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Modulation of brain Na^+ channels by a G-protein-coupled pathway

(whole-cell voltage clamp/electrical excitability/pertussis toxin)

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ABSTRACT **Na^+ channels in acutely dissociated rat hippocampal neurons and in Chinese hamster ovary (CHO) cells transfected with a cDNA encoding the α subunit of rat brain type IIA Na^+ channel (CNaIIA-1 cells) are modulated by guanine nucleotide binding protein (G protein)-coupled pathways under conditions of whole-cell voltage clamp. Activation of G proteins by 0.2–0.5 mM guanosine 5′-[γ-thio]triphosphate (GTP[γS]), a nonhydrolyzable GTP analog, increased Na^+ currents recorded in both cell types. The increase in current amplitude was caused by an 8- to 10-mV negative shift in the voltage dependence of both activation and inactivation. The effects of G-protein activators were blocked by treatment with pertussis toxin or guanosine 5′-[β-thio]diphosphate (GDP[βS]), a nonhydrolyzable GDP analog, but not by cholera toxin. GDP[βS] (2 mM) alone had effects opposite those of GTP[γS], shifting Na^+-channel gating 8–10 mV toward more-positive membrane potentials and suggesting that basal activation of G proteins in the absence of stimulation is sufficient to modulate Na^+ channels. In CNaIIA-1 cells, thrombin, which activates pertussis toxin-sensitive G proteins in CHO cells, caused a further negative shift in the voltage dependence of Na^+-channel activation and inactivation beyond that observed with GTP alone. The results in CNaIIA-1 cells indicate that the α subunit of the Na^+ channel alone is sufficient to mediate G protein effects on gating. The modulation of Na^+ channels via a G-protein-coupled pathway acting on Na^+-channel α subunits may regulate electrical excitability through integration of different G-protein-coupled synaptic inputs.**

Voltage-sensitive Na^+ channels are responsible for initiation and conduction of the neuronal action potential and thus play a central role in nervous system function. The Na^+ channel from rat brain is a heterotrimeric complex of α (260 kDa), β1 (36 kDa), and β2 (33 kDa) subunits. Three α-subunit genes, designated types I, II, and III, are expressed in rat brain, and the type II subunit is present in alternatively spliced forms designated type II and type IIA (1). Expression of the α subunit alone is sufficient to form functional voltage-gated Na^+ channels in *Xenopus* oocytes (2–4) or Chinese hamster ovary (CHO) cells (5, 6). Na^+ channels in cell bodies and axon initial segments determine the threshold for action potential generation and affect the duration and frequency of repetitive neuronal firing. Na^+ channels in nerve terminals (7, 8) can potentially influence neurotransmitter release from presynaptic nerve endings. These integrative functions of Na^+ channels are likely to be subject to neuromodulation. One possible mechanism is via protein phosphorylation. Neuronal Na^+ channels are modulated by phosphorylation in intact rat brain neurons and *Xenopus* oocytes expressing whole brain mRNA as well as in *Xenopus* oocytes and CHO cells expressing the rat brain type IIA α subunit (9–13). Another likely mechanism is through guanine nucleotide binding proteins (G proteins).

G proteins couple hormone and neurotransmitter receptors to effectors including neuronal K^+ and Ca^{2+} channels through multiple pathways that may involve membrane-delimited G-protein–ion channel interactions or diffusible second messengers (for review, see refs. 14–16). In heart, the opening of an inwardly rectifying K^+ channel by muscarinic acetylcholine receptors requires GTP and a pertussis toxin (PTX)-sensitive G protein (17–20), and β-adrenergic receptors modulate Na^+ channels by parallel pathways involving cAMP-dependent protein phosphorylation and possibly direct interaction with G_s (21, 22). Brain Na^+ channels expressed in *Xenopus* oocytes can be inhibited by guanosine 5′-[γ-thio]triphosphate (GTP[γS]) (23). In this report we show that activation of brain Na^+ channels is enhanced by activation of G proteins in both hippocampal neurons and in CHO cells expressing the α subunit of the type IIA Na^+ channel (ref. 6; CNaIIA-1 cells). The results suggest that activation of G proteins in brain in response to neurotransmitters can modulate Na^+-channel function and alter the excitability of neurons.

EXPERIMENTAL PROCEDURES

Cell Culture. Hippocampal neurons were acutely dissociated from 7- to 21-day-old Sprague–Dawley rats as described by Kuo and Bean (24). CNaIIA-1 cells were maintained as described (6).

Electrophysiological Recording. Whole-cell voltage-clamp recordings (25) were obtained as described (6) using micropipettes with resistances of 1–2 MΩ in our internal solutions. Capacity transients were canceled and series resistance was compensated (>80%) using the internal voltage-clamp circuitry. Remaining linear capacity transients as well as leakage currents were subtracted by the P/4 procedure. Conductance–voltage (g–V) relationships were calculated from peak current vs. voltage (I–V) relationships according to $g = I/(V - V_r)$, where I is the peak current measured at voltage V, and V_r is the measured reversal potential. Normalized conductance–voltage relationships and inactivation curves were fit with a Boltzmann relation, $1/(1 + \exp[(V - V_{1/2})/k])$, where $V_{1/2}$ is the voltage of half activation and k is a slope factor. Pooled data are reported as means ± SEM in the text and figure legends.

For recording from CNaIIA-1 cells, the external solution contained 140 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 10 mM Hepes (pH 7.4) with NaOH. The intracellular solution contained 140 mM potassium aspartate, 5 mM NaCl, 3 mM $MgCl_2$, 10 mM Hepes, and 2.5 mM EGTA (pH 7.4 with KOH). To reduce errors due to the large Na^+ currents in acutely dissociated hippocampal neurons, extracellular [Na^+] was reduced to 20 mM. The external solution contained 120 mM tetraethylammonium chloride (TEA-Cl), 20 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Hepes, 5 mM

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Abbreviations: GTP[γS], guanosine 5′-[γ-thio]triphosphate; GDP[βS], guanosine 5′-[β-thio]diphosphate; PTX, pertussis toxin; CTX, cholera toxin; G protein, guanine nucleotide binding protein.

4-aminopyridine, and 0.1 mM $CdCl_2$ (pH 7.4 with TEA-OH). For recording from hippocampal neurons the intracellular solution contained 120 mM cesium aspartate, 5 mM NaCl, 2 mM $MgCl_2$, 10 mM Hepes, 10 mM EGTA, and 4 mM MgATP (pH 7.3 with CsOH). MgATP (Sigma), guanine nucleotides (Sigma), thrombin (Calbiochem), and cholecystokinin (Sigma) were stored as concentrated aliquots at −70°C and diluted to final concentrations in recording solutions on the day of the experiment. PTX, A-protomer of PTX, and cholera toxin (CTX) (PTX and CTX were from List Biological Laboratories, Campbell, CA) were reconstituted in water and stored at 4°C. Toxin treatment of acutely dissociated hippocampal neurons was accomplished by inclusion of A-protomer of PTX (1 μg/ml) and 10 mM NAD in the pipette solution. CNaIIA-1 cells were incubated overnight with whole PTX (0.2 μg/ml) or CTX (0.2 μg/ml) before recording.

RESULTS AND DISCUSSION

Increased Na^+ Currents Due to Activation of PTX-Sensitive G Proteins. After establishing the whole-cell voltage-clamp configuration, time-dependent increases in Na^+-current amplitude were observed in hippocampal neurons without adding guanine nucleotides to the intracellular solution (Fig. 1*A Left*; mean increase at −20 mV = 80% ± 17%; n = 5). The increase in current was usually complete within 15 min. It results primarily from reversal of slow inactivation at the holding potential of −70 mV, which is more negative than the resting membrane potential of the cells. For hippocampal neurons, addition of the nonhydrolyzable GTP analog GTP[γS] (0.2–0.5 mM) to the intracellular solution caused an additional time-dependent increase in Na^+ current (Fig. 1*A Center*; mean increase = 216% ± 22%; n = 7). Effects of high GTP concentrations (>2 mM) were similar to those of GTP[γS] (data not shown). Similarly, CNaIIA-1 cells in which 1 mM GTP was present in the intracellular solution had much larger increases in Na^+ current (Fig. 1*B Center*; mean increase at −10 mV = 293% ± 55%; n = 5) than in control cells (Fig. 1*B Left*; mean increase = 62% ± 15%; n = 4). GTP[γS] (0.5 mM) had effects similar to those of GTP in CNaIIA-1 cells.

PTX and CTX were used to identify the class of G proteins involved in this Na^+-channel modulation. PTX acts on G_i and G_o to block GTP exchange by NAD-dependent ADP-ribosylation of their α subunits and thereby reduces the level of the activated form of these G proteins (26). When the

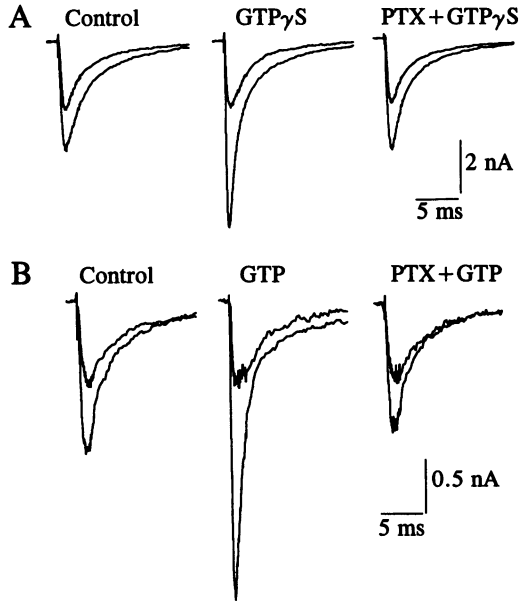

FIG. 1. Activation of PTX-sensitive G proteins increases Na^+ current in acutely dissociated hippocampal neurons and CNaIIA-1 cells. Na^+ currents from individual cells were elicited either immediately after breaking the membrane to achieve the whole-cell configuration (smaller traces) or 15 min later (larger traces). (*A*) Na^+ currents from hippocampal neurons recorded during 20-ms pulses to −20 mV after 50-ms-long prepulses to −110 mV from a holding potential of −70 mV. Pairs of currents are shown with control pipette solution (*Left*), with the addition of 0.5 mM GTP[γS] to the pipette (*Center*), or with GTP[γS] plus 1 μg of the A-protomer of PTX per ml (*Right*). Pair of control traces has been scaled to 0.8 times original current magnitude so that all initial Na^+ currents are equivalent in size. (*B*) Similar experiments in CNaIIA-1 cells. Currents were recorded during 20-ms pulses to −10 mV after 50-ms prepulses to −100 mV from a holding potential of −80 mV. Pairs of current traces are shown with control pipette solution (*Left*), with the addition of 1 mM GTP to the pipette (*Center*), or with 1 mM GTP after overnight treatment of the cells of 0.2 μg of PTX per ml (*Right*). Pair of GTP traces has been scaled to 1.4 times original current magnitude so that all initial Na^+ currents are equivalent in size.

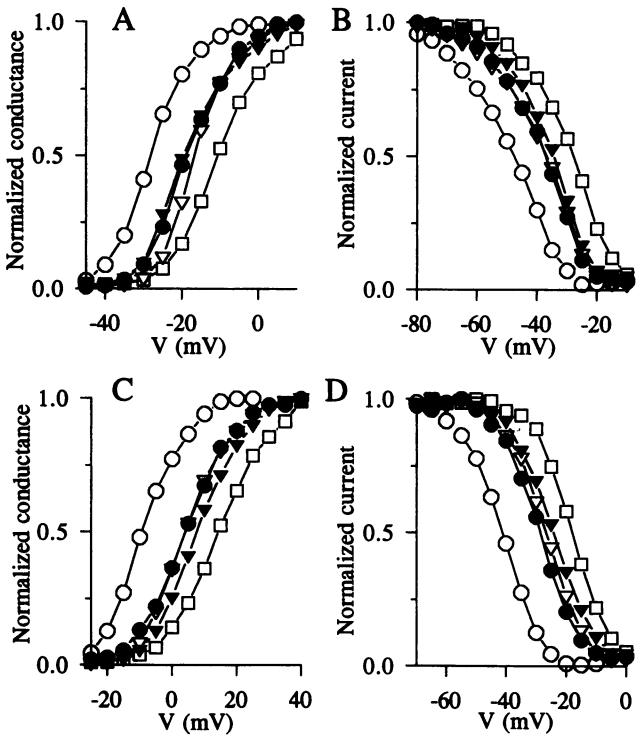

FIG. 2. G-protein activation shifts the voltage dependence of Na^+-channel kinetics toward negative membrane potentials. Conductance–voltage and inactivation curves were determined with the indicated additions to the pipette solution. Each curve is from an individual cell recorded 10–15 min after achieving the whole-cell configuration. Sample cells were chosen with half-activation or inactivation voltages near the mean for cells in a particular condition (Table 1). (*A*) Normalized conductance–voltage relationships for each of the indicated pipette solutions from hippocampal neurons with control pipette solution (●) or with pipette solutions containing GTP[γS] (○), GTP[γS] plus the A-protomer of PTX (▽), GTP[γS] plus GDP[βS] (▼), or GDP[βS] alone (□). (*B*) Normalized steady-state inactivation curves from hippocampal neurons generated using 80-ms prepulses of variable potential followed by test pulses to 0 mV. Symbols refer to the same conditions as in *A*. (*C*) Normalized conductance–voltage relationships for each of the indicated conditions from CNaIIA-1 cells with control pipette solution (●) or with pipette solutions containing GTP (○), GTP after overnight treatment with PTX (▽), GTP plus GDP[βS] (▼), or GDP[βS] alone (□). (*D*) Normalized steady-state inactivation curves from CNaIIA-1 cells generated using 80-ms-long prepulses of variable potential followed by test pulses to +15 mV. Symbols refer to the same conditions as in *C*.

active A-protomer of PTX (1 μg/ml) was included in the pipette solution along with 10 mM NAD^+, GTP[γS] failed to increase current amplitude further than was observed in control hippocampal neurons (Fig. 1*A Right*; mean increase = 68% ± 9%; $n = 6$). Similarly, the GTP-dependent increase in Na^+ current was reduced if the CNaIIA-1 cells were preincubated overnight with PTX (0.2 μg/ml) before recording (Fig. 1*B Right*; mean increase = 93% ± 12%; $n = 7$). CTX blocks the GTPase activity of G_s α subunits, locking them in an activated form. In contrast to PTX, pretreatment of CNaIIA-1 cells with CTX (0.2 μg/ml) overnight had no effect on the increase in Na^+ current caused by GTP (data not shown). These results indicate that PTX-sensitive G proteins such as G_o or G_i modulate Na^+ channels and that this modulation occurs through interaction with the α subunit of the Na^+ channel, which is expressed alone in CNaIIA-1 cells.

G-Protein Activation Shifts the Voltage Dependence of Na^+-Channel Activation and Inactivation Toward Negative Membrane Potentials. Maximum conductance increased with time in most cells (see Fig. 1), but the magnitude of the increase was unaffected by inclusion of GTP or GTP[γS] in the pipette or by treatment with PTX. The additional increase in Na^+ current recorded at −10 or −20 mV with GTP[γS] or GTP-containing internal solutions was caused by a negative shift in the voltage dependence of activation and inactivation for both hippocampal neurons and CNaIIA-1 cells (Fig. 2). Conductance–voltage relationships were determined from current–voltage relationships in multiple cells (Table 1). Mean values for half-maximal steady-state activation ($V_{1/2}$) in the presence of GTP or GTP[γS] were 8.9 mV more negative than control in hippocampal neurons and 10.6 mV more negative in CNaIIA-1 cells (Fig. 2 *A* and *C*; Table 1). PTX prevented the negative shift in the voltage dependence of activation caused by GTP or GTP[γS] (Fig. 2 *A* and *C*; Table 1).

The voltage dependence of steady-state inactivation determined with 80-ms prepulses was also shifted toward negative membrane potentials by ≈8 mV in the presence of GTP and GTP[γS] for both cell types (Fig. 2 *B* and *D*; Table 1). As for activation, PTX prevented the shift in the voltage dependence of steady-state inactivation (Fig. 2 *B* and *D*).

Time Dependence of G-Protein Effects. Time-dependent negative shifts in the voltage dependence of Na^+-channel activation occur in whole-cell recordings from many cell types beginning as soon as the electrode breaks through the plasma membrane (27, 28). However, with control intracellular solution only 2- to 3-mV shifts in the voltage dependence of Na^+-channel activation were observed during the 15 min after breaking the plasma membrane in hippocampal neurons (Fig. 3*A*; Table 1) and in CNaIIA-1 cells (Fig. 3*B*; Table 1). In contrast, 10- to 12-mV shifts were observed in GTP[γS] or GTP-containing internal solutions. This time-dependent shift was prevented in cells treated with PTX (Fig. 3; Table 1), confirming that PTX-sensitive G proteins are responsible for the time-dependent modulation of the voltage dependence of Na^+-channel gating.

Inhibition of Basal G-Protein Activation Shifts Na^+-Channel Gating to More-Positive Membrane Potentials. In many cases, G proteins are active in the absence of agonist (16). The basal activity of G proteins is greater for PTX-sensitive G proteins such as G_k, which couples muscarinic acetylcholine receptors to K^+ channels (20), and G_o, which couples opioid receptors to Ca^{2+} channels (29). We examined Na^+-channel modulation due to basal G-protein activity using guanine 5′-[β-thio]diphosphate (GDP[βS]), which stabilizes the inactive form of G proteins and prevents their activation. GDP[βS] prevented the effects of GTP and GTP[γS] on Na^+-channel activation and inactivation (Fig. 2; Table 1). Moreover, with 2 mM GDP[βS] in the pipette without GTP[γS] or GTP, both activation and inactivation curves were shifted to more-positive membrane potentials than control soon after breaking the cell membrane of hippocampal neurons or CNaIIA-1 cells and remained relatively unchanged thereafter (Fig. 3; Table 1). In hippocampal neurons, GDP[βS] shifted $V_{1/2}$ for activation by +8 mV and $V_{1/2}$ for inactivation by +9 mV as compared to control. In CNaIIA-1 cells, the corresponding shifts in voltage dependence were +12 and +8 mV for activation and inactivation, respectively. Consistent with the preceding data, the initial value of $V_{1/2}$ at the beginning of experiments in CNaIIA-1 cells after overnight treatment with PTX was 6 mV positive to that of untreated cells (Fig. 3; Table 1). These results indicate that basal activation of G proteins in these two cell types is sufficient to significantly shift the voltage dependence of Na^+-channel gating toward negative potentials and that this shift can be reversed by GDP[βS] or PTX. Such regulation by basal G-

Table 1. Voltage dependence of activation and inactivation in control and G-protein-modulated cells

	Hippocampal neurons				CNaIIA-1 cells			
	Activation		Inactivation		Activation		Inactivation	
	0–2 min	10–15 min	0–2 min	10–15 min	0–2 min	10–15 min	0–2 min	10–15 min
Control	−17.9 ± 0.7 ($n = 5$)	−19.0 ± 0.5 ($n = 6$)	−35.6 ± 0.3 ($n = 5$)	−37.1 ± 1.3 ($n = 4$)	+1.6 ± 2.3 ($n = 4$)	−2.2 ± 2.3 ($n = 4$)	−29.4 ± 3.1 ($n = 4$)	−32.6 ± 3.2 ($n = 4$)
GTP[γS] or GTP*	−19.2 ± 0.8 ($n = 12$)	−27.9 ± 0.4 ($n = 12$)	−35.2 ± 0.8 ($n = 11$)	−45.8 ± 1.7 ($n = 11$)	−0.3 ± 0.8 ($n = 5$)	−12.8 ± 0.7 ($n = 6$)	−33.2 ± 1.3 ($n = 5$)	−39.9 ± 1.6 ($n = 5$)
PTX + GTP[γS] or GTP*	−16.3 ± 0.6 ($n = 9$)	−18.4 ± 0.7 ($n = 8$)	−32.8 ± 0.6 ($n = 7$)	−36.9 ± 0.6 ($n = 8$)	+7.5 ± 1.1 ($n = 8$)	+3.5 ± 1.7 ($n = 9$)	−28.5 ± 1.4 ($n = 7$)	−29.4 ± 1.3 ($n = 10$)
GDP[βS]	−13.2 ± 1.1 ($n = 5$)	−11.2 ± 0.5 ($n = 8$)	−28.6 ± 0.3 ($n = 5$)	−28.6 ± 1.1 ($n = 8$)	+10.9 ± 1.4 ($n = 7$)	+10.7 ± 0.1 ($n = 4$)	−24.6 ± 1.9 ($n = 6$)	−24.8 ± 1.9 ($n = 9$)
GDP[βS] + GTP[γS] or GTP*	−19.5 ± 1.5 ($n = 3$)	−20.5 ± 1.0 ($n = 5$)	−33.7 ± 0.4 ($n = 3$)	−37.2 ± 2.1 ($n = 4$)	+9.2 ± 1.3 ($n = 4$)	+4.8 ± 1.3 ($n = 4$)	−24.0 ± 0.1 ($n = 4$)	−26.5 ± 1.4 ($n = 5$)
Thrombin + GTP					+1.5 ± 1.5 ($n = 12$)	−18.4 ± 2.2 ($n = 12$)	−29.2 ± 1.1 ($n = 13$)	−47.3 ± 1.5 ($n = 12$)

To compare G-protein effects on Na^+-channel gating in hippocampal neurons and in CNaIIA-1 cells, current–voltage curves and inactivation curves were recorded immediately after attaining the whole-cell configuration (0–2 min) or after 10–15 min to demonstrate the time dependence of effects of G-protein modulators. Half-activation and inactivation voltages ($V_{1/2}$, mV) at these times were determined by fitting Boltzmann relationships to the data as described. Data are presented as means ± SE.

*Data from hippocampal neurons were obtained with 0.2–0.5 mM GTP[γS] added to the pipette solution. Data from CNaIIA-1 cells were obtained with 1 mM GTP.

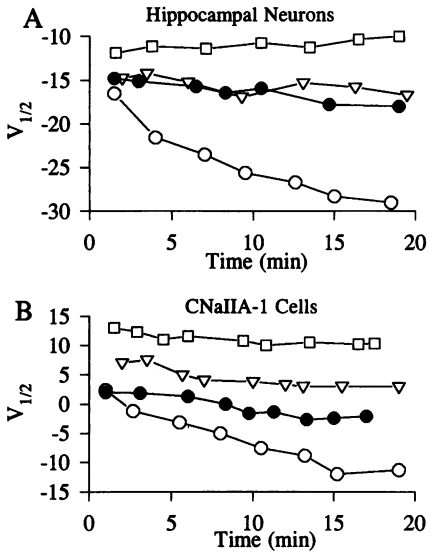

FIG. 3. Time dependence of Na^+-current modulation by G proteins. Half-activation voltage, $V_{1/2}$, for a representative cell with each pipette solution is plotted as a function of time after establishing the whole-cell configuration. (*A*) Hippocampal neurons. Time course of shifts in $V_{1/2}$ in control (●) or with pipette solutions containing GTP[γS] (○), GDP[βS] (□), or GTP[γS] plus the A-protomer of PTX (▽). (*B*) CNaIIA-1 cells. Time course of shifts in $V_{1/2}$ in control (●), or with pipette solutions containing GTP (○), GDP[βS] (□), or with GTP after overnight treatment with PTX (▽).

protein activity allows bidirectional regulation of Na^+-channel function.

Role of Diffusible Second Messengers. G proteins modulate ion channels through both membrane-delimited and diffusible second messenger pathways (15, 16). In the CNaIIA-1 cell experiments, ATP was not included in the recording pipettes. In hippocampal neurons, inclusion of 4 mM ATP had no effects on Na^+ currents and, therefore, was used routinely to improve cell stability. The lack of an ATP requirement suggests that protein phosphorylation is not involved. To test for the involvement of Ca^{2+} as a second messenger mediating GTP effects, the fast Ca^{2+} chelator bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetate (BAPTA; 10 mM) was included in recording pipettes, and the effects of GTP in CNaIIA-1 cells were measured (n = 6). No effect of Ca^{2+} chelation was observed. While more detailed studies are needed to firmly exclude a role for these processes, our results suggest that neither protein phosphorylation nor intracellular Ca^{2+} is involved in the G-protein effects on Na^+-channel gating reported here.

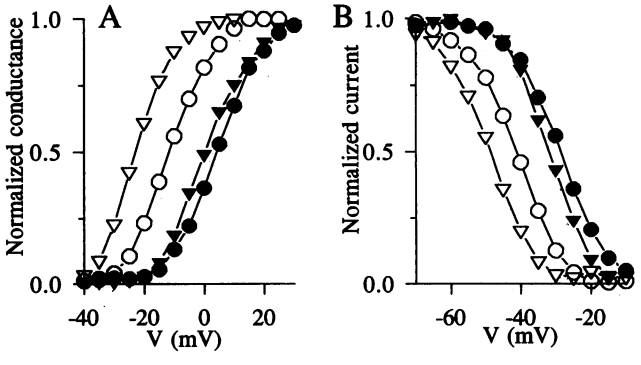

FIG. 4. Activation of thrombin receptor shifts voltage dependence of Na^+-channel kinetics toward negative membrane potential in CNaIIA-1 cells. Data were obtained from individual cells 15 min after achieving the whole-cell configuration. (*A*) Examples of normalized conductance–voltage relationships obtained with control pipette solution (●) or with GTP in the pipette solution with no additional treatment (○), with GTP in the pipette 10 min after bath application of thrombin (▽), or in a PTX-treated cell with GTP in the pipette after bath application of thrombin (1 unit/ml; ▼). (*B*) Normalized steady-state inactivation curves generated using 80-ms prepulses of variable potential followed by a test pulse to +10 mV from the same experiments as in *C*.

Functional Coupling of Membrane Receptors to Na^+ Channels in CNaIIA-1 Cells. G proteins couple many hormone and neurotransmitter receptors to a variety of effectors including ionic channels. We explored the possibility that modulation of expressed Na^+ channels through activation of G-protein-coupled receptors might occur in CNaIIA-1 cells. In the parent CHO cells, thrombin receptors have been shown to activate a PTX-sensitive G protein leading to inositol phosphate hydrolysis, and cholecystokinin receptors are coupled to the same pathway through a PTX-insensitive G protein (30). In the presence of GTP (1 mM), bath application of cholecystokinin (10 units/ml) showed no greater effect than GTP alone (n = 3; data not shown). In contrast, bath application of thrombin (1–5 units/ml) shifted the conductance–voltage relationship ≈6 mV further in the negative direction than 1 mM GTP (Fig. 4*A*; Table 1) and shifted the voltage dependence of steady-state inactivation 7 mV further in the negative direction (Fig. 4*B*; Table 1). These effects were blocked by PTX (n = 3). Activation of thrombin receptors increases intracellular Ca^{2+} concentration through activation of phospholipase C in CHO cells (30). However, the increased Ca^{2+} does not mediate the effects of thrombin since they were unaltered when Ca^{2+} was chelated by inclusion of BAPTA (10 mM) in the recording pipette (n = 6). These results indicate that brain Na^+ channels can be modulated by stimulation of an endogenous receptor coupled to a PTX-sensitive G protein in CNaIIA-1 cells and imply that they would be modulated by any receptor coupled to an appropriate G protein in neurons.

Possible Mechanisms for Modulation of Na^+ Channels by G Proteins in Brain. Numerous neurotransmitters modulate Ca^{2+} and K^+ channels in neurons via PTX-sensitive G proteins (for review, see refs. 14–16). Muscarinic stimulation triggers Na^+ fluxes in several systems (31–33), and G proteins are likely to be involved (34–36). Activation of D_1 and D_2 dopamine receptors modulates Na^+ currents in rat neostriatal neurons through both membrane-delimited and diffusible second messenger pathways (37), and G proteins may be associated with both pathways. Thus, it seems likely that Na^+ channels in brain neurons are modulated by neurotransmitters acting through PTX-sensitive G proteins.

It is not clear which subtypes of PTX-sensitive G proteins are involved in Na^+-channel modulation. The relevant G proteins in CHO cells are probably G_{i2} and G_{i3} because they are PTX sensitive and are present at substantial levels (38, 39). In brain neurons, G_o and G_{i1} are expressed at high levels, while G_{i2} and G_{i3} are expressed at lower levels (40). All four of these G-protein types may modulate Na^+ channels in the brain. Brain Na^+ channels are also functionally modulated by phosphorylation by protein kinases A and C (9–13). Our finding of G-protein-mediated modulation adds another potent pathway for regulation. Interactions between these pathways may play an important role in information processing and transmission in the central nervous system.

Na^+ currents through channels expressed from whole brain poly(A)$^+$ mRNA in *Xenopus* oocytes were reduced by GTP[γS] without effects on voltage dependence, but these effects were largely PTX insensitive (23). G proteins are also implicated in regulation of cardiac Na^+ channels by isoproterenol, which may occur through both membrane-delimited and cAMP-dependent pathways (21, 22, 41–43). However, in contrast to our results, the effects of G proteins on the principal cardiac Na^+-channel subtype are mediated through

CTX-sensitive G proteins (G_s). The evidence for regulation of Na^+-channel activity by G proteins in both brain and heart suggests a widespread mechanism for regulation of electrical excitability through G-protein-coupled pathways.

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