FEBS Letters 427 (1998) 220–224 FEBS 20191

Ca^{2+} channel sensitivity towards the blocker isradipine is affected by alternative splicing of the human α_{1C} subunit gene

R.D. Zühlke[1,*], A. Bouron, N.M. Soldatov[2], H. Reuter

Department of Pharmacology, University of Bern, Friedbühlstr. 49, CH-3010 Bern, Switzerland

Received 1 April 1998

Abstract L-type Ca^{2+} channels are important targets for drugs, such as dihydropyridines (DHPs), in the treatment of cardiovascular diseases. Channel expression is regulated by alternative splicing. It has been suggested that in the cardiovascular system tissue-specific expression of different L-type Ca^{2+} channel splice variants may underlie the observed differences in sensitivities to channel block by DHPs. We investigated the sensitivity of Ca^{2+} channel splice variants derived from the human α_{1C} gene to the DHP isradipine. Among seven α_{1C} channels we observed up to 10-fold differences in IC_{50} values for isradipine, as well as changes in the voltage dependence of DHP action.

Key words: Ca^{2+} channel; Splice variant; Channel blocker; Dihydropyridine; *Xenopus* oocyte

1. Introduction

Voltage-gated Ca^{2+} channels are heteromeric protein complexes where the main subunit, α_1, forms the voltage-sensitive functional pore structure [1–3]. It is composed of four homologous domains (I–IV), each of which contains six putative transmembrane segments (S1–S6). The α_{1C} subunit is a part of the dihydropyridine (DHP)-sensitive L-type Ca^{2+} channel. Sites of DHP interaction with α_{1C} subunits have been located in and around domains IIIS5–S6 and IVS6 [4–10]. More recently, high affinity DHP sensitivity could be conferred to non-L-type Ca^{2+} channels by transferring only 8–9 amino acids [11–13], which therefore provide the minimal constituents of the DHP binding site. However, amino acid sequences located outside these regions [14–16] as well as the interaction between α_{1C} subunits and auxiliary subunits [17–20] appear to modulate DHP sensitivity.

We have studied the DHP sensitivity of several Ca^{2+} channels which are splice variants of the human α_{1C} gene that was originally cloned from fibroblasts [21], but is virtually identical to that in other tissues, such as heart and brain. These channel α_{1C} subunits were coexpressed in *Xenopus* oocytes with the auxiliary α_2/δ and β_1 subunits. We show that amino acid sequences located in segments IIIS2 and IVS3 can slightly affect the interaction between the DHP isradipine and Ca^{2+} channels. However, the strongest effects on the isradipine sensitivity were seen in isoforms where alternative splicing modifies sequences before and in IS6 and in the C terminus of α_{1C}. Differences in IC_{50} values for isradipine spanned an up to 10-fold range. This adds additional evidence to previous results [14–16] indicating that structures distant from the high affinity DHP binding site are involved in the modulation of channel block by DHPs.

2. Materials and methods

2.1. Preparation of human α_{1C} subunit isoforms

The construction of the recombinant plasmids pHLCC77, pHLCC70, pHLCC72, and pHLCC86, encoding $\alpha_{1C,77}$, $\alpha_{1C,70}$, $\alpha_{1C,72}$, and $\alpha_{1C,86}$, respectively, has been described elsewhere [15,22]. However, please note that in our earlier paper [15] the two isoforms $\alpha_{1C,77}$ and $\alpha_{1C,70}$ were called $\alpha_{1C,22}$ and $\alpha_{1C,21}$, respectively, which emphasized the different exons present in the two constructs.

Partial clones and nucleotide positions (in parentheses) in the following description refer to the human fibroblast Ca^{2+} channel cDNA [21]. The recombinant plasmid pHLCC69 (EMBL Database accession number Z34809), encoding $\alpha_{1C,69}$, was prepared by using the *Mro*I (3462)/*Aat*II (4275) fragment from f39, and *Aat*II (4275)/*Sph*I (4747) fragment from uf11 in the scheme leading to pHLCC70 [15]. The plasmid pHLCC78 (EMBL Database accession number Z34816), encoding $\alpha_{1C,78}$, was obtained by the replacement of the *Mun*I (721)/*Sfu*I (3726) fragment of pHLCC69 with the respective fragment of pHLCC70. For the construction of pHLCC105 (EMBL Database accession number AJ224873), the plasmid that encodes the $\alpha_{1C,105}$ subunit, a fragment (1404–1738) containing the alternative exon 8a was first cloned by RT-PCR using poly(A)$^+$ RNA from human hippocampus (Clontech, Palo Alto, CA) as template. The *Rsa*I (1421)/*Sph*I (1711) fragment from this amplification product (clone P23/45) was further subcloned into pHLCC77. The construction of the mutant $\alpha_{1C,77}$ containing two amino acid substitutions in IIIS3 (G954F, Y958I) has been described [15].

2.2. Electrophysiological measurements

Xenopus laevis oocytes were prepared as described before [15,23]. For microinjection, cRNAs were transcribed in vitro after linearization of template cDNA [15,24]. cRNAs encoding the different α_{1C} subunit isoforms were always coinjected with cRNAs encoding the auxiliary subunits $\alpha_2\delta$ [25] and β_1 [26,27]. Electrophysiological experiments were carried out at room temperature 4–7 days after the injection. The oocytes were continuously superfused with a solution containing (in mM): $Ba(OH)_2$ 40, NaOH 50, KOH 1, HEPES 10, pH 7.4 (methanesulfonic acid). The DHP sensitivity was studied by using the DHP Ca^{2+} channel blocker (+)-isradipine, kindly provided by Dr. R.P. Hof (Sandoz, Basel, Switzerland). Isradipine-containing media were prepared fresh from a concentrated stock solution (1 mM in ethanol) and superfused until a steady-state inhibition of the Ba^{2+} current (I_{Ba}) was reached. The two-electrode voltage-clamp technique was applied to measure I_{Ba}, either with an Axoclamp 2-A amplifier (Axon Instruments, Foster City, CA, USA) or with a Warner Oocyte Clamp OC-725C amplifier (Warner Instruments, Hamden, NJ, USA). Cumulative dose-response curves were plotted and IC_{50} values and slopes were estimated by applying a double logarithmic regression through data points between IC_{80} and IC_{20}.

In some batches of injected oocytes we observed an endogenous, DHP-resistant Ca^{2+} current [28], which could influence the dose-response curves obtained in *Xenopus* oocytes when only small currents

*Corresponding author. Fax: (41) (31) 632 49 92.
E-mail: roger.zuehlke@pki.unibe.ch

[1]R.D.Z. and A.B. contributed equally to this work.

[2]Present address: Department of Pharmacology, Georgetown University Medical Center, 3900 Reservoir Rd., WN, Washington, DC 20007, USA.

PII S0014-5793(98)00425-6

through the heterologously expressed α_{1C} isoforms were recorded. We therefore excluded all recordings from oocytes with less than 500 nA I_{Ba} at a test pulse to +20 mV applied from a holding potential (V_h) of −90 mV. Based on this criterion, we also re-analyzed data presented before [15].

3. Results

3.1. Structural and electrophysiological properties of human α_{1C} splice variants

The putative transmembrane topology of the human α_{1C} subunit is schematically presented in Fig. 1A. The gene encoding the human α_{1C} subunit is composed of at least 50 exons [29] and its expression is characterized by alternative splicing [21,29,30]. Based on our reference channel $\alpha_{1C,77}$, we constructed cDNAs encoding six isoforms of the α_{1C} subunit, containing various combinations of alternatively spliced exons in four regions of the α_{1C} gene. Three of these alternative regions, located in segments IIIS2, IVS3, and the C-terminus (Fig. 1A), have already been documented [21,29]. The isoforms $\alpha_{1C,70}$, $\alpha_{1C,69}$, $\alpha_{1C,78}$, $\alpha_{1C,72}$, and $\alpha_{1C,86}$ contain alternative exons within these three regions (Fig. 1B). Another site of diversity, identified in α_{1C} from human hippocampus (N. Soldatov, unpublished) and encoding segment IS6 (Fig. 1A), is due to equally sized alternative exons 8a and 8. In the human α_{1C} gene, exon 8a precedes exon 8 and both are separated by a 303-bp intron (EMBL Database accession number Z26263). The amino acid sequences encoded by exons 8 and 8a, respectively, are identical to those found in α_{1C} from rabbit smooth muscle [31] and rabbit cardiac Ca^{2+} channels [32]. The isoform $\alpha_{1C,105}$ contains exon 8a instead of exon 8 in $\alpha_{1C,77}$ (Fig. 1B).

In *Xenopus* oocytes, when coexpressed with auxiliary subunits $\alpha_2\delta$ and β_1, all constructs gave rise to functional Ca^{2+} channels with some distinct electrophysiological features (Fig. 2A–C). The isoforms $\alpha_{1C,69}$, $\alpha_{1C,70}$, and $\alpha_{1C,78}$ exhibit Ba^{2+} currents similar to $\alpha_{1C,77}$, with almost the same voltage ranges of activation (I-V curves, Fig. 2B) and inactivation (Fig. 2C). This indicates that sequences in segments IIIS2 and IVS3, encoded by alternatively spliced exons 21/22 and 31/32, do not seem to be critically involved in channel gating. In contrast, $\alpha_{1C,105}$, $\alpha_{1C,72}$, and $\alpha_{1C,86}$ show differences in gating behavior when compared to $\alpha_{1C,77}$. While the I-V curve of $\alpha_{1C,86}$ is shifted towards more positive potentials, the isochronic inactivation curves of all three constructs are more negative than $\alpha_{1C,77}$ (Fig. 2C). In addition, the two isoforms $\alpha_{1C,72}$ and $\alpha_{1C,86}$, which contain alterations in C-terminal sequences because of alternative splicing of exons 40–43, showed significantly faster inactivation kinetics of I_{Ba} (Fig. 2A and [22]). Thus, certain amino acid residues near and within IS6 as well as in the C-terminus affect the I-V relationship and/or inactivation of α_{1C} isoforms.

3.2. Isradipine sensitivity of α_{1C} splice variants

Our main interest in this study concerned the pharmacological properties of the α_{1C} isoforms. Injected oocytes were voltage-clamped from $V_h = -90$ mV or −40 mV. Step depolarizations to +20 mV were applied every 30 s, as described before [15]. We have observed that endogenous, DHP-insensitive currents may occur in some batches of *Xenopus* oocytes injected with cRNA combinations containing $\alpha_2\delta$ and β_1. Therefore, we re-analyzed data obtained before [15] and performed new experiments with channel subunits $\alpha_{1C,77}$ (formerly $\alpha_{1C,22}$) and $\alpha_{1C,70}$ (formerly $\alpha_{1C,21}$).

Fig. 3A shows cumulative dose-response curves for $\alpha_{1C,77}$ and its isoforms encoded by cDNAs containing different

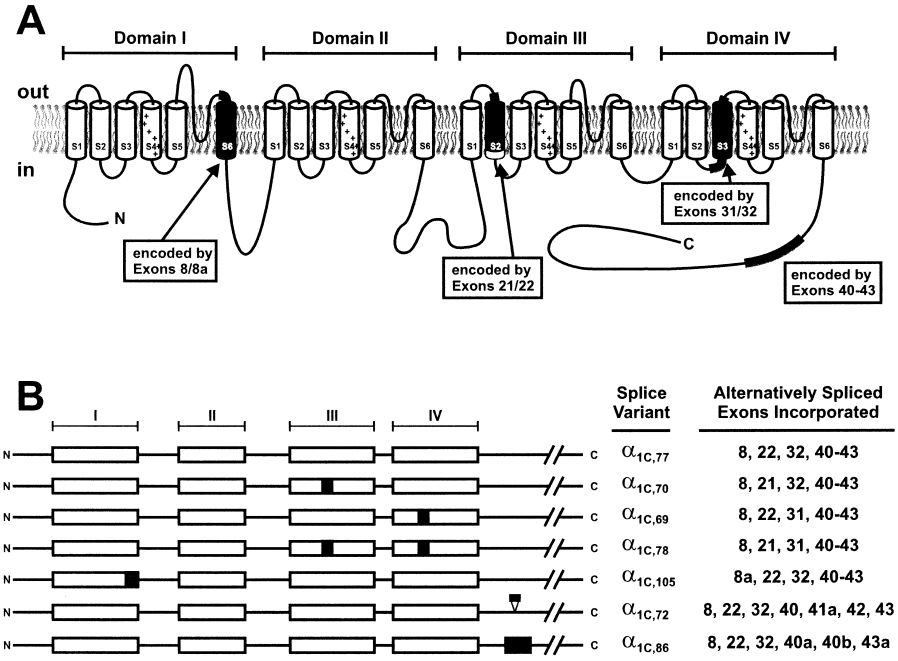

Fig. 1. Schematic representation of the α_{1C} subunit and its isoforms. A: The human α_{1C} subunit is characterized by four homologous domains (I–IV), each containing six transmembrane segments (S1–S6). Segments encoded by alternatively spliced exons are indicated. B: The cDNA constructs with alternatively spliced exons are schematically presented, with the respective exon combinations indicated in the right column. Exons 8 and 8a, 21 and 22, as well as 31 and 32 are pairs of mutually exclusive homologous exons. The presence of exon 41a in $\alpha_{1C,72}$ leads to an insertion of 19 amino acids in the C-terminus. In $\alpha_{1C,86}$, the combination of exons 40a, 40b, and 43a encodes a segment of 81 amino acids replacing 80 non-identical amino acids in $\alpha_{1C,77}$.

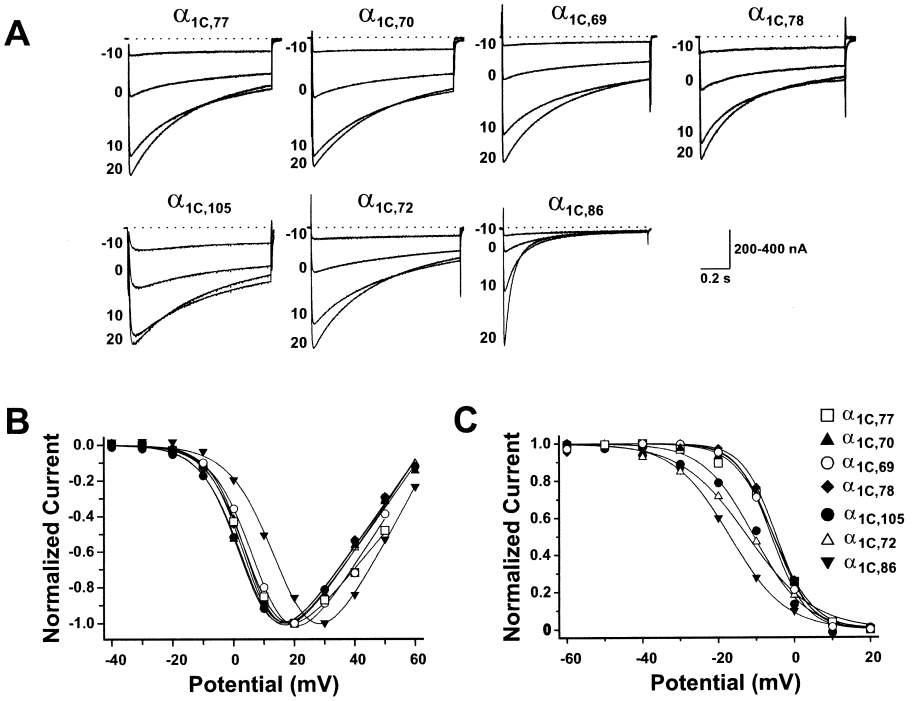

Fig. 2. Expression of human L-type Ca^{2+} channels isoforms in *Xenopus* oocytes. A: Representative families of current traces elicited from $V_h = -90$ mV are shown. The values of the depolarizing test potential are indicated on the left. B: Normalized current-voltage (I-V) curves. C: Isochronic inactivation curves for all α_{1C} isoforms. The inactivation curves were obtained by using a double-pulse protocol consisting of a 2-s conditioning prepulse of increasing amplitude (10 mV increment) followed by a test pulse to +20 mV. Lines are non-linear curve fits by the Boltzman equation of 3–21 experiments. For clarity, error bars were not added.

exons in IIIS3 and IVS2 coding regions. The presence of exon 21 (filled symbols) in combination with either exon 32 or 31, encoding $\alpha_{1C,70}$ or $\alpha_{1C,78}$, respectively, shifted the dose-response curves slightly to the right when compared to corresponding channels $\alpha_{1C,77}$ and $\alpha_{1C,69}$ encoded by cDNAs containing exon 22 (Fig. 3A, open symbols, and Table 1). The largest IC_{50} was obtained for $\alpha_{1C,70}$ at −90 mV, with a significant reduction of the slope for the dose-response curve (Table 1). The data obtained for $\alpha_{1C,77}$ and $\alpha_{1C,70}$ are qualitatively similar to the previous results [15], although the differences are less pronounced. Our former results [15] with $\alpha_{1C,77}$, where two amino acids in segment IIIS2 had been mutated (G954F, Y958I), could only be partially reproduced. The IC_{50} value at V_h −90 mV was significantly larger in $\alpha_{1C,77(G954F,Y958I)}$, which qualitatively resembles our previous results. Unlike before, however, slopes of dose-response curves and voltage dependence (IC_{50} ratio) of isradipine action at both holding potentials were very similar to those in wild type $\alpha_{1C,77}$ (Table 1). Our explanation for the discrepancies with the published data [15] is a larger contribution of the endogenous current in the earlier experiments, resulting in a reduced sensitivity to isradipine with higher IC_{50} values at V_h −90 mV.

Replacement of exon 32 (Fig. 3A, dotted lines) by exon 31 (Fig. 3A, solid lines), in combination with either exon 21 or 22, shifted the dose-response curves toward slightly higher concentrations of isradipine (compare $\alpha_{1C,77}$ with $\alpha_{1C,69}$ (open symbols) and $\alpha_{1C,70}$ with $\alpha_{1C,78}$ (closed symbols), and Table 1).

In Fig. 3B dose-response curves are presented with channel subunits $\alpha_{1C,105}$, $\alpha_{1C,72}$, and $\alpha_{1C,86}$. Changes of amino acid sequences around segment IS6, encoded by exons 8/8a ($\alpha_{1C,77}$, $\alpha_{1C,105}$) or in C-terminal segments encoded by exons 40–43 ($\alpha_{1C,72}$, $\alpha_{1C,86}$) had a pronounced effect on the sensitivity of the channels towards isradipine. The dose-response curve for channel $\alpha_{1C,105}$ is shifted to the right compared to channel $\alpha_{1C,77}$ (dotted lines in Fig. 3B), with 2–3-fold increases of the IC_{50} values at the two holding potentials (Table 1). In contrast, $\alpha_{1C,72}$ and $\alpha_{1C,86}$ show an increase of the isradipine sensitivity with more than 3-fold reduction in IC_{50} values compared to $\alpha_{1C,77}$ (Table 1). In addition to IC_{50} values for isradipine dose-response curves, Table 1 also indicates differences in slopes and in voltage dependence of isradipine action. Thus, the strongest voltage-dependent effect on IC_{50} values was seen with $\alpha_{1C,78}$ and the weakest with $\alpha_{1C,105}$.

4. Discussion

L-type Ca^{2+} channels are important targets for drug treatment of human cardiovascular disorders. Among a variety of known Ca^{2+} channel blockers [33], DHPs are the most specific and most potent drugs to inhibit L-type Ca^{2+} currents. This channel block occurs in a voltage-dependent manner [34,35]. Channel structures for high affinity DHP binding have been identified in segments IIIS5, IIIS6, and IVS6 [11–13,33]. The molecular mechanisms by which the potency of DHP action is affected by the membrane potential, however, have yet to be elucidated in detail. A recent report [36] suggests that residues which are part of the DHP binding sites are also involved in the voltage dependence of DHP block. Our results partially confirm and extend previous evidence presented by us [15] and by others [14,16], indicating that additional structural components modulate this voltage dependence. Our data also show that alternative splicing of α_{1C} genes can have important im-

Table 1
Isradipine sensitivity of α_{1C} channel splice variants and mutants

	HP = −40 mV			HP = −90 mV			IC_{50} ratio (−90)/(−40)
	Slope	IC_{50} (nM)	n	Slope	IC_{50} (nM)	n	
$\alpha_{1C,77}$	−0.409 ± 0.022	10.1 ± 1.0	20	−0.384 ± 0.016	109.9 ± 8.0	29	10.9
$\alpha_{1C,77(G954F,Y958I)}$	−0.406 ± 0.030	14.0 ± 1.9	11	−0.360 ± 0.02	150.4 ± 12.1**	16	10.7
$\alpha_{1C,70}$	−0.388 ± 0.027	18.3 ± 4.2**°	8	−0.265 ± 0.017*	251.6 ± 27.1**°	13	13.8
$\alpha_{1C,69}$	−0.514 ± 0.033*	6.7 ± 0.9	6	−0.389 ± 0.006	91.2 ± 10.7	6	13.6
$\alpha_{1C,78}$	−0.494 ± 0.028	8.6 ± 1.5	6	−0.357 ± 0.035	148.6 ± 20.8**	6	17.3
$\alpha_{1C,105}$	−0.306 ± 0.022*	28.0 ± 6.2**°	6	−0.364 ± 0.015	230.7 ± 18.4**°	6	8.2
$\alpha_{1C,72}$	−0.661 ± 0.050*°	2.7 ± 0.5**	4	−0.399 ± 0.020	31.1 ± 4.9**°	10	11.5
$\alpha_{1C,86}$	−0.538 ± 0.079*	2.9 ± 0.5**	4	−0.368 ± 0.040	30.5 ± 3.8**°	9	10.5

For the estimation of the IC_{50} and the slope values, data between IC_{80} and IC_{20} were used for double-logarithmic regression: $\log y = S \times \log x + c$; y being the fraction of residual current, S the slope of the regression line, x the isradipine concentration, and c a constant.
*$P < 0.05$; **$P < 0.01$ (vs. $\alpha_{1C,77}$ by t-test). °$P < 0.05$ (vs. $\alpha_{1C,77}$ by ANOVA and Dunnett test).

plications for the DHP sensitivities of L-type Ca^{2+} channels. The channel subunits $\alpha_{1C,70}$, $\alpha_{1C,69}$, and $\alpha_{1C,78}$ are isoforms of $\alpha_{1C,77}$ originally derived from human fibroblasts [21], while transcripts leading to $\alpha_{1C,72}$, $\alpha_{1C,86}$, and $\alpha_{1C,105}$ were identified in human hippocampus ([29] and unpublished). Notably exons 8 and 8a, encoding sequences in constructs $\alpha_{1C,77}$ and $\alpha_{1C,105}$ respectively, are interesting because the distinct spatial distribution of the homologous exons in the rat cardiovascular system underlies the tissue specificity for DHP action [16]. Although our channel isoform $\alpha_{1C,105}$ differs from that studied by Welling et al. [16] in containing exon 22 instead of exon 21, our results confirm the lower DHP sensitivity of α_{1C} subunits with an amino acid sequence encoded by exon 8a. It would be interesting to see whether the combined sequences encoded by exons 8a and 21 would lead to an even lower affinity for isradipine binding, since each sequence alone increases IC_{50} values several-fold (compare $\alpha_{1C,70}$ and $\alpha_{1C,105}$ in Table 1).

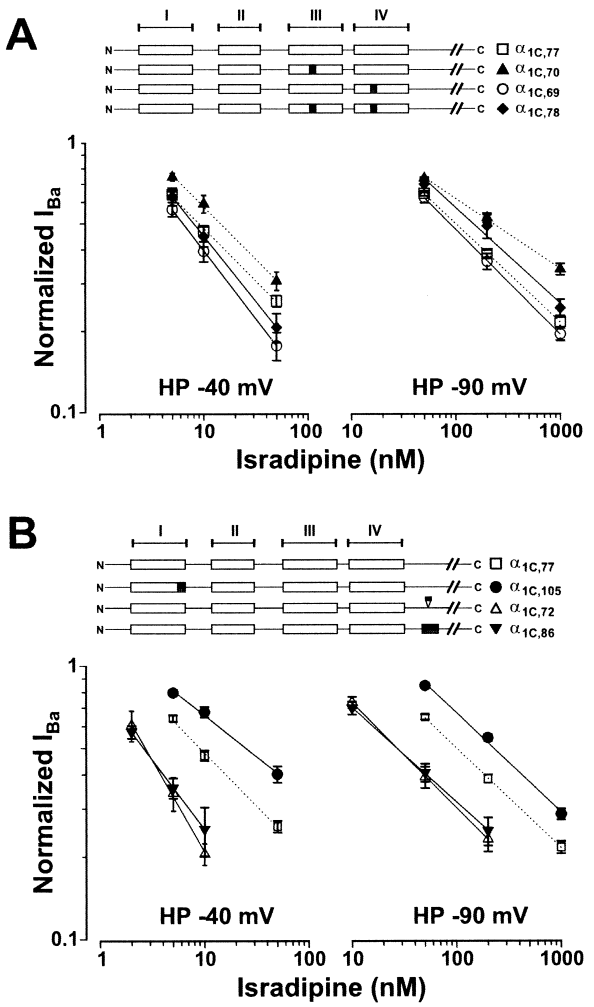

Fig. 3. (+)-Isradipine sensitivity of the human L-type Ca^{2+} channel isoforms. Above each pair of panels, the α_{1C} isoforms for which dose-response curves are presented are depicted schematically according to Fig. 1. These panels show dose-response curves which were obtained at $V_h = -40$ (left panels) or −90 mV (right panels). The curves are plotted on double-logarithmic scales. Effects of exon 21/22 and 31/32 substitutions (A) and of exon 8/8a substitution and C-terminal sequence modifications in α_{1C} channel isoforms (B). The solid lines represent double-logarithmic regression lines. The number of experiments is identical to Table 1.

In conclusion, our results show substantial variability in isradipine sensitivity between several splice variants of human L-type Ca^{2+} channels. Additional studies of their tissue-specific expression and function would be desirable. They could provide further information about the clinical potency and specificity of Ca^{2+} channel blockade in different organs.

Acknowledgements: This work was supported by grants from the Swiss National Science Foundation (31-45093.95) and from the Sandoz Foundation. We thank Ms. Heleen van Hees for excellent technical assistance.

References

[1] Catterall, W.A. (1991) Science 253, 1499–1500.
[2] Hofmann, F., Biel, M. and Flockerzi, V. (1994) Annu. Rev. Neurosci. 17, 399–418.
[3] Hosey, M.M., Chien, A.J. and Puri, T.S. (1996) Trends Cardiovasc. Med. 6, 265–273.
[4] Tang, S., Yatani, A., Bahinski, A., Mori, Y. and Schwartz, A. (1993) Neuron 11, 1013–1021.
[5] Peterson, B.Z. and Catterall, W.A. (1995) J. Biol. Chem. 270, 18201–18204.
[6] Grabner, M., Wang, Z., Hering, S., Striessnig, J. and Glossmann, H. (1996) Neuron 16, 207–218.
[7] Schuster, A., Lacinova, L., Klugbauer, N., Ito, H., Birnbaumer, L. and Hofmann, F. (1996) EMBO J. 15, 2365–2370.
[8] Mitterdorfer, J., Wang, Z.Y., Sinnegger, M.J., Hering, S., Striessnig, J., Grabner, M. and Glossmann, H. (1996) J. Biol. Chem. 271, 30330–30335.
[9] He, M., Bodi, I., Mikala, G. and Schwartz, A. (1997) J. Biol. Chem. 272, 2629–2633.
[10] Peterson, B.Z., Johnson, B.D., Hockerman, G.H., Acheson, M., Scheuer, T. and Catterall, W.A. (1997) J. Biol. Chem. 272, 18752–18758.

[11] Ito, H., Klugbauer, N. and Hofmann, F. (1997) Mol. Pharmacol. 52, 735–740.
[12] Sinnegger, M.J., Wang, Z.Y., Grabner, M., Hering, S., Striessnig, J., Glossmann, H. and Mitterdorfer, J. (1997) J. Biol. Chem. 272, 27686–27693.
[13] Hockerman, G.H., Peterson, B.Z., Sharp, E., Tanada, T.N., Scheuer, T. and Catterall, W.A. (1997) Proc. Natl. Acad. Sci. USA 94, 14906–14911.
[14] Welling, A., Kwan, Y.W., Bosse, E., Flockerzi, V., Hofmann, F. and Kass, R.S. (1993) Circ. Res. 73, 974–980.
[15] Soldatov, N.M., Bouron, A. and Reuter, H. (1995) J. Biol. Chem. 270, 10540–10543.
[16] Welling, A., Ludwig, A., Zimmer, S., Klugbauer, N., Flockerzi, V. and Hofmann, F. (1997) Circ. Res. 81, 526–532.
[17] Mitterdorfer, J., Froschmayr, M., Grabner, M., Striessnig, J. and Glossmann, H. (1994) FEBS Lett. 352, 141–145.
[18] Lacinova, L., Ludwig, A., Bosse, E., Flockerzi, V. and Hofmann, F. (1995) FEBS Lett. 373, 103–107.
[19] Wei, X.Y., Pan, S., Lang, W.H., Kim, H.Y., Schneider, T., Perez-Reyes, E. and Birnbaumer, L. (1995) J. Biol. Chem. 270, 27106–27111.
[20] Suh-Kim, H., Wei, X.Y. and Birnbaumer, L. (1996) Mol. Pharmacol. 50, 1330–1337.
[21] Soldatov, N.M. (1992) Proc. Natl. Acad. Sci. USA 89, 4628–4632.
[22] Soldatov, N.M., Zühlke, R.D., Bouron, A. and Reuter, H. (1997) J. Biol. Chem. 272, 3560–3566.
[23] Sigel, E. (1987) J. Physiol. 386, 73–90.
[24] Zühlke, R.D. and Reuter, H. (1998) Proc. Natl. Acad. Sci. USA 95, 3287–3294.
[25] Singer, D., Biel, M., Lotan, I., Flockerzi, V., Hofmann, F. and Dascal, N. (1991) Science 253, 1553–1557.
[26] Ruth, P., Röhrkasten, A., Biel, M., Bosse, E., Regulla, S., Meyer, H.E., Flockerzi, V. and Hofmann, F. (1989) Science 245, 1115–1118.
[27] Mori, Y., Friedrich, T., Kim, M., Mikami, A., Nakai, J., Ruth, P., Bosse, E., Hofmann, F., Flockerzi, V., Furuchi, T., Mikoshiba, K., Imoto, K., Tanabe, T. and Numa, S. (1991) Nature 350, 398–402.
[28] Bouron, A., Soldatov, N.M. and Reuter, H. (1995) FEBS Lett. 377, 159–162.
[29] Soldatov, N.M. (1994) Genomics 22, 77–87.
[30] Schultz, D., Mikala, G., Yatani, A., Engle, D.B., Iles, D.E., Segers, B., Sinke, R.J., Weghuis, D.O., Klöckner, U., Wakamori, M., Wang, J., Melvin, D., Varadi, G. and Schwartz, A. (1993) Proc. Natl. Acad. Sci. USA 90, 6228–6232.
[31] Biel, M., Ruth, P., Bosse, E., Hullin, R., Stühmer, W., Flockerzi, V. and Hofmann, F. (1990) FEBS Lett. 269, 409–412.
[32] Mikami, A., Imoto, K., Tanabe, T., Niidome, T., Mori, Y., Takeshima, H., Narumiya, S. and Numa, S. (1989) Nature 340, 230–233.
[33] Hockerman, G.H., Peterson, B.Z., Johnson, B.D. and Catterall, W.A. (1997) Annu. Rev. Pharmacol. Toxicol. 37, 361–396.
[34] Bean, B.P. (1984) Proc. Natl. Acad. Sci. USA 81, 6388–6392.
[35] Kokubun, S., Prod'hom, B., Becker, C., Porzig, H. and Reuter, H. (1986) Mol. Pharmacol. 30, 571–584.
[36] Bodi, I., Yamaguchi, H., Hara, M., He, M., Schwartz, A. and Varadi, G. (1997) J. Biol. Chem. 272, 24952–24960.