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COMMENTARY

ANALYSIS AND FUNCTIONAL CHARACTERISTICS OF DIHYDROPYRIDINE-SENSITIVE AND -INSENSITIVE CALCIUM CHANNEL PROTEINS

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The understanding of the molecular properties and structure–function relations of ion channels has advanced tremendously during the past few years. In this review I will try to summarize briefly the current advances in the field of calcium channel proteins and outline the problems facing researchers at present. I will concentrate exclusively on *vertebrate voltage-dependent* Ca^{2+} channels, because much less is known about the invertebrate Ca^{2+} channels and about receptor-operated Ca^{2+} channels. Recent reviews may be consulted for more detailed information on molecular properties of ion channels in general (e.g. Refs. 1–4) and of Ca^{2+} channels in particular [1, 5–15].

DIVERSITY OF Ca^{2+} CHANNELS

The first indications of Ca^{2+} channel protein heterogeneity were electrophysiological and pharmacological (reviews: [5, 6, 8, 9]). At present, it is conventional to divide the vertebrate voltage-dependent calcium channels into three main classes, which will be termed in this review for simplicity T,N and L, following Nowycky *et al.* [16]. Although the channels within each class display tissue- and species-related heterogeneity, the features described below are generally considered as specific and tissue-independent enough to serve as criteria for identifying a channel as belonging to one of the three classes:

(i) *L-channels* are the best studied ones, found in all excitable and several unexcitable cells. They are activated by relatively large depolarizations, inactivate slowly, and are thought to have the largest single-channel conductance among Ca^{2+} channels, in the range of 16–24 picosiemens (pS) in high-barium solutions [6, 9].

The most outstanding feature of L-channels is their sensitivity to a class of organic compounds, dihydropyridines (DHPs), some of which ("agonists") promote the channel opening, whereas the others ("antagonists") block it (see Refs. 10, 17 and 18). Separate allosterically interacting sites for agonists and antagonists have been proposed [19, 20] (but see a recent single-site model [12]). The affinity of potent DHP antagonists is less than nanomolar for inactivated channels (at depolarized membrane potentials), but is in the micromolar range for closed channels (at the cell's resting potential) [20, 21].

Other classes of organic Ca^{2+} channel blockers acting on L-channels are benzothiazepines (diltiazem), phenylalkylamines [(PAAs): verapamil, D600, D888], bepridil, and more recently described diphenylbutylpiperidines (pimozide, fluspirilene) and benzolactams (HOE-166) [7, 10, 17, 18]. The specificity of these compounds is less well established, and their effects on T and N channels have not been studied systematically. Among metallic L-channel blockers, cadmium is usually more effective than nickel [9].

L-channels are modulated by phosphorylation: enhanced by activation of cAMP-dependent protein kinase (PKA) [22], and both activated and inhibited by activators of the calcium/phospholipid-dependent protein kinase C (PKC) [23]. It is not clear to what extent these regulations are unique to L-channels. Activation of G-proteins (regulatory proteins which are activated by ligand-bound neurotransmitter receptors and transduce the signal to target enzymes or ion channels [24]) may enhance (G_s acting directly on cardiac L-channel, see Refs. 25 and 26) or suppress (G_o and/or G_i) Ca^{2+} channel activity, and alter the effects of DHPs [27–32]. In many studies, the current affected by the G-proteins has not been identified clearly; it is possible that, in neurons, G-proteins affect N- rather than L-channels [31, 33]. It is also unclear whether the coupling of the G-proteins to neuronal Ca^{2+} channels is direct, or proceeds through second messenger pathways. cAMP-dependent phosphorylation and G-proteins may play an important role in maintaining L-channel function and preventing the "run down" of the channels observed when the cytoplasmic surface of the membrane is exposed to an artificial physiological solution [8, 25, 34].

(ii) *T-channels*, found in heart, cultured skeletal muscle cells, smooth muscle, and in neurons, are "low-voltage activated," i.e. opened (and then inactivated) by mild depolarization. The current through these channels is fast and transient. Single channel conductance is about 8 pS in high-barium solutions. T-channels are usually considered insensitive to DHPs and, in sensory neurons, to verapamil. The currents through these channels do not run-down in excised patches. Nickel is usually more effective than cadmium in blocking the T-channels [6, 9].

(iii) *N-channels* appear to be exclusively neuronal.

They activate in the same voltage range as the L-channels but inactivate at more negative potentials. In most reports, it is claimed that the N-channel current is transient and fully inactivates within tens or hundreds of milliseconds. However, there is controversy as to what extent, if at all, N-channels are different from L-channels [35, 36]. Thus, the deactivation of the two channel types is indistinguishable [36]; single channel conductances of about 13 pS [16] or 20 pS [33] have been reported for the N-channel, and it has been proposed that the observed 13 pS N-channel represents a conductance substate of the L-channel [35].

N-channels are inhibited by a group of snail toxins, ω-conotoxins; the most widely used is ω-conotoxin GVIA [37, 38]. It is still controversial whether some channels are sensitive to both DHPs and ω-conotoxin (see Ref. 9). In some neurones, GVIA irreversibly blocks a fraction of the N-channel current and reversibly blocks another fraction, suggesting the existence of two sub-populations of N-channels; the current through the channel less sensitive to ω-conotoxin is sustained like the L-current [33]. The possibility of the existence of a Ca^{2+} channel of N-type with low sensitivity to ω-conotoxin is supported by the finding that a sustained, DHP-insensitive Ca^{2+} channel current expressed in *Xenopus* oocytes injected with brain RNA (see below) is insensitive to ω-conotoxin [39]. The current through the N-channels runs down in excised patches but is less vulnerable than the L-channel [8]. Among metallic blockers, Gd^{3+} was reported as selectively acting on the N-channel [40]. Verapamil inhibits the N-current to some extent [41]. As mentioned above, the N-channel may be a target for modulation by G-proteins; modulatory effects of protein kinases are less understood and controversial [6]. In summary, no good tools exist yet for unequivocal separation of L- and N-channel currents; pharmacological and electrophysiological data suggest the existence of more than one N-channel type.

At present, since the primary structures of most Ca^{2+} channel proteins are not known, a major problem with Ca^{2+} channel diversity is the confusion concerning the number of Ca^{2+} channel types and the extent of differences among them. Several Ca^{2+} channels do not fit into any one of the three categories described above. On the other hand, sometimes a certain channel displays features widely believed to be unique to another channel type. Below are several examples of the ambiguities of this kind:

(i) Examples of channels that are neither T, N or L: A fast-activating, non-inactivating Ca^{2+} current is present in adult muscle cells [42], whereas the "normal" T-channel current, found in cultured muscle cells, has not been observed. A slow, long-lasting Ca^{2+} current, relatively insensitive to block by Cd^{2+}, has been described in guinea pig ventricular cells [43]. A 7 pS "B"-type Ca^{2+}-permeable channel, open at negative potentials, has been detected when purified cardiac membranes are incorporated into an artificial lipid bilayer [44].

(ii) A DHP-sensitive T-channel: The low voltage-activated Ca^{2+} current in rat hypothalamic neurons has all the unmistakable electrophysiological "fingerprints" of T-channel current; yet, it is effectively suppressed by DHP antagonists and even by the DHP agonist Bay K 8644, and by other L-channel organic blockers (flunarizine, diltiazem). The DHPs block the resting (closed) channels in the micromolar range, exactly as one would expect for an L-channel [45]. Affinity of DHPs to inactivated T-channels has not been examined.

(iii) Proteins other than voltage-dependent L-type Ca^{2+} channels sensitive to DHPs: The activity of an 8 pS Ca^{2+} channel found in T lymphocytes, which is not voltage-dependent and appears to be activated by the intracellular second messenger inositol 1,4,5-trisphosphate, is strongly potentiated by the DHP agonist, Bay K 8644 [46]. Binding studies show that several proteins apparently unrelated to the Ca^{2+} channel, such as the multidrug resistance protein, also bind DHPs with high affinity [7, 11].

THE DHP RECEPTOR (DHPR)

Of all Ca^{2+} channel proteins, only the structure of the DHP-sensitive one is known at present. The subject has been reviewed extensively [1, 7, 10–15], and only a brief summary is given here. High-affinity DHP binding sites are present in all tissues in which the L-channels are found. The other Ca^{2+} channel antagonists (benzothiazepines, PAAs, bepridil, HOE 166) bind to distinct sites allosterically interacting among themselves and with the divalent cation binding site(s) [7, 10, 11, 17, 18]. The term "DHP receptor" is widely used as a synonym for Ca^{2+} (L-) channel protein, although, as already mentioned, other proteins also bind DHPs. Skeletal muscle T-tubule membranes are the richest source of DHPRs and thus were used to purify the DHP-binding protein. The DHPRs seem to be arranged in the T-tubule membrane in a highly ordered fashion, a tetrad per each pair of "feet" tetrads [47] ("feet" are structures connecting the membranes of T-tubules and the sarcoplasmic reticulum; they have been shown recently to contain the Ca^{2+} release channel of the sarcoplasmic reticulum (see Ref. 48).

Paradoxically, it becomes clear that the main function of DHPR in skeletal muscle is not the transport of Ca^{2+} into the sarcoplasm from T-tubules. Only a few percent of skeletal muscle DHP binding proteins appear to be functional Ca^{2+} channels [49]. There is good evidence [50] (see also below) that a DHP-sensitive molecule, possibly the same as one of the subunits of the Ca^{2+} channel itself, is the voltage sensor of excitation–contraction coupling (ECC) in skeletal muscle, i.e. the molecule that responds with a conformational change to depolarization of T-tubule membrane and transmits the signal to the Ca^{2+} release channels of sarcoplasmic reticulum membrane, which open and let the stored Ca^{2+} rush to the sarcoplasm [48, 50, 51].

Biochemical properties of the skeletal muscle DHP receptor. It is now generally agreed that the purified skeletal muscle DHPR consists of 5 subunits [52–57; see reviews in Refs. 1, 7, 13, 14, and 58]. The α_1 *subunit* (165 kD) is poorly if at all glycosylated and unaffected by disulfide reduction; it is the DHP-binding subunit. It also contains binding sites for other classical organic Ca^{2+} channel blockers. The

α_2 (140 kD) and δ *subunits* (33 kD) are linked by disulfide bonds and run together on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as a single 175 kD band under non-reducing conditions. Both subunits appear to be glycosylated, which is characteristic for integral membrane proteins. The β *subunit* (55 kD) is not glycosylated, and is quite labile and sensitive to proteolysis. γ *subunit* is heavily glycosylated and probably contains membrane-spanning regions. The stoichiometry of the five subunits is usually 1:1:1:1:1 [13, 15], but variable stoichiometry was observed in the course of muscle development [59, 60].

Although the subunit structure described above is generally accepted, one report [61] suggests a different scheme, whereby the Ca^{2+} channel consists of a channel (C) and a regulatory (R) component. C contains a 180 kD subunit which is DHP-binding, phosphorylated by PKA, and participates in ion transport (may correspond to α_1 described above), and 145 and 35 kD subunits linked by disulfide bonds (probably corresponds to α_2/δ). The R component, conferring the sensitivity of the channel to verapamil, contains a 165 kD and a 55 kD subunit, both phosphorylated by a polypeptide-dependent protein kinase (and lose sensitivity to verapamil when phosphorylated) but not by PKA. The flux of Ca^{2+} mediated by the C component can be inhibited by the G-protein, G_o, but only in the absence of the R component. Among other discrepancies with the current dogmas, this scheme implies that the 165 kD subunit (α_1) described in other works may not be homogeneous and contains two proteins, 180 and 165 kD, having different functional roles. Confirmation or rejection of the model must await more experimental work.

Reconstitution of functional L-type Ca^{2+} *channels in artificial membranes.* Purified T-tubule membranes and purified DHPRs have been incorporated into artificial membranes (lipid bilayers) and produced functional Ca^{2+} channels observed by direct single channel recordings or into lipid vesicles in which they were able to mediate Ca^{2+} fluxes (see Refs. 62 and 63 and references therein). Like the native L-channel, the reconstituted channel usually demonstrated sensitivity to DHP agonists and antagonists, divalent cation blockers and PAAs, high selectivity for divalent over monovalent cations, and high conductance for monovalent cations in the absence of divalents. In some cases, dependence of the open probability of the channel on membrane voltage has been reported [61, 64].

Purification of DHPRs in the presence of Bay K 8644 was sometimes required to obtain functional channels [61, 65]; even then, only 2–3% of the incorporated complexes acted as functional channels [62, 65]. However, under conditions permitting phosphorylation by PKA, the number of functional channels increased several-fold [62, 63, 66]. Although it is possible that phosphorylation by PKA only increases the chances of a channel to open [67], it is quite probable that there is an absolute requirement for phosphorylation for the channel's opening, and the few percent of active channels observed without PKA have remained phosphorylated in the course of purification [62, 63].

The reported values of single channel conductance of reconstituted T-tubular Ca^{2+} channels in 80–100 mM barium fall into two categories, 7–12 pS and about 20 pS (see Ref. 12). Several groups suggested that the two conductances represent substates of the same Ca^{2+} channel (e.g. Ref. 64, but see Ref. 68). Recently, it was reported that incorporation into bilayers of the purified 5-subunit complex gave rise to channels of 9 and 20 pS (only the latter was DHP-sensitive), whereas incorporation of purified α_1 subunit *alone* produced functional DHP-sensitive 20 pS channels [68].

In a series of experiments in which the amount of incorporated channels was "titrated," subconductance levels between 0.9 and 60 pS were observed, which depended on the rate or extent of incorporation of the channel into the bilayer [12, 62]. The most stable levels were 3.5, 7 and 15 pS [62]. The opening of the large, but not of the smallest conductance states was voltage-dependent. The authors suggested that the smallest conductance corresponds to a single conducting unit, "monochannel" (e.g. one 5-subunit complex of DHPR), and the rest are multiples of this value which are due to formation of "oligochannels," representing clusters of cooperatively gated monochannels, observed in other studies as conductance substates. The voltage dependence of a channel is proposed to be "rooted" in the oligochannel structure. Some substates are stabilized *in vivo* (and bilayers heavily loaded by DHPRs) by as yet unidentified factors, one of them possibly being their association with the feet structures [12]. Subconductance states are characteristic of many channel types [69]; if the oligochannel hypothesis is correct, our views on how ion channels work may have to be modified.

Although the reconstitution studies described above provide a functional proof that the purified DHPR is indeed the T-tubule L-type Ca^{2+} channel, uncertainties still exist. The estimates of the various groups of what exactly was the subunit composition of their purified and reconstituted DHPR vary considerably. The oligomeric structure of the channel remains to be understood. The roles of individual subunits are not clear, although α_1 seems to be the most important in pore formation (additional evidence comes from the expression experiments; see below).

Cloning and primary structure of skeletal muscle DHPR subunits. The cDNA of the rabbit skeletal muscle DHPR subunits α_1 and α_2 has been cloned, and the primary structure of the protein deduced [70, 71]. The predicted structure of the α_1 protein is strikingly similar to that of the channel-forming subunits of the voltage-dependent sodium and potassium channels, suggesting that α_1 is itself the channel-forming subunit (reviews: [1, 4]). The α_1 subunit contains 1873 amino acid residues, and the calculated molecular weight is 212 kD. The main features of the predicted structure are as follows. Both N- and C-termini are cytoplasmic. The protein contains four homologous internal repeats, each containing six α-helical segments (S_1–S_6) most probably spanning the membrane. Five of them are hydrophobic and one, S_4, is amphipatic: it contains a positively charged amino acid (arginine or lysine)

usually at every third position, rendering the whole S_4 segment positively charged. It is widely believed that this segment is the voltage sensor of the channel, which moves when the membrane voltage is changed and alters the conformation of the channel, causing its opening or closure. At present, this belief is based on analogy with the voltage-dependent Na^+ channel, where modeling (see Ref. 2) and experimental data [72] strongly suggest this role for the S_4 segment. Molecular modeling [12] indicates that one of the S_4 segments of the α_1 subunit of DHPR also contains the binding site for DHPs.

The intracellular (cytoplasmic) fragments connecting the internal repeats, and the C-terminal part, contain several putative PKA phosphorylation sites (serines 687, 1502, 1575, 1757, 1772 and 1854 and threonines 1854 and 1552) [70]. One of them, Ser 687, is rapidly phosphorylated by PKA *in vitro* and was proposed to be the *in vivo* site of regulation by PKA [73]. Although the α_1 subunit is a good substrate for protein kinase C, for calcium/calmodulin-dependent protein kinase, and for cGMP-dependent protein kinase [74–76], putative sites for PKC have not been identified yet. There are no clues as to the location of the other sites of regulation (by G-proteins, etc.).

The α_1 and α_2 subunits are coded for by distinct genes [71]. The predicted primary structure of α_2, in contrast with α_1, does not display homology with any known protein [71]. Its primary structure is less well modeled than that of α_1; it has been proposed to contain three transmembrane domains, eight extracellular N-glycosylation sites, and two intracellular sites for PKA phosphorylation [71]. The functional role of the latter is obscure, since α_2 is not a good substrate for PKA [15, 74].

The recently reported primary structure of the β subunit [77] supports the notion that it is a peripheral membrane protein, possibly interacting with cytoskeletal proteins. Abundance of phosphorylation sites for different protein kinases is in line with previous studies with purified DHPR [74] and suggests that this subunit may mediate some of the regulatory effects of phosphorylating agents on the L-channel (cf. Refs. 1 and 15].

Molecular properties of DHPR in other tissues. Immunological, RNA hybridization, and biochemical data demonstrated that cardiac α_2 is very similar to that of skeletal muscle (see Refs. 58 and 71). RNA homologous to that of cardiac, but not skeletal, α_1 has been detected in brain and smooth muscle [78, 79]. Recently, purification of a 185 kD DHP-binding protein from chick heart, similar to skeletal muscle α_1, has been achieved [80]. It co-purified with a protein highly similar to skeletal muscle α_2/δ subunit and possessed DHP and PAA binding sites. Co-purification of smaller peptides that could correspond to β and γ subunits of skeletal muscle DHPR has not been observed.

The evidence presented thus far suggests that heart and skeletal muscle L-type Ca^{2+} channels are distinct molecular entities, especially the DHP-binding α_1 subunits. This has been now confirmed by cloning of cardiac α_1 subunit cDNA [78, 79]. The predicted primary structure of this protein (calculated molecular weight, 243 kD) is 66% homologous to its skeletal muscle counterpart, and has the same general design. There are clear-cut differences, though. Most of the cytoplasmic domains show relatively low homology, and both N- and C-terminal regions are larger in cardiac α_1. Out of six predicted intracellular phosphorylation sites in cardiac α_1 (Ser 124, 1575, 1627, 1700, 1848 and 1928), only two (1627 and 1700) are conserved in skeletal muscle [78, 79]. The skeletal muscle Ser 687, the most relevant PKA target *in vitro* [73], has no counterpart in cardiac α_1 [78], although modulation of the cardiac L-channel by this kinase *in vivo* is conspicuous. The purified cardiac DHPR is a surprisingly poor substrate for phosphorylation by PKA [80]. These findings call for further studies and for caution in interpreting the results of *in vitro* phosphorylation studies to explain the phosphorylation phenomena *in vivo* [58].

Partial sequences of brain and smooth muscle DHPR cDNAs (presumably of α_1-like subunits) have been reported only very recently; they demonstrate overall good homology with skeletal and cardiac DHPR, but there are also clear differences [58, 81]. Since the differences are spread over the entire coding sequence of the cDNA, it is unlikely that they are due to alternative splicing of mRNA; rather, it is probable that tissue specificity is determined by expression of different genes [78].

RNA- (DNA)-DIRECTED EXPRESSION OF Ca^{2+} CHANNELS IN MODEL SYSTEMS

Many cells can synthesize foreign proteins when exogenous RNA, cDNA of a known protein, or the RNA transcribed from it ("cRNA") is introduced into them. Such expression systems are invaluable for the study of molecular properties of the proteins, because the cDNA can easily be manipulated by methods of molecular biology. One can engineer the cDNA in such a way that specific changes in the protein it codes for will take place (deletions, changes of single amino acids, etc.), and compare the behavior of the "mutated" protein with that of the native one, in the expression system (see Refs. 3, 72, and 82).

Ca^{2+} channels have been expressed in *Xehopus* oocytes injected with heterologous RNA extracted from mammalian heart, brain, skeletal muscle, and *Torpedo* electric lobe [39, 83–86]. RNA from heart directed expression of two Ca^{2+} channel currents, one (dominant) with the distinctive features of the DHP-sensitive L-type, and another one resembling the T-channel current but with a slower inactivation [83]. The oocytes possess a similar (though much smaller) "native" DHP-insensitive Ca^{2+} channel current even when they are not injected with RNA; thus, although unlikely, the possibility that injection of RNA causes and "overexpression" of this T-like current cannot be excluded [87]. N-channel is expressed from *Torpedo* electric lobe: it is blocked by ω-conotoxin but is insensitive to DHPs [85]. The Ca^{2+} channel current from rat brain has most of the characteristics of N-type but is blocked by neither ω-conotoxin nor DHPs [39]. Two currents are expressed from skeletal muscle RNA, one resembling the T-type and the other the L-type* [88].

* Dascal N, unpublished observations.

Before the primary sequence of cardiac α_1 subunit of DHPR was known, the identity of cardiac Ca^{2+} channels was probed by hybrid arrest of expression [86]: heterologous RNA was extracted from rat or rabbit heart, hybridized with DNA oligonucleotides corresponding to segments of the cDNA coding for fragments S_4 and S_6 of the rabbit skeletal muscle α_1 subunit of DHPR, and injected into *Xenopus* oocytes. After such treatment, the RNA hybridized with the oligonucleotide is degraded by the enzymatic apparatus of the oocyte (RNAase H-like activity), whereas all other RNA species remain intact and direct the synthesis of the corresponding proteins [89]. The hybridization suppressed the expression of the L-type, but not of the T-type, channel in the oocytes, suggesting that the major (α_1) subunit of heart Ca^{2+} L-type channel is coded by a DNA homologous to that of skeletal muscle's α_1, and that the T-channel is a separate molecule coded by a distinct RNA [86].

Recently, cRNA (i.e. "cloned" RNA) coding for the cardiac α_1 subunit was expressed in *Xenopus* oocytes; the ensuing current had all the features of the L-type [79]. Injection of cRNA coding for skeletal muscle α_2 subunit did not produce any currents, but co-injection of this cRNA with that of cardiac α_1 gave rise to larger (but otherwise identical) currents than with α_1 alone. Thus, cardiac α_1 alone can form the Ca^{2+} L-type channel, whereas α_2 may play a supportive role in its expression or help to preserve the stability of the channel [79].

Attempts to express skeletal muscle α_1 in oocytes have not been successful, but have succeeded in two other expression systems.

(i) Skeletal muscle cells of dysgenic (mdg/mdg) mice lack the L-type Ca^{2+} current, the α_1-subunit of DHPR, and the voltage sensor of ECC, have abnormal T-tubule and sarcoplasmic reticulum-T-tubule junction morphology, and have a defective DHPR α_1 gene [90–94]. Injection of cDNA coding for α_1 into the mdg/mdg myocyte nuclei restored all the missing features including morphology [94]. These findings supported the hypothesis [50, 70] that α_1 subunit of skeletal muscle DHPR is an essential part of both the L-type Ca^{2+} channel and the voltage sensor of ECC (although it is still possible that the reappearance of either L-current or ECC is due to restoration of normal morphology). Restoration of L-current and ECC in dysgenic myocytes was also induced by co-culture with normal neurons, and involvement of a trophic factor has been proposed [92]; however, these results may be due to the presence in the culture of fibroblasts that could fuse with myocytes and "donate" normal α_1 [95]. More intriguingly, in aged dysgenic myocytes, the L-current but not ECC is often present, implying the possibility of an additional gene coding for an L-channel in skeletal muscle [96].

(ii) Functional DHP-sensitive Ca^{2+} channels have been expressed in a mammalian cell line (normally lacking any Ca^{2+} currents) transfected with skeletal muscle α_1 cDNA [97]. This result strongly supports the notion that skeletal muscle α_1 is indeed the pore-forming subunit. However, the current through the expressed channels was exceedingly slow and non-inactivating, suggesting that either other subunits or the normal T-tubule "environment" is necessary for the channel to function normally [97].

ω-CONOTOXIN RECEPTORS

It is hoped that purification of the neuronal N-channel protein(s) will be possible, employing a strategy similar to that employed with DHPRs, using ω-conotoxin as a highly specific probe. Indeed, ω-conotoxin GVIA binds with high affinity to brain, but not skeletal or cardiac muscle, membranes [98–100]. Two populations of binding sites, with apparent affinities of 3–10 pM and 0.5–3.5 nM, have been reported in rat and bovine brain synaptosomes [99, 101, 102], which is consistent with the existence of two populations of N-channels with different sensitivity to ω-conotoxin. However, the existence of two sites and the values of affinities are controversial ([100]; discussed in [11]). Interestingly, binding of ω-conotoxin to the high-affinity binding site in bovine brain was stereospecifically inhibited by diltiazem but not by DHPs [102], suggesting that the N-channel may be sensitive to benzothiazepines, or that L- and N-channels may share common subunits. Yet, all groups agree that ω-conotoxin and DHP-binding sites reside on different proteins.

The size and subunit composition of the ω-conotoxin receptor are debatable. Cross-linking of ω-conotoxin to its binding sites by different techniques provided controversial results. Peptides of 210–220 kD and 170 kD have been labeled in one work [100], 135 kD in another [103]. The 170 kD protein [100] could be separated into 140 and 30 kD components under reducing conditions, and it has been proposed that it is similar or identical to the α_2/δ subunit of DHPR, and that the 210–220 kD subunit may be homologous to the α_1 subunit of the L-channel. Varying data were also obtained by using the photoaffinity labeling technique with photoreactive derivatives of ω-conotoxin: in rat brain synaptosomes, labeling of a single component of about 220 kD was reported by one group [104] and of three components (310, 230 and 34 kD) by another group [101]. Two ω-conotoxin binding proteins (310 and 230 kD) could be solubilized from brain; the 34 kD protein and the low-affinity binding site were lost in the process [102]. Further studies must be carried out to solve these controversies and to begin understanding the molecular structure of the ω-conotoxin receptor, presumably the N-type Ca^{2+} channel.

CONCLUSIONS

Voltage-dependent Ca^{2+} channels display great functional diversity. Each of the three main channel types defined by electrophysiological and pharmacological criteria (L, N and T) most probably represents a class of similar channels, that differ among different tissues. In addition, there are several Ca^{2+} channels that do not fit in any one of these classes. Although functional distinction does not necessarily imply structural difference, and although theoretically the behavior of a channel protein may be differently influenced by the composition of the membrane, combination of different subunits, and

by cytoplasmic factors, there are good reasons to believe that there are many different albeit homologous types of Ca^{2+} channel molecules. The tissue-related diversity of Ca^{2+} channels of the L-type (DHP-binding) has been unequivocally proven on the molecular level. Only initial steps towards understanding of the molecular properties of N-type channels have been made, and no clues exist concerning the T-type channels. In contrast, the study of the molecular properties of the DHP-sensitive L-channels is quite advanced. The availability data imply a role for DHP receptors both as Ca^{2+} channels of L-type and voltage sensors of ECC (in skeletal muscle). However, such essential issues as the roles of individual subunits, the oligomeric structure of a functional channel, and the structural basis of channel regulation by intracellular factors and by drugs are still poorly understood, which promises exciting perspectives for future research.

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