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A short history of voltage-gated calcium channels

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Introduction

Voltage-gated calcium channels play a major role both in the normal functioning and also in various pathological processes that occur in neuronal, neurosecretory and muscle cells. Indeed, their presence has been said to define an excitable cell (Hille, 2001). They were first identified by Paul Fatt and Bernard Katz in crustacean muscle, when they left the Na^+ out of their bathing medium and found that the muscle still generated action potentials (Fatt & Katz, 1953) (Figure 1a). The significance of this observation was subsequently investigated in detail by Fatt & Ginsborg (1958). Susumu Hagiwara (Figure 1b) then continued an in-depth characterization of the calcium conductances in various invertebrate tissues. The early work in invertebrates is comprehensively reviewed by Hagiwara & Byerly (1981) in an article entitled, with an unusual use of the singular: 'Calcium channel', perhaps reflecting the prevailing view at the time that all calcium channels were very similar. Work on mammalian tissues began later, and continued in parallel with the invertebrate work. Calcium action potentials and subsequently calcium currents were identified in mammalian skeletal and cardiac muscle, and subsequently in all excitable cells. The early work on calcium channel discovery and the role of calcium in synaptic transmission has recently been comprehensively reviewed and set in historical context (Barrett & Tsien, 2004). Calcium channels are now known also to be present at low levels in many cells not traditionally considered excitable, such as cells of the immune system, although their function here remains unclear (see e.g. Cahalan *et al*., 2001).

Pharmacology and the identification of calcium channel subtypes

Pharmacology has been key to understanding the function of calcium channels and to identifying the existence of multiple subtypes of calcium channel. Verapamil was the first organic molecule to be described as a 'calcium antagonist' in the 1960s, by Albrecht Fleckenstein (Figure 1c). He coined the term calcium antagonist for any drug that blocked excitation–contraction coupling in the same way as removal of external calcium ions (Fleckenstein, 1983). He subsequently also identified nifedipine as a calcium antagonist, the first molecule of many in the therapeutically important class of 1,4-dihydropyridines (DHPs). These drugs also proved to be invaluable for the isolation of purified voltage-gated calcium channels, an essential step on the path to the cloning era. The various classes of calcium antagonists were found to block calcium currents with differential selectivity in cardiac and smooth muscle in a state-dependent manner, and this forms the basis of their therapeutic role as antihypertensive and antianginal drugs. Harald Reuter (Figure 1d) was one of the pioneers in establishing the essential role of calcium channels in cardiac function (Reuter, 1967).

Identification of multiple calcium channel subtypes in native tissues

The first evidence that there might be more than one type of calcium channel came from the work of Hagiwara, using starfish eggs (Hagiwara *et al*., 1975). Subsequently Carbone & Lux (1984) and Fedulova *et al*. (1985) classified two current components in mammalian sensory neurons according to their biophysical properties. Carbone and Lux first used the term low- and high-voltage-activated (LVA and HVA) channels to describe these components, and showed that the single channels underlying them had different conductances. The additional aid of pharmacological tools led to the classification of certain HVA channels as 'long lasting' or L-type channels which were sensitive to the DHPs, and present in skeletal muscle, heart, smooth muscle and neurons (Hess *et al*., 1984) (Figure 2b). The use of DHP agonists, particularly the Bayer compound BayK 8644, were key to the unequivocal identification of single L-type channels, because they strongly enhanced the mean open time, a state termed 'mode 2' opening (Hess *et al*., 1984).

In skeletal muscle, there is a very high concentration of DHP receptors, representing L-type calcium channels, situated in the T-tubules. Muscle depolarization results in large gating currents, but anomolously low calcium flux, which is now explained by the very slow activation of the ionic currents. For this reason, little or no Ca^{2+} passes across the T-tubule membrane during a single action potential. The study of how these skeletal muscle calcium channels are now thought to couple by a direct mechanical process to the release of intracellular Ca^{2+} from the sarcoplasmic reticulum has enhanced our understanding of excitation–contraction coupling in muscle. One of the key players in this area has been Kurt Beam, who conclusively identified voltage-gated calcium

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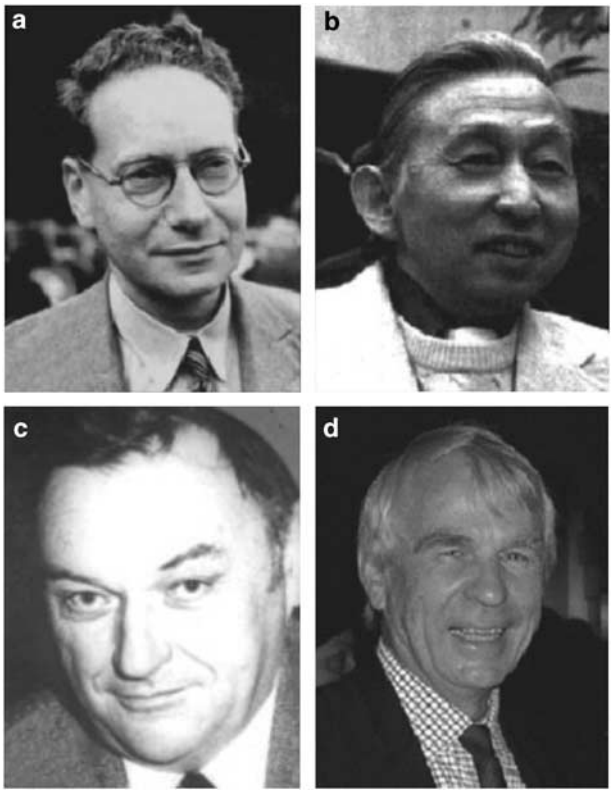

Figure 1 Some key figures in the early discovery of calcium channels and their pharmacology. (a) Bernard Katz. (b) Susumu Hagiwara. (c) Albrecht Fleckenstein. (d) Harald Reuter (photographs b and d obtained with permission from Richard Tsien and Curtis Barrett, from Barrett & Tsien (2004).

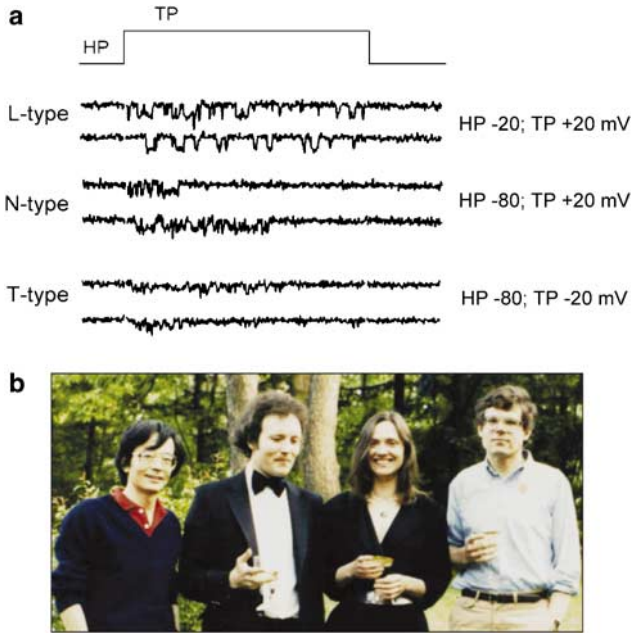

Figure 2 (a) Initial identification of a third component of voltage-gated calcium channels (N-type) from the biophysical properties of single channel currents observed in cell attached patches on dorsal root ganglion neurons. Redrawn from Nowycky *et al.* (1985) (with thanks to Richard Tsien and Curtis Barrett). TP = test potential, HP = holding potential. (b) Some of the members of Richard Tsien's laboratory at Yale at the time of discovery of N-type calcium channels. Right–left: Richard Tsien, Peter Hess, Martha Nowycky and Bruce Bean. Other key figures at that time, not present in the photograph were Ed McCleskey and Aaron Fox (photograph courtesy of Richard Tsien).

channels as the voltage sensors in skeletal muscle, by expressing them in myotubes from dysgenic mice that lacked these channels (Beam *et al*., 1992).

In neurons it was clear that a component of the HVA calcium current was not L-type, as it was not blocked by DHPs. This current was inferred to be particularly prevalent at presynaptic terminals, as synaptic transmission was generally found to be DHP insensitive (see e.g. Stanley & Atrakchi, 1990). The additional non-L-type current component was then subdivided according to its biophysical properties (Nowycky *et al*., 1985) (Figure 2a and b), and subsequently explored with the aid of several invaluable toxins (for review see Olivera *et al*., 1991). Two additional subtypes of calcium channel were thus identified. Firstly, N-type (for Non-L and Neuronal) channels were blocked by a toxin fraction from the fish-eating cone shell mollusc *Conus geographus*, termed ω-conotoxin GVIA. However, a very slowly inactivating calcium current was identified in Purkinje cells of the cerebellum, which was both DHP and ω-conotoxin GVIA insensitive (Hillman *et al*., 1991). These channels were dubbed P-type (for Purkinje), and were found to be sensitive to a component of the venom from the American funnel web spider, *Agelenopsis aperta*. Initially the nature of the blocking toxin was hotly contested, as one group suggested it to be a polyamine (Cherksey *et al*., 1991), but eventually it was established as a peptide, ω-agatoxin IVA (Mintz *et al*., 1992). Another ω-agatoxin IVA-sensitive current component, which showed more rapid inactivation and had a lower affinity for the toxin, was subsequently identified in cerebellar granule cells. This was originally thought to represent a different channel, and termed Q-type (Randall & Tsien, 1995). These two components are now usually combined as P/Q, and probably arise due to different splicing of the same molecular entity or association with different β subunits (Bourinet *et al*., 1999). There is also a Residual or R-type calcium current component that is resistant to DHPs and the N and P/Q channel toxins (Randall & Tsien, 1995).

Molecular subtypes of calcium channel

An understanding of the molecular basis for the physiological subtypes of calcium channel first required the identification of voltage-gated calcium channels as large heteromeric proteins (Figure 3a–d). This era started with purification of the skeletal muscle calcium channel complex, also termed the DHP receptor, which is highly enriched in T-tubules. Several groups including those of Bill Catterall (Figure 3a) and Franz Hofmann, were involved in these studies. The purified DHP receptor complex was found to contain five components, which were termed α_1 (170 kDa), α_2 (150 kDa), β (52 kDa), δ (17–25 kDa) and γ (32 kDa), in an approximately stoichiometric ratio (for reviews see Catterall, 2000; Dolphin, 2003b) (Figure 3b). The α_1 protein was identified as the component that bound 1,4-DHPs, and was therefore provisionally established as the pore-forming subunit.

Following identification of individual subunits, the cloning of the cDNA for the DHP receptor was accomplished, initially from skeletal muscle (Tanabe *et al*., 1987), and subsequently

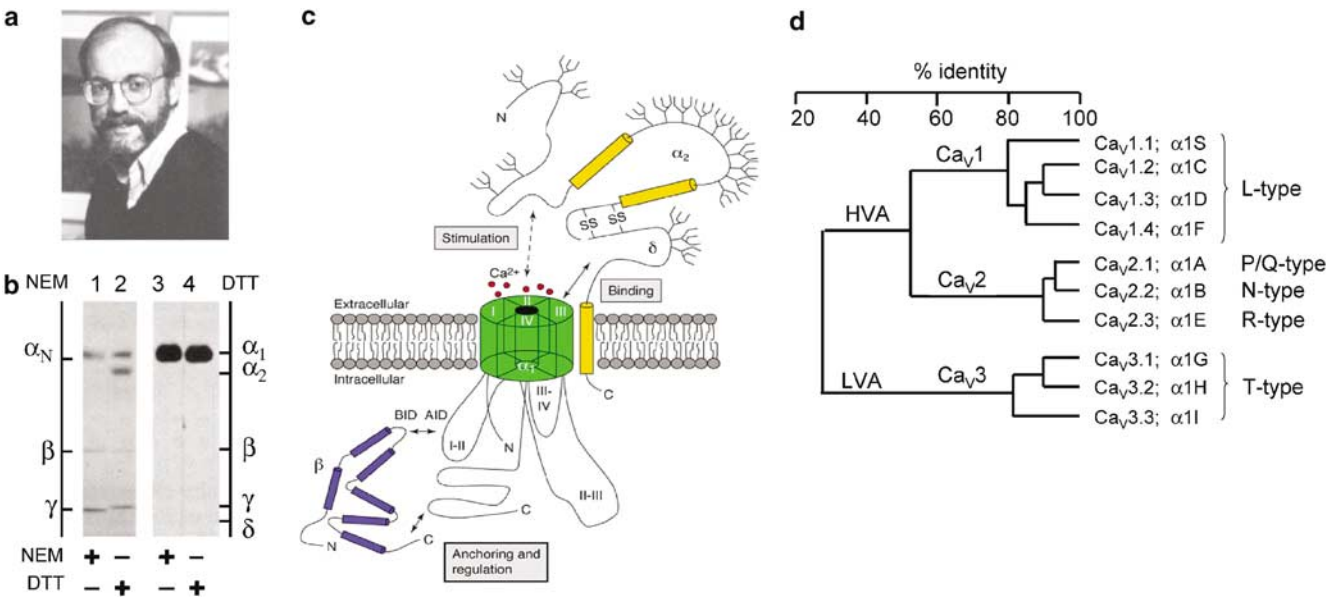

Figure 3 (a) William Catterall, whose laboratory was key to the purification and identification of the subunit structure of L-type calcium channels. (b) Purification of skeletal muscle calcium channels from Takahashi *et al*. (1987). DHP receptors purified with the use of ^{3}H-DHPs; and run on an SDS–PAGE gel under alkylating conditions (+N-ethyl maleimide, NEM, lanes 1 and 3) or reducing conditions (+dithiothreitol, DTT, lanes 2 and 4), and revealed by silver staining (lanes 1 and 2) or using an antibody raised against the DHP receptor (lanes 3 and 4) (redrawn from Takahashi *et al*., 1987). (c) Calcium channel heteromeric complex (redrawn from Walker & De Waard, 1998), taken from 'Calcium channel diversity' by ACD in Encyclopaedia of Life Sciences 2003. (d) Calcium channel dendrogram, showing the per cent identity between the different cloned calcium channels.

from heart, by homology with the skeletal muscle sequence (Mikami *et al*., 1989) (Figure 3c and d). This was a tour-de force at the time, given the size of the gene, and was achieved by the same group, directed by Shosaku Numa, that had cloned the first voltage-gated sodium channel gene. Hydropathy analysis indicated that the α_1 subunits have 24 putative transmembrane segments, arranged into four homologous repeated domains, with intracellular linkers and N- and C-termini (Figure 3c). In all, 10 α_1 subunits have now been cloned, and these all have specialized functions and distributions (for review of nomenclature, see Ertel *et al*., 2000) (summarized in Figure 3d). The four members of the Ca_V1 family are all L-type channels, with $Ca_V1.1$ being the skeletal muscle isoform and $Ca_V1.2$ being particularly prevalent in cardiac muscle; whereas the more recently cloned $Ca_V1.3$ and 1.4 are activated at lower voltage thresholds, and have a more restricted distribution. $Ca_V2.1$ (initially termed α_1A) is the molecular counterpart of P/Q-type calcium channels (Mori *et al*., 1991), $Ca_V2.2$ or α_1B (Dubel *et al*., 1992), is the molecular counterpart of the neuronal N-type calcium channels. $Ca_V2.3$ or α_1E was initially thought to be a low-voltage-activated channel, and it is certainly more inactivating than the other HVA channels cloned (Soong *et al*., 1993). However, it is now thought to contribute to the molecular counterpart of the R-type calcium current. The Ca_V3 group of channels were the last to be cloned, all by the group of Ed Perez-Reyes, and comparison of the homology shows that these channels are clearly divergent in terms of structure from the HVA channels (for review see Perez-Reyes, 2003) (Figure 3d).

All these channels have large numbers of potential splice variants, and one of the challenges of the future will be identify which isoforms are expressed in different tissues, how their expression is regulated and how the expression of different isoforms contributes to specific physiological or pathological processes.

The identification of the voltage sensor, pore and gate

Structural and structure-function studies on the large and unwieldy voltage-gated calcium channels will inevitably lag behind, and rely on analogy with, the elegant structural work on the smaller K^+ channel subunits (see also Jenkinson, this issue). In common with voltage-gated sodium and potassium channels, the sequence of voltage-gated calcium channels revealed that they have positively charged lysine and arginine residues in the S4 transmembrane segment in each domain, which collectively are thought to form the voltage sensor. In terms of permeation, calcium channels have different challenges to overcome from K^+ channels, and appear to have solved the problem rather differently. The calcium channel pore is exquisitely calcium selective, and this property has been attributed to key negatively charged residues, usually glutamate, in each pore loop (Yang *et al*., 1993). It is these side chains that are thought to coordinate Ca^{2+}, rather than the carbonyl backbones of the GYG signature sequence in K^+ channels. Further understanding of permeation will await structural information on calcium channels, which might come initially from prokaryotic single domain homologues. The location of the gate, that regulates the opening and closing of the pore, is still unknown. While there is evidence for the gate in K^+ channels being associated with the bundle crossing 'inverted teepee' formed by the inner S5 and S6 transmembrane α-helices, there may be both internal and external gates in Ca^{2+} channels (for a discussion of this and other unanswered questions see the stimulating review by Jones,

2003). The molecular basis for inactivation in voltage-gated calcium channels is also unclear, and does not appear to involve the same short intracellular loop, between domains III and IV, that moves to obstruct the ion permeation pathway in voltage-gated sodium channels.

Auxiliary subunits

β *subunits*

Four intracellular β subunits have been cloned (β1–4), with β1a being the skeletal muscle isoform of β1, and β1b being its neuronal and cardiac isoform. β2 was initially cloned from cardiac muscle, β3 is present in cardiac and smooth muscle and in neuronal tissue and β4 was cloned from brain. A number of splice variants have been identified, with one particular splice variant of β_2, the β_2a, isoform being N-terminally palmitoylated in certain species, giving it distinctive properties (for recent review see Dolphin, 2003b).

The intracellular $Ca_V\beta$ subunits have marked effects on the properties of HVA α_1 subunits (of the Ca_V1 and Ca_V2 families), including trafficking of calcium channel complexes to the plasma membrane and modification of kinetic- and voltage-dependent properties (for recent review see Dolphin, 2003b). The converse also applies, in that antisense-induced knock-down of $Ca_V\beta$ subunits from native neurons results in a reduction of the amplitude of endogenous calcium currents, and slowed kinetics of activation (Berrow *et al*., 1995).

Most research indicates that all $Ca_V\beta$ subunits, except certain truncated splice variants, increase the functional expression of HVA α_1 subunits (for review see Dolphin, 2003b). Initial studies did not agree whether there was an increase in number of channels at the plasma membrane, measured as charge moved in gating currents, with either no increase (Neely *et al*., 1993), or an increase (Josephson & Varadi, 1996) being reported. Now the consensus of results from most groups would agree that trafficking of α_1 subunits is one of the major roles of $Ca_V\beta$ subunits.

The increase in current density brought about by $Ca_V\beta$ subunits is also the result of influences on a number of biophysical properties, as well as the important influence on trafficking. $Ca_V\beta$ subunits generally shift the activation curve of HVA calcium channels to more hyperpolarized potentials (Birnbaumer *et al*., 1998). Furthermore, where it has been studied, β subunits all produce an increase in channel open probability. While $Ca_V\alpha_1$ subunits contain inherent determinants of voltage-dependent inactivation, association with different $Ca_V\beta$ subunit isoforms influences their overall kinetics of inactivation. Related to this, all β subunits, except the β_2a splice variant that is N-terminally palmitoylated, hyperpolarize the voltage dependence of steady-state inactivation (for review see Dolphin, 2003b).

$Ca_V\beta$ subunits have been found to bind with very high affinity to the cytoplasmic intracellular linker between domains I and II of all HVA calcium channels, *via* an 18 amino-acid motif, dubbed the α interaction domain (AID) on this linker (Pragnell *et al*., 1994). The AID sequence of rabbit $Ca_V2.2$ is QQIERELNGYLEWIFKAE, and the consensus sequence present in both $Ca_V1.x$ and $Ca_V2.x$ subfamilies is QQxExxLxGYxxWIxxxE. The mechanism whereby the binding of $Ca_V\beta$ subunits to this motif regulates trafficking was originally proposed to involve masking an endoplasmic reticulum (ER) retention signal in the I–II linker of the α_1 subunit (Bichet *et al*., 2000), but to date no specific ER retention motif has been found here, and the $Ca_V\beta$ subunit may be acting as a chaperone protein, binding to several regions of the α_1 subunit and promoting its folding, using the I–II linker interaction for tethering. The affinity between $Ca_V\beta$ subunits and a I–II linker fusion protein has been measured to be between 10 and 20 nM (for review see Dolphin, 2003b).

$Ca_V\beta$ subunits contain two conserved domains bounded by N-terminal, C-terminal and centrally located nonconserved regions. In a modelling study, we predicted that the first conserved domain of $Ca_V\beta$ subunits was a *src* homology-3 (SH3) domain linked by a flexible loop to the second conserved domain, which was a guanylate kinase (GK)-like domain (Hanlon *et al*., 1999). Thus $Ca_V\beta$ subunits belong to the family of proteins called membrane-associated GK (MAGUK) proteins, which often have scaffolding functions. Within proteins of the MAGUK family there is known to be an intramolecular interaction between their SH3 and GK domains (Mcgee & Bredt, 1999). Just 17 years after the first β subunit was cloned, in 2004 three groups solved the crystal structures of isolated SH3 and GK domains or the combined SH3-GK core domain of β2, β3 and β4 (Chen *et al*., 2004; Opatowsky *et al*., 2004; Van Petegem *et al*., 2004). A 41 amino-acid sequence that was termed the β interaction domain (BID) in $Ca_V\beta$ subunits was originally identified as the minimal motif required to influence α_1 subunit expression and to bind to the AID region on the α_1 subunit (De Waard *et al*., 1994). However, one of the main findings of the structural studies is that the key BID amino acids make up the hydrophobic core of the GK domain of the β subunit and do not interact directly with the AID α-helix, which lies in a deep binding groove within the GK domain.

The $\alpha_2\delta$ *subunits*

The topology of the α_2 and δ auxiliary proteins, and their relationship, took some time to determine, but eventually it was established that they are encoded by a single gene, whose polypeptide product is post-translationally cleaved into an α_2 and a δ moiety. The α_2 subunit has an N-terminal signal sequence, indicating that it has an extracellular N-terminus. Topology mapping with site-directed antibodies also showed that α_2 is entirely extracellular. Four $\alpha_2\delta$ subunit genes have now been cloned, $\alpha_2\delta$-1 being the original skeletal muscle $\alpha_2\delta$ subunit, whose distribution is fairly ubiquitous, while $\alpha_2\delta$-2 and $\alpha_2\delta$-3 are more selectively found in neurons and a small number of other tissues. The most recently cloned $\alpha_2\delta$-4 is largely non-neuronal (for recent reviews see Arikkath & Campbell, 2003; Canti *et al*., 2003; Klugbauer *et al*., 2003).

Following the identification of $\alpha_2\delta$ subunits as stoichiometric components of skeletal muscle calcium channels, they have also been shown to be associated with native cardiac (L-type) and neuronal N- and P/Q-type channels. Thus it would appear that HVA calcium channels all associate with $\alpha_2\delta$ subunits, although it has not been determined whether functional native HVA α_1 subunits always exist in the plasma membrane in association with $\alpha_2\delta$ subunits.

Parallel purification studies have not been performed on native LVA or T-type calcium channels, hampered by the lack of selective ligands. Since the expression of cloned T-type

channels occurs readily in the absence of $\alpha_2\delta$ subunits, it is assumed that native T-type channels probably exist in their absence in the plasma membrane. However, the expression of T-type currents is enhanced by both $\alpha_2\delta$ and β subunits (Dolphin *et al*., 1999; Dubel *et al*., 2004).

Although there is only limited structural information available for voltage-gated calcium channels, with the exception of the β subunits, a number of electron microscopic single particle studies of purified skeletal muscle calcium channels have been published recently, which show $\alpha_2\delta$ to be a bent structure (reviewed in Wang *et al*., 2004).

Gamma subunits

Skeletal muscle calcium channels also copurify with a γ subunit (Takahashi *et al*., 1987). Whether any of the recently cloned novel γ-like subunits (γ_2–γ_8) are tightly associated with other types of calcium channels, and could be termed subunits, remains controversial (Chu *et al*., 2001; Moss *et al*., 2002), as the closely related γ_{2-4} and γ_8 subfamily interact with AMPA glutamate receptors (see also Watkins & Jane, this issue).

Modulation of calcium channels by G-proteins and second messenger pathways

There are several means by which calcium channels may be both up- and downregulated by second messenger pathways (reviewed in Dolphin, 1999; Catterall, 2000). These include regulation by kinases, for example upregulation of cardiac L-type channels by cyclic AMP-dependent protein kinase (Reuter, 1987). One fascinating conundrum is that whereas L-type channels in cardiac cells are very strongly upregulated by cyclic AMP-dependent phosphorylation (Cachelin *et al*., 1983), this has not yet been entirely reproduced in expression systems, indicating that additional components and post-translational modifications must be necessary.

For the neuronal channels of the Ca_V2 class, particularly N- and P/Q-types, a major mechanism of inhibitory modulation occurs *via* the activation of heterotrimeric G-proteins by G-protein-coupled receptors (GPCRs). GPCR activation was first found to reduce action potential duration in dorsal root ganglion neurons in the 1970s (Dunlap & Fischbach, 1978). Subsequently this effect was shown to result from inhibition of voltage-gated calcium channels (Dunlap & Fischbach, 1981). Such modulation was found to involve the activation of pertussis toxin-sensitive G-proteins (Holz *et al*., 1986; Scott & Dolphin, 1986) (Figure 4A), and has since been observed in many cell types (reviewed in Dolphin, 2003a; see also Milligan & Kostenis, this issue).

The GPCRs typically involved in this type of inhibitory modulation include α2-adrenoceptors, μ- and δ-opioid receptors, GABA-B receptors and adenosine A1 receptors (Figure 4A). A key feature that characterizes the inhibition is that the current activation kinetics are slowed, and this slowing can be mimicked by GTP analogues, also implying the involvement of a G-protein (Dolphin & Scott, 1987; Scott & Dolphin, 1986) (Figure 4A). Stephen Ikeda (Figure 4B) as well as Bill Catterall and Bertil Hille were the main figures involved in the important step of identifying that the G-protein subunits involved in this modulation were the G$\beta\gamma$ dimers (reviewed in Dolphin, 2003a). Bruce Bean (Figure 2b) coined the term

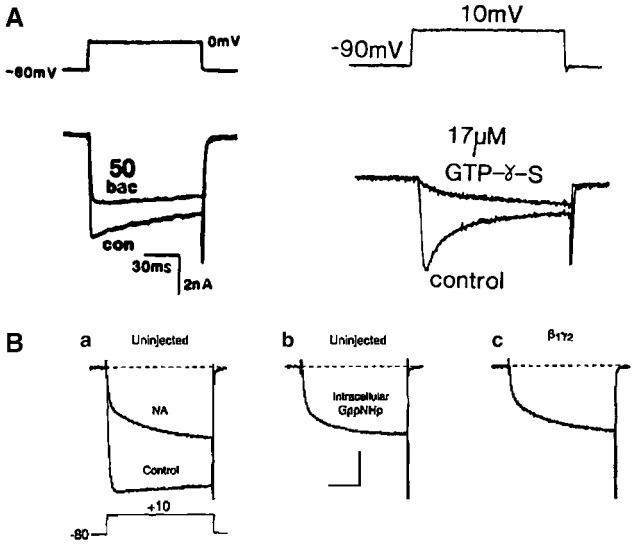

Figure 4 (A) Identification that the GTP analogue GTPγS mimicked the effect of activation of receptors, which contributed to showing that a G-protein was essential for this effect (Scott & Dolphin, 1986). Left: calcium channel currents recorded from dorsal root ganglion neurons, showing inhibition by the GABA-B agaonist baclofen (bac, 50 μM). Right: irreversible inhibition of currents by the GTP analogue GTPγS. (B) Identification by Stephen Ikeda that G$\beta\gamma$ mediated the effects of receptor activation to produce G-protein modulation of calcium channels. Inhibition of calcium channel currents recorded from superior cervical ganglion neurons by noradrenaline (NA, a), an effect mimicked by a GTP analogue GppNHP (b) and by expression of G$\beta\gamma$ dimers (c). Reprinted with permission from Ikeda (1996).

'reluctant' for G-protein-modulated channels, in contrast to the 'willing' control channels (Bean, 1989). The 'reluctance' is manifested by a shift to more depolarized potentials of the current activation–voltage relationship, and the loss of inhibition at large depolarizations, because of a shift from 'reluctant' to 'willing' channels (Bean, 1989). The prevailing view is that channels associated with G$\beta\gamma$ cannot open, despite movement of the voltage sensors. However, depolarization results in slow unbinding of the G$\beta\gamma$ subunits, presumably because they have a lower affinity for the channels in this state, following which the channels open normally, so that the whole process results in the observed slow current activation. Removal of inhibition can also be induced more rapidly by a large depolarizing prepulse applied just before the test pulse (Ikeda, 1991).

Following their activation by depolarization, calcium currents generally show substantial inactivation, in a mechanism that is partially voltage dependent. However, a Ca^{2+}-dependent process was also identified as being a physiologically very important means by which certain Ca^{2+} channels are inactivated (Eckert & Chad, 1984). The role of calmodulin in this process for both L- and P/Q-type channels has been elucidated by a number of groups including those of David Yue, Dick Tsien and Bill Catterall.

Functional roles of calcium channels in synaptic transmission

N- and P/Q-type channels are the main subtypes of calcium channel that support synaptic transmission and they are concentrated at nerve terminals. P/Q-type channels are most

important for transmitter release at central terminals, although N-type channels are also present, and particularly contribute earlier in development. In contrast, N-type channels are more prevalent in peripheral nerve terminals, and are largely responsible for synaptic transmission in autonomic and sensory terminals. L-type channels of the $Ca_V1.3$ and 1.4 class support synaptic transmission at particular specialized terminals, for example those that are designed to release transmitter continuously with low depolarizations in the retina and auditory hair cells.

Role of calcium channels in pathology

Pathological changes in calcium channel expression have been shown to occur in several disease states including neuropathic pain, epilepsy and congestive heart failure. The use of drugs targeting calcium channels now extends far beyond the original discoveries of Fleckenstein (for recent review see Elmslie, 2004). For example, the antiepileptic drugs gabapentin and pregabalin are now known to bind to the $\alpha_2\delta$-1 and $\alpha_2\delta$-2 subunits of calcium channels and are also effective treatments for neuropathic pain. A novel peptide antinociceptive drug based on ω-conotoxin is now in use for the relief of intractable neuropathic pain, just 20 years after the first identification of N-type channels, and this provides hope that orally active small molecule blockers of N-type channels will be available in the future. Several antiepileptic drugs are known to block T-type channels, although it is still a matter of debate whether this represents their major therapeutic mode of action (Perez-Reyes, 2003). In this regard, it will be enormously useful to obtain more selective T-type channel blocking drugs, both for experimental purposes, and for potential therapeutic use.

A number of human and mouse channelopathies that have been identified, for example in familial hemiplegic migraine and several cerebellar ataxias, both of which involve $Ca_V2.1$. An understanding of the mechanism of such channelopathies, while important in itself, may also shed light on novel therapeutic approaches to the treatment of idiopathic forms of migraine (reviewed in Pietrobon, 2002). Furthermore in recent years, knock-out mice have been made for many of the calcium channel subunits (for review see Liu *et al*., 2003), and these have proved to be of enormous importance in enhancing our understanding of the functions of these fascinating channels.

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