Partial Characterization of the rH1 Sodium Channel Protein From Rat Heart Using Subtype-Specific Antibodies

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Three subtype-specific antisera were generated against peptides corresponding to portions of the amino terminus, interdomain 1-2, and carboxy terminus of the rH1 sodium channel primary sequence to confirm the expression of this protein in the adult rat heart and to determine selected biochemical properties of this protein that might contribute to its subtype-specific characteristics. All three antisera identify a 240-kD band on Western blots of partially purified cardiac membrane proteins and by immunoprecipitation of iodinated partially purified membrane proteins. Unlike other characterized mammalian sodium channels, no β subunit is detected in association with the rH1 α subunit. The rH1 α subunit is a complex sialoglycoprotein as evidenced by its interaction with wheat germ agglutinin–Sepharose and by reduction in its apparent molecular weight after treatment with neuraminidase; deglycosylation with N-glycanase confirms that the rH1 protein contains significantly less carbohydrate than other sodium channel proteins characterized to date (5% versus 25% to 30%). Consistent with electrophysiological studies indicating a role of phosphorylation in channel regulation, the rH1 α subunit can be phosphorylated by the catalytic subunit of cAMP-dependent protein kinase A. The possible functional significance of these findings is discussed. (Circ Res. 1993;73:735-742.)

KEY WORDS • ion channels • cardiovasculature • phosphorylation • glycosylation • β subunits

Electrophysiological, toxin binding, and molecular studies provide evidence for the existence of multiple sodium channel subtypes in cardiac tissues.1 Multiple subtypes are thought to provide functional diversity to excitable cells. Although the predominant sodium current in the heart is tetrodotoxin insensitive and is responsible for the rapid upstroke phase of the action potential, electrophysiological studies suggest the presence of at least two additional sodium currents,2-6 whereas radiolabeled toxin-binding studies identify two distinct toxin-binding phenotypes in cardiac tissues.7,8 Molecular studies using Northern blotting, RNase protection assays, and the sequencing of clones have identified five distinct sodium channel transcripts in rat cardiac tissues and, to date, two related sodium channel transcripts in human cardiac tissues. The predominant transcript in rat (rH1) and human (hH1) cardiac tissues appear to be homologues of the same subtype9,10; both possess electrophysiological characteristics virtually identical to the dominant channel seen electrophysiologically.11,12 mRNA corresponding to a second sodium channel subtype originally cloned from rat glial cells has also been shown to be abundantly expressed in rat cardiac tissues13; a related channel has been cloned and sequenced from human heart and myometrium.14 A third subtype (CSC-1) has only been partially sequenced but appears to be rat heart specific.15 Finally, transcripts corresponding to two sodium channel subtypes that are identical to channels originally cloned from rat brain (rB1 and rB3) have also been identified in rat cardiac tissues.10,16

Sodium channel subtypes differ in their flux rates, channel kinetics, voltage sensitivity, and drug and toxin sensitivity.17-19 Although the common features of sodium channel function are thought to reside in portions of the primary sequence that demonstrate high homology among subtypes, it is not clear whether the unique characteristics of each subtype are due to differences in channel primary sequence, posttranslational modification, presence of associated βi and/or βs subunits, or a combination of the above factors.

Two previous studies provide insight into the biochemical/biophysical characteristics of sodium channels in the rat heart. In the first, an antibody against a portion of the conserved interdomain 3-4 region was used to identify a 230-kD α subunit that contained approximately 8 kD of sialic acid and that could be phosphorylated by the catalytic subunit of protein kinase A.20 In the second study, the same authors used a rat brain β-specific antibody to demonstrate the presence of β, subunits in cardiac tissues.21 Because the antibody used in the first study was generated against a region that is nearly 100% conserved in all sodium channels, because the second study used a rat brain β1-specific antibody, and because multiple sodium channel subtypes are expressed in the rat heart, it is unclear which sodium channel subtype(s) was identified in these previous studies.

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Because of this uncertainty and in order to further characterize each of the subtypes whose messages have been identified in cardiac tissues, we have begun to develop subtype-specific antipeptide antibodies for use in biochemical and immunocytochemical studies. The present study details our efforts with the rh1 subtype. These studies confirm the expression of the rh1 protein in the adult rat heart and elucidate several unique characteristics that may be important in regulating the synthesis, membrane insertion, and half-life of this channel and in modulating its electrophysiological properties.

Materials and Methods

Materials

Materials for the preparation of oligopeptides and antibodies, isolation of crude membranes, and partial purification of membrane proteins were obtained from sources previously identified. G-25 Sephadex, DEAE Sephadex (A 25-120), wheat germ agglutinin–agarose, immobilized protein A, the catalytic subunit of cAMP-dependent protein kinase from bovine heart (PKA), protease inhibitors, neuraminidase, and one set of pre-stained molecular weight standards (26,000 to 180,000 D) were obtained from Sigma Chemical Co, St Louis, Mo. All electrophoresis materials including a second set of pre-stained molecular weight standards (14,400 to 97,400 D) were from Bio-Rad Laboratories, Richmond, Calif. Agarose-immobilized dianinodipropylamylone (DADP-agarose), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), and Amino Link coupling gel were obtained from Pierce Chemical Co, Rockford, Ill. [125I]-Protein A, [125I]- Bolton Hunter reagent, and [32P]ATP were from ICN Radiochemicals, Irvine, Calif. Recombinant N-glycanase was obtained from Genzyme Corp, Boston, Mass.

Preparation of Site-Directed Antisera

Polyclonal antibodies were prepared to synthetic oligopeptides as described previously. All oligopeptides correspond to portions of the rh1 cardiac sodium channel sequence as given in Fig 1. A carboxy-terminal cysteine residue was added to assist in coupling peptides to the carrier protein.

Preparation of Crude Surface Membrane

Freshly frozen rat heart (100 g) was pulverized with a mortar and pestle and extracted five times (to remove blood) with 0.25 mol/L sucrose in 0.1 mol/L Tris at pH 7.4 (4°C) containing the following protease inhibitors: 10 mmol/L EDTA, 10 mmol/L EGTA, 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 mmol/L indoacetamide, and 0.1 μg/mL pepstatin A. The procedure was otherwise as previously described except that the final sucrose gradient was omitted. Membrane fragments were kept frozen at −70°C until use.

Partial Membrane Protein Purification

Membranes were solubilized in buffer containing 180 mmol/L KCl, 20 mmol/L potassium phosphate (pH 6.5), 0.5 mmol/L MgCl₂, 1 mmol/L EGTA, 0.1 mol/L PMSF, and 0.1 μg/mL pepstatin A (buffer A) by the addition of 10% Nonidet P-40 (NP-40) to achieve a final concentration of 1% detergent and then placed on a rotating platform at 4°C for 15 minutes. After centrifugation at 125,000 × g for 30 minutes, the supernatant was batch-adsorbed onto DEAE Sephadex (A 25-120), which was preequilibrated with buffer A containing 0.1% NP-40 (buffer B). The adsorbed resin was washed extensively with buffer B and eluted with buffer containing 400 mmol/L KCl, 20 mmol/L potassium phosphate (pH 6.5), 0.5 mmol/L MgCl₂, 1 mmol/L EGTA, 0.1 mmol/L PMSF, 0.1 μg/mL pepstatin A, and 0.1% NP-40 (buffer C). The eluate from the DEAE Sephadex was applied to a column of wheat germ agglutinin immobilized on agarose. The wheat germ agglutinin column was extensively washed with buffer C, equilibrated with buffer containing 100 mmol/L NaCl, 50 mmol/L sodium phosphate (pH 7.5), 0.5 mmol/L MgCl₂, 1 mmol/L EGTA, and 0.05% NP-40 (buffer D), and eluted with buffer D containing 100 mmol/L N-acetylglucosamine. Two-step purified membrane proteins were kept at 4°C in 0.03% sodium azide and used within 1 week.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) sample buffer (5×) contained 5% SDS, 0.43 mol/L dithiothreitol, 0.28 mol/L Tris (pH 6.8), and 44% glycerol. Proteins were denatured with 1× SDS-PAGE sample buffer at 65°C for 20 minutes and resolved by electrophoresis using the buffer system of Cleveland et al. The stock solution contained 30% acrylamide and 0.2% bisacrylamide. The separating gels were 7% to 20% linear acrylamide gradients, and the stacking gels were 5% acrylamide made from a stock solution of 30% acrylamide and 0.8% bisacrylamide. Gels containing labeled protein were dried and subjected to autoradiography using Kodak XAR-5 film with a Cronex (DuPont) intensifying screen.

Two sets of molecular weight standards were run on all gels. The first set contained α₁-macroglobulin (subunit M₆, 180,000), β-galactosidase (subunit M₇, 116,000), fructose-6-phosphate kinase (M₈, 84,000), pyruvate kinase (M₉, 58,000), fumarase (M₁₀, 48,500), lactate dehydrogenase (M₁₁, 36,500), and triosephosphate isomerase (M₁₂, 26,600). The second set contained phosphorylase B (M₁₃, 97,400), bovine serum albumin (BSA; M₁₄, 66,000), ovalbumin (M₁₅, 45,000), carbonic anhydrase (M₁₆, 29,000), soybean trypsin inhibitor (M₁₇, 21,500), and lysozyme (M₁₈, 14,400). Adult rat skeletal muscle sodium channel (M₁₉, 276,000) was also used for the phosphor-
ylation and iodination studies and served both as a control as well as a molecular weight standard.

Transblots

Transblots were performed as described previously.²² For detection of immunoreactivity, the nitrocellulose transblots were incubated in antiserum diluted 1:100 in 2% BSA, 50 mmol/L sodium phosphate (pH 7.4), and 150 mmol/L NaCl (buffer E) for 2 to 3 hours at room temperature. The blots were washed five times with buffer containing 50 mmol/L sodium phosphate (pH 7.4), 150 mmol/L NaCl, and 0.25% Tween 20 (buffer F). All blots were finally incubated with ¹²⁵I-labeled protein A (250 μCi/mL, ICN Radiochemicals) diluted 1:200 in buffer D for 1 hour at room temperature. Blots were washed extensively in buffer F and wrapped in saran wrap to prevent drying. Bound antibody was visualized by autoradiography using Kodak XAR-5 film with a Cronex (DuPont) intensifying screen.

Iodination With ¹²⁵I-Bolton Hunter Reagent

Partially purified membrane proteins were concentrated 40-fold to a final volume of 25 μL, adjusted to pH 8.7 with 5 μL of 1 mol/L sodium bicarbonate, and added to a reaction vessel containing 50 μCi of ¹²⁵I-Bolton Hunter reagent. The reaction was allowed to proceed for 3 hours at room temperature before being terminated by the addition of 10 μL of 0.1 mol/L glycine. Iodinated protein was separated from reactants by chromatography using G-25 Sephadex and immunoprecipitated using affinity-purified antibody bound to protein A-Sephadex (see below).

Phosphorylation

Partially purified membrane proteins were dialyzed against deionized water, lyophilized, and resuspended in buffer containing 25 mmol/L HEPES (pH 7.4), 5 mmol/L MgCl₂, 5 mmol/L EGTA, and 0.05% NP-40 and phosphorylated by the addition of 0.4 μg PKA and 20 μCi [γ⁻³²P]ATP (3000 Ci/mmol) at 37°C. Reactions were terminated by the addition of 10 mmol/L EDTA in 25 mmol/L sodium phosphate buffer (pH 7.4). The labeled channel was then immunoprecipitated with purified antibody bound to protein A-Sepharose (see below).

Immunoprecipitation

Antibodies were affinity-purified on columns containing peptide immobilized to either DAPD-agarose or Amino Link resin (Pierce). Affinity-purified antibody was bound to protein A-Sepharose and incubated overnight at 4°C with either iodinated or phosphorylated partially purified membrane proteins and 1 mg/mL BSA. Resin was recovered by gentle centrifugation, washed extensively with phosphate-buffered saline, and either used directly for deglycosylation or for SDS-PAGE as described above.

Deglycosylation With Neuraminidase or N-Glycanase

Phosphorylated partially purified membrane proteins were immunoprecipitated with affinity-purified D-492 antibody immobilized on protein A-Sepharose. Immunoprecipitated protein was then deglycosylated by treatment with either 20 U/mL neuraminidase or 28 U/mL N-glycanase in 0.1 mol/L sodium phosphate buffer, pH 7.0, at 37°C for 4 hours before being subjected to SDS-PAGE.

Results

Antibodies directed against synthetic peptides corresponding to three sites distributed along the rH1 channel primary sequence were used to identify the rH1 protein (Fig 1). Each peptide generated antisera that was reactive with its respective peptide in radioimmunoassays (data not shown) and that specifically labeled surface, t-tubular, and intercalated disc membranes in immunocytochemistry of the rat heart.²⁴ A crude membrane preparation was used as the starting material for these studies because purified membrane preparations did not retain sufficient amounts of channel protein for biochemical characterization. Partial membrane protein purification was also necessary because of the low density of this channel in cardiac membranes.

All three antisera specifically identify an identical 240-kD band on Western blots of partially purified rat heart membrane proteins (Fig 2, left). Occasionally, and especially when protease inhibitors were omitted, additional lower molecular mass bands were visible on blots probed with each of the rH1-specific antisera (Fig 2, right). The number and migration of these lower molecular mass bands differ with the antibody used for visualization; the pattern obtained with each antibody resembles that produced by endogenous proteolysis of the rat skeletal muscle sodium channel protein.²⁵ Thus, the 240-kD band represents the intact rH1 α subunit, whereas the lower molecular mass bands correspond to proteolytic fragments of the α subunit.

Studies of mammalian sodium channels have consistently demonstrated that they contain noncovalently associated β, subunits and, in brain tissue, covalently associated βγ subunits.²⁶ To determine whether β subunits are associated with the rH1 α subunit, partially purified membrane proteins were first iodinated using Bolton Hunter reagent and then immunoprecipitated with each of the rH1-specific antisera. Each antiserum immunoprecipitated a 240-kD band identical to the α-subunit band obtained on Western blots; no lower molecular mass bands were visualized on the autoradiogram (Fig 3). The absence of lower molecular mass bands strongly suggests that the rH1 α subunit is not associated with either β1 or β2 subunits. However, the possibility that β subunits might not be sufficiently labeled to be visualized on autoradiograms was further investigated.

Scanning densitometry was used to determine the relative intensity of the α and β subunit bands of the rat skeletal muscle sodium channel positive controls; a ratio of 6:1 (α:β) was obtained. This ratio more closely reflects the relative quantity of carbohydrate than of protein in these subunits (6:1 versus 8:1, respectively). Confirming the suggestion that radiolabeled iodine is preferentially incorporated into carbohydrate rather than protein is the observation that neuraminidase treatment results in the release of most of the radioactivity associated with iodinated skeletal muscle sodium channel protein (data not shown).

Because the rH1 sodium channel contains significantly less carbohydrate than is present on adult skeletal muscle channels (see below), it is possible that any...
rHl-associated β subunits might also contain less carbohydrate than their nerve and skeletal muscle counterparts and would thus be difficult to discern on autoradiograms. To test for this, autoradiograms were prepared in which the intensity of iodinated and immunoprecipitated skeletal muscle sodium channel bracketed the intensity of the rHl α-subunit bands. β subunits were easily visualized on these autoradiograms (see lanes 6 and 7 in Fig 3). Scanning densitometry of both these and the rHl autoradiograms demonstrates both the ability to visualize β-subunit bands when less radioactivity is present and the absence of a discernible β-subunit band in the rHl-containing lanes (see bottom of Fig 3). In addition, autoradiograms were systematically overexposed to ensure that β subunits would be visualized even if they were not efficiently iodinated by Bolton Hunter reagent. In each of these experiments, no specific immunoprecipitated radiolabeled bands were visible in the region where β subunits would be expected to be located (≈ 20 to 40 kD). These data support the conclusion that the rHl sodium channel does not contain associated β subunits.

Sodium channel phosphorylation by cAMP-dependent PKA has been reported to both regulate the expression of functional channels and modulate the activity of voltage-dependent sodium channels. To determine whether the rHl protein possesses sites capable of being phosphorylated by PKA, partially purified membrane proteins were phosphorylated using the catalytic subunit of PKA and then immunoprecipitated using each of the subtype-specific antisera. Again, an identical 240-kD rHl protein was specifically immunoprecipitated (Fig 4), confirming the availability of sites for PKA phosphorylation on the rHl α subunit.

The rHl α subunit appears as a relatively compact band on SDS-PAGE (Figs 3 and 4). This is in contrast to previously characterized sodium channel α subunits, which have molecular masses of 260 to 280 kD and appear as diffuse bands on SDS-PAGE. All sodium channel α subunits characterized to date contain approximately 25% to 30% (wt/wt) complex carbohydrate, of which approximately half is sialic acid. The diffuse character of the α-subunit band on Western blots reflects the known microheterogeneity of glycosylation found in complex glycoproteins. Based on a core protein molecular weight of 227 417 and the relatively compact appearance of the 240-kD band, it appears that the rHl channel contains only 12.6 kD of carbohydrate compared with 50 to 60 kD of carbohydrate for sodium channel α subunits from cel electrophax, rat brain, and rat skeletal muscle.

The carbohydrate of the rHl channel contains either N-acetylgalactosamine or terminal sialic acid, as evidenced by the specific interaction of this glycoprotein with immobilized wheat germ. To determine whether this sugar is simple or complex and to determine the core protein molecular mass of the rHl protein, partially purified, phosphorylated, and immunoprecipitated rHl protein was treated with neuraminidase or N-glycanase. Treatment with neuraminidase resulted in an ≈6-kD decrease in electrophoretic mobility, whereas treatment with N-glycanase produced an ≈12-kD decrease in electrophoretic mobility (Fig 5).
Discussion

The predominant sodium channel identified in cardiac tissues in vivo is tetrodotoxin and conotoxin insensitive, is sensitive to divalent cations, demonstrates use-dependent behavior with local anesthetics and antiarrhythmic agents, and is located diffusely throughout atrial and ventricular muscle. The rH1 rat cardiac channel was initially cloned from both a newborn rat heart cDNA library and a denervated rat skeletal muscle cDNA library. The rH1 channel includes an open reading frame of 6058 nucleotides that encodes for a protein of 2019 amino acids with a predicted Mr of 227,417. Northern blot analysis and RNase protection assays confirm that message for this channel is abundantly expressed in heart and denervated skeletal muscle but is not detectable in brain, kidney, spleen, liver, uterus, or adult skeletal muscle. Electrophysiological examination of the rH1 channel in a heterologous expression system both demonstrates kinetics closely resembling the predominant channel identified in vivo and confirms the tetrodotoxin and conotoxin insensitivity of this channel, its use-dependent behavior with type-I antiarrhythmic agents, and its sensitivity to divalent cations. In the present study, we confirm the expression of the protein product of this gene in the adult rat heart and biochemically characterize selected properties of the rH1 protein that may be important in regulating both channel expression and function. In a related abstract, we describe the immunocytochemical localization of the rH1 channel to the surface and t-tubular membranes of atrial and ventricular muscle cells and to the intercalated discs between adjacent ventricular muscle cells.

rH1 Molecular Mass

The rH1 α subunit appears as a compact band on SDS-PAGE with a molecular mass of 240 kD compared with 260 to 280 kD for the eel electroplax, rat brain, and rat skeletal muscle sodium channels. With a core protein molecular mass of 227.4 kD, this implies the presence of only 12.6 kD of carbohydrate compared with 50 to 60 kD of carbohydrate in other characterized sodium channel proteins. The finding that the molecular mass of the deglycosylated protein approximates the calculated core protein molecular mass indicates that minimal, if any, posttranslational proteolysis of the.
channel α subunit has occurred. This is confirmed by the ability of both amino- and carboxy-terminal antibodies to immunoprecipitate the 240-kD protein. rH1 has 14 potential N-linked glycosylation sites compared with up to 18 in other sodium channels; 5 of these sites are common to all sodium channels sequenced to date. The reduced quantity of carbohydrate associated with the rH1 α subunit implies either that less carbohydrate is attached per potential glycosylation site or that not all potential sites are glycosylated. Assuming 2 to 6 kD of sugar per potential glycosylation site,22 then anywhere from 2 to 6 of the 14 potential glycosylation sites may be posttranslationally modified by carbohydrate. This can be compared with the adult skeletal muscle sodium channel protein, in which nearly every potential glycosylation site contains carbohydrate (S.A. Cohen, S.J. Zwerling, and R.L. Barchi, unpublished observations).

Core glycosylation appears to be required for the normal synthesis, processing, and membrane insertion of sodium channels3,22; terminal sialic acid groups are thought to play a role both in determining the magnitude of the electric field affecting sodium channel activation and in stabilizing the normal conducting conformation of the ionic pore.39 The present study demonstrates that, similar to other sodium channels, carbohydrate attached to the rH1 protein is complex, as evidenced by an ≈6-kD reduction in molecular mass after neuraminidase treatment. However, although the rH1 channel resembles other sodium channel α subunits in being a complex sialated glycoprotein, it is unique in that it contains 5% rather than 25% to 30% carbohydrate by weight.

Finally, rH1 channel cDNA expressed in oocytes has a linearly shaped current-voltage curve and a reduced single-channel current amplitude. These unique electrophysiological characteristics are thought to result from decreased negative charge near the extracellular opening of the rH1 channel. Although differences in local amino acid residue charge have been suggested as being responsible for this effect,35,38 it is also possible that the reduced glycosylation and resulting fewer negatively charged sialic acid residues present on the rH1 channel might contribute to this finding. The exact role of the reduced levels of glycosylation in the expression and function of this channel will need to be determined by further studies.

Absence of β Subunits

Although our autoradiograms clearly demonstrate no radiolabeled bands in the region where one would expect to see channel β subunits, it is possible that β subunits might have become dissociated from α subunits during biochemical manipulation. We think this unlikely since β subunits are not easily dissociated from either rat brain or rat skeletal muscle α subunits, the only other β-associated α subunits that have been extensively biochemically characterized. Rat brain β1 subunits require treatment with 1.0 mol/L MgCl2 followed by sedimentation through sucrose gradients to be separated from rat brain α subunits, whereas treatment with disulfide reducing agents is required to separate rat brain β1 subunits from rat brain α subunits.27 Rat skeletal muscle β subunits require ionic detergents to be separated from α subunits, and α:β stoichiometry in adult rat skeletal muscle was determined to be 1:1,28 whereas α:β1, β1 stoichiometry in rat brain was shown to be 1:1:1.38 In no case in the literature21,29 or in our experience with skeletal muscle sodium channels has solubilization with nonionic detergents or immunoprecipitation resulted in dissociation of β from α subunits. Therefore, it is unlikely that unrecognized loss of β subunits occurred during any of our biochemical manipulations.

An alternate possibility for our results is that β subunits might be minimally labeled, thus making it difficult or impossible to be visualized on autoradiograms. Fig 3 demonstrates that simply reducing the extent of radioactive labeling still provides an easily discernable β-subunit band on autoradiograms. This analysis and the systematic overexposure of autoradiograms make it extremely unlikely that minimally labeled β subunits were present but were undetected.

Various roles have been assigned to channel β subunits, including those of stabilizing α-subunit structure29,40 and modulating channel kinetics.41,42 Although initial reconstitution studies of purified cest electroplax sodium channels (which lack β subunits) demonstrated that α subunits alone were sufficient to mediate ion fluxes with the voltage dependence, ion selectivity, and
pharmacological properties expected of native sodium channels,43 the rat brain and adult rat skeletal muscle channels could only be successfully reconstituted as complexes of α and β subunits.40,44

Recent heterologous expression studies of α-subunit cRNA in oocytes support the notion that these small subunits may not be required for the production of fully functional channels but indicate that they may play a modulating role in both channel expression and function. Heterologous expression of rat brain and rat skeletal muscle sodium channels produces channels with abnormal inactivation kinetics. Coinjection of either low molecular weight mRNA from rat brain or of cRNA prepared from a subgroup of cDNAs that does not itself encode for sodium channel α subunits reduces the time course of channel inactivation to values comparable to those seen in vivo and increases the cell surface expression of functional sodium channels almost fourfold.41,42

The recent elucidation of the primary structure and the functional expression of the β, subunit of the rat brain sodium channel has confirmed most of these suggestions.45 Coexpression of β, subunits with α subunits increased the size of the peak inward sodium current, accelerated its inactivation, and shifted the voltage dependence of inactivation to more negative membrane potentials. These results indicate that β subunits play a role in sodium channel assembly, expression, and functional modulation. It is tempting to speculate that the normal channel kinetics of rH1 cRNA expressed alone in oocytes is both consistent with the lack of need for β subunits by rH1 and with their absence as demonstrated in this study.

Channel Phosphorylation

Each sodium channel studied to date is capable of being rapidly and specifically phosphorylated by cAMP-dependent PKA. All sodium channels have high levels of basal phosphorylation, and activation of endogenous PKA induces phosphorylation of the same residues that are phosphorylated in vitro.46,47 Electrophysiological effects of PKA activation include regulation of the surface expression of functional channels,29,48 reduction in channel conductance,27,49 and shifting of the voltage dependence of channel inactivation to more negative potentials.50 The rH1 protein contains five potential sites for PKA phosphorylation: one in the amino terminus, two in the interdomain 1-2 region, and two in the interdomain 2-3 region. Our results demonstrate that the rH1 channel protein can be phosphorylated by PKA in at least one of these sites.

Two previous studies address the question of the biochemical characteristics of sodium channels in mammalian cardiac membranes. In the first study, antibody SP-19, directed against an 18-residue sequence in the 100% conserved interdomain 3-4 region, was used to survey the biochemical characteristics of sodium channels in several tissues including rat heart.20 A 230-kD phosphorylated α subunit containing ≈8 kD of sialic acid and lacking a β subunit was immunoprecipitated by this antiserum from rat heart. In the second study, the same investigators specifically immunoprecipitated a β, subunit from solubilized cardiac membranes using antisera directed against purified nerve β subunits;21 this finding was recently corroborated by Northern blot analysis of total RNA from rat heart using a rat brain β, subunit–specific probe.45 One difference between these and the present study is that the former studies used a single antibody against a highly conserved sodium channel sequence, whereas the present study uses three sequence- and subtype-specific antisera directed against different regions of the rH1 protein. Our subtype-specific antisera confirm that the biochemical characteristics observed are specific for the rH1 channel. In addition, the ability to immunoprecipitate a β subunit from solubilized cardiac membranes or to identify β subunit mRNA on Northern blots does not identify the α subunit with which it is associated; the β subunit could be associated either with sodium channels present on autonomic nerves that innervate the heart or with any of the other sodium channels whose messages have been identified in cardiac tissues. Antisera specific for each of the other transcripts identified in cardiac preparations will need to be generated to confirm the presence, determine the tissue localization, and elucidate the characteristics of each of these distinct channels.

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