**Minireview**

# Structural Implications in the Function of L-type Voltage-Dependent Calcium Channels

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**Abstract.** The calcium channels play a key role in controlling many physiological processes in the body. Voltage dependent calcium channels have been extensively characterized in terms of their electrophysiological and pharmacological properties. The L-type voltage-dependent calcium channel is composed of several subunits, from which the $\alpha_1$ subunit is the most important. Recent interest has been focused more on the functional implication of the subunit structures and/or their parts. Therefore, this review will concentrate on the structure-functional studies of the voltage-dependent calcium channel rather than on the progress in the study of their structure and tissue specificity.

**Key words:** L-type Ca-channel — Structure-function — Expression

Voltage-dependent calcium channels have been in the center of interest for a long time, since they form the basis for fundamental cellular activity and regulation. It took about 30 years from the first electrophysiological recordings of a $Ca^{2+}$ action potential in crustacean muscle (Fatt and Ginsborg 1958) to the analysis of the complete amino acid sequence of the first voltage-dependent calcium channel (Tanabe et al. 1987). Recent interest has been focused more on the functional implication of these structures and/or their parts. Therefore, this review will concentrate on the structure-functional studies of the voltage-dependent calcium channel rather than on the progress in the study of their structure and tissue specificity.

The importance of voltage-dependent calcium channels lies in their ability to link electrical activity of the cell to biological effects. The most important physiological processes controlled by $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels include muscle contraction, action potential propagation (Fleckenstein 1983), secretion (McCleskey et al. 1987), regulation of gene expression (Murphy et al. 1991), and neuronal migration (Komuro and Rakic 1992). Individual processes are regulated by miscellaneous $Ca^{2+}$ channels, differing in their electrophysiological and

pharmacological properties (for a review see Perez-Reyes and Schneider 1994). The bulk of our knowledge about these channels comes from in depth studies of the L-type voltage-dependent calcium channel, so named because of its specific electrophysiological characteristics and sensitivity to organic calcium antagonists. In these type of channels, structure-functional implications are the best studied so far. Therefore, this minireview will mainly focus on the L-type of voltage dependent calcium channels.

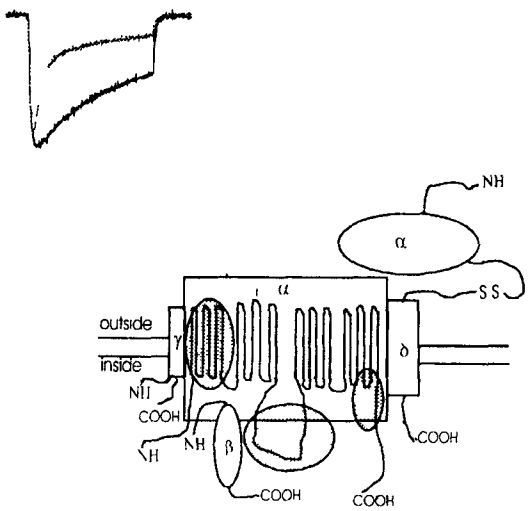
## Structure-function of the L-type calcium channel $\alpha_1$ subunit

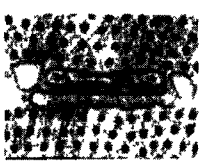
Voltage-dependent calcium channels (VDCCs) of L-type are multisubunit complexes comprising five subunits: $\alpha_1, \alpha_2/\delta, \beta, \gamma$ (Fig. 1). The essence of the L-type VDCC is formed by the $\alpha_1$ subunit, which possesses the main functional characteristics of a calcium channel, i.e. the capability to form a channel pore selective for calcium, sensitivity to Ca-antagonists and Ca-agonists (for a review see Rampe and Kane 1994), etc. As originally determined from its sequencing, the $\alpha_1$ subunit is composed of four repeating motifs, each containing six transmembrane segments (Fig. 1). While the transmembrane segments are highly conserved in all $\alpha_1$ subunits cloned from different tissues, a high divergence occurs in extracellular and intracellular loops as well as in amino and carboxy termini.

Binding of $Ca^{2+}$ antagonists, especially dihydropyridines (DHP) is the typical feature of the $\alpha_1$ subunit of L-type VDCC, and the channel has therefore been dubbed the DHP-receptor. Chemically, $Ca^{2+}$ antagonists are divergent compounds (for a review see Triggle, 1991), of which 1,4-dihydropyridines (DHP), phenylalkylamines and benzothiazepines are the most popular. Sensitivity to these compounds is often used to define whether a voltage-dependent $Ca^{2+}$ channel belongs to the L-type class. Dihydropyridines not only modulate the calcium flux through this channel pore, but a majority of them also have therapeutic implications and are employed to treat a number of cardiovascular disorders, most notably angina pectoris and hypertension. DHP binding regions have also been identified using biochemical and molecular biological approaches. By chimeras constructed between DHP sensitive and insensitive $\alpha_1$ subunit, Tang et al. (1993) showed that one of the DHP interaction sites is near the domain IV pore region (Fig. 1).

DHP receptor has been found in a variety of species and also in a variety of mammalian tissues. The $\alpha_1$ subunit of the L-type VDCC also occurs in crayfish (Krizanova et al. 1990), carp (Grabner et al. 1991), and also in Drosophilla brain membranes. An abundance of the L-type VDCC in mammals occurs in the skeletal muscle, to a lesser extent in various brain regions, heart, smooth muscle, kidney, etc.

Big effort was generated to evaluate the most important regions on the $\alpha_1$ subunit which may be responsible for tissue and functional specificity. A very valu-



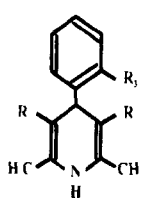



**Figure 1.** Schematic representation of the L-type voltage dependent calcium channel from skeletal muscle This channel is composed from five subunits, from which the $\alpha_1$ subunit is able to function as a channel pore This subunit contains four transmembrane motifs I II, III and IV Motif I was found to be involved in channel inactivation, while intracellular loop between motifs II and III in skeletal muscle is responsible for E-C-coupling The $\beta$ subunit as the only cytosolic protein is located close to the region between motifs I and II The specific feature of this channel is the binding of dihydropyridines The binding site for these compounds has been proposed to be somewhere between motif IV and the carboxy terminus of the $\alpha_1$ subunit

able approach, construction of chimeras from the individual subunits which differ in certain regions, was used First chimeras of the $\alpha_1$ subunit were constructed from skeletal and cardiac muscles (Tanabe et al 1990, 1991) These authors have found that motif I (Fig 1) is responsible for the $Ca^{2+}$ channel activation process and that the intracellular loop between motifs II and III is a major determinant

of skeletal muscle E-C coupling. Using similar approaches the motif I S6 segment was shown to be involved in voltage-dependent inactivation (Zhang et al. 1994).

L-type $Ca^{2+}$ channels manifest $Ca^{2+}$ sensitive inactivation, a biological feedback mechanism in which elevation of intracellular $Ca^{2+}$ concentration speeds up channel inactivation. A structural determinant of this $Ca^{2+}$ sensitive inactivation was revealed by chimeric $Ca^{2+}$ channels (cardiac and neuronal, which is lacking $Ca^{2+}$ inactivation). A consensus $Ca^{2+}$ binding motif (EF hand), located in cardiac $\alpha_1$ subunit, was required for $Ca^{2+}$ inactivation (de Leon et al. 1995). These results strongly suggest that $Ca^{2+}$ binding to the skeletal muscle $\alpha_1$ subunit initiates $Ca^{2+}$ inactivation.

Although structure-function studies are still in the preliminary stages, they already have provided exciting fundamental information. Several expression systems have been established. Among these are *Xenopus laevis* oocytes, primary cell cultures from dysgenic mice, and certain mammalian cell lines.

## Expression systems form the basis for functional studies

Of the systems available, the *Xenopus laevis* oocyte system is the most widely used for transient expression of mRNA, since it does not express its own L-type VDCC. Oocytes possess a rather universal translational apparatus, where injected foreign mRNA can be successfully translated and they correctly perform many posttranslational modifications such as proteolytic modification, glycosylation or acetylation. Therefore, the expressed proteins can be studied with a variety of electrophysiological and biochemical techniques. With vaccinia virus, cDNA has recently been used instead of mRNA. This modification can markedly facilitate expression experiments. The $\alpha_1$ subunits of L-type VDCC from the brain (Mori et al. 1991), smooth muscle (Biel et al. 1991) and heart (Mikami et al. 1989) have been expressed in oocytes. It is peculiar that functional expression of the skeletal muscle $\alpha_1$ subunit has thus far been unsuccessful.

Another expression system, the primary *mdg* cell culture has been derived from murine dysgenic skeletal muscle. This muscle type is not able to contract because it lacks the $\alpha_1$ subunit of the L-type VDCC. All other channel subunits however, have been identified in these cells. These cells are being used to investigate the important parts of the $\alpha_1$ subunit (Tanabe et al. 1990, 1991).

The *mdg* cell culture can be easily contaminated with fibroblasts. For this and other reasons, an *mdg* cell line has been created. The advantage of the cell line is that it can be grown indefinitely as a single cell type. The immortalized *mdg* cells display the same properties as their primary culture counterparts, i.e. lack of contraction, slow $Ca^{2+}$ current and triadic differentiation.

Stable transfection in mammalian cell lines has been used for studies of the skeletal muscle $\alpha_1$ subunit. For this purpose mouse L-cells have been established

as a convenient system, because of their lacking of all calcium channel subunits. Transfection of L-cells with $\alpha_1$ subunits of the skeletal muscle results in the expression of 600–1000 dihydropyridine binding sites per cell (Perez-Reyes et al. 1989). Accordingly, this system forms the basis for biochemical and electrophysiological studies.

## Function of the individual subunits of the L-type VDCC

Numerous experiments have been performed in order to estimate the function of other channel subunits. Although the exact role of the $\alpha_2/\delta$ subunit complex remains unknown, it is likely that it facilitates the incorporation of the $\alpha_1$ subunit into the membrane. This is analogous to the role proposed for the $\beta$ subunit of the $Na^+/K^+$ ATPase. In this system, the $\beta$ subunit is a glycosylated protein with a single membrane spanning region, which may be required for the insertion of the subunit of $Na^+/K^+$ ATPase into the membrane (Noguchi et al. 1987). Heterologous coexpression of the skeletal muscle $\alpha_2$ with the $\alpha_1$ in *Xenopus* oocytes increased the amplitude of the Ca current without changes in its kinetics.

The $\beta$ subunit has been shown by several laboratories to modulate activity of the $\alpha_1$ subunit not only in the skeletal muscle (Lacerda et al. 1991; Varadi et al. 1991), but also in the brain and the heart. DHP receptor function increases dramatically (3–15 fold) upon homologous coexpression of $\alpha_1$ and $\beta$ subunits was performed (Varadi et al. 1991). In order to clarify whether the change upon coexpression of $\alpha_1$ and $\beta$ originated from enhanced synthesis and/or stabilization of the DHP receptor complex in the cell membrane, or from facilitated processing of the $\alpha_1$ subunit by $\beta$, the receptor levels in the cell membrane were qualified (Krizanova et al. 1995). The results clearly show that upon coexpresssion of skeletal muscle $\alpha_1$ and $\beta$ subunits the increased $Ca^{2+}$ channel current density and DHP-receptor density are, at least partly, the consequence of an increased channel/receptor protein complex in the cell membrane.

The sites of $\alpha_1$–$\beta$ subunit interaction have been recently localized into the cytoplasmic domains of both subunits. The $\alpha_1$ subunit interaction domain is an 18-amino-acid conserved region located between motifs I and II on all $\alpha_1$ subunits which is essential for the binding of $\beta$ subunits (Witcher et al. 1995). Site on the $\beta$ subunit that interacts with the $\alpha_1$ subunit has also been mapped (De Waard et al. 1994). In these studies the $\beta$ subunit was truncated into progressively smaller pieces, then assayed for their ability to stimulate the $\alpha_1$ induced (coexpressed with the $\alpha_2$) currents in oocytes. The smallest fragment that stimulated currents contained residues 211 to 245. Additionally, the amino terminus of $\beta$ subunits is capable of interacting with the $\alpha_1$ and this interaction controls the effect of $\beta_s$ on the kinetics (Olcese et al. 1994).

The $\gamma$ subunit might also be involved in the regulation of the inactivation

process (Singer et al. 1991), making it faster and perhaps more sensitive to voltage. This appears to be a voltage-rather than a current-dependent phenomenon, since in the presence of the $\gamma$ subunit barium current inactivated at voltages that do not cause any current flow through the channel.

All of the results discussed here highlight the importance of the $\beta$ subunit, and probably of other subunits as well, in regulation of the $\alpha_1$ subunit of calcium channels.

## Developmental studies and modulation of the Ca-channel gene expression

Individual subunits of the VDCC are encoded by different genes and give rise to highly homologous but structurally diverse channel polypeptides. The $\alpha_1$ subunit as the major functional component of the L-type VDCC possesses a high homology with other VDCC, but also with $Na^+$ and $K^+$ voltage-dependent calcium channels. These genes originated from one common ancestral gene (Hille 1984). Therefore, the transmembrane structure has been relatively well preserved during the evolution. However, several variants have been developed during evolution in different tissues.

The localization of genes for the individual Ca-channel subunits in the human gene map has been of a great interest. Although the $\alpha_1$ subunit of VDCC has evolved from a common ancestral gene (Hille 1984), genetic mapping of only one of these genes has been reported, except of the $\alpha_1$. The results of Chin et al. (1991) represent the first chromosomal localization for the $Ca^{2+}$ channel genes. These authors mapped the brain N-type $\alpha_1$ subunit gene to the proximal region of chromosome 3 in humans, to the regions p21-p23, or p-26. More recent studies mapped the L-type human skeletal muscle $\alpha_1$ gene to chromosome 1q-31 -q32 (Gregg et al. 1993). In contrast, both the $\beta$ and the $\gamma$ subunit of the human skeletal muscle L-type VDCC genes are located to chromosome 17q.

As revealed by S1 nuclease protection analysis, two variant forms of the IVS3 region were equally expressed in the newborn and fetal rat heart, while only a single isoform was expressed in adult rat heart. This phenomenon is the product of mutually exclusive alternative splicing, and illustrates the presence of a developmentally regulated switch of the $\alpha_1$ subunit in cardiac tissue (Diebold et al. 1991). Additionally, RNA-hybridization analysis revealed that primary cultures derived from neonatal rat hearts expressed two types of mRNA encoding the skeletal muscle as well as the cardiac calcium channel $\alpha_1$ subunit, while neonatal rat heart tissue only expressed the message for cardiac calcium channel. These results provide evidence for the functional expression of skeletal muscle-type calcium channel subunits during the cultivation of neonatal rat heart cells (Haase et al. 1994).

Very little is known about factors which induce and regulate the expression of genes encoding dihydropyridine receptors. A temporal difference in the induction of

the DHP receptor subunit mRNAs in developing muscle has been shown, suggesting that these genes may be under the control of distinct endogenous factors. Ray et al. (1995) demonstrated that nerve plays a critical role in regulating the expression of DHP receptors *in vivo*. Nerve-derived factors can enhance the transcriptional activity of the DHP receptor subunit genes and of their respective mRNA levels. Other group of factors which can directly or indirectly affect the expression of the VDCC's subunits, are hormones. Insulin was also shown to decrease the expression of VDCC in the rat aorta (Thurzová et al. 1995).

## Conclusion

The rapid progress in calcium channel research in the past few years has resulted in an boom of information. Current research is now concentrated on the connection between the structure and the function. The result of these experiments can be expected to reveal specific roles for various regions of the channel proteins as well as for the function of subunits and how the channels are regulated. Additional work on this and other aspects can be expected to reveal the interesting and unusual interplay of the subunits in controlling the activity of calcium channels.

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