Q_{on}\) and \(Q_{off}\) at \(+80\) mV was \(1.19 \pm 0.07\) \((n = 14)\). In experiments where the duration of the voltage steps was diminished to 1 ms, the values for \(Q_{on}\) and \(Q_{off}\) were nearly equal \((Q_{on}/Q_{off} = 1.05 \pm 0.02, n = 5)\), whereas pulses of 10 ms enhanced the inequality of charges (data not shown). Therefore, in the experiments shown in Figs. 2, 4, and 7, only \(Q_{on}\) was analyzed.

Given the ratio of \(Q_{on}\) and \(Q_{off}\) (Fig. 1C), the time and voltage dependence, and the saturation of mobile charge at high depolarizations (Fig. 1D), we concluded that most of this nonlinear charge movement represents the gating charge movement that precedes or accompanies the opening of voltage-gated ion channels (2, 6, 12). Judging by the relative fast time course of the total nonlinear capacitive current, we hypothesized that it may be associated with the gating charge of fast voltage-activated channels (i.e., \(Na^+\), \(Ca^{2+}\), and transient type-A \(K^+\) channels).

What channels are actually contributing to total gating charge movement? There are no pharmacological tools to isolate and/or identify gating charge movements arising from a specific channel population. To obtain an estimation of the channels that are actually contributing to the total charge movement, we followed two approaches: 1) the use of charge movement immobilization and voltage-gated channel inactivation protocols and 2) nonstationary fluctuation analysis of \(Na^+\) and \(Ca^{2+}\) currents.

**Charge movement immobilization and voltage-gated channel inactivation.** If gating charge of \(Na^+\), fast-transient type-A \(K^+\), and \(Ca^{2+}\) channels at least contribute to the total charge movement that we recorded, distinct fast and slow components should be observed. However, as can be appreciated in Fig. 1A, nonlinear capacitive currents did not exhibit distinct fast and slow components. One possibility could be that series resistances are not completely compensated; therefore, nonlinear capacitive currents might be filtered. This limits the possibility to establish a kinetic separation of distinct components. In an alternative attempt to separate such distinct components, we explored charge movement immobilization and voltage-gated channel inactivation. Inequality of moved charges (Fig. 1, C and D) might be due to a time- and voltage-dependent gating charge immobilization associated with fast voltage-dependent inactivation (5, 12). To test this possibility, we first evaluated charge movement immobilization and then voltage-gated channel inactivation by using double-pulse protocols. Figure 2A
shows nonlinear capacitive currents traces elicited after a 500-ms conditioning prepulse (PP) to voltages from −100 to 0 mV. Increasing the magnitude of PP resulted in a reduction of \( Q_{on} \) charge movement, elicited by a 4-ms pulse to +20 mV. In four neurons, the reduction of \( Q_{on} \) averaged 64\% ± 3\% when nonlinear capacitive currents were preceded by a PP to −40 mV (Fig. 2A; trace c). The immobilization curve is shown as \( Q_{on} \) versus PP voltage in Fig. 2E (solid circles). \( Q_{on} \) began to decrease at PP potentials positive to −90 mV and was partially immobilized by a 500-ms PP to 0 mV. This raises the possibility that the component(s) of gating charge movement in SCG neurons, in which amplitude decreases after a PP (Fig. 2A), in fact arises from rapidly inactivating Na\(^+\) channels and transient type-A K\(^+\) channels since these neurons have large Na\(^+\) \( (I_{Na}) \) and transient type-A K\(^+\) \( (I_{A}) \) currents (51, 52). We tested this possibility by comparing the voltage dependence of charge immobilization with that of both \( I_{Na} \) and \( I_{A} \) steady-state inactivation. Figure 2, B and C, shows experiments in which voltage dependence of steady-state inactivation of both Na\(^+\) and transient type-A K\(^+\) channels was determined by voltage protocols illustrated at the top of each panel. \( I_{Na} \) and \( I_{A} \) evoked by depolarizing steps to −20 mV after a 500-ms PP are shown in Fig. 2. B and C, respectively. Increasing the amplitude of PP resulted in the inactivation of both \( I_{Na} \) and \( I_{A} \) (Fig. 2, B and C). The \( I_{Na} \) steady-state inactivation curve is shown as peak current versus PP voltage (Fig. 2E; open squares). \( I_{Na} \) began to inactivate at potentials positive to −90 mV and was almost fully inactivated by a 500-ms PP to −40 mV. At −20 mV, \( I_{A} \) component was a little contaminated by other K\(^+\) currents, obviating the need for digital subtraction. As the PP was depolarized, the \( I_{A} \) amplitude decreased until it was completely inactivated at potentials more depolarized than −60 mV. The \( I_{A} \) steady-state inactivation curve is shown as peak current versus PP voltage (Fig. 2E; solid triangles). The voltage dependence of \( I_{Na} \) and \( I_{A} \) inactivation and charge immobilization could be fit well by a modified Boltzmann function:

\[
A/A_{max} = [1 + \exp((V - V_h)/k)]^{-1}
\]  

(3)

where \( A \) is the peak current or charge evoked from the PP potential \( V \), \( A_{max} \) is the maximal current or maximal charge, \( V_h \) is the half-deactivation voltage, and \( k \) a measure of steepness. Mean Boltzmann values of \( V_h \) and \( k \) for \( Q_{on} \) from four neurons were −67.2 ± 1.6 and 10.6 ± 1.4 mV, respectively. Mean values of \( V_h \) and \( k \) for \( I_{Na} \) from the same four neurons were −67.4 ± 0.3 and 6.7 ± 0.3 mV, respectively. Mean values of \( V_h \) and \( k \) for \( I_{A} \) from six neurons were −78.7 ± 1.7 and 8.9 ± 1.6 mV, respectively. As shown in Fig. 2E, the voltage dependence of \( Q_{on} \) charge availability is most similar to that of the availability of \( I_{Na} \), suggesting that most of the charge movement that immobilizes over this voltage range comes from Na\(^+\) channels with perhaps a small contribution for A-type K\(^+\) channels. Also, a component of gating charge arising from Ca\(^{2+}\) channels would certainly be expected since SCG neurons express Ca\(^{2+}\) currents (26, 49). In data illustrated in Fig. 2D, we evaluated the Ca\(^{2+}\) current inactivation by using a three-pulse voltage protocol consisting of two 40-ms test pulses to the voltage producing peak-inward current before (P1) and after (P2) a 500-ms conditioning PP over a wide range of voltages (from −100 to 40 mV; Fig. 2E, open diamonds). As a result of the relatively shorter conditioning PPs (500 ms), this protocol specifically examines the voltage dependence of fast inactivation. We use the first test pulse (P1) to correct the relations between P2 and PP voltage for rundown and for a component of inactivation that recovers slowly (see voltage protocol in Fig. 2D). Note that maximal inactivation is observed at voltages near those yielding maximal inward current and less inactivation at more positive and negative voltages (Fig. 2E, open diamonds). This results from U-type inactivation, which is characterized by U-shaped voltage dependence of inactivation and an absence of current dependence to inactivation, likely the result of a pure voltage-dependent and close-state preferential inactivation (38, 48). The voltage protocol designed to study N-type Ca\(^{2+}\)-channel current steady-state inactivation promotes −40\% reduction of peak Ca\(^{2+}\) current recorded after depolarized PP in the range of −80 to −40 mV (Fig. 2E; open diamonds), whereas an almost full inactivation of both \( I_{Na} \) and \( I_{A} \) was observed after depolarized PP in the range of −90 to −40 mV (Fig. 2E). Note the −60–80\% reduction of peak Ca\(^{2+}\) current recorded after depolarized PP in the range of −40 to 10 mV (Fig. 2E). These results suggest that also a portion of total gating charge that is not reduced by depolarized PP in the range of −80 to −40 mV (≤1/3 of the total) might arise from Ca\(^{2+}\) channels. The steady-state separation of two components of total gating charge movement by digital subtraction is illustrated in Fig. 2A. The current traces a–c show the PP-sensitive component, presumably the gating charge arising from both Na\(^+\) and transient type-A K\(^+\) channels, whereas the current trace c shows the PP-insensitive component of the total charge movement, presumably the gating charge arising from Ca\(^{2+}\) channels. Our immobilization/inactivation experiments suggest that, from a host of voltage-gated ion channels present in the cell body of a SCG neuron, primarily Na\(^+\) and Ca\(^{2+}\) channels might contribute to the total gating charge movement.

Nonstationary fluctuation analysis of Na\(^+\) and Ca\(^{2+}\) currents. We use ensemble fluctuation analysis to obtain an estimation of the number of Na\(^+\) and Ca\(^{2+}\) channels present in the cell body of the rat SCG neurons. Nonstationary fluctuation analysis typically involves measuring an ensemble of responses to a repeated activating stimulus. The theory is based on three general assumptions about voltage-gated channels: 1) channels are composed of a homogeneous population, 2) this channel population is composed of a fixed number of independently gated channels, and 3) channels are assumed to exist in either a conducting or nonconducting state. When the probability of being in the conducting state is maximal, the theory predicts a parabolic relation between the current variance and mean that can be used to estimate the number of channels, \( N \), and their unitary current amplitude, \( i \) (3, 55).

Figure 3, A and C, shows representative macroscopic recordings of both \( I_{Na} \) and \( I_{Ca} \) (gray traces), as well as the corresponding time courses of the mean current (bold traces). Figure 3, A,b and C,b, shows the time of course of the variance \( \sigma^2 \) of both \( I_{Na} \) and \( I_{Ca} \) respectively, estimated at the voltages indicated in each panel from three different neurons. Figure 3, B and D, shows plots of variance versus mean current corresponding to data shown in Fig. 3, A and C, respectively. Continuous lines are fit to the data by using Eq. 1, allowing the estimation of the single channel current and the number of the channels, indicated in each panel. These results show
the number of Na\(^+\) (7.8 \times 10^4 \pm 0.39 \times 10^4, n = 7) and Ca\(^{2+}\) (3.8 \times 10^4 \pm 0.27 \times 10^4, n = 5) channels in the cell body of 1-day cultured SCG neurons.

G protein activation by GTP\(\gamma\)S inhibits gating charge movement. A general strategy of implicating G proteins in the modulation of voltage-gated ion channels is to introduce non-hydrolysable guanine nucleotide analogs into the cell via the patch pipette (32). Figure 4A shows that averaged total non-linear capacitive current records obtained in SCG neurons dialyzed with GTP\(\gamma\)S (300 \mu M) are reduced in amplitude compared with the control (Fig. 4A; traces labeled as GTP\(\gamma\)S, n = 9). The magnitude of the inhibition induced by GTP\(\gamma\)S depends on the magnitude of the test depolarization. Maximal inhibition (~60%) was present around −30 mV, whereas only 37% is seen at extreme test depolarizations (Fig. 4B; solid inverted triangles).

Fig. 3. Nonstationary noise analysis of macroscopic currents from Na\(^+\) and Ca\(^{2+}\) channels. Ensembles of currents were generated by a series (80–200) of identical voltage pulses from 100 to voltages indicated in each panel, delivered every 4 s. Data were sampled at 20 \mu s after filtering at 3 kHz. The variance at each time point was calculated for each series and then averaged over the set of series. Background variance at the holding potential was subtracted from the variance during the test pulse (\(A,a\) and \(C,a\)). Time courses of the series of macroscopic Na\(^+\) (\(A\)) and Ca\(^{2+}\) (\(C\)) currents (\(I_{\text{Ca}}\); gray traces). Bold traces represent the mean current (\(A,b\) and \(C,b\)). B and D: time courses of the variance for each condition. \(\sigma A^2\), variance. Variance vs. mean current plot for data is shown in \(A\) and \(C\), respectively. Smooth lines are best fits to the Eq. 1 (see Ref. 55). For \(I_{\text{Na}}\), fit shown is \(i = 0.23\) pA and \(N = 7.8 \times 10^4\); and for \(I_{\text{Ca}}\), fit shown is \(i = 0.20\) pA, \(N = 3.8 \times 10^4\), where \(N\) is the number of channels and \(i\) is unitary current.

Fig. 4. G protein activation modulates gating charge movement. A: nonlinear capacitive currents evoked by 4-ms depolarizing pulses from a holding potential of −100 mV to membrane potentials indicated on each trace. Current traces represent the average nonlinear capacitive current from control (\(n = 14\)), GTP\(\gamma\)S-dialyzed neurons (\(n = 9\)), norepinephrine (NE) exposed (\(n = 14\)), and G protein \(\beta_1\gamma_4\) subunit (G\(\beta_1\gamma_4\)) cDNA-injected neurons (\(n = 5\)), where \(n\) is the number of observations. Same calibration bars for all traces. B: \(Q_{\text{on}}-V\) relationships for control, GTP\(\gamma\)S-dialyzed, NE-exposed, and G\(\beta_1\gamma_4\) cDNA-injected neurons from \(A\). For each condition, the continuous line through the symbols is a best fit to Eq. 2 (see parameters in Table 1).
The voltage-dependence of gating charge movement inhibition arises from a positive shift in its activation curve. Figure 4B summarizes experimental data for Q-V curves of control and GTPγS-dialyzed neurons. Three main significant effects are observed upon GTPγS dialysis: 1) a ~34% decrease in \( Q_{\text{max}} \); 2) a ~10 mV positive shift in \( V_h \); and 3) a ~63% increase in \( k \) (\( n = 9; P < 0.05 \), see Table 1). This suggests that nonselective G protein activation by GTPγS modifies both the voltage dependence and the number of available channels of one or more populations of voltage-gated ion channels present in the cell body of SCG neurons.

Selective inhibition of gating charge movement. Gating charge movement is also inhibited by NE and an overexpression of G protein \( \beta_3\gamma_4 \)-subunits (\( \beta_3\gamma_4 \)). Figure 4A shows that total nonlinear capacitive currents recorded at moderated test depolarizations are much more inhibited by NE than those recorded at more positive potentials (traces labeled as NE). Gating charge movement is inhibited by NE ~25% at 20 mV compared with just ~3% at 40 mV (Fig. 4B; solid triangles). \( \beta_3\gamma_4 \) overexpression mimics part of the effects of NE. Gating charge movement is inhibited by \( \beta_3\gamma_4 \) overexpression ~41% at ~20 mV in contrast to 14% at 40 mV (Fig. 4B; solid diamonds). A slight prolonged decaying phase of the “on” nonlinear capacitive currents is also evident at moderate test potentials, especially with NE, but we did not analyze this effect in more detail. In neurons dialyzed with GDPβS, a nonhydrolysable analog of GDP that minimizes G protein activation, NE was without effect (data not shown). Therefore, NE-induced effects are G protein dependent.

In rat SCG neurons, GDPβS has a fairly large effect on N-type Ca\(^{2+}\) current (see Table 2). Intracellular dialysis with solutions containing 2 mM of GDPβS removes tonic G protein-mediated Ca\(^{2+}\) current inhibition, prevents the actions of GPCR activated by neurotransmitters, greatly reduced facilitation, enhanced inactivation, and shifted tail Ca\(^{2+}\) current activation curves to more hyperpolarized potentials (26, 33). However, on gating charge movement, dialysis with GDPβS induces small and nonsignificant effects (see Table 1).

Single Boltzmann fits to Q-V relationships (Fig. 4B, continuous lines) reveal that the main effect of both NE (\( n = 14 \)) and \( \beta_3\gamma_4 \) (\( n = 5 \)) is a significant 6-9 mV positive shift in \( V_h \), respectively (\( P < 0.05 \), see Table 1). In NE-exposed neurons, a significant decrease was found in the steepness (\( k \)) of the Q-V curve and \( Q_{\text{max}} \) remained essentially unchanged (see Table 1). It is important to note that, in \( \beta_3\gamma_4 \)-overexpressed neurons, \( Q_{\text{max}} \) was reduced ~13% compared with control neurons, which may indicate that overexpression of \( \beta_3\gamma_4 \) induces both voltage-dependent and -independent inhibition.

The following reasons suggest that a possible candidate to underlie gating charge movement inhibition by G protein activation is the N-type Ca\(^{2+}\) channel population. First, in rat SCG neurons, most of the whole cell Ca\(^{2+}\)-channel current flows through N-type Ca\(^{2+}\) channels (26, 46, 49). Second, N-type Ca\(^{2+}\) channels are a typical target of inhibitory modulation by several signaling pathways activated by different GPCRs (32, 37). Third, N-type Ca\(^{2+}\) channel inhibition by distinct GPCR-dependent signaling pathways alters channel-current properties in a voltage-dependent or -independent manner (23, 32, 37, 39). Fourth, at least part of the voltage-dependent inhibition of N-type Ca\(^{2+}\) current arises from an inhibition of their gating charge movement (36).

### Table 2. Parameters for single Boltzmann fit to \( I_{\text{Ba tail}} \)-voltage relationships of data from Fig. 5B.

<table>
<thead>
<tr>
<th>( n )</th>
<th>( Q_{\text{max}}, \text{nC}/\mu \text{F} )</th>
<th>( V_h, \text{mV} )</th>
<th>( k, \text{mV} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>57.4 ± 1.0</td>
<td>−110 ± 1.1</td>
</tr>
<tr>
<td>NE</td>
<td>16</td>
<td>54.7 ± 2.2</td>
<td>6.2 ± 2.7*</td>
</tr>
<tr>
<td>( \beta_3\gamma_4 )</td>
<td>5</td>
<td>47.3 ± 1.6*</td>
<td>3.5 ± 2.3*</td>
</tr>
<tr>
<td>GTPγS</td>
<td>9</td>
<td>38.4 ± 0.6*</td>
<td>2.5 ± 1.0*</td>
</tr>
<tr>
<td>GDPβS</td>
<td>6</td>
<td>67.8 ± 0.5*</td>
<td>−13.4 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), number of observations. \( I_{\text{max}} \), maximum current. See RESULTS, Eq. 4, for Boltzmann approximation. * \( P < 0.05 \), significantly different from control.
of \(I_{Ba}\) is absent at extreme positive potentials (45% at \(-10\) mV vs. 6% at extreme depolarizations), and it is therefore mostly voltage dependent (10, 11, 23). Consequently, all NE-induced effects can be ascribed to a significant +17-mV shift in \(V_h\) and a \(-9\)-mV increase in \(k\) of the activation curve, with little if any effect on \(I_{max}\) (\(n = 16; P < 0.05\), see Table 2).

Previous reports strongly support the idea that voltage-dependent inhibition of N-type \(Ca^{2+}\) channels results from a direct interaction between activated G protein \(\beta\gamma\)-subunits and the \(\alpha_1\)-subunit of these channels (25, 28, 34). Figure 5, A and B, shows that a modulation of \(I_{Ba}\) similar to the inhibition caused by NE is produced after overexpression of \(G\beta_1\gamma_4\) by intranuclear injection of cDNA in SCG neurons (\(n = 5; P < 0.05\), see Table 2). As has been shown before (25, 28), we find that a microinjection of DNA for \(G\beta\) subunits, with and without DNA for \(G\gamma\) subunits, into the nucleus of SCG neurons inhibited \(Ca^{2+}\) currents mostly in a voltage-dependent manner, increased the facilitation ratio, and partially occluded the actions of NE (data not shown). However, it is important to note that, in \(G\beta_1\gamma_4\)-overexpressed neurons, \(I_{max}\) was significantly reduced \(-17\%\) compared with control neurons (see Table 2). This effect may indicate that overexpression of \(G\beta_1\gamma_4\) induces both voltage-dependent and -independent inhibition.

From these results, and in agreement with previous interpretations, we conclude that nonselective G protein activation by GTP\(\gamma\)S induces two independent inhibitory patterns on N-type \(Ca^{2+}\) channels. One inhibitory pattern is voltage dependent, whereas the other is voltage independent. It can be inferred that different G proteins might induce different combinations of voltage-dependent and -independent effects.

As it can be appreciated in Figs. 4 and 5, there is a qualitative correlation between the actions of GTP\(\gamma\)S, NE, and \(G\beta_1\gamma_4\) on both N-type \(Ca^{2+}\)-channel currents and gating charge movement. On the one hand, GTP\(\gamma\)S and NE induce a predominant voltage-dependent inhibition of both \(I_{Ba}\) and gating charge movement, arising from a positive shift on both \(I_{Ba}\) and \(Q\)-\(V\) relationships (see Figs. 4B and 5B). Also, Fig. 7A shows a complete recovery of total gating charge movement at +40 mV, but a strong inhibition of \(I_{Ba}\) at this same voltage. One possibility, to explain the fact that NE is still inhibiting N-type \(Ca^{2+}\) current at +40 mV, could be that at such voltage, G proteins acts on transitions (near to the open state) (12) where little charge moves; however, such transitions may be strongly affected, and the excitation-conduction coupling could be less efficient.

On the other hand, note that both GTP\(\gamma\)S and \(G\beta_1\gamma_4\) also inhibit \(I_{Ba}\) and gating charge movement by a constant proportion in addition to altering their voltage dependence (Figs. 4, 5, and 7A). Therefore, based on the above comparisons, we suggest that part of the effects on gating charge movement induced by G protein activation may arise from the inhibition of gating charge of N-type \(Ca^{2+}\) channels. In a previous report, Jones et al. (36) suggest that G proteins act to inhibit both voltage-sensor movement and the transduction of voltage-sensor activation into channels opening. Our results with native channels are consistent with this hypothesis.

**Inhibition of Na\(^+\) currents by GTP\(\gamma\)S and angiotensin II.** Several observations suggest that not all modulation of gating charge movement can be ascribed to the regulation of N-type \(Ca^{2+}\) channels and that one or more different populations of voltage-gated ion channels can be involved in the observed...
effects. First, maximal asymptotic effects of GTP\textsubscript{S} are very similar in \( I_{\text{Ba}} \) but quite different on gating charge movement. Second, if all GTP\textsubscript{S}-observed effects were due solely to the modulation of N-type Ca\textsuperscript{2+} channels, it would imply that these channels are responsible for at least 50% of the total gating charge movement recorded in these cells, a very unlikely condition (see DISCUSSION).

Two observations led us to suspect that the modulation of a fast-inactivating voltage-gated ion channel population can contribute to the observed effects of G proteins on gating charge movement. First, as noted above, the inequality of \( Q_{\text{on}} \)-to-\( Q_{\text{off}} \) ratio (Fig. 1C) suggests that rapidly inactivating channels contribute to total gating charge movement. Second, at least two-thirds of the gating charge movement is immobilized by PP to \(-40 \) mV (Fig. 2, A and E). The same protocol produces a complete inactivation of both macroscopic \( I_{\text{Na}} \) and \( I_{\text{A}} \) (Fig. 2, B and C).

Several reports have shown that voltage-gated Na\textsuperscript{+} channels of central and sensory neurons are targets of inhibitory GPCR-dependent signaling pathways (15). The above observations and findings prompted us to look for electrophysiological evidence of \( I_{\text{Na}} \) modulation by GPCR-dependent signaling pathways in rat sympathetic neurons.

To evaluate a possible rundown of \( I_{\text{Na}} \), we assessed changes in \( I_{\text{Na}} \) amplitude during repetitive depolarizing pulses (up to 200 sweeps) from a holding potential of \(-100 \) mV to a test potential of \(-20 \) mV, applied 10 s apart. The amplitude of \( I_{\text{Na}} \), recorded in this manner, was minimally reduced (<5%) over the 10-min course of a typical experiment (Fig. 6A). Step depolarizations beyond \(-40 \) mV activated a fast transient-inward \( I_{\text{Na}} \) (Fig. 6E, solid circles). Figure 6B (trace labeled as control) shows a \( I_{\text{Na}} \) elicited by a 10-ms depolarizing pulse to \(-20 \) mV from a holding potential of \(-100 \) mV. Superfusion of the cell with 100 nM TTX completely blocked the current at all

![Fig. 6. GTP\textsubscript{S} and ANG II inhibit whole cell \( I_{\text{Na}} \). A: peak current amplitudes elicited by a 10-ms pulse depolarization to \(-20 \) mV delivered every 10 s from a holding potential of \(-100 \) mV. Control (\( n = 6 \)) and GTP\textsubscript{S}-dia
dyzed (\( n = 7 \)) neurons are shown. TTX (100 nM) was bath applied during the time indicated by the bar. B: \( I_{\text{Na}} \) elicited by a 10-ms pulse depolarization to \(-20 \) mV. \( I_{\text{Na}} \) traces represent the average \( I_{\text{Na}} \) from control (\( n = 12 \)) and GTP\textsubscript{S}-dia
dyzed neurons (\( n = 7 \)). C: representative peak Na\textsuperscript{+} current amplitudes elicited by the protocol (top) before, during, and after ANG II application. ANG II (500 nM) was applied to the recording chamber during the time indicated by the bar. D: \( I_{\text{Na}} \) elicited by a 10-ms pulse depolarization to \(-20 \) mV before (a), during (b), and after ANG II (c) bath application recorded from the same neuron illustrated in C. E: \( I_{\text{Na}} \) vs. voltage relationships (\( I_{\text{Na}}-V \) average of control (\( n = 6 \)), GTP\textsubscript{S}-dia
dyzed (\( n = 7 \)), ANG II-exposed (\( n = 7 \)), and TTX-exposed (\( n = 3 \)) neurons. F: Na\textsuperscript{+} conductance-voltage (\( G_{\text{Na}}-V \) relationships obtained from the same neurons as in E. For each condition, the continuous line through the symbols is the best fit to Eq. 5. G: average inhibition by ANG II with the control internal solution (0.3 GTP, \( n = 12 \)) or with an internal solution in which 0.3 GTP was replaced by 2 mM GDP\textsubscript{S} (\( n = 8 \)).

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tested voltages (Fig. 6E, solid squares). The current versus voltage (I-V) relationship shown in Fig. 6E (solid circles) shows that the currents activated at potentials positive to approximately -50 mV reached maximum amplitude near -20 mV and reversed near +20 mV. An activation curve plotted as normalized conductance versus test potential for control neurons is shown in Fig. 6F (solid circles). Conductance was calculated from peak \( I_{Na} \) values from the I-V relationship (Fig. 6E; solid circles) using the equation \( G_{Na} = I_{Na} / (V - V_{Na}) \), where \( V \) is the membrane potential and \( V_{Na} \) the \( I_{Na} \) are reversal potential. The solid line through the activation curve in Fig. 6F (solid circles) was a least squares fit of the data to the modified Boltzmann equation:

\[
G / G_{max} = \left\{ 1 + \exp\left[ (V_h - V) / k \right] \right\}^{-1}
\]

where \( G_{max} \) is the maximal conductance. Mean values of \( V_h \) and \( k \) analyzed in this manner were -23.1 ± 0.5 and 4.3 ± 0.4 mV, respectively.

Consistent with a previous report (56), we found that the TTX-sensitive \( I_{Na} \) of SCG neurons is insensitive to both NE and acetylcholine (data not shown). However, we found that GTPyS (\( n = 7 \)) inhibits \( I_{Na} \). Figure 6A and B, shows an experiment with a significant inhibition (38% ± 3%, \( n = 7 \); \( P < 0.05 \) compared with control) of \( I_{Na} \) in GTPyS-dialyzed neurons (solid inverted triangles). Figure 6F shows that inhibition affects the maximal relative conductance but does not alter its voltage dependence. Therefore, judging by the lack of effect on the current kinetics, and the voltage dependence, we can classify GTPyS-mediated inhibition as voltage independent.

Bath application of 500 nM angiotensin II (ANG II) reduced \( I_{Na} \). Figure 6C shows a representative experiment with a ~16% inhibition of \( I_{Na} \) by ANG II; since it was typical, the inhibition reversed only slightly after washout of the agonist. ANG II did not induce a marked change in the activation kinetics and voltage dependence of \( I_{Na} \) (Fig. 6, D and F, open diamonds). The speed of the action of ANG II on \( I_{Na} \) was variable. In the experiment shown in Fig. 6C, ~100 s elapsed between the start of the change in current (a rough estimation of when ANG II had reached its receptors) and when the inhibition had reached half its maximal level. The speed of action of ANG II on \( I_{Na} \) was qualitatively similar to that observed on both M-type K"+ and Ca"+ currents (54) but slower than that induced by NE on Ca"+ currents in SCG neurons (11, 60). To test whether modulation of \( I_{Na} \) by ANG II was initiated by G proteins, we compared neurons dialyzed with either a control GTP-containing internal solution (0.3 mM GTP) or a GTP-free solution containing GDP[βS (2 mM), an antagonist of G protein activation. Neurons were dialyzed for >5 min using pipettes of 1.2 MΩ before ANG II application. ANG II had a negligible effect in neurons dialyzed with GDP[βS. ANG II inhibited \( I_{Na} \) by ~16 ± 6% in control neurons (\( n = 12 \)) but only by 4 ± 2% in GDP[βS-dialyzed neurons (\( n = 8 \)) (Fig. 6C). Therefore, we conclude that ANG II-induced effects involve the activation of G proteins.

Interestingly, the inhibition of \( I_{Na} \) by G protein activation parallels the inhibition of gating charge movement. As it can be appreciated in Fig. 7B, there is a correlation between the actions of GTPyS on both \( I_{Na} \) and gating charge movement. On both the \( Q-V \) and \( G_{Na-V} \) curves, GTPyS induced a net decrease in \( Q_{max} \) and maximal relative sodium conductance (\( G_{Na} \), respectively (Fig. 7B). Based on the above comparisons, we suggest that part of the effects on gating charge movement induced by G protein activation may arise from the inhibition of gating charge of Na"+ channels.

Taking all the above observations together, we conclude that G protein-induced effects on gating charge movement might underlie the mixture of G protein-induced inhibitory patterns that target, at least, Na"+ and N-type Ca"+ channels. The most striking result of the present study is that gating charge movement is modulated by G protein-dependent biochemical routes. This conclusion is supported by both voltage-dependent and -independent inhibition of gating charge movement induced by GTPyS, NE, and Gβ3/γ4.

**DISCUSSION**

Gating charge movement was predicted by Hodgkin and Huxley (30) and was first recorded in native preparations (1, 4, 19, 41, 45, 50). Modulation of gating charge movement has been shown previously in both native and heterologous preparations (7, 36).

Most of the charge movement comes from Na"+ and Ca"+ channels. A host of voltage-gated ion channels contributes to the total gating charge movement recorded in our experiments. We used immobilization and inactivation pulse protocols and nonstationary noise measurements to approximate the relative contribution of particular channel populations to the total gating charge movement. Our results shown in Figs. 2 and 3 suggest that most of the charge movement arises from Na"+ and Ca"+ channels, with perhaps a smaller contribution from transient type-A K"+ channels. Delayed-rectifier K"+ channels and Ca"+-dependent K"+ channels seem unlikely to contribute significantly to our measured gating charge movement. These
channels both activate and deactivate very slowly with time constants on the order of tens of milliseconds (51). If the gating charge arising from these channels were to move with a similar time course, it would be lost in the baseline unless it was enormous (9).

**Voltage-dependent and -independent inhibition of N-type Ca<sup>2+</sup> channels.** In our experimental conditions, only N-type Ca<sup>2+</sup> channels are inhibited by NE. In rat SCG neurons, neither Na<sup>+</sup> channels nor type-A K<sup>+</sup> channels are sensitive to NE (56). Therefore, it is tempting to speculate that all NE-induced effects on the gating charge movement are the result of the voltage-dependent inhibition of N-type Ca<sup>2+</sup> channels. This fits well with a previous study that demonstrated GTPγS-mediated voltage-dependent inhibition on gating currents of N-type Ca<sup>2+</sup> channels expressed in HEK293 cells (36). In the present study, the demonstration of the same observation in a native neuronal system with a putative transmitter and Gβγ subunits is an important advance.

The magnitude of the inhibition of both total gating charge movement and N-type Ca<sup>2+</sup> channels by NE correlates well with the estimated contribution of these channels to the total gating charge movement. With an assumption of a contribution of one-third of these channels to the total gating charge movement, the ~13% inhibition by NE at 0 mV correlates with the observed ~40% inhibition of the N-type Ca<sup>2+</sup>-channel current at that same potential. The value of *Q*<sub>max</sub> in unmodulated (GDPβS-dialyzed) neurons is about 6.2 nC/μF (Table 1). With an assumption of a specific membrane capacitance of 1 μF/cm<sup>2</sup>, the saturating gating charge movement of surface membrane is 6.2 nC/cm<sup>2</sup> or, translated into elementary charges (e<sup>+</sup>), 390 e<sup>+</sup>/μm<sup>2</sup>. With a consideration of an equivalent gating charge of ~12 e<sup>+</sup> per each voltage-gated ion channel (12, 29, 47, 53), the predicted density of voltage-gated ion channels in rat SCG neurons would be 32 channels/μm<sup>2</sup>. A SCG neuron with a capacitance of 40 pF and a total area of ~4,000 μm<sup>2</sup> would have ~12.8 × 10<sup>4</sup> channels. Nonstationary noise analysis of N-type Ca<sup>2+</sup> currents suggests ~3.8 × 10<sup>4</sup> channels in a rat SCG neuron of average size. This value is in agreement with the number of Ca<sup>2+</sup> channels reported by McDonough et al. (44). Taking together, the above arguments suggest that gating charge arising from Ca<sup>2+</sup> channels comprises approximately one-third of total gating charge movement.

It is well established that a direct protein-protein interaction of the Gβγ complex with the α<sub>1</sub>-subunit of N-type Ca<sup>2+</sup> channel mediates voltage-dependent inhibition of these channels (23, 25, 28, 34). Our results strengthen this conclusion at the level of gating charge movement. Overexpression of Gβγ subunits mimics much of the effects induced by NE on both N-type Ca<sup>2+</sup>-channel current and gating charge movement. In particular, both NE and Gβγ subunits decrease the steepness of activation curves for both N-type Ca<sup>2+</sup> channels and gating charge movement. This suggests a reduced sensitivity of modulated N-type Ca<sup>2+</sup> channels to the membrane potential. However, several observations do not fit well with solely voltage-dependent effects of Gβγ subunits on N-type Ca<sup>2+</sup> channels. Overexpression of Gβγ subunits was used to induce a tonic and voltage-dependent inhibition of N-type channels. Unexpectedly, this procedure appears to induce both voltage-dependent and -independent inhibitions on *Q*<sub>max</sub> (see table 1).

Voltage-independent inhibition of N-type Ca<sup>2+</sup> channels may contribute to the observed voltage-independent inhibition of total gating charge movement. Our results show that activation of G proteins by GTPγS induces a reduction of *Q*<sub>max</sub> even by extreme depolarization. There are two possible explanations for this observation. First, in channels modulated by a voltage-independent pathway, channel opening is blocked at a very early step where much of the gating charge moves (12). This mechanism may agree with the observations made by Carabelli et al. (16) and Lee and Elmslie (42), who reported an increase of “null sweeps” in records of single N-type Ca<sup>2+</sup> channels exposed to neurotransmitters. Second, Tombler et al. (58) recently reported a novel mechanism of GPCR-mediated modulation of N-type Ca<sup>2+</sup> channels involving destabilization and subsequent removal of Ca<sup>2+</sup> channels from the plasma membrane. Concurrently with the above, our results support the initial hypothesis of Dunlap and Fischbach (21), who first suggested a reduction in the number of functionally available Ca<sup>2+</sup> channels by neurotransmitter-mediated inhibition.

Voltage-independent inhibition of N-type Ca<sup>2+</sup> channels is quite complex and has been much less studied than the voltage-dependent pathway (23). At least two forms of voltage-independent inhibition, fast and slow, can be distinguished in sympathetic neurons by the speed of inhibition and by the involvement of a final effector(s) (23). Clues as to which final effector might be involved in one slow form came from experiments showing that G protein-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) causes voltage-independent N-type Ca<sup>2+</sup>-channel inhibition (24, 59). The final effector of fast voltage-independent inhibition of N-type Ca<sup>2+</sup> channels is still unknown. Nevertheless, our data in Fig. 4 provide an additional piece of evidence in favor of the Gβγ subunit as this final effector. Further experiments are required to establish whether PIP<sub>2</sub> or Gβγ binds to and modulates N-type Ca<sup>2+</sup> channels and gating currents in a voltage-independent manner.

**Modulation of Na<sup>+</sup> channels by G protein-dependent pathways.** Several pieces of evidence suggest that N-type Ca<sup>2+</sup> channels are not the only target of voltage-independent modulation by activated G proteins. For example, with the assumption that N-type Ca<sup>2+</sup> channels contribute one-third of the total gating charge movement, the inhibition of nonlinear capacitive currents should be at most 67% smaller than that of N-type Ca<sup>2+</sup> current. However, GTPγS inhibits 50% of both total gating charge movement and N-type Ca<sup>2+</sup> current (see Figs. 4 and 5). Thus the only possible conclusion is that activated G proteins do not specifically inhibit N-type Ca<sup>2+</sup>-channel gating charge movement.

Early studies in rat sympathetic neurons led to the idea that Na<sup>+</sup> channels are not modulated by GPCR-dependent signaling pathways (56). This study is the foremost report in showing that TTX-sensitive Na<sup>+</sup> channels of rat sympathetic neurons are modulated by G protein activation. There are two observations in support of this: 1) GTPγS (a nonselective and irreversible G protein activator) and ANG II (a GPCR agonist) induce an inhibition of Na<sup>+</sup> channel-current amplitude; and 2) ANG II had no effect in neurons dialyzed with GDPβS, which is expected to minimize the activation of G proteins. The observed effects of GTPγS and ANG II on *I*<sub>Na</sub> show a voltage-independent inhibition at all potentials tested and poor reversibility. Our results might echo the reported voltage-independent inhibition of Na<sup>+</sup> channels in central neurons by GPCR linked to PKA and/or PKC signaling pathways (Refs. 43 and...
57; for review, see Ref. 15). Nevertheless, further work is required to explore details about the final effector(s) of voltage-independent inhibition of Na\(^+\) channels observed in SCG neurons.

Finally, we cannot conclude that all the observed effects on gating charge movement inhibition arise exclusively from the regulation of Na\(^+\) and N-type Ca\(^{2+}\) channels. In particular, there is evidence that transient type-A K\(^+\) channels are modulated by GPCR through an indirect mechanism in central neurons (31, 40). Whether this modulation affects their gating movement may precede or accompany some forms of ion channel changes in channel voltage dependence. Nature 340: 153–156, 1989.


