Motif III S5 of L-type Calcium Channels Is Involved in the Dihydropyridine Binding Site

A COMBINED RADIOLIGAND BINDING AND ELECTROPHYSIOLOGICAL STUDY*

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Voltage-dependent Ca\(^{2+}\) channels (VDCC\(^1\)) are divided into two subfamilies, the DHP-sensitive L-VDCC subfamily and the DHP-insensitive VDCC subfamily (1). Electrophysiological studies using charged DHPs pointed out that the binding site is accessible exclusively from the outside membrane (2, 3). Previous experimental evidence using photoaffinity labeling and peptide antibody mapping suggested that S6 of motif III and IV plus part of their adjacent extracellular loops (4, 5) as well as a putative “E-F hand” flanking region (6) are involved in forming the binding “pocket”. These results were in part supported by molecular studies. Because there is a considerable sequence homology between the residues of the DHP-sensitive VDCC \(\alpha\)\(_1\) and the DHP-insensitive VDCC \(\alpha\)\(_1\) subunits, construction of chimeric channels proved to be a logical method to identify regions involved in the drug binding sites. It was shown that part of IV S6 and the linker of IV S5/S6 were critical for the DHP agonist effect (7). In order to further define the regions responsible for DHP binding and pharmacological action, single amino acids in III S6 and IV S6 were mutated, and radioligand binding demonstrated that these mutants had reduced binding affinity to DHPs (8, 9). Moreover, electrophysiological studies suggested that the amino acids in IV S6 may contribute differentially to the effect of DHP agonist and antagonist (7, 9, 10). The importance of these regions was further confirmed by introducing III S5-III S6 and IV S6 along with part of IV S5/S6 linker of \(\alpha\)\(_1\) channel into the \(\alpha\)\(_{1A}\) channel (10). This alteration was sufficient to confer sensitivity to both DHP agonist and antagonist to the \(\alpha\)\(_{1A}\) channel.

At the same time, we found that by replacing the III S5-S6 of the \(\alpha\)\(_{1C}\) with that of the corresponding region of \(\alpha\)\(_{1A}\), the resulting Ca\(^{2+}\) channels lost sensitivity to both DHP agonist (−)BayK 8644 and antagonist (+)BayK8644 (11). If III S6 was replaced by the corresponding region of \(\alpha\)\(_{1A}\), the chimeric channel became insensitive to (−)BayK8644, and the EC\(_{50}\) of (−)BayK8644, the agonist, was increased 10-fold compared with the wild type channel (11). We speculated that even though III S5 was not photoaffinity labeled, it may form an important part of interaction sites for DHPs distal from the photoproactive group. We feel that these mutations in III S5 cause alterations in the binding of DHPs to the channel, which in turn decreases the response of the channels to these drugs. Because DHP binding is done on membranes that are electrically neutral, and electrophysiological effects are voltage- and state-dependent, it is difficult to interpret whether mutations cause only an alteration of the binding pocket and/or changes in other functional properties of the channel occur. It is critical, therefore, to study the coupling between DHP binding and modulation of current. Thus, we mutated nonconserved amino acids of III S5 in the \(\alpha\)\(_{1C}\) channel (12, 13) to those in the \(\alpha\)\(_{1A}\) channels (14) and tested the mutants using both radioligand binding and electrophysiological studies. We found consistent results in terms of binding affinity and pharmacological sensitivity of these mutants to DHPs, strongly suggesting the importance of S5 in repeat III of the \(\alpha\)\(_1\) subunit of the L-VDCC.

The \(\alpha\) subunit of L-type voltage-dependent Ca\(^{2+}\) channels (\(\alpha\)\(_{1C}\)) has been shown to harbor high affinity binding sites for the Ca\(^{2+}\) channel dihydropyridine (DHP) modulators. It has been suggested by a number of investigators that the binding site may be composed of III S6 and IV S6. Evidence with chimeric channels indicated the possible involvement of III S5 in DHP binding. Site-directed mutations were introduced in motif III S5 region of the \(\alpha\)\(_{1C}\), changing the amino acids to their counterparts in the DHP-insensitive \(\alpha\)\(_{1A}\) channel. The mutant channels were expressed both in HEK 293 cells and in Xenopus oocytes. Equilibrium binding and electrophysiological studies showed that the Thr\(^{1006}\) to Tyr substitution produced a mutant channel with at least 1000-fold decreased affinity in \[^{3}H\](+)(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-(2,6-dimethyl-5-methoxy carbonyl)pyridine-3-carboxylate (PN200-110, isradipine) binding and in sensitivity of R(−)-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridincarboxylic acid isopropyl ester (R202-791) in terms of inhibition of current through the L-type voltage-dependent Ca\(^{2+}\) channels. Replacing Gln\(^{1016}\) with Met resulted in more than a 10-fold decrease in binding affinity for \[^{3}H\](+)(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-(2,6-dimethyl-5-methoxy carbonyl)pyridine-3-carboxylate (PN200-110) and in the potency of channel modulation by S202-791. Four additional mutations in this region also lead to a slight but statistically significant increase of \(K_D\) values for \[^{3}H\](+)(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridincarboxylic acid isopropylester (R202-791) in terms of inhibition of current through the L-type voltage-dependent Ca\(^{2+}\) channels. The binding and electrophysiological results show that certain residues of the transmembrane segment III S5 are important in contributing to the DHP binding “pocket” and are critical for DHP binding and for its calcium channel effect.
Fig. 1. DHP binding characteristics of selected mutants in III S5 of the cardiac Ca\(^{2+}\)-channel. A, upper, schematic representation of \(\alpha_1\), motif III of the voltage-dependent, Ca\(^{2+}\) channel. Lower, alignment of III S5 amino acid sequences of \(\alpha_1\) and \(\alpha_{1A}\). The numbers refer to the \(\alpha_{1A}\) sequence. B, the saturation binding curves of wild type, T1007M, and Q1010M to the DHP antagonist [\(\text{H}]\)PN200-110. C, the \(K_d\) values from the saturation curve fittings are presented by horizontal bars to the right. The names of the mutants are indicated to the left. The data are means \(\pm\) S.E. A p value smaller than 0.05 is represented by an asterisk. Two asterisks represent a p value < 0.005. The open end of the horizontal bar of T1007Y represents an estimated value. D, Scatchard plots of the saturation binding data of wild type, T1007M, and Q1010M.

EXPERIMENTAL PROCEDURES

Construction of \(\alpha_1\) cDNA Clones—The wild type and mutant rabbit heart L-VDCC \(\alpha_1\) subunit cDNAs were constructed in pBluescript II SK(−) in the T7 orientation for expression in the Xenopus oocyte system as described (7). Site-directed mutations were introduced by the PCR (Hoffman-LaRoche) in two steps. NsiI and PstI restriction sites were used to insert the PCR products. Oligonucleotides carrying the PstI site or having the desired mismatching bases served as forward primers, whereas oligonucleotides having the NsiI site served as the reverse primer. The cassette in the wild type \(\alpha_1\) subunit was replaced by the PCR products, and the existence of point mutations was verified by dideoxynucleotide sequencing (Amersham Corp.). For mammalian HEK 293 cell expression, mutant and wild type \(\alpha_1\) cDNAs were transferred from pBluescript II SK(−) into the mammalian expression vector pAGS-3 (15) at HindIII and NotI sites.

Transient Expression of Ca\(^{2+}\) Channels in HEK 293 Cells—HEK 293 cells were transfected with \(\alpha_1\), \(\beta_3\), \(\alpha_2\) (16), and human \(\beta_3\) (17) subunits at a 1:2:2 molar ratio by the calcium phosphate precipitation technique (18). Cells were harvested by scraping 45–72 h post transfection. Cells were washed twice with phosphate-buffered saline and disrupted in homogenizing buffer (10 mM Tris, pH 7.4, 2.0 \(\mu\)g/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride). The membranes were pelleted by centrifugation at 36,000 rpm for 40 min and resuspended in storage buffer (50 mM Hepes-NaOH, 1.37 mM MgCl\(_2\), 1 mM EDTA, pH 7.4, 2 \(\mu\)g/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride). Protein concentrations were measured using a BCA assay kit (Pierce).

Radioligand Binding—Saturation binding assays of [\(\text{H}\)](+)-PN200-110 (Amersham Corp.) were performed according to Varadi and coworkers (19) using 100 \(\mu\)g of protein for each sample in the binding buffer (100 mM Tris, 1.4 mM MgCl\(_2\), 2.4 mM CaCl\(_2\), pH 7.4) at 25 °C for 1 h. Non-specific binding was determined using 1 \(\mu\)M unlabeled ([\(\text{H}\)](+)-PN200-110). Membranes were collected on glass fiber filters (FF-200 GF/C) and then washed with 50 mM Tris buffer (pH 7.4). Bound radioactivity was measured by liquid scintillation counting.

Xenopus Oocyte Expression—Expression of the wild type and mutant calcium channels in oocytes was done as described previously (7). In order to achieve maximal expression, the \(\alpha_1\) subunit cRNAs were coinjected with cRNAs specific for the \(\alpha_2\beta_3\) (16) and human \(\beta_3\) (20) subunits at a 1:1:1 molar ratio. Ca\(^{2+}\)-activated Cl\(^-\) channel contamination of Ba\(^{2+}\) currents was eliminated by microinjecting the oocytes with 50 \(nL\) of a solution containing 100 mM 1,2-bis(2-aminophenoxy)-ethane-N\(_2\)N\(_2\)N\(_2\)N\(_2\)-tetraacetate, 1 mM Tris, pH 7.4, 60 min preceding current recording. Whole cell currents were recorded with a two-electrode voltage-clamp amplifier (Axoclamp 2A). Voltage pulses were applied from a holding potential (HP) of −30 mV to test potentials between −30 mV and +40 mV in 10 mV increments. Whole cell leakage and capacitive currents were subtracted on line using the P/4 procedure. Currents were digitized at 1 kHz after being filtered at 1 kHz. The pClamp software (Axon Instruments, Burlingame, CA) was used for data acquisition and analysis. Only oocytes that showed less than 15% rundown over the first minutes were included in the analysis. The peak inward Ba\(^{2+}\) currents in the control solution and after applying each concentration of DHP compounds were plotted against DHP concentrations, and the curves were fitted to the following equation:

\[
I_{Ba^{2+}}/I_{Ba^{2+}}(\text{control}) = 1/(1 + (C/C_{50})^h)
\]  

where \(I_{Ba^{2+}}\) is Ba\(^{2+}\) current, \(C\) is the concentration of DHP compounds, \(C_{50}\) is the half-effective (inhibitory) concentration, and \(h\) is the Hill coefficient.

Chemicals—Stereosomers of S202-791 were purchased from Biomol Research Laboratories Inc. (Plymouth Meeting, PA). [\(\text{H}\)](+)-PN200-110 was purchased from Amersham Corp. All other chemicals were from Sigma.

Statistical Analysis—The results were analyzed by the Radlig program version 4.0 (Biosoft) and Kaleidagraph software. The data are presented as the means \(\pm\) S.E. The statistical analyses necessary were performed using Student's paired t test, with p values lower than 0.05 considered as indicator of significant difference.

RESULTS AND DISCUSSION

Our previous work investigated the contribution of motif IV to DHP effects by testing chimeric \(\alpha_1\) subunits engineered between the DHP-sensitive rabbit heart L-VDCC (Ca9) and the DHP-insensitive brain B12 (\(\alpha_{1A}\) subunits (7). We then further studied the contribution of other motifs to DHP effects. Our preliminary data (11) indicated that by replacing motif III SS-S6 of Ca9 (wild type) with that of the corresponding region of the \(\alpha_{1A}\), DHP sensitivity was lost.

A two-pronged experimental approach was launched. Following site-directed mutagenesis to change nonconserved amino acids in \(\alpha_{1C}\) (Ca9) into the corresponding amino acids of \(\alpha_{1A}\) subunit, each mutant was transiently coexpressed in HEK 293 cells with the accessory \(\alpha_2\beta_3\) subunits, and the "cardiac DHP receptor" was tested for DHP affinities using [\(\text{H}\)](+)-PN200-110 by equilibrium binding assays. In parallel, the same mutants were expressed in Xenopus oocytes, and the Ca\(^{2+}\) channel sensitivity to DHP agonists and antagonists was...
tested. [3H](+)-PN200-110 binds to the membranes harboring wild type channel with a dissociation rate \( K_D \) of 0.125 ± 0.019 nM. A hyperbolic saturation curve and a linear Scatchard plot indicate a single class of binding sites (Fig. 1B). We initiated our study by mutagenizing Thr\(^{1006}\), which starts the second putative \( \alpha \)-helical turn of III S5. Mutation of Thr\(^{1006}\) to Tyr caused a dramatic decrease in the apparent affinity of the Ca\(^{2+}\) channel toward DHPs. At 25 nM [3H](+)-PN200-110, only slight specific binding was detectable. At 75 nM of [3H](+)-PN200-110, there was an increase in specific binding. These results indicate that the \( K_D \) of T1006Y may be close to 100 nM. However, because specific binding can only be observed when high concentrations of ligand is used, it is difficult to make a quantitative analysis for the binding properties of DHPs to this mutant. We next mutated Gln\(^{1010}\), which may be adjacent to Thr\(^{1006}\) if one assumes an \( \alpha \)-helical structure for III S5. Q1010M had a \( K_D \) of 1.612 ± 0.395 nM, which is also significantly different from that of the wild type channel. These results suggest that Thr\(^{1006}\) and Gln\(^{1010}\) might be part of the tertiary structure forming the "DHP binding pocket."

In order to investigate other nonconserved residues of III S5, we then mutated Cys\(^{1015}\) and Gly\(^{1017}\) to their counterparts Val and Ala in the DHP-insensitive \( \alpha_A \) channel, because they may participate in disulfide bridges or turns, thereby contributing to the overall structure of the DHP binding domain. C1015V and G1017A mutants revealed a \( K_D \) value for [3H](+)-PN200-110 of 0.261 ± 0.016 nM and 0.180 ± 0.011 nM, respectively. This demonstrates that Cys\(^{1015}\) and Gly\(^{1017}\) are probably not important structural members for DHP binding. Because the residues Thr\(^{1006}\) and Gln\(^{1010}\) played an important role in DHP binding, we further mutated the adjacent amino acids Thr\(^{1007}\), Leu\(^{1009}\) and Met\(^{1012}\) to the corresponding amino acids in the \( \alpha_A \) channel. T1007M, L1009F, and M1012I mutant membranes had \( K_D \) values indicating a 2–3-fold decrease in affinity. We speculate that these mutations may only slightly change the conformation of the DHP binding pocket.

In order to see whether the mutations that are associated with the changes described reflect corresponding changes in the channel action, T1006Y, T1007M, and Q1010M channels were expressed in Xenopus oocytes. Every mutant expressed functional voltage-dependent Ca\(^{2+}\) channels. All of the mutants exhibited a current activation threshold between −30 and −20 mV, and the peak current was reached at +10 to +20 mV test potential. The basic properties of current activation and inactivation were not affected by the mutations. The average of the peak inward current amplitude was 489.4 ± 39.3 nA (n = 48), 930.3 ± 248.1 (n = 13), 601.4 ± 64.3 (n = 22), and 390.2 ± 57.3 nA (n = 25) for the wild type, T1006Y, T1007M, and Q1010M channels, respectively.

The effects of the DHP agonist (+)-S202-791 and antagonist (−)-R202-791 were tested on T1006Y, T1007M, Q1010M, and the wild type. T1006Y completely lost the sensitivity to both agonist and antagonist (Fig. 2). After applying 10 μM agonist (+)-S202-791 or antagonist (−)-R202-791, we observed an approximately identical 23% decrease in the peak current that is likely due to a run-down during the experiment (Table I). T1007M showed a slight decrease of sensitivity to the agonist, but this difference was not statistically significant (Table I). The sensitivity to the antagonist of this mutant was slightly decreased compared with the wild type channel. After application of 1 μM and 10 μM (−)-R202-791, the peak current was decreased by 40 and 78%, respectively (Table I), and it was associated with an accelerated inactivation similar to the wild type. Q1010M partially retained sensitivity to both agonist and antagonist. The peak current was increased by 1.65-fold after application of 10 μM (+)-S202-791 and decreased by 37% after

**Table I**

|        | Wild type | T1006Y | T1007M | Q1010M |
|--------|-----------|--------|--------|--------|
| (+)-S202-791 | 1 μM | 3.79 ± 0.37 | 0.98 ± 0.45 | 1.14 ± 0.30 |
|         | 10 μM | 3.32 ± 0.53 | 0.63 ± 0.82 | 1.65 ± 0.09 |
| (−)-R202-791 | 1 μM | 0.39 ± 0.05 | 0.60 ± 0.05 | 0.89 ± 0.04 |
|         | 10 μM | 0.17 ± 0.04 | 0.23 ± 0.03 | 0.63 ± 0.08 |

*p < 0.05 versus wild type.
10 μM (-)R202-791. However, these effects were significantly smaller than those seen with the wild type. The calculated EC_{50} values were 0.33, >10, 0.45, and 3.2 μM for the wild type, T1006Y, T1007M, and Q1010M, respectively. The calculated IC_{50} values were 0.13, 7.25, 0.66, and 3.82 μM for the above listed clones. In Fig. 3, the current-voltage relations of the Ba^{2+} current for the wild type and selected mutant channels are shown. Like the wild type channel, the T1007M responded to (+)S202-791 with both enhancement of current amplitude as well as by a shift of the current-voltage relations to hyperpolarized potentials.

DHP antagonist activity increases when more channels are shifted to inactivated states (21). It is possible that the mutations change the distribution of Ca^{2+} channels active in a certain conformation. The decrease in DHP binding affinity that was determined at 0 mV membrane potential may be due to a loss of a channel's ability to become inactivated. However, our data showed that these mutants had normal basic biophysical properties. In addition, these mutants responded to DHP agonists and antagonists in parallel, even though the DHP agonist effect is not dependent on membrane potential. We believe that the decrease in binding affinity of the DHPs to these mutants is caused by a direct alteration at the interaction site between the DHP and the Ca^{2+} channel and not a general nonspecific conformational change. If the latter were true, we would expect electrophysiological changes in the basic properties of the channel.

In our study, we used a mammalian cell line for the binding assay and *Xenopus* oocytes for electrophysiological measurements. One must therefore apply caution in comparing binding dissociation constants and EC_{50}/IC_{50} values. In the study of quinidine's effect on cloned K^{+} channels, it was reported that at least a ten times higher drug concentration was needed to achieve the same effect in the oocyte system than in mammalian cells; however, normal sensitivity was seen in excised patches (22). In mammalian cells, the IC_{50} of α_{1C} to DHP antagonists is approximately 10 nm. Considering this factor, our results from the oocyte system are in good agreement with the data obtained from mammalian cells in which the binding was done. In addition, our electrophysiological measurements were performed at −30 mV holding potential in which some channels (−30%) will be in the inactivated state. Thus, the results under these conditions should be reasonably comparable with those obtained from the binding studies that were performed at 0 membrane potential.

PN200-110 is an intrinsically photoactive DHP compound. The benzofurazan group at the para-position of the DHP ring can cross-link with amino acids. Photoaffinity labeling studies using PN200-110 showed that this DHP was mainly incorporated into motif III S6 (4). This suggests that the benzofurazan group of PN200−100 may associate with III S6. It is still not clear where the dihydropyridine ring binds. Radioligand binding studies suggest a common binding site for both DHP agonists and antagonists (8), whereas electrophysiological studies support the concept of different sites or different conformations of the same site (7, 9, 10). It is possible that the DHP ring of both agonists and antagonists bind to the same site but the 4-aryl ring interacts with a different component of the channel. The Q1010M and T1006Y mutant channels had a decreased sensitivity to agonists and antagonists and to a similar degree for both. Therefore, we now need to consider the importance of III S5 as being involved in the interaction with a domain shared between DHP agonists and antagonists.

Dihydropyridines bind to and modulate L-VDCC functions in a highly specific, voltage-dependent manner. Mutagenesis studies revealed a complex binding site that also emphasizes the importance of cooperative interactions. It is intriguing to compare these data with the ones obtained studying the binding site of local anesthetics on the Na^{+} channel (23, 24). The hydrophobic portion of local anesthetics binds to IV S6, whereas the charged portion may interact with the P regions of motifs II and III (25). Additionally, mutations that are characteristic of the disease paramyotonia congenita also modulate local anesthetic effects (L_{II,IV}, IV S2, and IV S4) (26). The binding site of DHPs on the L-type Ca^{2+} channels may be composed of III S5, III S6, and IV S6. Additional regions that appear to influence some aspects of DHP binding are III S2 and IV S3 (voltage dependence) (27) and the motif III P-region (Ca^{2+} dependence) (28, 29). The complexity of drug binding sites and regions that modulate drug effects indicate that these drugs bind to a "dynamic pocket" in the channel, and one must be alert to monitor several aspects of channel function to be certain that the mutations under investigation actually alter one aspect and not all of them.

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Note Added in Proof—After submitting this manuscript (November 27, 1996), a paper appeared in the Journal of Biological Chemistry by Mitterdorfer et al. (Mitterdorfer, J., Wang, Z., Sinnegger, M. J., Hering, S., Striessnig, J., Grabner, M., and Glossmann, H. (1996) J. Biol. Chem. 271, 30330–30335), using molecular reagents we supplied in part. Point mutations in motif III S5 of a brain channel (BI, a1A), engineered to be DHP-sensitive, using an oocyte expression system, revealed the importance of the same threonine and glutamine residues described in our paper.

REFERENCES
1. Zhang, J. F., Randall, A. D., Ellinor, P. T., Horne, W. A., Sather, W. A., Tanabe, T., Schwarz, T. L., and Tsien, R. W. (1993) Neuropharmacology 32, 1075–1088
2. Strubing, C., Hering, S., and Glossmann, H. (1993) Br. J. Pharmacol. 108, 884–891
3. Catterall, W. A., and Striessnig, J. (1992) Trends Pharmacol. Sci. 13, 256–262
4. Striessnig, J., Murphy, B. J., and Catterall, W. A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10769–10773
5. Nakayama, H., Taki, M., Striessnig, J., Glossmann, H., Catterall, W. A., and Kanaoka, Y. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9203–9207
6. Regulla, S., Schneider, T., Nastainczyk, W., Meyer, H. E., and Hofmann, F. (1991) EMBO J. 10, 45–49
7. Tang, S., Yatani, A., Bahinski, A., Mori, Y., and Schwartz, A. (1993) Neuron 11, 1013–1021
8. Peterson, B. Z., Tanada, T. N., and Catterall, W. A. (1996) J. Biol. Chem. 271, 5293–5298
9. Schuster, A., Lacinova, L., Klugbauer, N., Ito, H., Birnbaumer, L., and Hofmann, F. (1996) EMBO J. 15, 2365–2370
10. Grabner, M., Wang, Z., Hering, S., Striessnig, J., and Glossmann, H. (1996) Neuron 16, 207–218
11. He, M., Wakamori, M., and Schwartz, A. (1996) Biophys. J. 70, 184 (abstr.)
12. Slish, D. F., Engle, D. B., Varadi, G., Lotan, I., Singer, D., Dascal, N., and Schwartz, A. (1989) FEBS Lett. 250, 509–514
13. Itagaki, K., Koch, W. J., Bodi, I., Klockner, U., Slish, D. F., and Schwartz, A. (1992) FEBS Lett. 297, 221–225
14. Morii, Y., Friedrich, T., Kim, M. S., Mikami, A., Nakai, J., Ruth, P., Bosse, E., Hofmann, F., Flockerzi, V., Furushi, T., Mikoshiha, K., Imoto, K., Tanabe, T., and Numa, S. (1991) Nature 350, 398–402
15. Miyazaki, J., Takaki, S., Araki, K., Tashiro, F., Tominaga, A., Takatsu, K., and Yamamura, K. (1989) Gene (Amst.) 79, 269–277
16. Ellis, S. B., Williams, M. E., Ways, N. R., Brenner, R., Sharp, A. H., Leung, A. T., Campbell, K. P., McKenna, E., Koch, W. J., Hui, A., Schwartz, A., and Hampond, M. M. (1988) Science 241, 1661–1664
17. Klockner, U., Mikala, G., Varadi, M., Varadi, G., and Schwartz, A. (1995) J. Biol. Chem. 270, 17306–17310
18. Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752
19. Varadi, G., Leny, P., Schultz, D., Varadi, M., and Schwartz, A. (1991) Nature 352, 159–162
20. Collin, T., Wang, J. J., Nargeot, J., and Schwartz, A. (1993) Circ. Res. 72, 1337–1344
21. Bean, B. P. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 6388–6392
22. Yatani, A., Wakamori, M., Mikala, G., and Bahinski, A. (1993) Circ. Res. 73, 351–359
23. Qu, Y., Rogers, J., Tanada, T., Scheuer, T., and Catterall, W. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11839–11843
24. Ragsdale, D. S., McPhee, J. C., Scheuer, T., and Catterall, W. A. (1994) Science 265, 1724–1728
25. Sunami, A., Dudley, S. D., Kyle, J. W., and Fozzard, H. A. (1996) Circulation 94, Suppl. I, 256 (abstr.)
26. Pan, Z., George, A. L., Kyle, J. W., and Makielski, J. C. (1996) Biophys. J. 70, 319 (abstr.)
27. Soldatov, N. M., Bouron, A., and Reuter, H. (1995) J. Biol. Chem. 270, 10540–10543
28. Petersen, B. Z., and Catterall, W. A. (1995) J. Biol. Chem. 270, 18201–18204
29. Mitterdorfer, J., Sinnegger, M. J., Grabner, M., Striessnig, J., and Glossmann, H. (1995) Biochemistry 34, 9350–9355