Mutations associated with sodium channel-linked inherited Long-QT syndrome often result in a gain of channel function by disrupting channel inactivation. A small fraction of channels fail to inactivate (burst) at depolarized potentials where normal (wild type) channels fully inactivate. These noninactivating channels give rise to a sustained macroscopic current. We studied the effects of protein kinase A stimulation on sustained current in wild type and three disease-linked C-terminal mutant channels (D1790G, Y1795C, and Y1795H). We show that protein kinase A stimulation differentially affects gating in the mutant channels. Wild type, Y1795C, and Y1795H channels are insensitive to protein kinase A de-pendent phosphorylation. Our results suggest that the charge at position 1790 of the C terminus of the channel modulates the response of the cardiac sodium channel to protein kinase A stimulation and that phosphorylation of residue 36 in the N terminus and residue 525 in the cytoplasmic linker joining domains I and II of the channel α subunit facilitate destabilization of inactiva-tion and thereby increase sustained current.

Voltage-gated Na⁺ channels are integral membrane proteins (1, 2) that not only govern cell excitability but may determine the vulnerability of the heart to the development of abnormal rhythms. Typically, Na⁺ channels open in response to membrane depolarization and rapidly enter an absorbing inactiva-tion state (3–6). However, a small fraction of channels may fail to inactivate, a phenomenon termed “bursting.” Bursting channels allow passage of Na⁺ at depolarized potentials, resulting in sustained macroscopic current (Iₚₛₛ). A number of Na⁺ channel-linked mutations that underlie Long-QT syndrome (LQT-3) have been shown to promote channel bursting (7–9), and computational analysis has confirmed that this activity underlies action potential prolongation and increased risk of arrhythmia (10). Hence modulation of bursting in general and alteration in modulation by inherited mutations are of considerable interest.

The Na⁺ channel α subunit forms the ion-conducting pore and contains multiple consensus sites for protein kinase A (PKA)-dependent phosphorylation (11–16). PKA-dependent phosphorylation of the α subunit modulates Na⁺ channel activity in an isoform- and tissue-specific manner (17, 18). Furthermore, the C terminus of the Na⁺ channel α subunit modulates gating of brain and heart Na⁺ channels (19) including Iᵥᵥₛₛ cardiac Na⁺ channels (20, 21). In this study we investigate the ability of mutations in the heart Na⁺ channel C terminus to modulate the functional response of the channel to PKA-dependent phosphorylation. We focused on the effects of PKA on Iᵥᵥₛₛ in wild type (WT) and three previously reported C-terminal mutations: D1790G (22), Y1795C (23), and Y1795H (23). We were particularly interested in the response of D1790G channels to PKA stimulation because this mutation has been reported to promote Iᵥᵥₛₛ by some investigators (24) but not by others (22), raising the possibility that Iᵥᵥₛₛ may be subject to regulation.

We indeed found marked differences in the response of the mutant channels to PKA stimulation. Bursting activity was insensitive to PKA in WT, Y1795C, and Y1795H channels but was significantly enhanced in a PKA-dependent manner in D1790G channels. We extended our inquiry to try to elucidate the mechanism of this differential response of the mutations by carrying out site-directed mutagenesis. Our analysis suggests that PKA-dependent modulation of bursting is enhanced by alteration of the negative charge at residue 1790 and may require the phosphorylation of both Ser³⁶ and Ser⁵²⁵ in the channel α subunit. The results support the idea that the C terminus of the Na⁺ channel plays an important role in modulating gating of the channel and suggest that multiple factors are likely to affect the cellular and systems phenotypes of disease-linked mutations of the Na⁺ α subunit.

MATERIALS AND METHODS

Mutagenesis and Transient Transfection—The C-terminal mutations of SCN5A were engineered into WT cDNA cloned in pCDNA3.1 (Invitrogen) by overlap extension using mutation-specific primers and a QuikChange site-directed mutagenesis kit (Stratagene). The presence of the mutation was confirmed by sequence analysis (23). We were particularly interested in the response of D1790G channels to PKA stimulation because this mutation has been reported to promote Iᵥᵥₛₛ by some investigators (24) but not by others (22), raising the possibility that Iᵥᵥₛₛ may be subject to regulation.

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FIG. 1. Selective increase of $I_{\text{sus}}$ in D1790G channels by cAMP dialysis. TTX-sensitive (“Materials and Methods”) currents are shown at high gain in the absence and presence of intracellular cAMP (200 μM) in cells expressing WT, D1790G (DG), Y1795C (YC), and Y1795H (YH) channels. A, averaged current traces are shown for each construct with numbers indicated below. Currents measured in the presence of cAMP were recorded 10 min after establishing whole cell recording conditions with pipettes containing cAMP (200 μM). cAMP-free recordings were made under identical conditions but without cAMP in the pipette. B, the bars represent the percentages of $I_{\text{sus}}$ normalized (to peak current) in the absence (open bars) or presence (closed bars) of cAMP. The following values were obtained: WT: without cAMP, 0.08 ± 0.02%, $n = 5$, and with cAMP, 0.08 ± 0.02%, $n = 6$; D1790G: without cAMP, 0.12 ± 0.02%, $n = 11$, and with cAMP, 0.26 ± 0.06%, $n = 12$, $p < 0.05$; Y1795C: without cAMP, 0.37 ± 0.03%, $n = 10$, and with cAMP, 0.37 ± 0.07%, $n = 7$; Y1795H: without cAMP, 0.24 ± 0.04%, $n = 4$, and with cAMP, 0.21 ± 0.02%, $n = 4$, $^*, p < 0.05$. 

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FIG. 2. The protein phosphatase inhibitor okadaic acid (0.2 μM) selectively increases sustained current ($I_{\text{sus}}$) in D1970G channels. 

**A**, averaged TTX-sensitive current (“Materials and Methods”) recorded in cells expressing WT, D1790G (DG), Y1795C (YC), and Y1795H (YH) channels under conditions with (arrow) and without okadaic acid (0.2 μM) included in the recording pipette. The recordings were made 10 min after establishing whole cell recording conditions. **B**, the bar graph plots percentages of normalized (to peak current) $I_{\text{sus}}$ in the absence and presence of okadaic acid (0.2 μM) in the pipette. The results are as follows: WT: without OA, 0.09 ± 0.02%, $n = 7$, and with OA, 0.09 ± 0.03%, $n = 5$; DG: without OA, 0.09 ± 0.02%, $n = 6$, and with OA, 0.33 ± 0.07%, $n = 7$, $p < 0.01$; YC: without OA, 0.36 ± 0.04%, $n = 6$, and with OA, 0.31 ± 0.04%, $n = 7$; YH: without OA, 0.21 ± 0.05%, $n = 7$, and with OA, 0.23 ± 0.04%, $n = 6$. **, $p < 0.01$. **
dependence of inactivation not recorded for the aspartate/Cl
MgCl2, pH 7.4, adjusted with CsOH. In some experiments, the major
external solution contained 130 mmol/liter NaCl, 2 mmol/liter CaCl2,5
mmol/liter HEPES, pH 7.4, adjusted with 25 mmol/liter CsOH. The
NaF, 110 mmol/liter CsF, 20 mmol/liter CsCl, 10 mmol/liter EGTA, 10
anion in the pipette solution was replaced by fluoride: 10 mmol/liter
channel recordings was 140 mM KCl, 5 mM HEPES, 10 mM MgCl2,p H
mmol/liter HEPES, pH adjusted to 7.4). The external solution for single
filled with single channel internal solution (110 mmol/liter NaCl, 10

Electrophysiology—The membrane currents were measured using
whole cell and single channel patch clamp procedures with Axopatch
200B amplifiers and the Pclamp 8 program (Axon Instruments, Foster
City, CA). All of the measurements were obtained at room temperature
(22 °C). Macroscopic whole cell Na+ current was recorded using the
following solutions. The normal internal solution contained 50 mmol/
liter aspartic acid, 60 mmol/liter CsCl, 5 mmol/liter Na2-ATP, 11 mmol/
liter EGTA, 10 mmol/liter HEPES, 1 mmol/liter CaCl2, 10 mM MgCl2, pH 7.4, adjusted with CsOH. In some experiments, the major
anion in the pipette solution was replaced by fluoride: 10 mmol/liter
channel current (I∑) was determined by subtracting background currents measured in the presence of tetrodotoxin
(TTX; 30 μM, Molecular Probes) from TTX-free records. The amplitude of I∑ was measured 150 ms after depolarization to −100 to −150 mV for aspartate/Cl− intracellular solution (26); consequently holding potentials were changed from −100 to −150 mV for aspartate and F− pipette solutions. The data were discarded when the series resistance was more than 7 MΩ. Noninacti-
vated sustained Na+ channel current (I∑) was determined by subtracting background currents measured in the presence of tetrodotoxin (TTX; 30 μM, Molecular Probes) from TTX-free records. The amplitude of I∑ was measured 150 ms after depolarization to −100 mV to avoid
channel re-openings that occur in the voltage range in which activation and inactivation overlap (window current) (25, 26). The voltage dependence of channel availability was determined by relative current ampli-
tude after the application of conditioning pulses (500 ms) applied to a series of voltages once every 2 s. Boltzmann relationships were fit to the
data using Origin (Microcal, Northhampton, MA) software to extract the voltage of half-maximal inactivation (V1/2) and slope factor (κ) for this relationship.

For single channel experiments, the pipettes were coated with Syl-
gard (Dow Chemical Co., Midland, MI) to decrease noise and capa-
tiance of the glass. Electrode resistance was typically 5–7 MΩ when
filled with single channel internal solution (110 mM NaCl, 10 mmol/liter HEPES, pH adjusted to 7.4). The external solution for single channel recordings was 140 mM KCl, 5 mM HEPES, 10 mM MgCl2, pH adjusted to 7.4. After establishing the cell-attached configuration (seal resistance > 10 GΩ), the membrane was held at a holding potential of −120 mV. Test pulses (−20 mV for 100 ms) were applied every 0.5 s. Single channel currents were filtered by a low pass filter in the clamp amplifier with a cut-off frequency of 5 kHz and digitized for storage on computer at a sampling frequency of 20 kHz. Capacity and leak cur-
rents were eliminated by digital subtraction of averaged null sweeps.

Burusting activity was defined as repetitive (more than three times) and
long lasting (more than 30 ms) channel openings (see Fig. 6). Bursting probability (Pb) for less than 11 channel patches was calculated by the following equation.

\[
P_b = 1 - (1 - b)^{t/n}
\]

where \( b, t, n \) represent the number of non-bursting sweeps, total sweeps (1500–3000), and channels, respectively. The number of channels included in each patch was estimated by counting the overlap of channel openings. The data are represented as the means ± S.E. The statistical significance of control and treated group was evaluated by the unpaired Student’s t test. Statistical significance of treated groups against control was first verified by analysis of variance (for multiple groups) and then evaluated by Dannet’s test; \( p < 0.05 \) was considered statistically significant.

Okadaic acid, okadaic acid methyl ester (Calbiochem, San Diego, CA) and cyclic AMP (Sigma) were dissolved with internal solution and kept at −20 °C. The concentration of the stock solution was 2 μM for okadi-
acid acid and okadaic acid methyl ester and 20 μM for cAMP. The protein
kinase inhibitor peptide (PKI) was also purchased from Sigma and
dissolved in pipette solution (20 μM). TTX was purchased from Molec-
ular Probes (Eugene, OR) and dissolved in the bath solution (30 μM).

RESULTS

To focus on possible changes in channel bursting induced by
PKA, we measured currents in response to prolonged (150 ms)
pulses to −10 mV, a voltage that is sufficiently positive to avoid
measurement of channel reopening that may occur as a conse-
quence of the overlap steady-state activation and inactivation
curves (25, 27). In whole cell recordings, bursting channels are
reflected in sustained inward (I∑sus) current under these condi-
tions. As illustrated in Fig. 1, dialysis of cells with cAMP (200
μM) has no effect on I∑sus recorded in cells expressing WT,
Y1795H, or Y1795C channels but markedly increases I∑sus in
cells expressing D1790G channels.

The results summarized in Fig. 1 suggest that cAMP-de-
pendent phosphorylation may selectively increase I∑sus in
D1790G channels. We next tested for activity of endogenous
phosphatase activity that might exist and buffer phosphorylation-dependent changes in channel activity. Similar to the results summarized in Fig. 1, we found that dialysis of cells with the nonspecific phosphatase inhibitor okadaic acid (0.2 μM) significantly increased $I_{\text{sus}}$ of D1790G channels but had no significant effect on $I_{\text{sus}}$ recorded in cells expressing WT, Y1795C, or Y1795H channels (Fig. 2). Similarly we found but did not illustrate that $I_{\text{sus}}$ recorded in cells expressing two other disease linked mutant channels (ΔKPQ7 and S1103Y28) was unaffected by okadaic acid dialysis (ΔKPQ, 0.40 ± 0.06% (control, $n = 6$), 0.37 ± 0.08% (okadaic acid, $n = 6$, n.s.); S1103Y, 0.09 ± 0.01% (control, $n = 5$), 0.09 ± 0.02% (okadaic acid, $n = 5$, n.s.)). Thus, the results of these experiments suggest that okadaic acid and/or dialysis of cells with cAMP can increase $I_{\text{sus}}$ specifically in cells expressing D1790G channels.

The effects of okadaic acid on $I_{\text{sus}}$ were due to dephosphorylation of a PKA-mediated reaction because 1) dialysis of cells with the inactive methylester of okadaic acid did not have any effect and 2) concomitant dialysis of a PKA inhibitory peptide with okadaic acid ablated okadaic acid-enhanced $I_{\text{sus}}$ in cells expressing D1790G channels (Fig. 3). Further evidence supporting the effect of okadaic acid on D1790G channel current is due to inhibition of endogenous phosphatase activity is provided by the data in Fig. 4, which show that dialysis of cells with F−, which is also a nonspecific phosphatase inhibitor (29–31) known also to activate $G_s$ (32, 33), increases $I_{\text{sus}}$ in cells expressing D1790G channels (Fig. 4A, D1790G traces). This effect of F− is inhibited by concomitant dialysis with PKI (Fig. 4B, hatched bar) and is also specific for D1790G versus WT channels (Fig. 4, see wild type traces in A and bars in B).

Augmentation of $I_{\text{sus}}$ may occur as a result of a change in Na+ channel window current and/or via an increase in the activity of channels in an inactivation-deficient bursting mode (7, 25). The voltage protocols used in the experiments summarized above were chosen to minimize possible effects on window currents; however, experiments summarized in Figs. 5 and 6 confirm that the effects of okadaic acid (and/or cAMP) on D1790G channel activity are a consequence of an increase in the bursting frequency of the expressed channels. An increase
in sustained current that is caused by bursting should be detected over a broad range of voltages in response to a slow voltage ramp protocol. This is in contrast to window current, which occurs over a narrow voltage range, causing peak of current in response to a voltage ramp as illustrated in Fig. 5 for WT channels. The voltage dependence of WT channel current in response to the voltage ramp is clearly distinct from that of D1790G channels using the same protocol (Fig. 5, lower traces).

In the case of D1790G channels, the non-zero current has a broad voltage dependence, and this current is more than doubled by dialysis with okadaic acid (0.2 μM) plus cAMP (200 μM).

Single channel recordings (Fig. 6) confirm an effect on channel bursting. In these experiments, D1790G channel activity measured in cell-attached patches is altered by exposure of cells to okadaic acid. In these experiments cells were incubated in okadaic acid (2 μM) containing solution for 30 min, and then, in the continued presence of okadaic acid (2 μM), single channel recordings were carried out. Okadaic acid significantly (p < 0.05) increased the frequency for which bursting was detected compared with recordings made under identical experimental conditions but in the absence of okadaic acid. These data are summarized as the bar graph in Fig. 6B, which compares bursting frequency in the absence (0.004 ± 0.001%, control, n = 9) and presence (0.011 ± 0.003%, 2 μM OA, n = 10) of okadaic acid.

Taken together, these data strongly suggest that D1790G channels are modulated by cAMP-dependent in a manner that increases the frequency of bursting for these channels and that this effect appears specific for D1790G versus WT and all other (four) disease-linked mutant channels we investigated. We carried out additional analysis by mutagenesis to determine 1) whether or not the change in charge that occurs via the D1790G mutation contributes to the cAMP sensitivity of channel bursting and 2) whether or not previously identified consensus PKA phosphorylation sites might be involved in this process.

Residue 1790 falls within a region of the C-terminal tail of the α subunit, which is predicted to be highly structured and enriched with negative charges (20). Because the naturally occurring mutation D1790G neutralizes a negative charge at residue 1790G, we tested the possibility that this alteration in charge may, at least in part, underlie the apparently unique responsiveness of D1790G channels to cAMP-dependent modulation. As such, we replaced the aspartate at this residue by a lysine (D1790K) that, instead of neutralizing the charge at this residue, changes its polarity. As is the case for D1790G channels, cells expressing D1790K channels do not exhibit marked I_{sus} under control conditions (not shown) but share virtually an identical response to okadaic acid with D1790G channels (Fig.
7). Similar to currents for D1790G channels, D1790K \(I_{\text{sus}}\) is more than doubled by okadaic acid (0.2 \(\mu\)M) dialysis, and the effect is completely inhibited by concomitant dialysis with PKI (20 \(\mu\)M) (Fig. 7). Importantly, replacing the aspartate at residue 1790 by another negatively charged amino acid, glutamate, has no effect on the sensitivity of channel bursting to cAMP. In fact both the basal bursting level and the lack of cAMP-dependent regulation of the D1790E channel are remarkably similar to those of the wild type (Asp1790) channel. Thus, it appears that mutation-induced alteration in charge at position 1790 plays an important role in conferring a cAMP sensitivity to bursting of the expressed channels.

Mutagenesis, as summarized in Fig. 8, suggests that the effects of cAMP and okadaic acid on D1790G channels are mediated via PKA phosphorylation of specific sites on the \(\alpha\) subunit. Using the D1790G mutant as a backbone, we then used alanine mutations to analyze the roles of three consensus PKA sites, Ser\(^{36}\), Ser\(^{525}\), and Ser\(^{528}\), in the response of D1790G channels to cAMP (200 \(\mu\)M) and okadaic acid (0.2 \(\mu\)M). The response of D1790G channels to cAMP was not changed when Ser\(^{528}\) (in the D1790G backbone) was mutated to alanine. Similarly, the individual mutations S36A and S525A in the D1790G backbone reduced but did not significantly alter cAMP-dependent D170G channel bursting. However, mutation of both Ser\(^{36}\} and Ser\(^{528}\} to alanine within the D1790G backbone significantly reduced the effects of cAMP and okadaic acid on D1790G channel bursting (Fig. 8). In fact there is no significant difference between \(I_{\text{sus}}\) measured in the presence of okadaic acid and cAMP for the D1790G double mutant (S36A, S525A) channels compared with D1790G channels without these mutations but in the presence of the inhibitory peptide PKI.

**Fig. 7.** PKA-dependent bursting of D1790 mutant channels. Replacement of the negatively charged residue Asp\(^{1790}\} by a positively charged lysine (D1790K), but not a negatively charged glutamate (D1790E), reconstitutes PKA-dependent bursting of expressed channels. A, intracellular OA and intracellular OA plus PKI have similar effects on D1790G and D1790K channel currents but not on D1790E channel currents. Shown are averaged TTX-sensitive D1790G (\(n = 5\) and 10), D1790K (\(n = 9\) and 8), and D1790E (\(n = 6\)) currents measured at \(-10 \text{ mV}\) in the presence of OA (0.2 \(\mu\)M) and OA (0.2 \(\mu\)M) plus PKI (20 \(\mu\)M). B, the bar graph summarizes the effects of the two intracellular solutions on normalized sustained current. The results are D1790G (DG): OA, 0.36 \(\pm\) 0.05\%, \(n = 5\); OA and PKI, 0.15 \(\pm\) 0.02\%, \(n = 10\) (\(p < 0.05\)); D1790K (DK): control, 0.12 \(\pm\) 0.02\%, \(n = 8\); OA, 0.29 \(\pm\) 0.04\%, \(n = 9\); OA and PKI, 0.14 \(\pm\) 0.03\%, \(n = 8\), (\(p < 0.05\)); D1790E (DE): control, 0.08 \(\pm\) 0.01\%, \(n = 6\); OA, 0.07 \(\pm\) 0.01\%, \(n = 6\) (not significant). *, \(p < 0.05\).
PKA-dependent Regulation of I_{sus} Is Modulated by the C-terminal Domain—The Na⁺ channel α subunit expressed in multiple tissues has been reported to be a substrate for both PKA and protein kinase C-dependent phosphorylation (11–16, 34), and various effects have been reported as consequences of phosphorylation of specific sites on the channel. Activation of protein kinase C has been reported to promote bursting of Na⁺,1.2 channels, but PKA stimulation has been reported previously to have little or no effect on bursting of WT or of ΔKPQ mutant Na⁺,1.5 channels (35). Thus, this is the first report of significant PKA-dependent enhancement of Na⁺ channel bursting.

Attention has focused increasingly on a role of the Na⁺ channel C terminus in modulation of channel inactivation. Kinetic and voltage-dependent properties of brain (Na⁺,1.2) channel gating can be conferred upon heart (Na⁺,1.5) channels by exchange of C-terminal domains (19). A recent study showed that truncation of a distal region of the C-terminal domain of the heart channel (Na⁺,1.5) markedly stimulates the channel bursting (20). Our results are consistent with these studies and suggest not only an important role of the C-terminal domain in determining the effects of PKA on channel bursting but that discrete regions are involved in this regulation.

Diversity in Sodium Channel Structure May Contribute to Multiple Effects of PKA—Diversity in the sequences in the I and II cytoplasmic loops of Na⁺,1.2 (brain) and Na⁺,1.4 (skeletal muscle Na⁺ channels), which contain multiple PKA consensus phosphorylation sites, has been postulated to contribute distinct functional effects of PKA in these Na⁺ channel isoforms (12, 13, 18, 36–38). PKA-dependent reduction of Na⁺,1.2 channel current was ablated or converted into a PKA-dependent increase in current by mutation of PKA consensus serines in the I-II linker. These effects were not seen when the I-II linker was replaced by the Na⁺,1.4 I-II linker (37). Similarly experiments provide evidence for a key role of the I-II linker in the response of heart (Na⁺,1.5) channels to cAMP (15).

In this study five putative PKA consensus sites (Thr¹⁷, Ser⁴⁸³, Ser⁵⁰⁸, Thr⁹⁷⁹, and Thr¹⁰²⁸) shown not to be involved in PKA-dependent modulation of Na⁺ channel (15) were not investigated. However three other consensus sites, including Ser²⁵ in the I-II loop, were investigated, and we did find a role for the I-II linker (S525) in the PKA-dependent control of bursting. However, interestingly, this is not the only cytoplasmic segment implicated in our work. We find an equal contribution of an N-terminal serine (Ser³⁶). Furthermore, the functional roles of these loops depend critically on the sequence of the C-terminal domain, suggesting that interactions among multiple cytoplasmic components of the channel contribute to the functional consequences of PKA-dependent modulation of the channel. Interestingly, similar interactions have been predicted previously for cytoplasmic loops and for direct interactions between the C- and N-terminal domains of rat skeletal muscle Na⁺ channels (39). Clearly our data suggest a similar possibility for Na⁺,1.5 channels.

Insight from Structural Studies of the Na⁺,1.5 C-terminal Domain—A recent computational and experimental investigation of the secondary structure of the Na⁺,1.5 C-terminal domain provided evidence for a proximal region of the C terminus that is highly structured and enriched in negative charges (20). Residue Asp¹⁷⁹⁰ falls within this region, as does residue Tyr¹⁷⁸⁵. More distal in the C-terminal domain is a putative helical positively charged region that, when truncated, results in promotion of channel bursting (20). PKA promotes bursting for Asp¹⁷⁹⁰ mutants (D1790G and D1790K) but not Tyr¹⁷⁸⁵ mutant channels (Y1795H and Y1795C), despite that fact that both residues 1790 and 1795 are included in first α helix (H1)

The results of this study strongly suggest that the D1790G mutation results in an increased sensitivity of channel bursting to PKA-dependent phosphorylation. Furthermore, the effects of cAMP on channel bursting appear to depend critically upon the negative charge at position 1790 of the C-terminal tail of the channel, because alteration of this charge by inherited (D1790G) or experimentally imposed (D1790K) mutation significantly increases PKA-dependent modulation of bursting, whereas replacement of Asp¹⁷⁹⁰ by a negatively charged glutamate residue has no effect either on basal bursting activity or on the response of the channel to PKA stimulation. Recording conditions that inhibit endogenous phosphatase activity promote bursting of D1790G channels, whereas in the absence of phosphatase inhibitions, bursting of these channels is not significantly different from wild type channels.

**DISCUSSION**

The results of this study strongly suggest that the D1790G mutation results in an increased sensitivity of channel bursting to PKA-dependent phosphorylation. Furthermore, the effects of cAMP on channel bursting appear to depend critically upon the negative charge at position 1790 of the C-terminal tail of the channel, because alteration of this charge by inherited (D1790G) or experimentally imposed (D1790K) mutation significantly increases PKA-dependent modulation of bursting, whereas replacement of Asp¹⁷⁹⁰ by a negatively charged glutamate residue has no effect either on basal bursting activity or on the response of the channel to PKA stimulation. Recording conditions that inhibit endogenous phosphatase activity promote bursting of D1790G channels, whereas in the absence of phosphatase inhibitions, bursting of these channels is not significantly different from wild type channels.

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**Insight from Structural Studies of the Na⁺,1.5 C-terminal Domain**—A recent computational and experimental investigation of the secondary structure of the Na⁺,1.5 C-terminal domain provided evidence for a proximal region of the C terminus that is highly structured and enriched in negative charges (20). Residue Asp¹⁷⁹⁰ falls within this region, as does residue Tyr¹⁷⁸⁵. More distal in the C-terminal domain is a putative helical positively charged region that, when truncated, results in promotion of channel bursting (20). PKA promotes bursting for Asp¹⁷⁹⁰ mutants (D1790G and D1790K) but not Tyr¹⁷⁸⁵ mutant channels (Y1795H and Y1795C), despite that fact that both residues 1790 and 1795 are included in first α helix (H1).
of the C terminus (20). However, according to the structural model, these residues face opposite directions. Asp<sup>1790</sup> faces extraglobular and Tyr<sup>1795</sup> faces intraglobular regions. This model predicts that Asp<sup>1790</sup> can interact with other unknown proteins or internal loops of the Na<sup>+</sup> channel α subunit, but Tyr<sup>1795</sup> cannot because of the asymmetry of their orientation. Because deletion of the negative charge at 1790 residue promotes PKA-dependent modulation of modal gating, this negative charge may play an important role in stabilizing the inactivation state through electrostatic interactions with cytoplasmic loops of the channel. In rat skeletal Na<sup>+</sup> channels, an electrostatic interaction between the N terminus (13–30, negative-rich) and C terminus (1716–1737, positive-rich) has been suggested (39). The finding that the charge of residue 1790 appears to have a distinct role in PKA-dependent modulation of channel bursting suggests that its orientation or location in the structured proximal portion of the C-terminal domain may confer privileged access to key structures responsible for channel inactivation.

Na<sup>+</sup> channel inactivation is due to rapid block of the inner mouth of the channel pore by the cytoplasmic linker between domains III and IV that occurs within milliseconds of membrane depolarization (3–6), and bursting is an inactivation-deficient or unstable state (10). Modulation of bursting, or inactivation, thus suggests modification of these interactions. Our data implicate the N-terminal domain (Sec<sup>36</sup>), the I-II linker (Sec<sup>525</sup>), as well as the C-terminal domain (Asp<sup>1790</sup>) as modulators of this process. Complex interactions between the cytoplasmic loops of the channel, which may be altered either by mutation and or phosphorylation state, provide a broad range of possibilities for the roles of sympathetic-mediated modulation of Na<sup>+</sup> channels in the heart.

In summary, our data indicate that cAMP-dependent modulation of sodium channel gating can be affected by mutations in regions of the channel, such as the C-terminal domain, that do not directly contain PKA consensus sites and that the activity of endogenous kinases and phosphatases may contribute to channel activity recorded in heterologous expression systems.

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