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# Guanine Nucleotides Modulate Steady-State Inactivation of Voltage-gated Sodium Channels in Frog Olfactory Receptor Neurons

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**Abstract.** The voltage for half-inactivation ($V_{1/2}$) of $Na^+$ currents in frog olfactory receptor neurons (ORNs) under whole-cell voltage clamp showed a shift to more negative potentials with time. Inclusion of guanosine triphosphate (GTP) or its nonhydrolyzable analogue, guanosine-5′-*O*-3-thiotriphosphate (GTP-γ-S), which activates G proteins, in the recording pipette, not only gave a more positive $V_{1/2}$, but also reduced and delayed the negative shift observed in the absence of nucleotides. Guanosine-5′-*O*-2-thiodiphosphate (GDP-β-S), a nonhydrolyzable analogue that prevents the binding of GTP to G proteins, did not affect the $V_{1/2}$ significantly by itself but blocked the positive shift induced by GTP. Since the steady-state activation was not affected, our results indicate that a G protein or a G-protein-dependent process may be important in regulating the steady-state inactivation of $Na^+$ channels in ORNs of the frog.

**Key words:** Voltage-dependent $Na^+$ currents — G-protein modulation — Perforated-patch recording — Whole-cell voltage clamp — Olfaction

## Introduction

G proteins can alter cellular excitability either directly, by opening ion channels, or indirectly by phosphorylating the ion channels via the activation of an enzyme cascade, or both (Brown, 1990; Brown & Birnbaumer, 1990, 1988). There is supporting evidence that G proteins may play a role in modulating the voltage-dependent $Na^+$ channels. In neonatal rat cardiac myocytes β-adrenergic stimulation leads to an inhibition of $Na^+$ currents which was mimicked by intracellular application of GTP or GTP-γ-S (Schubert et al., 1989, 1990). Furthermore, a negative shift in the voltage for half-inactivation ($V_{1/2}$) and a decrease in current amplitudes with time were found. However, it was not determined if the nucleotides had any effects on the negative shift in $V_{1/2}$.

In olfactory receptor neurons (ORNs) of vertebrates, there is much evidence that a G-protein-dependent process is involved in the odorant transduction mechanism (Firestein, Darrow & Shepherd, 1991; Firestein, 1992; Reed, 1992; Ronnett & Snyder, 1992; Anholt, 1993). Most studies on the modulatory effects of G proteins and second messengers (e.g., cyclic AMP) have concentrated on the odorant responses and the nucleotide-dependent channels, and little is known of the possible modulatory effects of G proteins on voltage-dependent channels in this system. In a previous communication we reported that the voltage for half-inactivation ($V_{1/2}$) of the $Na^+$ channels in frog ORNs was about 20 mV more negative than the resting membrane potential (Pun & Gesteland, 1991; *see also* Rajendra, Lynch & Barry, 1991, for rat ORN) and proposed that the $Na^+$ channels may be under some form of modulation. To determine whether the steady-state inactivation of $I_{Na}$ in ORNs is under the influence of G proteins, we examined the effects of various guanine nucleotides and adenine nucleotides on the steady-state inactivation of $I_{Na}$, and the effects of GTP-γ-S on steady-state activation of $I_{Na}$.

## Materials and Methods

Electrophysiological experiments were performed on freshly isolated cells using the EPC-7 amplifier (Adams-List) as described in Pun and Gesteland (1991). Briefly, the epithelia were removed after decapitation and pithing of the frog *Rana pipiens*. The epithelia were then

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pressed gently between two glass coverslips and the cell suspension (in Ringer's solution) was then transferred onto a 35 mm culture dish. ORNs were identified by the presence of motile cilia on the cells.

Recording medium containing the following salts (mM): NaCl, 120; $CaCl_2$, 2; $MgCl_2$, 1; KCl, 3; $CoCl_2$, 3 (to block $Ca^{2+}$ currents); HEPES, 5; pH 7.2; osmolarity, 230–250 mOsm. Some experiments were performed with the addition of 5–10 mM TEACl. Results obtained in the presence and absence of extracellular TEA were similar. Pipette solution was a mixture of (mM) CsCl, 120; TEACl, 20; and NaCl, 10; buffered with HEPES, 5; and EGTA, 1.1. The pH was 7.0 and osmolarity was 215–225 mOsm. Nucleotides and their analogues were introduced into the cell by including these compounds in the pipette solution. Following the establishment of whole-cell recording, these compounds diffuse from the pipette into the cells. Concentrations of the nucleotides were 100 μM, with the exception of ATP, which was used at a concentration of 2 mM.

For perforated-patch recording, the solution was modified to account for the difference in permeability of the pores created by nystatin to cations and anions (Gillis, Pun & Misler, 1991). The composition of the solution was (in mM): CsCl 64; $Cs_2SO_4$, 28; NaCl, 12; EGTA, 0.5; $MgCl_2$, 1; HEPES, 20; pH was adjusted to 7.2 with CsOH and osmolarity was 250 mOsm. The nystatin was dissolved in DMSO at a concentration of 5 mg/100 μl and made fresh daily. Final concentration of nystatin used was 400 μg/ml. Resistances of the pipettes were between 6–12 MΩ when filled with either pipette solution.

The command voltage is nominally used as the membrane potential. Junction potential was usually less than 2 mV, and was corrected as described in Hagiwara and Ohmori (1982) and Barry and Lynch (1991) only if greater than 5 mV. The mean series resistance in whole-cell recording was 24 MΩ with 40% compensation. Thus, the true voltage of the membrane potential could differ by a maximum of 5 mV (maximum mean peak current was 330 pA). The series resistance for the perforated patch studies was much higher, averaging 44 MΩ with similar peak current amplitudes and degree compensation. Therefore, the membrane potentials for this series of studies were corrected before use in the curve-fitting routines. Current records were filtered (between 3–5 kHz depending on sampling time), digitized, stored on a PDP-23 computer and analyzed later.

Steady-state inactivation and activation results were analyzed as described by Hodgkin and Huxley (1952). Steady-state inactivation was obtained as follows: peak inward currents to a depolarizing step of −20 mV were preceded by a 500 msec pulse from −140 to −30 mV. The currents at various prepulse potentials were then normalized to the maximum evoked current. The normalized data were then fit to the Boltzmann equation

$$h_\infty = \{1 + \exp[(V - V_{1/2})/k]\}^{-1} \quad (1)$$

where $V$ is the prepulse potential, $V_{1/2}$ is the voltage at which the elicited current was half-maximal, and $k$ is the slope factor.

Steady-state activation was obtained from the peak evoked currents to depolarizing steps (−60 to +60 mV) from a holding potential of −100 mV. The conductance ($G_{Na}$) at each potential step was calculated using the relation.

$$G_{Na} = I/(V - V_r) \quad (2)$$

where $V$ is the membrane potential, and $V_r$ is the reversal potential obtained from the current-voltage relation curve (about +50 mV). The conductance for each potential was then normalized to the maximal conductance and the data fit to the Boltzmann equation

$$m_\infty = (G_{Na}/G_{max})^{-1/3} = \{1 + \exp[(V_{1/2} - V)/k]\}^{-1} \quad (3)$$

where $V_{1/2}$ is the potential for half-activation, and $k$ is the slope constant.

The steady-state inactivation and activation curves were fit with routines based on a sum of least-squares algorithm. Each data point shown in the plots is the mean value obtained under the same condition for the population of cells studied. Only results collected within the initial two minutes of establishing whole-cell recording were used for constructing the steady-state inactivation curves. Lines drawn through the points are Boltzmann fit through the data points. The $V_{1/2}$ obtained from this curve is similar to the mean $V_{1/2}$ obtained for the same population of cells. The $V_{1/2}$ value reported in the text is the mean $V_{1/2}$ value, and is obtained by averaging the $V_{1/2}$ measured for each cell independently. All data shown are the mean ± SEM. Analysis of variance test was used for statistical comparison between each series of studies and controls. A value of less than 0.05 was considered significant.

## Results

### Steady-State Inactivation

In the absence of nucleotides in the recording pipette solution, the $V_{1/2}$ for steady-state $I_{Na}$ inactivation was −80.5 ± 2.6 mV (mean ± SEM; $n = 10$) with a slope factor of 10.5 ± 0.7 (Fig. 1). These values are similar to those reported earlier (Pun & Gesteland, 1991). The data were obtained within the first 2 min after establishing whole-cell recording. Within the same time frame but with GTP-γ-S (100 μM), a nonhydrolyzable analogue of GTP, in the pipette solution, the $V_{1/2}$ measured was significantly more positive (Fig. 1). The mean value was −61.5 ± 3.0 mV ($n = 9$; $P < 0.01$). The slope factor was also significantly affected (8.2 ± 0.7; $P < 0.05$), indicating that both the voltage dependence of the channels and the sensitivity to changes in voltage were affected. GTP (100 μM) also produced a more positive value of $V_{1/2}$, with a mean of −61.7 ± 3.1 mV ($n = 10$; $P < 0.01$) and a slope factor of 8.3 ± 0.3 ($P < 0.01$; Fig. 1). The $V_{1/2}$ for $I_{Na}$ inactivation in the presence of another analogue, guanosine-5′-*O*-2-thiodiphosphate (GDP-β-S, 100 μM), which blocks the activation of G proteins, was −84.4 ± 2.2 mV ($n = 5$) with a $k$ value of 9.4 ± 1.1 (Fig. 2). These values were not significantly different from the $V_{1/2}$ and $k$ values obtained in the absence of nucleotides. When GDP-β-S and GTP were both present in the pipette solution (each at 100 μM), the $V_{1/2}$ measured was −78.5 ± 1.7 mV ($P > 0.05$; $n = 8$) with a slope factor of 10.5 ± 0.3 ($P > 0.05$; Fig. 2). The presence of an analogue that prevents the binding of GTP blocked the positive $V_{1/2}$ that was observed when GTP was present alone. These results indicate that a guanine nucleotide-mediated and/or a G-protein-mediated event may be responsible for regulating the steady-state inactivation of $Na^+$ channels in frog ORNs.

To determine whether nucleotides other than GTP might also be effective in altering the steady-state inactivation process of the $Na^+$ channels, we studied the effects of ATP and its nonhydrolyzable analogue. ATP-γ-

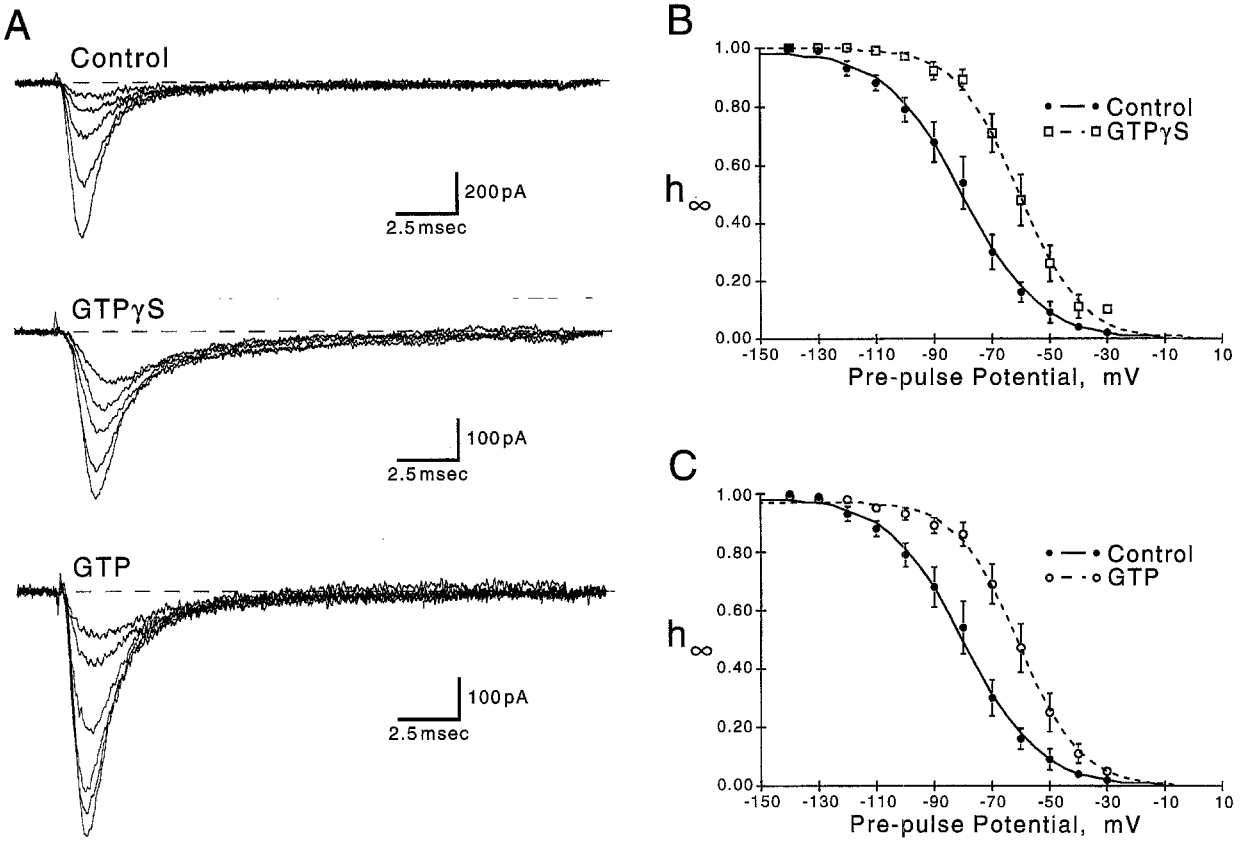


**Fig. 1.** Steady-state inactivation of sodium currents ($I_{Na}$) in frog olfactory receptor neurons measured in the absence and presence of guanine nucleotides. (*A*) Examples of $I_{Na}$ measured from three different cells in the absence of nucleotides (control, top panel), in the presence of GTP-γ-S (middle panel), and in the presence of GTP (lower panel) in the pipette solution. Current tracings shown were obtained from prestep voltages of −110, −90, −80, −70 and −60 mV for control; −120, −80, −70, −60, and −50 mV for GTP-γ-S; −140, −80, −70, −60, −50, and −40 mV for GTP. The respective $V_{1/2}$s for the individual cells were −87 mV (control), −62 mV (GTP-γ-S) and −57 mV (GTP). (B) Steady-state inactivation curves obtained in the absence (control, filled circles) and presence of GTP-γ-S (open squares). (C) Steady-state inactivation curves measured in the absence (control, filled circles) and presence of GTP (open circles). Each data point is the mean value obtained for each population of cells at various prepulse potentials. Results were collected within the initial 2 min after establishing whole-cell recording. Lines drawn through the points are Boltzmann fit through the data points. The $V_{1/2}$ from this curve is similar to the mean of the $V_{1/2}$ for all the cells. The mean $V_{1/2}$ is the $V_{1/2}$ value reported in the text, and is obtained from averaging the $V_{1/2}$ obtained for each cell independently. The nucleotides GTP-γ-S and GTP significantly shifted the $V_{1/2}$ of the steady-state inactivation curves positively. The slope factor was also altered by the nucleotides.

S was also effective in shifting the $V_{1/2}$ to more positive membrane potentials. The mean $V_{1/2}$ for the six cells studied was −67.8 ± 4.1 mV ($P < 0.05$) with a slope factor of 7.8 ± 0.5 ($P < 0.02$; Fig. 3). The results obtained with ATP were more variable. In four of nine cells $V_{1/2}$s were more negative than −80 mV, in the remaining cells their $V_{1/2}$s were more positive than −70 mV. Thus, some cells appeared to be affected by ATP while others were not. The mean $V_{1/2}$ obtained for all nine cells was −74.1 ± 3.2 mV ($P > 0.05$) with a slope constant of 10.6 ± 0.8 ($P > 0.05$; Fig. 3).

### Time-dependent Shift

We noticed that with time there was a reduction in peak current amplitudes that was accompanied by a shift in the $V_{1/2}$ of the steady-state inactivation towards more negative potentials. The reduction in peak amplitudes ranged between 30–50% and is reminiscent of the "run-down" described for $Ca^{2+}$ currents in other excitable cells (*see* Belles et al., 1988). The magnitude of the negative shift was about 10–15 mV and occurred over a 10 min period although the duration was somewhat variable (Fig. 4*A*). Negative shifts in activation and inactivation potentials have been described for $I_{Na}$ in bovine adrenal chromaffin cells (Fenwick, Marty & Neher, 1982), $GH_3$ (Fernandez, Fox & Krasne, 1984) and cardiac cells (Schubert et al., 1990; Hanck & Sheets, 1992). In frog ORNs, inclusion of nucleotides in the recording solution delayed or prevented the negative shift of $I_{Na}$ inactivation. Figure 4 shows examples of the effects of GTP-γ-S (Fig. 4*B*), and GTP (Fig. 4*C*) on the $V_{1/2}$ of $I_{Na}$ inactivation. The re-

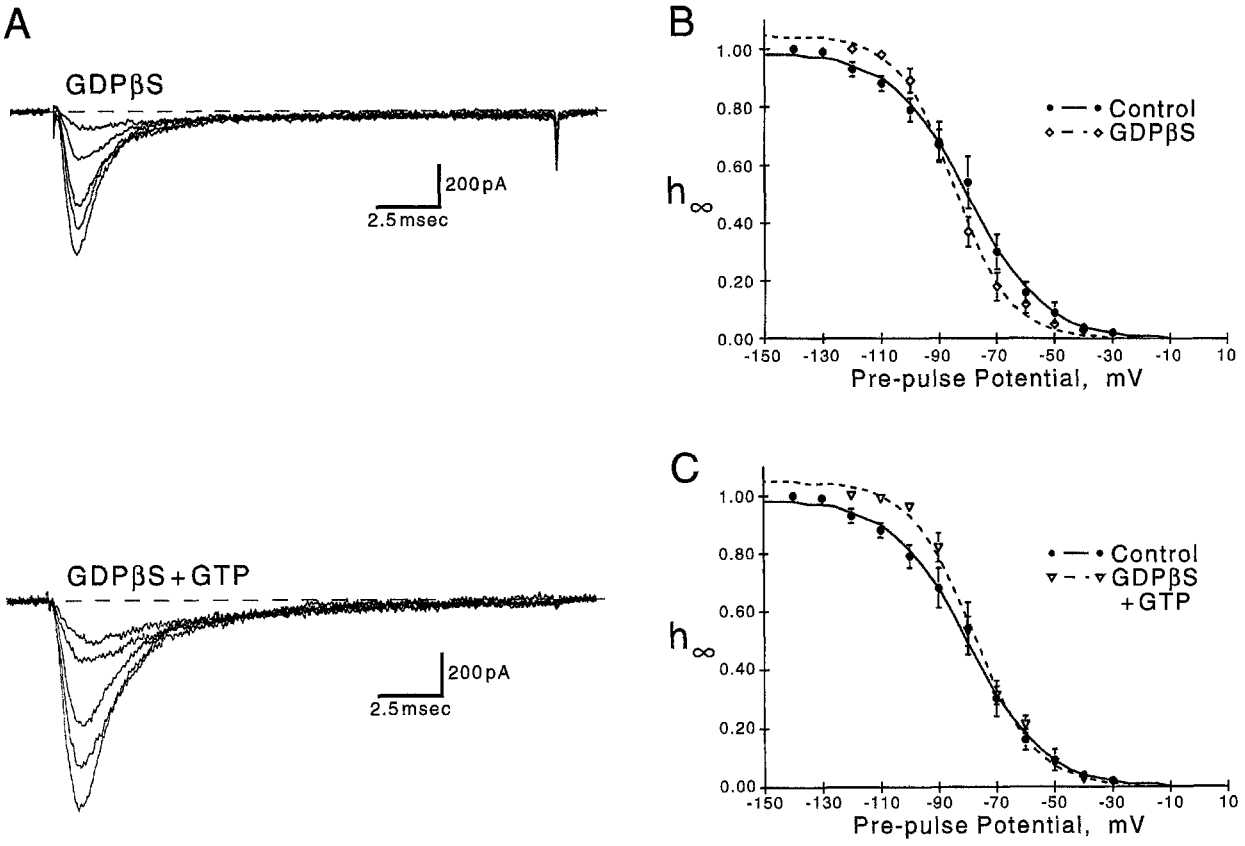


**Fig. 2.** Steady-state inactivation curves of $I_{Na}$ from frog olfactory receptor neurons measured in the presence of an inactive guanine nucleotide, GDP-β-S. (*A*) Current tracings obtained from two different cells with GDP-β-S in the pipette solution (top panel) and with GTP plus GDP-β-S in the pipette solution (lower panel). Currents were recorded from presteps of −110, −100, −90, −80 and −70 mV for GDP-β-S and −110, −90, −80, −70 and −60 mV for GTP + GDP-β-S. The $V_{1/2}$ values obtained with the illustrated cells were −88 and −78 mmV for GDP-β-S and GTP with GDP-β-S respectively. (*B*) Steady-state inactivation curves in the absence (control, filled circles) and presence of GDP-β-S (open diamonds). (*C*) Steady-state inactivation curves in the absence (control, filled circles) and in the presence of GDP-β-S plus GTP (open triangles). GDP-β-S itself did not affect the $V_{1/2}$ and apparently blocked the effects of GTP.

corded $V_{1/2}$ values were more positive in the presence of the guanine nucleotides, and over a duration of 10 min, the magnitude of the negative shift in the $V_{1/2}$ observed in the absence of nucleotides was greatly reduced. In most cases there still was a small shift of about 5 mV.

To determine whether the negative shift in the $V_{1/2}$ measured in the absence of nucleotides could possibly be related to a washout phenomenon, we used the perforated-patch method (Horn & Marty, 1988; Gillis et al., 1992) to examine the stability of recording with time. Under this mode of recording where the cytoplasmic contents are not intermixed with the recording solution, the $V_{1/2}$ was essentially unchanged for 30 min (Fig. 5*B*). Moreover, the value of $V_{1/2}$ is similar to that obtained in the presence of the guanine nucleotides (−61.6 ± 3.9, $n$ = 5 with a slope factor of 8.9 ± 0.5) and significantly different from that measured in the absence of nucleotides (Fig. 5*C*).

### I-V Relation and Activation

To determine if the positive shift in the $V_{1/2}$ of the steady-state inactivation could result from a nonspecific charge screening effect (Perozo & Bezanilla, 1990), we examined the effects of GTP-γ-S on the activation process. GTP-γ-S was chosen because of the consistency of its effect on the steady-state inactivation process. Figure 6 shows the results obtained from two separate populations of cells recorded in the absence and presence of GTP-γ-S. Figure 6*B* illustrates the normalized peak current-voltage relation in the absence and presence of GTP-γ-S. There was an apparent positive shift in the peak current-voltage relation of about 5 mV. When the steady-state activation was compared (Fig. 6*C*), there was a mean change in the potential for half-activation ($V_a$) of about 7 mV towards positive potentials (absence of nucleotides, −48.5 ± 2.9 mV, $n$ = 6; GTP-γ-S, −41.1 ± 2.4 mV, $n$ = 6).

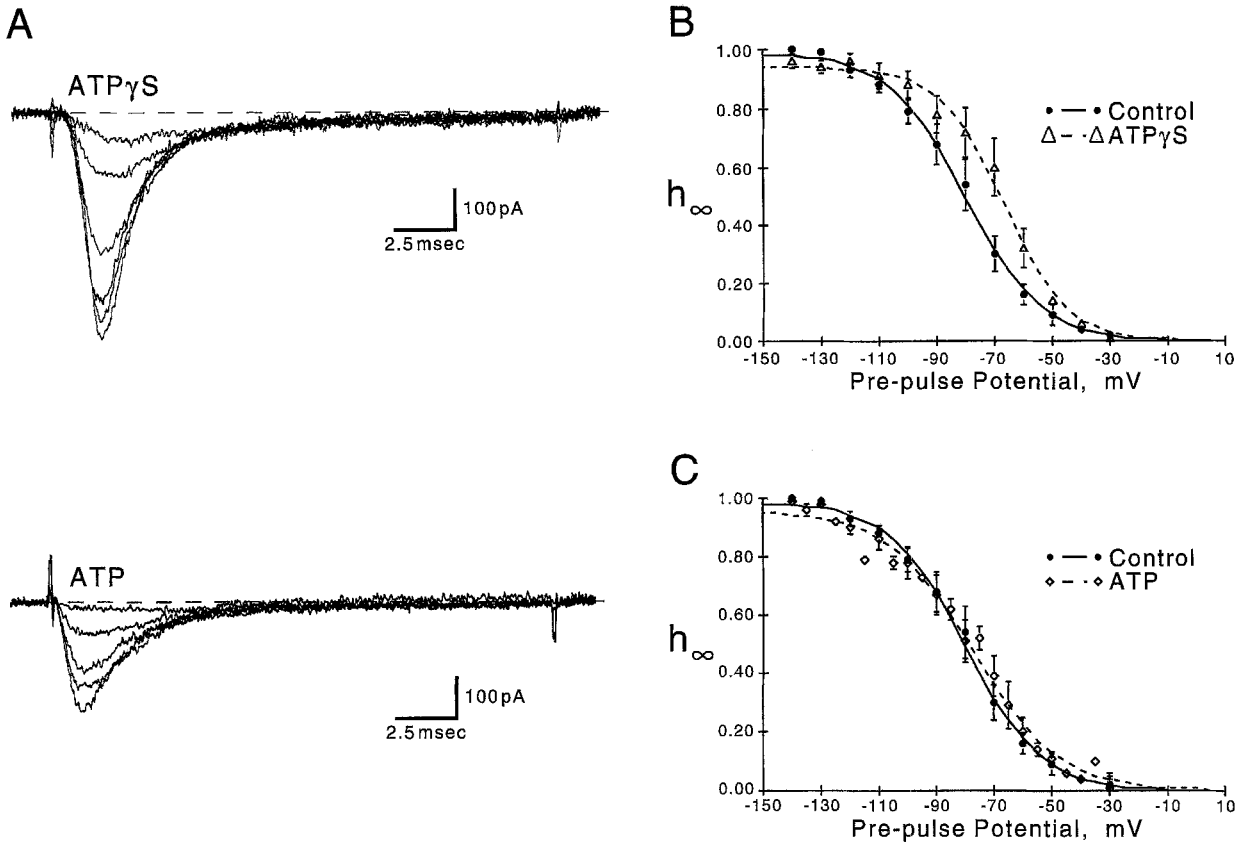


**Fig. 3.** Steady-state inactivation curves of $I_{Na}$ from frog olfactory receptor neurons measured in the absence and presence of adenine nucleotides. (*A*) Current tracings obtained from two different cells in the presence of ATP-γ-S (top panel) and ATP (lower panel). Currents were recorded from presteps of −120, −100, −80, −70, −60, and −50 mV for ATP-γ-S; and −140, −115, −90, −70, and −50 mV for ATP. The $V_{1/2}$ values obtained with the illustrated cells were −66 and −83 mV for ATP-γ-S and ATP, respectively. (*B*) Steady-state inactivation curves in the absence (control, filled circles) and presence of ATP-γ-S (open triangles). (*C*) Steady-state inactivation curves in the absence (control, filled circles) and presence of ATP (open diamonds). ATP-γ-S significantly shifted the $V_{1/2}$ value to more positive voltages, but ATP did not have an effect.

The 7 mV difference measured is much less than the 20 mV shift observed for the shift in $V_{1/2}$ for the steady-state inactivation, and was not statistically significant ($P > 0.05$). There also was no difference in the slope constants between absence and presence of GTP-γ-S (control, 7.1 ± 0.4; GTP-γ-S, 7.1 ± 0.7).

## Discussion

It is well established that the voltage-dependent ion channels in excitable cells can be influenced or modulated by second messengers, such as $Ca^{2+}$, and small biochemical compounds generated intracellularly, such as cyclic AMP. Modulation of ion channels can lead to an alteration in the activities of these channels and, subsequently, a change in the membrane electrical properties and behavior of the cell. In previous studies, we (Pun & Gesteland, 1991) and others (Rajendra et al., 1992) have shown that voltage-dependent $Na^+$ channels in ORNs have a negative $V_{1/2}$ of inactivation making them not available for generation of action potentials. To account for the generation of action potentials in ORNs following odorant stimulation, we examined whether the voltage-dependent $Na^+$ channels in frog ORNs are under any form of modulation. By comparing the steady-state activation and inactivation processes in the absence and presence of various nucleotides and their analogues, we obtained evidence that the steady-state inactivation of $I_{Na}$ is regulated by guanine nucleotides and perhaps a G-protein-related mechanism.

In the absence of nucleotides, the mean $V_{1/2}$ of $I_{Na}$ measured was about −80 mV, whereas the mean $V_{1/2}$ measured in the presence of GTP or GTP-γ-S was about −60 mV. Since the more positive values measured in the presence of the nucleotides are similar to those measured under the perforated-patch method, it appears that the

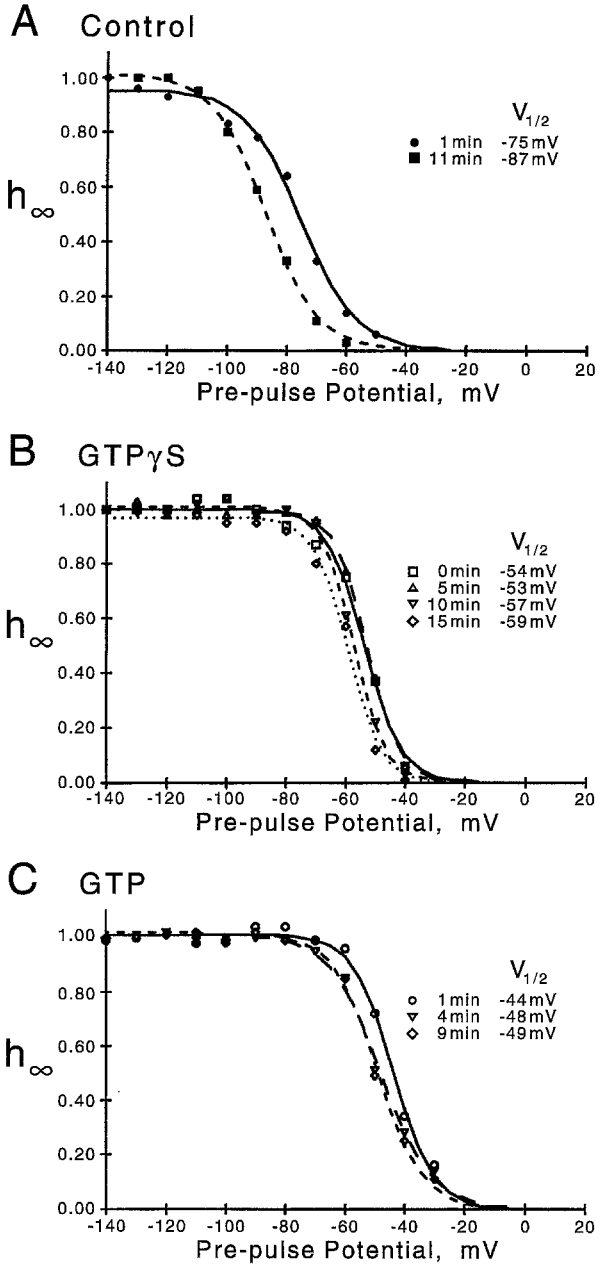


**Fig. 4.** GTP and GTP-γ-S delayed and reduced the negative shift of steady-state $I_{Na}$ inactivation. Illustrations are from three different cells. (*A*) In the absence of nucleotides (control), the steady-state inactivation curve shifted to more negative potentials ($V_{1/2}$ changed −12 mV) in about 10 min. Zero time denotes recordings made between 0 and 1 min after establishing whole-cell recording and after adjustments for $C_{slow}$, series resistance and $R_S$ compensation. Inclusion of GTP-γ-S (*B*), GTP (*C*), or ATP-γ-S (*data not shown*) stabilized the steady-state inactivation and reduced the magnitude of the negative shift in $V_{1/2}$ to less than 5 mV.

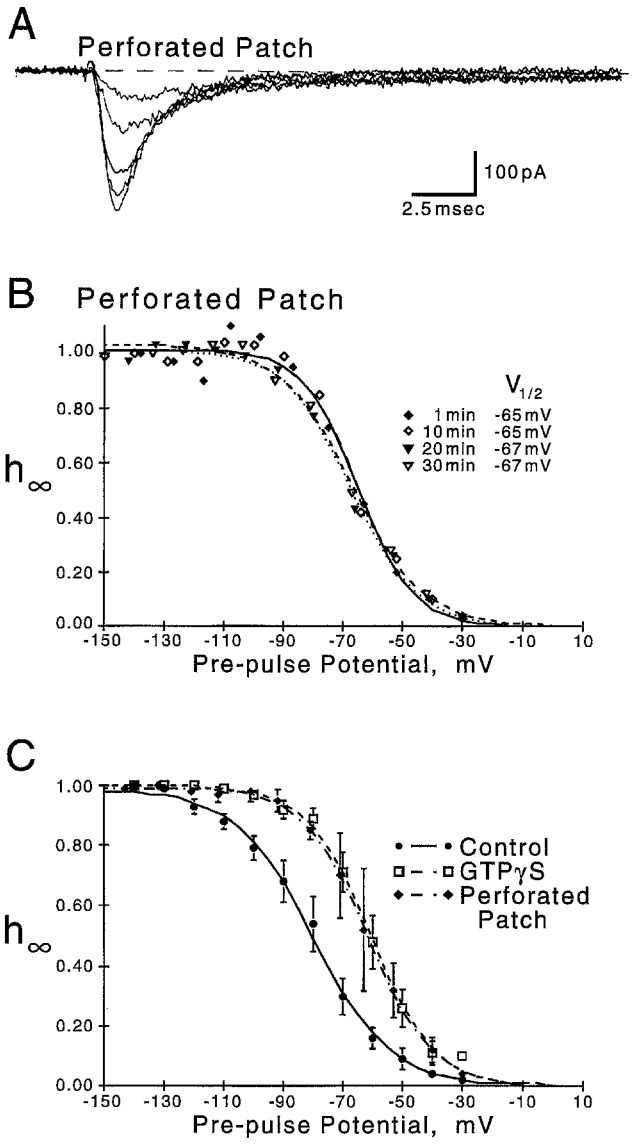


**Fig. 5.** Steady-state inactivation of $I_{Na}$ from olfactory receptor neurons obtained with the perforated-patch (nystatin) technique. (*A*) Current tracings obtained from a cell. Currents illustrated were from presteps of −137, −117, −75, −63, and −52 mV. (*B*) Steady-state inactivation curves measured at 1 min (filled diamonds), 10 min (open diamonds), 20 min (filled inverted triangles), and 30 min (open inverted triangles) of continuous recording. Voltages used in the curve-fit have been corrected for uncompensated access resistance. There was little or no change in the $V_{1/2}$ measured throughout the duration of recording. (*C*) Steady-state inactivation curves with whole-cell recordings from control (filled circles), in the presence of GTP-γ-S (open squares), and perforated-patch recording (filled diamonds). The $V_{1/2}$ measured under perforated-patch was similar to that measured for GTP-γ-S.

negative value measured in the absence of nucleotides results from a loss of intracellular substances, or washout, that may be functionally important in regulating $I_{Na}$ inactivation. Although the measurements were made within the first minutes of establishing whole-cell recording, the small size of these neurons (membrane capacitance 4–6 pF) made them particularly susceptible to washout. In addition, inclusion in the pipette solution of the analogue GDP-β-S, which blocks the binding of GTP

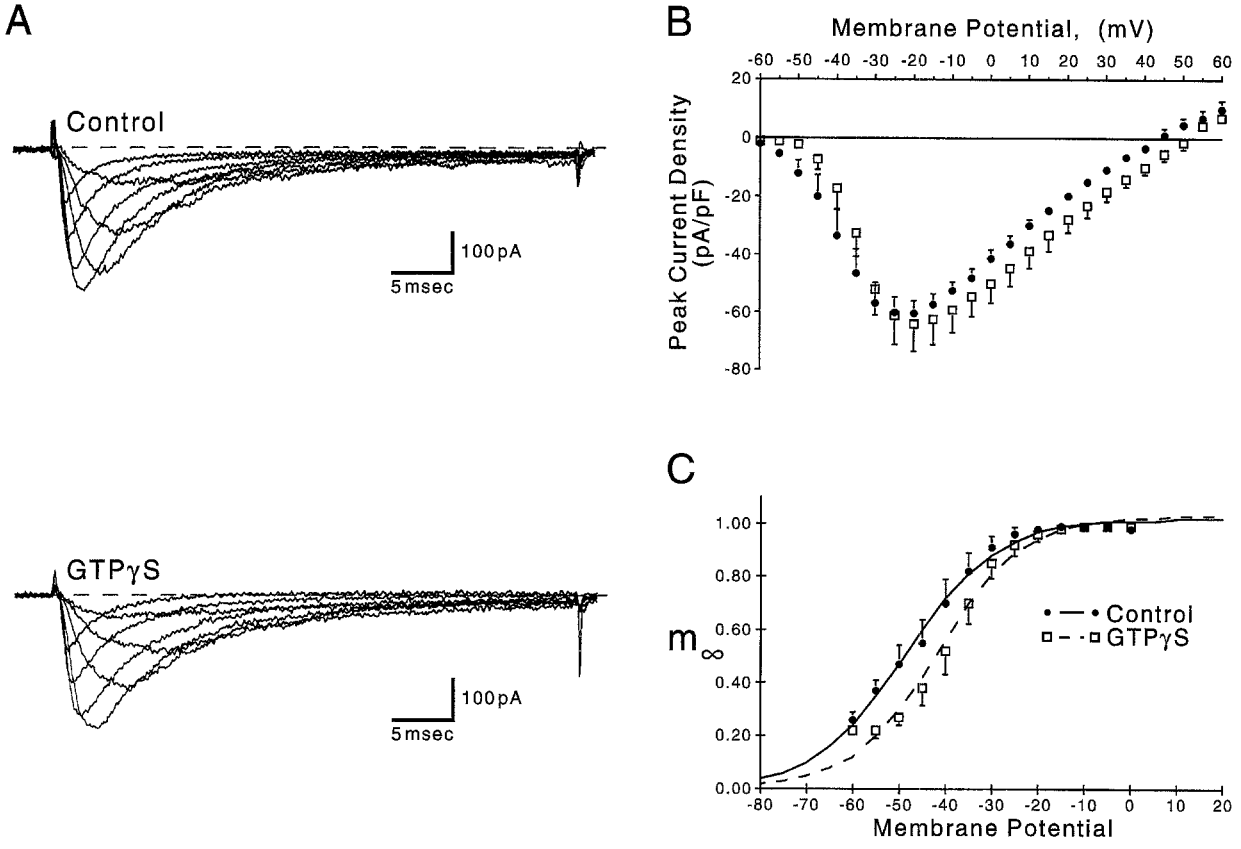


**Fig. 6.** Lack of effect of GTP-γ-S on steady-state $I_{Na}$ activation in frog olfactory receptor neurons. (*A*) Current tracings from two different cells obtained in the absence (control, top panel) and in the presence of GTP-γ-S (lower panel). Currents shown were elicited from a holding potential of −100 mV to depolarizing steps of −40, −35, −30, −20, −10, +5 and +20 mV for both illustrations. The $V_{1/2}$ values were −42 and −41 mV for control and in the presence of GTP-γ-S, respectively. (*B*) Current-voltage relation in the absence (control, filled circles) and presence of GTP-γ-S (open squares). Current amplitudes were normalized to membrane capacitance to account for variation in cell size. GTP-γ-S slightly shifted the peak current-voltage relation of voltage-dependent $Na^+$ currents to more positive potentials. (*C*) Steady-state activation curves were derived as outlined in Materials and Methods. GTP-γ-S did not have any effect on the steady-state activation curves of $I_{Na}$ in frog olfactory receptor neurons. Data were obtained from six cells in each category.

to G proteins, gave a $V_{1/2}$ value similar to that obtained under control conditions and prevented the effects of GTP. Taken together, these results strongly imply that a guanine nucleotide or a guanine nucleotide-dependent process is involved in the regulation of steady-state inactivation of $Na^+$ channels in frog ORNs. Whether the effects of the nucleotides observed here are mediated by a direct action of G proteins on the channels, or a cyclic nucleotide-dependent phosphorylation process as a result of G-protein activation, or a combination of both mechanisms, is not established in our studies.

There is precedent that voltage-dependent $Na^+$ channels are under the influence of G proteins and phosphorylation processes. In neonatal rat cardiac myocytes, activation of G proteins by isoprenaline and GTP-γ-S inhibited $Na^+$ channels by shifting the steady-state inactivation curve to negative potentials and reducing the probability of channel opening (Schubert et al., 1989, 1990). In contrast, isoprenaline and GTP-γ-S enhanced $Na^+$ currents in rabbit cardiac myocytes (Matsuda, Lee & Shibata, 1992). Phosphorylation of the voltage-dependent $Na^+$ channels in cardiac myocytes by protein kinase A (Sorbbera & Morad, 1991) or expressed brain $Na^+$ channels by protein kinase C (Dascal & Lothan, 1991; Numann, Catterall & Scheuer, 1991; West et al., 1991) led to inhibition of the $Na^+$ currents. Earlier, Catterall's group showed that coexpression of the α-subunit of the rat brain $Na^+$ channels with low molecular weight messenger RNAs from rat brain led to the modification of $I_{Na}$ inactivation kinetics, and suggested that perhaps the low molecular weight messenger RNAs code of the β-subunits (Auld et al., 1988). More recently, they demonstrated that coexpression of the α- and $\beta_1$-subunits not only increased the amplitudes of peak $I_{Na}$, but also accelerated the decay kinetics and altered the steady-state inactivation. In their experiments, however, steady-state inactivation was shifted to more negative potentials (Isom et al., 1992). When a longer prepulse was used, a positive shift in the $V_{1/2}$ was recorded with the coexpressed $Na^+$ channel (Kyle et al., 1993). If the shift in

the $V_{1/2}$ of $I_{Na}$ that we measured for frog ORNs can be attributed to a β-subunit effect, it is tempting to speculate that perhaps the guanine nucleotides interact with β-subunits to stabilize the $Na^+$ channel.

We also observed similar shifts in the $V_{1/2}$ of $I_{Na}$ with adenine nucleotides. The action of ATP on the steady-state inactivation of $I_{Na}$ was not consistent, but the analogue ATP-γ-S was effective. ATP-γ-S was also found to mimic GTP-γ-S in inhibiting $Na^+$ currents in cardiac myocytes (Schubert et al., 1989). One possible explanation for its effectiveness is the conversion of ATP-γ-S to GTP-γ-S by intracellular enzymes (Otero, Breitwieser & Szabo, 1988). Another possibility is that there is an unknown process in ORNs, perhaps a dephosphorylation step which involves a phosphatase, that may be important in terminating the modulatory effect. The variability of the actions of ATP could reflect a difference in the resting levels of phosphatase activity in ORNs. If this hypothesis is true, it implies that guanine nucleotides and adenine nucleotides act at different steps along a cascade of events, e.g., GTP-γ-S and GTP act by directly activating G proteins, whereas ATP-γ-S acts by phosphorylation of an enzyme or the $Na^+$ channel.

Under whole-cell recording and in the absence of nucleotides, there also was a tendency of the $V_{1/2}$ of $I_{Na}$ to shift towards more negative potentials with time. This negative shift was reduced when GTP or GTP-γ-S was present in the pipette solution, and was absent when the recordings were made with the perforated-patch method. The negative shift in potential for $I_{Na}$ with time under whole-cell recording has been attributed to a change in Donnan equilibrium as a result of exchange of the pipette solution with intracellular contents (Fernandez et al., 1984). Our results indicate that in addition to this re-equilibration effect, there may be an additional nucleotide-dependent process(es) which plays a role in causing the negative shift. A small shift in the *I-V* relation and the steady-state activation to more positive potentials (about 7 mV) was also observed in the presence of GTP-γ-S, though this change was not significantly different from control. Taken together, these results indicate that the actions of guanine nucleotides are specific for regulating the $Na^+$ channel inactivation process in ORNs of the frog.

One puzzling finding was that we did not record an alteration of holding current or detect a current response when GTP-γ-S was present in the pipette solution. If the odorant response is mediated via a cyclic nucleotide-gated current, it is to be expected that activation of G proteins will lead to increased production of cyclic AMP by adenylate cyclase, activation of the cyclic nucleotide-gated currents, and an "odorant response." The presence of cyclic AMP (Nakamura & Gold, 1987) or GTP-γ-S plus ATP (Kleene, Gesteland & Bryant, 1994) has been shown to evoke currents in recordings from isolated frog cilia. The voltage that we used for the prestep is rather negative, and the conductance generated by cyclic AMP at these voltages is low when external divalent cations are present (Dhallan et al., 1990; Frings, Lynch & Lindemann, 1992; Zufall & Firestein, 1993). Also, we used subtraction to remove the leak and holding currents before the data were collected. If there had been an activation of the cyclic nucleotide-gated channels we would have subtracted out the current.

A recent report indicated that in ORNs of catfish, mixtures of amino acids can shift activation and inactivation of $I_{Na}$ without generation of detectable currents (Ivanova & Caprio, 1992; *see also* Dionne, 1992, for silent modulation). Whether odorant stimulation in frog ORNs produces similar shifts is not established. If indeed odorant stimulation can shift the activation and inactivation curves, the parallel mechanisms in altering excitability, i.e., induction of an odorant current as well as altering voltage-dependent channels, are comparable to those described for G-protein action in cardiac myocytes following β-adrenergic receptor stimulation (*see* Brown & Birnbaumer, 1988; 1990; Brown, 1990). The presence of both direct (modulation of $Na^+$ channels) and indirect (generation of cyclic AMP which activates a channel) actions of G protein upon activation would ensure that once the mechanisms for odorant detection are triggered, transmission of the signal to the brain is highly probable.

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