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ABSTRACT

Calcium is a ubiquitous second messenger which plays key roles in numerous physiological functions. In cardiac myocytes, Ca^{2+} crosses the plasma membrane via specialized voltage gated Ca^{2+} channels which have two main functions: (i) carrying depolarizing current by allowing positively charged Ca^{2+} ions to move into the cell; (ii) triggering Ca^{2+} release from the sarcoplasmic reticulum. Recently, it has been suggested than Ca^{2+} channels also participate in excitation-transcription coupling. The purpose of this review is to discuss the physiological roles of Ca^{2+} currents in cardiac myocytes. Next, we describe local regulation of Ca^{2+} channels by cyclic nucleotides. We also provide an overview of recent studies investigating the structure-function relationship of Ca^{2+} channels in cardiac myocytes using heterologous system expression and transgenic mice, with descriptions of the recently discovered Ca^{2+} channels α_{1D} and α_{1E}. We finally discuss the potential involvement of Ca^{2+} currents in cardiac pathologies, such as diseases with autoimmune components, and cardiac remodelling.

Key words: Cardiac, myocytes, calcium current, L type calcium channel, T type calcium channel, modulation, auto-immune, remodelling.

1 INTRODUCTION

The importance of extracellular Ca^{2+} in cardiac contraction has been known since the classical experiments of Ringer at the end of the XIXth century, which demonstrated that frog cardiac muscle cannot contract in Ca^{2+} free solutions (Ringer, 1883). More than half a century was needed to elucidate the mechanisms behind this observation. In the 1950's, cellular electrophysiology was dominated by neuronal preparations and the discovery of a surprisingly long action potential in cardiac preparations was unexpected (Draper and Weidmann, 1951). These action potentials showed a "plateau" phase (~200 ms duration) clearly contrasting with action potentials recorded from the squid axon (~5 ms) (Hodgkin and Huxley, 1952a). Characterization of the current(s) involved in this plateau phase was possible by the development of voltage clamp techniques. By recording inward current using two microelectrode voltage clamp in Purkinje fibers, Reuter (1967) was able to demonstrate that depolarization to –40 mV inactivated the Na^+ current (I_{Na}) and further depolarization resulted in a small "slow inward" (*si*) current. This denomination arose by comparing the electrical characteristics of the *si* current with the major inward current described at this time, I_{Na} (Hodgkin and Huxley, 1952b), which is fast and large. Reuter also showed that *si* current was unaffected by tetrodotoxin but abolished by Ca^{2+} free solutions and concluded that *si* current was carried by Ca^{2+} (Reuter, 1967). This finding was confirmed in other cardiac preparations using other voltage clamp techniques (*e.g.* sucrose gap in frog trabeculae, Rougier et al., 1969).

The development of the isolated cell preparation led to improvement of the voltage clamp conditions, by increasing the quality of voltage clamp and reducing extracellular ionic accumulation-depletion in inter-cellular clefts in multi-cellular preparations. Insenberg and Klockner (1982) were the first to describe the *si* current as "large and fast" hence proposing that *si* was an inaccurate name for this current. A considerable further technical advance came with the development of the patch clamp technique (Hamill et al., 1981) which allows control of intracellular solutions (permitting the use of intracellular and extracellular channel blockers to separate the current of interest from other ionic currents). Tsien's laboratory proposed renaming *si* current as L-type Ca^{2+} current, for "Long Lasting" (Nowycky et al., 1985); indeed, L type Ca^{2+} current contrasted with another Ca^{2+} current present in some cardiac cell types (*e.g.* sinoatrial node, atrial and Purkinje cells) which is "Tiny and Transient" (T-type Ca^{2+} current, Nowycky et al., 1985). From the mid 1980's, investigation of Ca^{2+} current (I_{Ca}) in cardiac myocytes led to a profusion of data. The role of the Ca^{2+} current (I_{Ca}, L and T) as a depolarizing current was characterized in all cardiac cells types. Meanwhile, the development of fluorescent dyes to monitor intracellular ion concentrations helped to elucidate the role of

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the L type Ca^{2+} current in excitation-contraction coupling (EC coupling) as the major trigger for Ca^{2+} release from the sarcoplasmic reticulum (via the Ca^{2+}-induced Ca^{2+}-release mechanism, CICR). More recently, the development of molecular biology techniques has allowed the identification of the molecular components of cardiac Ca^{2+} channels identified in electrophyiological studies. Molecular biology now provides tools to alter gene expression in transgenic mice, allowing knockout or overexpression of specific targets and the discovery of new Ca^{2+} channels in cardiac preparations.

This review is subdivided into four parts. In part I, a description is given of the physiological functions of cardiac Ca^{2+} channels *i.e.* their role in membrane depolarization, EC coupling and excitation-transcription coupling (ET coupling). Part II deals with regulation of cardiac Ca^{2+} channels, with emphasis on recent information about local signaling and interplay between different regulatory pathways. Part III provides recent information on the structure-function relationship of cardiac Ca^{2+} channels investigated using molecular biology and expression systems. Part IV discusses the involvement of I_{Ca} in cardiac pathologies, such as diseases with autoimmune components, and remodeling.

2 PHYSIOLOGICAL FUNCTION OF CARDIAC Ca^{2+} CHANNELS

The view that cardiac I_{Ca} can be classified into two main types, L-type and T-type, has been challenged recently by work on transgenic mice. The major L type Ca^{2+} channel expressed in the heart is generally thought to contain α_{1C} (Ca$_V$1.2 in the new nomenclature, see section 4 for details and also table 1) as the pore-forming subunit. However, another high voltage activated channel (HVA), Ca$_V$1.3 (α_{1D}), is also present in cardiac myocytes. This channel has properties similar to Ca$_V$1.2, although it activates at slightly more negative potentials (see section 3.1.1 and see Figure 1). Mice that lack Ca$_V$1.2 expression (α_{1C} knockout, Ca$_V$1.2 $\alpha 1^{-/-}$) have been generated; this knockout mutation is embryonic lethal (Seisenberger et al., 2000), emphasising the crucial importance of Ca$_V$1.2 in cardiac physiology. However before the embryo dies Ca$_V$1.3 gene expression is upregulated, suggesting a compensatory regulation (Xu et al., 2003), although this does not lead to a viable mouse. The reverse also occurs: mice that lack Ca$_V$1.3$\alpha 1$ expression ($\alpha 1D$ knockout, Ca$_V$1.3 $\alpha 1^{-/-}$) show compensatory upregulation of Ca$_V$1.2 channel expression (Zhang et al., 2002b). In this mouse Ca^{2+} channel function is unaffected by the loss of one Ca$_V$1.3 allele (heterozygous mice), suggesting that there is upregulation to the same level of the output from the remaining functional allele (Zhang et al., 2002b). The same upregulation has been observed in Ca$_V$1.2 heterozygous mice (Seisenberger et al., 2000). The mechanism underlying this upregulation is not known. Recently, a third HVA channel has been described in rat and mouse cardiac myocytes (R type, α_{1E} or Ca$_V$2.3) (Weiergraber et al., 2000; Mitchell et al., 2002; Lu et al., 2004).

The low voltage activated (LVA, see Figure 1) family (the T type Ca^{2+} channel) has not expanded in a similar way, although the cloning of 3 genes coding for T-type Ca^{2+} channels has led to a better understanding of these channels in cardiac myocytes (Perez-Reyes, 2003).

In this section, we will review the potential physiological role of these Ca^{2+} channels in different types of cardiac myocytes. As described above, Ca^{2+} entering the cardiac cell has three main roles: carrying depolarizing current, activating contraction and regulating gene expression.

2.1 Depolarizing current

As Ca^{2+} enters the cell, it carries positive charges, which contributes to the electrical current responsible for depolarization. This function differs between cell types in the heart.

2.1.1 *Working cells (ventricular, atrial)*

Working cells in the heart are strongly contractile *i.e.* atrial and ventricular myocytes. I_{CaL} is present in all atrial and ventricular cells studied to date. I_{CaT} is not detectable in most ventricular myocytes *i.e.* frog, calf, cat, rabbit, ferret, mouse, rat, human (Argibay et al., 1988; Bean, 1989; Nuss and Houser, 1993; Yuan and Bers, 1994; Yuan et al., 1996; Nascimento, 1996; Li et al., 1999; Chen et al., 2002) but has been described in modest amounts in guinea-pig (Mitra and Morad, 1986; Sipido et al., 1998a). I_{CaT} is present in atrial myocytes from frog, dog, cat, rat and guinea pig (Wu and Lipsius, 1990; Romanin et al., 1992; Xu and Best, 1992; Alvarez et al., 1996; Yue et al., 1999), but not human (Ouadid et al., 1991), heart.

Although I_{CaL} is present on the sarcolemma of cardiac myocytes, it has become increasingly clear that I_{CaL} is not uniformly distributed on the cardiac cell membrane. The sarcolemma of mammalian ventricular myocytes contains invaginations called transverse (t)-tubules, whereas most mammalian atrial myocytes lack t-tubules (reviewed in Brette and Orchard, 2003). An early study used the properties of dihydropyridines (DHPs) to bind L-type Ca^{2+} channels (also named DHP receptors, DHPr) and showed that in the rabbit heart, the membrane fraction from the t-tubules had a higher density of DHPr than did membrane from the surface sarcolemma (Brandt, 1985). The same technique was used to show that in rat, DHPr are 3 times more concentrated in the t-tubules than on the surface sarcolemma (Wibo et al., 1991). Subsequently, the development of immunohistochemical techniques, coupling confocal microscopy and specific antibodies, has been widely used to investigate the location of proteins within cardiac myocytes. The first immunohistochemical study of Ca^{2+} channel distribution, in rabbit myocytes, showed that in ventricular cells immunostaining occurred primarily at the t-tubules (Carl et al., 1995) whereas in rabbit atrial myocytes, L type Ca^{2+} channel staining was observed in discrete spots along the sarcolemma but was absent from the interior of the fibers (Carl et al., 1995). Therefore, it appears that the L type Ca^{2+} channel is concentrated at the t-tubules; this has been confirmed in guinea pig (Gathercole et al., 2000) and rat (Scriven et al., 2000). Comparative studies suggest that the t-tubular concentration of the L-type Ca^{2+} channel is greater in rat ventricular myocytes than in those from the rabbit (Takagishi et al., 2000).

Thus a high density of L-type Ca^{2+} channel at the t-tubules has been reported in all immunohistochemical studies; the channel has, however, also been reported to be present in appreciable amounts on the surface membrane (Gathercole et al., 2000; Musa et al., 2002). Contrary to binding studies, where a value of Ca^{2+} channel density is achieved (t-tubules *vs* surface membrane), to date no study has quantified the Ca^{2+} channel density using

immunohistochemical technique. However, images from confocal microscopy are clear enough to confirm the concentration of Ca^{2+} channel at the t-tubules compare to cell surface.

Although binding studies and immunohistochemical data show preferential localization of the L type Ca^{2+} channel within the t-tubules of ventricular myocytes, this may not reflect the distribution of its function, which will also depend on local environment. This is particularly true for I_{Ca} since the number of channels activated when gating charge is being recorded is ~10 times higher than the number of channels recorded when monitoring membrane current (Bean and Rios, 1989; Hadley and Lederer, 1989; Hadley and Lederer, 1991b). This can be explained if ~ 10x more channels respond to the change in membrane potential with intra-molecular movement, leading to gating charge, than switch to the open state, resulting in ionic movement and hence current (Hadley and Lederer, 1991b).

Alternative approaches have therefore been used to study the functional localization of I_{Ca} on cardiac sarcolemma. The first study of localization of function, rather than protein, was made using a double barreled microperfusion system coupled to two patch-clamp pipettes (Jurevicius and Fischmeister, 1997), which showed that I_{Ca} is uniformly distributed on the sarcolemma membrane of frog ventricular myocytes. However these myocytes lack t-tubules and this technique do not have access to the t-tubules. An alternative approach has been to use the diffusion delay between changing the bulk extracellular solution and the change in the t-tubules. In guinea pig ventricular myocytes, a rapid change of extracellular $[Ca^{2+}]$ leads to a biphasic change in I_{Ca}, 36% of I_{Ca} changing rapidly (~20 ms time constant) and 64% slowly (~200 ms) (Shepherd and McDonough, 1998). This indicates ~64% of functioning Ca^{2+} channels are in the t-tubules. In atrial myocytes, which lack t-tubules, changing the bathing Ca^{2+} produced a monophasic rapid changes of I_{Ca} (Shepherd and McDonough, 1998).

Recently a new technique has been developed, derived from scanning ion-conductance microscopy (Hansma et al., 1989), in which a patch pipette is used to scan the cell surface and monitor membrane currents (Gorelik et al., 2002). It is unclear whether this technique can monitor current within the t-tubules, although currents at the t-tubule opening can be recorded. Using this technique it has been shown that I_{Ca} is not present on the cardiac cell surface membrane, but occurs only at the entrance of the t-tubules (Gu et al., 2002). This is surprising since cell-attached patches from cardiac cells routinely contain Ca^{2+} channels, suggesting that functional Ca^{2+} channels are present in the surface sarcolemma (*e.g.* the visualization of sparklets by Wang et al., 2001, described in section 2.2.1).

Another approach developed recently has been to adapt the "osmotic shock" technique used previously to detubulate skeletal muscle to disrupt the t-tubules of rat ventricular myocytes (Kawai et al., 1999). T-tubules seal off within the cell and hence are physically and

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electrically uncoupled from cell surface membrane. By comparing currents from detubulated and control myocytes it is possible to estimate the proportion of current within the t-tubules. Detubulation decreases cell capacitance by ~30% in good agreement with the proportion of cell membrane within the t-tubules (Page, 1978), while I_{Ca} decreases by 75% (Kawai et al., 1999). After correction for the presence of non-detubulated myocytes, this suggests that ~90% of I_{Ca} is concentrated within the t-tubules. A further study using the same technique, but using solutions designed to better isolate I_{CaL}, found ~80% of I_{CaL} within the t-tubules *i.e.* L type Ca^{2+} channel 6 times more concentrated at the t-tubules than at the cell surface (Brette et al., 2004b).

Thus there is both structural and functional evidence that I_{CaL} is concentrated at the t-tubules in mammalian ventricular myocytes. Study of the localization of T-type channels has been hampered by the lack of specific toxins or drugs, precluding binding studies, and the lack of specific antibodies. Furthermore, no functional study has been performed to date, hence it is unknown whether I_{CaT} is concentrated at the t-tubules or uniformly distributed.

I_{CaT} inactivates quickly (see section 3.1.1), consequently I_{CaL} is responsible for the long duration (plateau phase) of cardiac action potential from ventricular and atrial myocytes. In a given species, the plateau phase is longer in ventricular myocytes than in atrial cells, although it is likely that this is due to differences in currents other than I_{Ca} (e.g. I_{K1} guinea pig, (Hume and Uehara, 1985)).

The development of the patch clamp technique has led to a better understanding of the role of I_{CaL} during the plateau phase, by using action potential voltage clamp command waveforms. I_{Ca} during the action potential differs significantly from that elicited using square pulses (Yuan et al., 1996; Linz and Meyer, 1998a; Li et al., 1999). Figure 2 shows the time course of I_{CaL} in three different species widely used in cardiac physiology: I_{Ca} is more sustained during an action potential than during a square pulse, because the peak of the action potential occurs at positive potential (30-50 mV), hence Ca channels activate rapidly but the driving force for Ca^{2+} is low. As the membrane potential falls, I_{Ca} remains large since the membrane potential changes quicker than voltage dependent inactivation. This sustained I_{CaL}, explaining the plateau phase, has been recorded in rat (Yuan et al., 1996), rabbit (Yuan et al., 1996; Puglisi et al., 1999), guinea pig (Grantham and Cannell, 1996; Linz and Meyer, 1998a) and human (Li et al., 1999). Yuan et al., (1996) compared Ca^{2+} entry during step and action potential depolarizations in rat and rabbit myocytes. They showed that during square pulses the amount of Ca^{2+} entry is higher in rat than in rabbit. This reflects differences in I_{Ca} activation and inactivation. However when an action potential waveform was used Ca^{2+} entry was much higher in rabbit than in rat ventricular myocytes (21 *vs* 14 µmol/L cytosol respectively),

because of the short action potential in rat myocytes. The profile of I_{Ca} during an action potential is highly modulated by Ca dependent inactivation and this will be discussed in section 3.2.1

2.1.2 Nodal cells

Cardiac pacemaker cells exhibit automaticity as a result of gradual depolarization of the membrane potential during electrical diastole. Work from various laboratories has shown that multiple mechanisms contribute to pacemaker activity, although the relative contribution of each current to pacemaker function is controversial (reviewed in Lipsius et al., 2001).

In atrioventricular nodal cells, both T and L type Ca^{2+} currents have been found (Liu et al., 1993; Munk et al., 1996), which play a crucial role in conduction through the node (Noma et al., 1980) (see also section 5.1.1). Since there is a paucity of data regarding the atrioventricular node, the following discussion will focus on sinoatrial node cells.

A role for I_{CaT} in the generation of cardiac automaticity has been proposed because of the electrophysiological properties of this channel (low threshold of activation) and its high expression in this cell type. Current-clamp studies of spontaneous action potentials have shown that 40μM Ni^{2+}, which selectively blocks I_{CaT}, slows the late phase of depolarization, hence slowing the firing of rabbit sinoatrial node cells (Hagiwara et al., 1988; Doerr et al., 1989; Zhou and Lipsius, 1994; Satoh, 1995). The contribution of I_{CaT} is thought to result from the depolarizing effect of inward Ca^{2+} current. In addition, a window current (see section 3.1.2) from I_{CaT} might participate in this process during the late phase of the pacemaker potential. Recently, it has been proposed that I_{CaT} might also be involved in the generation of spontaneous activity by another mechanism: Huser et al., (2000) showed that I_{CaT} could trigger local Ca^{2+} release events (Ca^{2+} sparks, see section 2.2.1). Local increases in Ca will increase inward Na/Ca exchange current which will further depolarize the cell (Huser et al., 2000). This might explain early work showing that ryanodine significantly reduced the rate of spontaneous beating of guinea pig sinoatrial node preparation (Rigg and Terrar, 1996). In contrast, there is evidence that I_{CaT} might not be involved in sinus rhythm: a recent report showed that Ca sparks in sinoatrial pacemaker cells do not require membrane depolarization (Vinogradova et al., 2004). This is consistent with a recent report showing that mice deficient in α_{1H} T type Ca^{2+} channel ($Ca_V3.2$ see section 4.2) do not have a significantly different heart rate (Chen et al., 2003). Since the Ni^{2+} sensitivity of $Ca_V3.2$ is similar to that of native I_{CaT} it was thought that $Ca_V3.2$ was the major isoform in cardiac cells. However the mRNA of $Ca_V3.1$, another T type Ca^{2+} channel isoform, is present in abundance (Bohn et al., 2000) (see also section 4.2). This isoform recovers faster from inactivation (120 ms *vs* 400 ms for $Ca_V3.2$, Klockner et al., 1999)

which is more consistent with the kinetics of recovery of the native I_{CaT} (e.g. 140 ms in rabbit, Hagiwara et al., 1988). However, to the best of our knowledge no cardiac anomalies have been reported in transgenic mice lacking $Ca_V 3.1$ (-/-) (Kim et al., 2001). Thus the role and isoform of I_{CaT} in pacemaking cells remains unclear.

I_{CaL} may also participate in pacemaker activity. However, given the relatively positive membrane potential for activation (-40 mV) I_{CaL} is probably only relevant in the latter phase of pace making. I_{CaL} is also essential for the rapid phase of depolarization (upstroke, depending on the presence or absence of I_{Na} in the preparation) and may explain the much slower action potential upstroke in nodal cells compared to ventricular myocytes (~2 V/sec, sinoatrial node and ~20 V/sec atrioventricular node *vs* 200 to 300 V/sec in ventricular myocytes, (Schram et al., 2002)). In sinoatrial node cells, I_{CaL} appears to be modulated by Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII) under basal condition. Vinogradova *et al.* (2000) showed that KN93, a CaMKII specific blocker, slows spontaneous excitation in the sinoatrial node of rabbit myocytes. This effect appeared to be due to a decrease in I_{CaL} amplitude and a hyperpolarizing shift of the steady state inactivation curve, hence decreasing the I_{CaL} window current (Vinogradova et al., 2000).

Transgenic mice have provided new insights into the role of HVA in cardiac pacemaking. An early study of electrocardiogram recordings from α_{1D} deficient mice revealed sinoatrial node dysfunction with bradycardia and arrhythmia (Platzer et al., 2000) indicating an essential role for pacemaking in mouse heart. At this time, cell isolation from mouse sinoatrial node cells was not established. However it has since been shown by two groups that sinoatrial node cells isolated from α_{1D} deficient mice show decreases in spontaneous rate of firing (Zhang et al., 2002b; Mangoni et al., 2003) due to a significant depolarizing shift in the activation threshold and a decrease in the amplitude of I_{CaL} (Zhang et al., 2002b; Mangoni et al., 2003). Indeed, α_{1D} is characterized by a more negative activation threshold than α_{1C} (see Figure 1), allowing α_{1D} to mediate a substantial Ca influx in sinoatrial node cells (Zhang et al., 2002b).

These studies provide functional evidence that I_{CaL} in sinoatrial node cells is due, in part, to α_{1D} subunit activity and that this channel plays a role in the generation of pacemaking in mouse. To date no pharmacological tools that are selective for $Ca_V 1.3$ (α_{1D}) L-type Ca^{2+} channels have been described, hence it is not possible to extend this finding to other species. Interestingly, it has been shown that in sinoatrial node from rabbit I_{CaL} can be activated at more negative potentials (-60 mV) than the classic potential for α_{1C} (-40 mV) (Verheijck et al., 1999).

2.1.3   Conducting tissue

Conducting tissues in the heart are Purkinje and His cells. Both L and T type Ca^{2+} current are present in conducting tissue, with T type Ca current being fairly large compared with other cardiac preparations (Hirano et al., 1989; Tseng and Boyden, 1989; Zhou and January, 1998). For example, in dog Purkinje cells, I_{CaT} density is similar to I_{CaL} (Zhou and January, 1998). I_{CaL} density is smaller than in ventricular myocytes, consistent with the less positive plateau phase (Schram et al., 2002). I_{CaT} inhibition does not affect Purkinje fibre automaticity suggesting that I_{CaT} may not be important for conducting tissue pacemaking (Pinto et al., 1999), but, L and T type Ca^{2+} channels are important in the conduction of the action potential.

2.2   Excitation-contraction coupling

Contraction of striated muscle cells is due to Ca^{2+} binding to troponin C (see Bers 2001 for review). As described above, significant Ca^{2+} entry occurs during an action potential in cardiac myocytes and this Ca^{2+} might activate troponin C directly. However, in mammalian cardiac myocytes, the main mechanism is Ca^{2+} entry triggering Ca^{2+} release (CICR) from the sarcoplasmic reticulum.

2.2.1   Triggering sarcoplasmic reticulum Ca release

The phenomenon of CICR was initially demonstrated in skinned skeletal myocytes (Endo et al., 1970; Ford and Podolsky, 1970) and subsequently in skinned cardiac Purkinje cells (Fabiato and Fabiato, 1973; Fabiato and Fabiato, 1975), by abruptly increasing the bathing Ca^{2+} concentration. Fabiato extensively described CICR in cardiac preparations, demonstrating that CICR is graded both by the magnitude and the rate of change of the trigger Ca^{2+}, and provided compelling evidence for the existence of this mechanism in cardiac myocytes (Fabiato and Fabiato, 1978; Fabiato, 1982; Fabiato, 1983; Fabiato, 1985). The next step was to determine whether CICR constituted the normal Ca^{2+} release process in intact cardiac myocytes, and if so, which source of Ca^{2+} provided the trigger for release. During the 1980's it became clear that I_{Ca} provided the normal trigger for Ca^{2+} release via CICR in cardiac myocytes: several laboratories showed the similar bell shaped voltage dependence of Ca^{2+} release and I_{Ca} (Cannell et al., 1987; Beuckelmann and Wier, 1988; Callewaert et al., 1988). Further support came from the observation of tail currents and transients, due to I_{Ca} inducing Ca^{2+} release (Cannell et al., 1987; Beuckelmann and Wier, 1988): when voltage steps to +100 mV are used to induce Ca channel opening, but no Ca influx because the membrane potential is near the Ca^{2+} equilibrium potential, no Ca transient is observed (Cannell et al., 1987). However when the membrane potential is stepped back to negative voltages, Ca^{2+} enters the cell before the Ca^{2+} channels close and a Ca transient is observed. Further evidence for I_{Ca} as

the main trigger for CICR is voltage-dependent reduction of the "gain" of EC coupling (Wier et al., 1994), which was first described as maximum sarcoplasmic reticulum Ca^{2+} flux/ I_{Ca} peak (Wier et al., 1994), although most investigators now use a simplification of this definition (Ca^{2+} transient amplitude/ I_{Ca} peak) (see Bers, 2001 for more details). Gain decreases with voltage; Wier et al., (1994) suggested that this reflected the link between CICR and the unitary current flowing through a single Ca^{2+} channel (i_{Ca}).

By the end of the 1980's, there was compelling evidence that CICR was the major mechanism of Ca^{2+} release in the heart and the L type Ca channel was the major trigger. However, this led to a paradox since it was unclear how such a positive feedback mechanism could be graded (Fabiato, 1985). Stern (1992) showed mathematically that global entry of Ca^{2+} leading to global Ca^{2+} release from the sarcoplasmic reticulum (common pool theory) is inherently unstable and should result in all or nothing behavior. Instead, Stern proposed a local control theory to explain Ca^{2+} release from the sarcoplasmic reticulum, in which RyRs are under the control of a single Ca^{2+} channel. He envisaged that this one Ca^{2+} channel could either regulate a single RyR ("Ca^{2+} synapse" model) or a group of RyRs ("cluster bomb" model) in a single Ca^{2+} release unit (Stern, 1992). Each Ca^{2+} release unit operates independently and the whole-cell Ca^{2+} transient is the temporal and spatial summation of these individual localized release events.

Functional evidence supporting Stern's theory was provided with the development of confocal microscopy and new fast Ca^{2+} indicators with high contrast (*i.e.* fluo-3). Cheng et al (1993) described small, local and brief increases of intracellular Ca^{2+}. These discrete sarcoplasmic reticulum Ca^{2+} release events by RyRs were named Ca^{2+} sparks (Cheng et al., 1993). It appears now that they are due a cluster (4-20) of RyRs operating in concert (Wang et al., 2004b). Ca^{2+} sparks occur in a stochastic and discrete manner and are predominantly generated at the t-tubules in ventricular myocytes (Shacklock et al., 1995; Parker et al., 1996). In atrial myocytes, which lack t-tubules, Ca^{2+} sparks occur predominately at the cell periphery (Kockskamper et al., 2001; Woo et al., 2003a). Structural studies also support the local control theory. Electron microscopy and immunocytochemistry show co-localization of the L type Ca^{2+} channel and RyRs at the junctional sarcoplasmic reticulum in several species (guinea-pig, rabbit, rat, mouse; Carl et al., 1995; Franzini-Armstrong et al., 1999; Gathercole et al., 2000; Scriven et al., 2000). In ventricular myocytes this co-localization occurs at the t-tubules and at the surface sarcolemma, with no obvious difference at the two sites (Carl et al., 1995; Franzini-Armstrong et al., 1999). The junctional cleft between the sarcolemma and the sarcoplasmic reticulum membrane results in a restricted diffusion space, termed "fuzzy space" by Lederer et al., (1990). Interestingly, this gap is the same at the cell surface and the t-tubules (Page, 1978),

although junctional sarcoplasmic reticulum is more prominent at the t-tubules (Bers, 2001). In chick and atrial myocytes, which lack t-tubules, L type Ca^{2+} channel and RyR co-localization is seen only at cell surface membrane (Carl et al., 1995; Sun et al., 1995).

The functional relationship between single Ca^{2+} channels and RyRs was initially investigated indirectly. Early work by Lopez-Lopez et al., (1995) provided indirect evidence that a single channel activates Ca^{2+} sparks. Using verapamil to decrease the open probability, but not the amplitude, of single L type Ca currents, they showed that the probability of evoking a local release followed the voltage dependence of i_{Ca} (Lopez-Lopez et al., 1995). Direct demonstration that a single Ca^{2+} channel can trigger a Ca^{2+} spark was a technical challenge. Gigaseal formation during patch clamp experiments leads to the formation of Ω-shaped membrane in the pipette (Sakmann and Neher, 1995), which will disrupt the delicate coupling of Ca^{2+} channel with RyR. Wang et al., (2001) overcame this drawback by using the loose-seal patch clamp technique, which allows formation of sufficient resistance between the cell membrane and the pipette without destroying the coupling of the two channels. Although the success rate was low, they succeeded in recording Ca^{2+} sparks directly activated by the opening of a single Ca^{2+} channel. They recorded low amplitude Ca^{2+} events that were insensitive to ryanodine (opening of i_{Ca}), which they named Ca^{2+} sparklet (Wang et al., 2001), and high amplitude Ca^{2+} events, representing Ca^{2+} sparks, rising from the shoulder of an ongoing Ca^{2+} sparklet (see Figure 3). It is noteworthy that the demonstration of communication between the L type Ca^{2+} channel and RyRs was demonstrated at the cell surface. Indeed, to date no technique allows recording of single Ca^{2+} channel activity within the t-tubules.

Recently, a transgenic mouse overexpressing α_{1C} has been generated (Muth et al., 1999). I_{Ca} density was increased compared with control mice but interestingly the gain function of sarcoplasmic reticulum Ca^{2+} release remained unchanged (Song et al., 2002). This suggests that the overexpressed L type Ca^{2+} channels are able to form functional couplings with the RyRs which are as effective as the native L type Ca^{2+} channels in triggering sarcoplasmic reticulum Ca release.

Although any increase in Ca^{2+} in the fuzzy space is able to induce CICR from the sarcoplasmic reticulum, as demonstrated by the use of flash photolysis and caged Ca^{2+} (Valdeolmillos et al., 1989; Niggli and Lederer, 1990; Nabauer and Morad, 1990), it is now generally accepted that I_{Ca} is the major trigger for sarcoplasmic reticulum Ca^{2+} release. Alternative pathways, such as Na/Ca exchange, have been shown to be weak trigger under physiological condition (reviewed in Wier and Balke 1999).

The possibility that T-type Ca current might participate in CICR remains uncertain due to its absence in most ventricular myocytes (see section 2.1.1). In ventricular guinea-pig myocytes, in which I_{CaT} is present, it was found that sarcoplasmic reticulum Ca^{2+} release produced by I_{CaT} is delayed and slower than I_{CaL} (Sipido et al., 1998a). Similar results have been observed in Purkinje cells, where I_{CaT} is more substantial (Zhou and January, 1998). It appears therefore that a given Ca^{2+} influx through I_{CaT} is much less effective and slower than I_{CaL} in triggering sarcoplasmic reticulum Ca^{2+} release. This suggests that I_{CaT} does not belong to the junctional domain near the RyR. Specific antibodies against T-type Ca^{2+} channels are not available yet, but when available might confirm these functional studies. To the best of our knowledge, no functional investigation of I_{CaT} in CICR in atrial myocytes has been performed.

There is no information about the presence of α_{1D} in working cells, and hence whether this subunit participates in EC coupling. Indirect support of non-involvement of α_{1D} in EC coupling comes from $Ca_v1.3$ -/- transgenic mice, which show normal cardiac function except bradycardia (Seisenberger et al., 2000) (see also section 2.1.2). Similarly, no information is available about a potential role for α_{1E} in EC coupling (see also section 4.3).

Voltage activated Ca^{2+} released (VACR), as in skeletal muscle, has been proposed in the heart. Although in skeletal muscle, it is clear that the L type Ca^{2+} channel (α_{1S} subunit, see section 4.1.1) is the voltage sensor for VACR, in the heart, it remain unclear if any Ca^{2+} channel acts as the voltage sensor (see for review Ferrier and Howlett 2001). Evidence for VACR in the heart has been provided mainly by one laboratory and many technical issues have been raised against this mechanism (Wier and Balke, 1999). Most of the studies showing VACR have used a double step protocol to separately activate VACR and CICR: a first step to −40 mV to activate VACR and a second step to 0 mV to activate CICR (Ferrier and Howlett, 1995; Hobai et al., 1997; Ferrier et al., 1998; Howlett et al., 1998). However VACR requires internal cAMP, which will shifts the voltage dependence of activation of I_{CaL} to below −40 mV (see section 3.3.1); VACR has therefore been explained by CICR due to I_{CaL} in several studies (Piacentino, III et al., 2000; DelPrincipe et al., 2000; Griffiths and MacLeod, 2003; Trafford and Eisner, 2003; Brette et al., 2003b).

2.2.2 *Direct activation of contraction*

The role of Ca^{2+} influx in activating troponin C directly is difficult to investigate in cardiac myocytes since I_{Ca} also triggers Ca^{2+} release (above). Fabiato (1983) calculated that the role of Ca^{2+} influx in directly activating contraction is minor. Ryanodine, which blocks sarcoplasmic reticulum Ca^{2+} release, has frequently been used to investigate this issue. However, in the presence of ryanodine, Ca^{2+} dependent inactivation of I_{Ca} is smaller than

under normal conditions (see section 3.2.1), thus enhancing the possible role of I_{Ca} in directly activating contraction. Furthermore, most studies are performed at low stimulation frequency and room temperature, hence the value presented below might slightly differ from a more physiological context (higher stimulation rate and 37ºC). Ryanodine has no effect on frog ventricular myocytes, but reduces contraction of rabbit (by 30%) and rat (by 80%) ventricular myocytes (Bers, 2001). These results do not show that under physiological conditions 70% of contraction is activated by I_{Ca} in rabbit ventricular myocytes (20% in the rat), but suggest that when the sarcoplasmic reticulum is not functional I_{Ca} can provide a significant amount of Ca^{2+} for contraction (Bers, 2001). Closer analysis suggests that Ca from I_{Ca} can account for ~23% of contraction in rabbit and ~8% in rat ventricular myocytes (Delbridge et al., 1996; Delbridge et al., 1997). An approximate sequencing of cardiac muscle preparations, from most to least reliant on sarcoplasmic reticulum Ca^{2+} release: adult mouse ventricular > adult rat ventricular > dog ventricular ~ferret ventricular > cat ventricular > neonate rat ventricular ~ rabbit atrial > human ventricular > rabbit ventricular > guinea pig ventricular > neonate rabbit ventricular > fetal ventricular (human, cat and rabbit) > frog ventricular (see Bers 2001 for details). This sarcoplasmic reticulum dependence of contraction follows structural data. In frog ventricular myocytes, the sarcoplasmic reticulum is sparse, more developed in rabbit ventricular myocytes and even more so in rat myocytes. Such a change in sarcoplasmic reticulum structure also occurs during development. Ventricular myocytes from newborn animals shows little sarcoplasmic reticulum and enhanced I_{Ca}, hence contraction in these cells relies mainly on Ca^{2+} influx directly activating contraction (Fabiato and Fabiato, 1978; Cohen and Lederer, 1988; Haddock et al., 1999). Interestingly, in a given species, atrial muscle is more CICR dependent than ventricular muscle (Fabiato, 1982).

2.2.3 *Loading of the sarcoplasmic reticulum*

Fabiato suggested that the early part of I_{Ca} triggers sarcoplasmic reticulum Ca^{2+} release and that the late part of I_{Ca} loads the sarcoplasmic reticulum with Ca^{2+} for subsequent release (Fabiato, 1985). He proposed that the sarcoplasmic reticulum has two compartments, one for uptake, one for release with a lag for diffusion of Ca^{2+} between the two compartments (Fabiato, 1985). Indeed, the pioneering work of Beeler and Reuter (1970) showed that while I_{Ca} is fully activated and does not change during repetitive depolarization, the first contraction is very small and the force increases subsequently to reach a steady state (staircase phenomena). They proposed that I_{Ca} have to flow to fill intracellular stores from which Ca^{2+} can be released during subsequent depolarizations (Beeler and Reuter, 1970). More recently, the contribution of the late phase of I_{Ca} to sarcoplasmic reticulum Ca^{2+} loading has been

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demonstrated in action potential clamp experiments in guinea-pig ventricular myocytes (Linz and Meyer, 1998b), and a series of papers from Eisner's laboratory has shown that I_{Ca} participates substantially in sarcoplasmic reticulum Ca^{2+} loading (see for review Trafford et al., 2002). Trafford et al., (1997) demonstrated that during stimulation following sarcoplasmic reticulum Ca^{2+} depletion, integrated I_{Ca} was initially large but progressively become smaller (from ~14 µmol/L cell to 8 µmol/L cell). In parallel, Ca^{2+} efflux from the Na/Ca exchanger increased from ~3 µmol/L cell to 8 µmol/L cell (Trafford et al., 1997). This results to a net Ca^{2+} influx during the first few contractions until a steady state is reached where Ca^{2+} influx matches Ca^{2+} efflux (Eisner et al., 2000).

2.3   Excitation-transcription coupling

An emerging field in cellular physiology is the link between membrane ion channels and regulation of gene expression, namely excitation-transcription coupling (ET coupling). Genome-wide screens have identified over 300 different genes and approximately 30 transcription factors that are regulated by intracellular Ca^{2+} (Feske et al., 2001). Studies over the past decade suggest that most Ca^{2+} activated transcription factors require a rise in nuclear Ca^{2+} for activation. Recent evidence, however, mainly in neuronal studies, indicates that a rise of Ca^{2+} in the vicinity of sarcolemmal Ca^{2+} channels also plays a key role in regulating transcription (see for review Dolmetsch 2003).

One of the best studied transcription factors is the cAMP-responsive element binding protein (CREB). CREB binds to the cAMP response element (CRE) and to the Ca^{2+} response element (CARE), and is thus activated by both cAMP and Ca^{2+} via the calmodulin and CaMKII cascade. CREB is activated by phosphorylation of several serines, including Ser133, which recruits the coactivator CREB binding protein (CBP) (Shaywitz and Greenberg, 1999). In neurons, it has been shown that CREB phosphorylation on Ser133 can occur even in the presence of EGTA, a slow Ca^{2+} buffer that prevents nuclear Ca^{2+} elevation but allows intracellular Ca^{2+} elevation close to the plasma membrane (Deisseroth et al., 1996; Hardingham et al., 2001) (see also section 3.2.1). Calmodulin near the L-type Ca^{2+} channel appears to be the link between local Ca^{2+} influx and CREB activation, by translocation of calmodulin to the nucleus (Deisseroth et al., 1998). Whether Ca^{2+} or Calmodulin or CaMKII is translocated to the nucleus to activate CREB in cardiac cells has not yet been determined (see Figure 4). Another well characterized Ca^{2+}-dependent transcription factor is the nuclear factor of activated T cells (NFAT). NFAT plays a critical role in the activation of cells in the immune system and also in the development of the cardiovascular system (Crabtree, 2001). For example, NFAT3 translocates from the cytoplasm into the nucleus in response to

dephosphorylation of several of its serines by the Ca^{2+}-calmodulin activated phosphatase calcineurin (Clipstone and Crabtree, 1992). NFAT3 appears to be important also in the development of heart failure (see section 5.2.2). Ca^{2+} can also regulate transcription without the intermediate of kinases and phosphatases, as in the case of EF hand protein downstream regulatory element antagonistic modulator (DREAM) (Carrion et al., 1999). At resting nuclear Ca^{2+} levels, DREAM is bound to DNA and suppresses transcription, possibly by preventing the interaction of transcription factors and coactivators (Ledo et al., 2002). When a DREAM tetramer binds Ca^{2+} it disrupts the interaction between DREAM and other proteins relieving the transcriptional block. These events occur in the nucleus, suggesting that inactivation of DREAM requires a nuclear Ca^{2+} elevation. Interestingly, DREAM may also have a role outside the nucleus, because its sequence is nearly identical to that of a neuronal Ca^{2+} sensor acting on K^+ channels (Carafoli, 2002).

A central question in excitation-transcription coupling is how cardiac cells can distinguish changes of Ca^{2+} distinct from the cyclic variation of Ca^{2+} at each heartbeat and convert them into specific transcriptional response. One possible answer is that transcription factors have distinct response characteristics that may refine the message of intracellular Ca^{2+} oscillations; the time course, amplitude and spatial localization of a rise of Ca^{2+} conveys important information about the type and intensity of the stimulus. For example, in B lymphocytes, the transcriptional factor NFAT is differentially activated by brief Ca^{2+} signals of high magnitude compared with prolonged Ca^{2+} signals of lower amplitude (Dolmetsch et al., 1997). Location is also important since regulatory proteins may be confined to distinct intracellular domains (*e.g.* nucleus) or anchored by specific binding proteins (Pawson and Scott, 1997). For example, the nucleus experiences very different Ca^{2+} signals than the cytoplasm: a preliminary study has shown prolonged discrete Ca^{2+} release events (~2 sec duration) in the nucleus of rat ventricular myocytes, clearly contrasting with Ca^{2+} spark characteristics in the cytosol (Yang and Steele, 2004). In addition, specific isoforms of CaMKII-δ might have different locations within the cell (*i.e.* cytosol *vs* nucleus) and can be linked to the expression of gene during the hypertrophic response (Ramirez et al., 1997; Hoch et al., 1999). Privileged avenues are also important. In cultured neurons, an early study showed that Ca^{2+} influx through L type Ca^{2+} channels activates early gene expression (Murphy et al., 1991). In hippocampal slices, Ca^{2+} influx through L type Ca^{2+} channels leads to phosphorylation of CERB at Ser133 and activation of CERB-dependent transcription. It has been shown that the critical element in signaling from the L type Ca^{2+} channel is calmodulin since mutation of the calmodulin site of L type Ca^{2+} channels reduces their ability to activate CREB and mitogen-activated protein kinases (MAPK) pathway (Dolmetsch et al., 2001). A

very recent study estimated that the concentration of free calmodulin in the vicinity of L-type Ca^{2+} channel is 2.5 mM, *i.e.* ~5 times the calmodulin concentration in the bulk cytoplasm (Mori et al., 2004). This has clear implication in the transduction of Ca^{2+}/calmodulin signal (*e.g.* activation of CREB, above), but also raises the question of how calmodulin is concentrated in the region of the L type Ca^{2+} channel.

In the heart, there is an emerging picture about the importance of ET coupling however Ca^{2+}-dependent transcription factors are only now beginning to be explored.

3 MODULATION OF CARDIAC Ca^{2+} CHANNELS

Ca^{2+} channels play a major role in cardiac cell function; it is therefore not surprising that they can be modulated by a variety of physiological factors. In this section we will briefly describe how Ca^{2+} channels are modulated by voltage (for extensive review see McDonald et al., 1994). We will then focus on modulation by Ca^{2+}: interestingly, Ca^{2+} itself modulates Ca^{2+} channel activity, causing important negative and positive feedback. We will also describe modulation by cyclic nucleotides which occur physiologically when hormones or neurotransmitters bind to seven span transmembrane receptors. In the majority of cases, phosphorylation pathways are used to affect Ca^{2+} channel activity, however interplay between cyclic nucleotides also modifies the effects on Ca^{2+} channels.

3.1 Regulation by Voltage

3.1.1 *Activation*

Ca^{2+} channels are voltage-dependent with the open probability of the channel increases with depolarization. In cardiac myocytes, I$_{Ca}$ reaches a peak in 2~7 ms depending on the temperature and holding potential (McDonald et al., 1994). Recently, new insights into Ca^{2+} channel activation have been provided using molecular biology techniques (see section 4). The threshold potential for activation of T type Ca^{2+} channels is more negative than for L-type Ca^{2+} channels. Thus L and T type I$_{Ca}$ can be separated by using different holding potentials: positive to $-$ 40 mV I$_{CaT}$ will inactivate, I$_{CaL}$ will remain mainly unchanged, although the recent discovery of another L type Ca^{2+} channel in cardiac myocytes (α_{1D}), slightly alters this notion. Figure 1 shows the current voltage relations (I-V) of the 3 major Ca^{2+} channels in cardiac cells. The I-V curves are all bell-shaped, and T type Ca^{2+} current reaches a maximum ~30 mV negative to the L type Ca^{2+} channel (α_{1C}). α_{1D} Ca^{2+} channels show a more negative threshold and peak than α_{1C}, which explains their potential physiological role in nodal cells (see section 2.1.2).

Ca^{2+} channels can also deactivate (closure of the channel before inactivation). This occurs mainly during short square voltage clamp pulse experiments and has been used by some investigators to investigate tail currents (see section 2.2.1). T type deactivation is slower than L type, but the physiological role of I$_{Ca}$ deactivation during the action potential remains uncertain.

3.1.2   Inactivation

I_{Ca} inactivation was described with the first recording, since inactivation by definition is a decrease of the current during a maintained depolarization. Both T-type and L-type Ca^{2+} current show voltage dependent inactivation. Using a double pulse protocol, it has been shown that steady state inactivation of I_{CaT} occurs at more negative potential than I_{CaL} (see for review McDonald et al., 1994). I_{CaT} voltage dependent inactivation occurs quickly, which suggested the name "Transient" proposed by Tsien and collaborators (Nowycky et al., 1985). I_{CaL} inactivation is less marked, which accounts for the role of I_{Ca} during the plateau phase of the cardiac action potential. Species differences exist in voltage dependent inactivation. Yuan et al., (1996) showed that steady state inactivation of rat I_{CaL} occurs at more negative potentials than in rabbit. This has implications for window currents, when steady state activation and inactivation curves overlap. This defines a voltage window in which some Ca^{2+} channels will conduct (at ~ -25 mV). Since this window occurs during the plateau phase of the cardiac action potential it has been proposed as a mechanism for arrhythmias (January and Riddle, 1989; Ming et al., 1994). A rough sequence of I_{CaL} window currents from larger to smaller is: guinea pig > rat > rabbit (Josephson et al., 1984; Yuan et al., 1996).

An "ultra slow" inactivation has also been described for I_{CaL} because it occurs on a second time scale (Boyett et al., 1994; McMorn et al., 1996). The physiological role of this inactivation remains unclear.

3.1.3   Facilitation

Facilitation of a current is defined by an increase in the amplitude of the current. In cardiac myocytes, I_{CaL} facilitation can occur when increasing simulation frequency or when first depolarizing the cell to a high positive potential. Rate dependent facilitation is linked to Ca and will therefore be discussed in the next paragraph. The voltage dependent facilitation described in cardiac myocytes (Pietrobon and Hess, 1990; Sculptoreanu et al., 1993a; Xiao et al., 1994a; Hirano et al., 1999; Kamp et al., 2000) is similar to that described in chromaffin cells (Artalejo et al., 1990; Artalejo et al., 1992) and skeletal myocytes (Sculptoreanu et al., 1993b). This facilitation is observed when Ba^{2+} is used as the charge carrier (contrary to the frequency dependent facilitation, below). It appears to be mediated by cAMP dependent protein kinase and at the single channel level produces mode 2 gating behavior (long opening, Hirano et al., 1999) as does modulation by the cAMP/PKA pathway (see section 3.3.1). However, conflicting results exist: Dai et al (1999) reported that this facilitation is not affected by cAMP dependent phosphorylation. They also reported that the β_{2a} subunit supports this facilitation, whereas the α_2-δ subunit prevents it (Dai et al., 1999). The importance of the β

subunit in voltage-dependent facilitation has been confirmed by Kamp et al., (2000). However, this facilitation occurs only when β_1, β_3, β_4, but not β_2 are co expressed with α_{1C} (Cens et al., 1998). A similar voltage-dependent facilitation has been observed for I_{CaT} in frog atrial cells (Alvarez et al., 1996). A G protein mechanism has been proposed to explain such facilitation in neuronal Ca channels (see for review Dolphin 2003). However another study suggests that voltage directly affects channel activity without the involvement of second messenger signals (Publicover et al., 1995).

Clearly, the mechanism(s) behind voltage-dependent facilitation of both L and T type Ca^{2+} currents are not fully elucidated, and need further investigation. Furthermore, the unphysiological test pulses (to +100 mV) required to elicit such facilitation render a physiological role unlikely.

Recently, I_{Ca} facilitation induced by a moderate depolarization of the diastolic membrane potential (in the range of −80 to − 40 mV) has also been described in guinea pig, rat, and human myocytes (Barrere-Lemaire et al., 2000; Brette et al., 2003a). Modest depolarization induces an increase in I_{Ca} amplitude and a slowing of I_{Ca} inactivation, which was blocked by ryanodine, suggesting a role for sarcoplasmic reticulum Ca release (Barrere-Lemaire et al., 2000; Brette et al., 2003a). The mechanism and physiological role of this facilitation is not elucidated yet, although it has been suggested that it can regulate cardiac automaticity in sinoatrial node cells (Mangoni et al., 2000).

3.2 Regulation by Calcium

Ca^{2+}-dependent inactivation of Ca^{2+} channels was first described in *Paramecium* (Brehm and Eckert, 1978) and shortly after in cardiac cells (Mentrard et al., 1984). Subsequent work showed that cardiac L type Ca^{2+} channels exhibit both voltage and Ca dependent inactivation (Kass and Sanguinetti, 1984; Lee et al., 1985; Hadley and Hume, 1987). Although an increase in intracellular Ca^{2+} can decrease I_{Ca} amplitude, a second Ca^{2+}-dependent process has been shown to potentiate I_{Ca} in the heart: ouabain can stimulate I_{Ca} in ferret ventricular muscle (Marban and Tsien, 1982), and this effect is linked to an elevation of intracellular Ca^{2+}. Subsequently, direct demonstration of Ca^{2+}-dependent facilitation of L type Ca channels has come from studies using flash photolysis of caged Ca^{2+} (Gurney et al., 1989; Hadley and Lederer, 1991a). Molecular biology has provided evidence that the same molecular determinants are involved in this facilitation and Ca^{2+}-dependent inactivation (see below).

In contrast, T type Ca channels do not show modulation by Ca^{2+} (see Perez-Reyes 2003 and section 4.2). The following discussion will focus on I_{CaL}.

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3.2.1 Inactivation

Several studies have shown that Ca^{2+}-dependent inactivation of I$_{CaL}$ is mediated by Ca^{2+} binding to calmodulin, which is tethered to the L type Ca channel (see 4.1.1 for details). A recent study showed that adenoviral expression of mutant calmodulin, deficient in Ca^{2+} binding, in cultured rat myocytes results in slowing of inactivation (by ~2x) of I$_{CaL}$ (Yang et al., 2003). Ca^{2+} entering the cell via the channel can inactivate the channel, as demonstrated in frog myocytes, which have sparse sarcoplasmic reticulum (Argibay et al., 1988). However in mammalian heart cells the sarcoplasmic reticulum is well developed and releases a considerable amount of Ca^{2+} (see section 2.2.1). An early study suggested that sarcoplasmic reticulum Ca^{2+} release modulates I$_{CaL}$ in rat ventricular myocytes (Cohen and Lederer, 1988). The participation of sarcoplasmic reticulum Ca^{2+} release in Ca^{2+}-dependent inactivation of I$_{CaL}$ was demonstrated by two groups in 1995. Monitoring I$_{CaL}$ and intracellular Ca^{2+}, Sipido et al., (1995) showed that global sarcoplasmic reticulum Ca^{2+} release inactivates I$_{CaL}$ in a reversible fashion in guinea pig ventricular myocytes. Using very long voltage pulses (2.4 sec), I$_{CaL}$ inactivated (due to Ca^{2+}-dependent inactivation) and then recovered by the end of the pulse. Although the sarcoplasmic reticulum was extensively loaded with Ca^{2+} to record such reversible inactivation, it was the first demonstration that sarcoplasmic reticulum Ca^{2+} release modulates I$_{CaL}$ (Sipido et al., 1995). These authors also suggested the existence of Ca^{2+} gradients in the microdomain near the Ca^{2+} channel since early inhibition of I$_{CaL}$ exceeds intracellular Ca^{2+} measured using Fura-2. This was confirmed by Morad's laboratory which provided striking evidence for discrete Ca^{2+} dependent inactivation of L type Ca^{2+} channel independent of bulk cytosolic Ca^{2+} concentration (Sham et al., 1995). They proposed the term "functional coupling" between the L-type Ca^{2+} channel and RyR and suggested that Ca^{2+} dependent inactivation of I$_{CaL}$ is due to local Ca^{2+} signaling rather than global Ca^{2+} signaling. This functional coupling was confirmed by structural data showing co-localization of the L-type Ca^{2+} channel and RyR (see section 2.2.1). The "fuzzy space" between the L-type Ca^{2+} channel and RyR form a restricted ionic diffusion space. Sham et al (1995) showed that ryanodine significantly slowed I$_{CaL}$ inactivation and that this occured even in the presence of 10 mM EGTA in the pipette solution. Sham (1997) extended this study showing that I$_{CaL}$ decay was not affected by the presence of 10 mM EGTA, despite abolition of the Ca^{2+} transient and cell contraction. However, 10 mM BAPTA significantly slowed I$_{CaL}$ decay (Sham, 1997). This is consistent with the existence of local Ca^{2+} signaling, occurring in a restricted space and exceeding global Ca^{2+}. The inability of intracellular EGTA to prevent sarcoplasmic reticulum Ca^{2+} release in the fuzzy space is not surprising. Naraghi and Neher (1997) calculated that the length constant of 2 mM EGTA to chelate Ca^{2+} from the mouth of a

Ca^{2+} channel is 419 nm. This is clearly above the ~12 nm separating the Ca^{2+} channel and RyR at the dyadic junction (see section 2.2.1). Interestingly, the length constant of 2 mM BAPTA is 28 nm (Naraghi and Neher, 1997). Such a difference is explained by the on rate of Ca^{2+} binding (k_{on} (mol^{-1} sec^{-1}) EGTA < BAPTA by two order of magnitude). Hence, EGTA is not fast enough to chelate Ca^{2+} released from the sarcoplasmic reticulum (even at 10 mM) whereas BAPTA is. This observation was subsequently confirmed by several laboratories showing that sarcoplasmic reticulum Ca^{2+} release occurs and modulates I_{CaL} in the presence of EGTA (Masaki et al., 1997; Delgado et al., 1999; Barrere-Lemaire et al., 2000; Wu et al., 2001; Guo and Duff, 2003; Brette et al., 2003a; Brette et al., 2003b) whereas BAPTA prevents it (Sham et al., 1998; Brette et al., 2003a). The slow binding properties of EGTA have been used in conjunction with a fast, low-affinity indicator, (Oregon Green 488 BAPTA-5N) to directly measure local sarcoplasmic reticulum Ca^{2+} release flux named "Ca^{2+} spikes" (Song et al., 1998). These Ca^{2+} spikes peaked in ~15 ms at 0 mV and decayed in ~50 ms. Interestingly, the difference in I_{Ca} in the absence and presence of ryanodine showed similar kinetics (Brette et al., 2003a), emphasizing that I_{Ca} is an excellent sensor of local Ca^{2+} in the cell. When sarcoplasmic reticulum Ca^{2+} release is enhanced (*e.g.* in the presence of PKA stimulation), I_{Ca} decay can show a notch during this inactivation phase (Brette et al., 2003a; Brette et al., 2003b), indicating a recovery of Ca^{2+}-dependent inactivation, as described by Sipido et al., (1995), but on a more physiological time scales (tens of msec). This also suggests that Ca^{2+}-dependent inactivation is not an absorptive state (Brette et al., 2003b; Findlay, 2004).

Local Ca^{2+} modulation of I_{CaL} has also been demonstrated in atrial myocytes in which the fuzzy space is restricted to the surface membrane (see section 2.2.1) (Sun et al., 1997; Hatem et al., 1997). In ventricular myocytes, dyadic junctions are found both at the t-tubule and the cell surface and a recent report showed that modulation of I_{CaL} by sarcoplasmic reticulum Ca^{2+} release is more pronounced at the t-tubules (Brette et al., 2004b).

Ca^{2+} dependent inactivation provides an important negative feedback to limit the amount of Ca^{2+} entry via I_{CaL}, limiting the depolarizing current and further Ca^{2+} release from the sarcoplasmic reticulum. Using action potential waveform voltage clamp to record I_{Ca}, it has been shown that Ca^{2+} dependent inactivation resulting from sarcoplasmic reticulum Ca release reduced the influx of Ca during an action potential by 30% in guinea pig ventricular myocytes (Grantham and Cannell, 1996). Of course, this effect is species-dependent, however similar results have been found in rabbit (50%, Puglisi et al., 1999) and rat (~ 40%, Takamatsu et al., 2003) ventricular myocytes. Thus if sarcoplasmic reticulum Ca^{2+} is reduced, the increase in I_{Ca} will help reload the sarcoplasmic reticulum Ca^{2+} store (as described in section 2.2.3 and reviewed in Eisner et al., 2000). Beyond considerations of EC coupling, Ca^{2+}-dependent

inactivation is important in controlling cardiac excitation. Early studies showed that BAPTA, but not EGTA, inside a patch pipette, induced a significant prolongation of action potential duration, probably due to reduced Ca^{2+}-dependent inactivation which suggested a negative feed back role played by this phenomena (White and Terrar, 1992; Le Guennec and Noble, 1994). More recently, elimination of Ca^{2+}-dependent inactivation of native Ca channels by adenoviral transfection of guinea-pig ventricular myocytes with engineered calmodulins, rendered Ca^{2+} insensitive by mutation, resulted in a 4-5 fold increases in action potential duration (Alseikhan et al., 2002) consistent with this process regulating action potential duration.

3.2.2 *Facilitation*

At the same time that direct Ca^{2+}-dependent facilitation was being characterized (above) an intriguing phenomena was observed: an increase in stimulation frequency could increase I_{CaL} amplitude and slow inactivation. This was described first in frog atrium (Noble and Shimoni, 1981), and then in rat (Richard et al., 1990; Richard et al., 1993), guinea-pig (Mitra and Morad, 1986; Lee, 1987; Fedida et al., 1988a; Fedida et al., 1988b; Zygmunt and Maylie, 1990), rabbit (Hryshko and Bers, 1990), ferret (Yuan and Bers, 1994) dog (Tseng, 1988) and human (Piot et al., 1996) cardiomyocytes, although a pronounced decrease of I_{Ca} has been observed in mouse (Sipido et al., 1998b) in which the decrease might reflect insufficient time to recover from voltage dependent inactivation.

Depolarization from near physiological potential (~ -80 mV) to near the peak of the I_{Ca}-V curve (~0 mV) produces a gradual increase and slowing of decay of I_{Ca} (Lee, 1987), whereas when the same protocol is applied from –40 mV a progressive decline is observed (Tseng, 1988; Hryshko and Bers, 1990). Tseng (1988) characterized a holding potential threshold (-60 mV) above which increasing the frequency of stimulation leads to a decrease of I_{Ca} amplitude. Ca^{2+} influx through the Ca^{2+} channel is the basis for this facilitation since facilitation is eliminated when Ba, Sr or Na are used as the charge carrier (Lee, 1987; Fedida et al., 1988a; Zygmunt and Maylie, 1990; Tiaho et al., 1994; Xiao et al., 1994a; Dzhura et al., 2000).

It is striking that Ca^{2+} underlies frequency-dependent facilitation of I_{Ca}, as well as causing inactivation (above). However, the mechanism underlying this facilitation is still controversial although three hypotheses have been proposed. The first hypothesis is that this facilitation is due to calmodulin tethered to the Ca^{2+} channel at the IQ motif (calmodulin binding region, see section 4 for more details). Point mutations within the IQ motif results in marked effects on Ca-dependent facilitation. (Zuhlke et al., 1999; Zuhlke et al., 2000). The second hypothesis is phosphorylation of the L-type Ca channel by CaMKII (Yuan and Bers, 1994; Anderson et al.,

1994; Xiao et al., 1994a). Evidence supporting this mechanism includes the observation that CaMKII inhibitors such as KN-62 (Yuan and Bers, 1994) inhibit facilitation. Facilitation is also eliminated by CaMKII inhibitory peptides including CamKII290-309, CaMKII273-302 (Yuan and Bers, 1994) and ICK (Xiao et al., 1994a). Interestingly, a CaMKII inhibitory peptide (AC3-I) nearly abolished I_{Ca} facilitation but the facilitation was rescued by a peptide mimetic IQ domain, reinforcing the importance of IQ motif in Ca^{2+} channel modulation (Wu et al., 2001). At the single channel level, constitutively active CaMKII induced longer channel opening of the L type Ca^{2+} channel (Dzhura et al., 2000). The third hypothesis is that facilitation is related to reduced Ca^{2+} dependent inactivation due to sarcoplasmic reticulum Ca^{2+} release at high rates. This suggestion is based on the observation that sarcoplasmic reticulum Ca^{2+} release inhibitors blunt facilitation, highlighting the fact that facilitation and inactivation Ca^{2+} dependent share common properties. Facilitation occurs when EGTA is used as an intracellular Ca chelator but reduced by BAPTA (Tseng, 1988; Tiaho et al., 1994; Xiao et al., 1994a; Bates and Gurney, 1999) and sarcoplasmic reticulum Ca^{2+} release inhibitors (Ryanodine, thapsigargin, caffeine) (Tseng, 1988; Delgado et al., 1999; Wu et al., 2001; Guo and Duff, 2003). These observations emphasize the role of local Ca^{2+} signaling in Ca^{2+}-dependent modulation of I_{CaL}, but do not provide a clue which of these mechanisms, which are not mutually exclusive, underlies I_{CaL} facilitation.

Finally, the physiological role of this facilitation remains unclear. The increase in Ca^{2+} entry might overcome the decrease in I_{CaL} due to voltage dependent inactivation following an increase in stimulation frequency and offset Ca^{2+} dependent inactivation. In rat ventricular myocytes, it has been proposed that this facilitation may participate in the increase of action potential duration following an increase in pacing rate (Fauconnier et al., 2003).

3.3 Regulation by cyclic nucleotides

The most common cyclic nucleotides are cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Although there is a consensus on the modulation of I_{Ca} by cAMP (via cAMP-dependent protein kinase A (PKA),) cGMP modulation of I_{CaL} is more contraversial.

3.3.1 *cAMP*

It is well established that in cardiac myocytes, an elevation of cAMP activates cAMP protein kinase dependent (PKA), thus phosphorylating the L type Ca^{2+} channel (Bers 2001 and see also section 4.1). In contrast, the cAMP/PKA signal pathway has no effect on T-type Ca^{2+} currents recorded from canine atrial cells (Bean, 1985), rabbit sinoatrial node cells (Hagiwara

et al., 1988), canine Purkinje cells (Hirano et al., 1989; Tseng and Boyden, 1989) or guinea pig ventricular myocytes (Tytgat et al., 1988).

In the heart, the major pathway to increase cAMP is catecholamine binding to a seven span transmembrane (β-adrenergic) receptor, which couples the stimulatory guanosine triphosphate (GTP) regulatory protein (Gs), stimulating adenylyl cyclase (AC) to produce (cAMP) (Bers, 2001). Other hormones can use the same pathway: for example, histamine, acting on the H2 receptor, can increase I_{CaL} via the cAMP/PKA signal pathway (Hescheler et al., 1987), and serotonin, acting on the 5-HT$_4$ receptor, which is present in human atrial myocytes (Ouadid et al., 1992) (see also section 5.1.2).

PKA phosphorylation of the L-type Ca^{2+} channel causes a dramatic increase in global I_{CaL} (~2-7 fold) and shifts activation and inactivation to more negative potentials (~10 mV) (Tsien et al., 1986; Hartzell et al., 1991). At the single channel level, PKA phosphorylation increases open probability (by switching to gating mode 2) and increases the number of channels available, although the unitary current remains the same (Yue et al., 1990). There is extensive evidence that the β-adrenergic/cAMP/PKA pathway upregulates I_{CaL} via phosphorylation of the channel; this is reviewed elsewhere (Tsien et al., 1986; McDonald et al., 1994).

Although an increase of intracellular cAMP leads to activation of PKA, evidence is accumulating that compartmentalization of cAMP is essential for intracellular signaling. Early work showed that PKA activation by prostaglandin E and isoprenaline exerted differential effects on glycogen phosphorylase phosphorylation in rat heart (Keely, 1979). Similarly, cAMP accumulation stimulated via the glucagon-like peptide receptor was recently found to be completely uncoupled from inotropic effects in cardiomyocytes (Vila Petroff et al., 2001), whereas glucagon can increase I_{CaL} via the cAMP/PKA cascade in cardiac myocytes (Mery et al., 1990). The first direct evidence that cAMP signaling is spatially regulated in cardiac myocytes was obtained using the double barrelled microperfusion system described in section 2 on frog myocytes. Localized β-adrenergic receptor activation by isoproterenol caused only local activation of I_{Ca}, suggesting localized elevation of intracellular cAMP (Jurevicius and Fischmeister, 1996). However, when AC was directly activated with forskolin, distant L-type Ca channels were also activated by PKA phosphorylation. Similar results were obtained if isoproterenol and IBMX (IsoButyMethylXanthine, a phosphodiesterase (PDE) inhibitor) were applied simultaneously. Hence the authors concluded that PDE activity around the L-type Ca channels restricted cAMP diffusion. In a subsequent study, they characterized the PDE isoforms involved by using specific inhibitors. They concluded that PDE3 and PDE4 contribute equally to the functional coupling of β-adrenergic receptors to nearby Ca channels via local elevations of cAMP. Such local elevations of cAMP near the cell membrane and

restriction by PDE has also been demonstrated in HEK cells expressing cyclic nucleotide-gated channels to monitor cAMP concentration (Rich et al., 2001). However cAMP compartmentalization in mammalian cardiac myocytes has only recently been demonstrated directly, using FRET. Imaging of cAMP in neonatal rat cardiomyocytes showed that the noradrenaline-induced cAMP signal diffused only ~1 μm, but phosphodiesterase inhibition led to generalized cAMP elevation (Zaccolo and Pozzan, 2002). Thus, in mammalian myocytes cAMP degradation by phosphodiesterase may also spatially limit cAMP signals.

Other mechanisms have also been proposed to explain L-type Ca channel modulation by spatially compartmentalized cAMP signaling in mammalian cardiac myocytes. In mammalian heart, three different β-adrenoceptor subtypes have been cloned and identified pharmacologically to date: β_1, β_2, β_3. However, only two types of β-adrenergic receptors have been reported to modulate I_{CaL}; β_1 and β_2. β_1 is the major receptor in mammalian myocytes (*e.g.* ~ 70% in human see for review Brodde and Michel 1999). Both β_1- and β_2- adrenergic receptors couple to G_s to activate adenylyl cyclase, and stimulation of both receptor subtypes increases the intracellular level of cAMP, hence I_{CaL}. The activation of β_1-adrenergic receptors produces a robust increase of I_{CaL} and also leads to phosphorylation of phospholamban present on the sarcoplasmic reticulum and of troponin I (Bers, 2001). By contrast, β_2-adrenergic receptor stimulation increases I_{CaL}, but does not increase global cAMP levels or phosphorylate any non-sarcolemmal proteins. To explain this difference, Lakatta and colleagues proposed that in adult rat and dog ventricular cardiomyocytes, β_2- adrenergic receptors also couple to G_i and that this will spatially restrict the increase in cAMP (Xiao and Lakatta, 1993; Xiao et al., 1994b; Xiao et al., 1995; Kuschel et al., 1999; Xiao et al., 1999; Chen-Izu et al., 2000). Evidence that β_2-adrenergic receptor stimulation leads to local signaling and phosphorylation of I_{CaL} was obtained using the cell attached configuration of the patch clamp technique. β_2-adrenergic receptor agonists only activated I_{Ca} if it was included in the patch pipette (local signaling) whereas β_1-adrenergic receptors agonists outside the patch can phosphorylate I_{CaL} (diffusive signaling). This local signaling is achieved by the dual coupling of β_2-adrenergic receptors to Gs and Gi since treatment with pertussis toxin (PTX, disrupting Gi regulation) allowed β_2-adrenergic receptors to activate I_{CaL} via a diffusing signal (reviewed in Xiao 2001). Comparable data were recently obtained in murine ventricular cardiomyocytes in which the β_2-adrenergic receptor agonist zinterol failed to increase force of contraction or $[Ca^{2+}]_i$ transient amplitude. However, after treatment of the cardiomyocytes with PTX, zinterol, via β_2-adrenergic receptor stimulation, increased both parameters significantly (Xiao et al., 1999). Despite compelling evidences, the dual activation of Gs and Gi via β_2-adrenergic receptors

stimulation remains controversial (Kuznetsov et al., 1995; Laflamme and Becker, 1998) and whether human cardiac β_2-adrenoceptors also couple to G_i is unknown. In addition, recent studies identify compartmentalization of β_2-adrenergic receptors and AC to caveolae in neonatal and adult rat cardiomyocytes (Rybin et al., 2000). Caveolae are small (70-90 nm in diameter) invaginated foldings of the sarcolemma that are enriched in cholesterol and glycosphingolipids (see for review Razani et al., 2002). On the contrary, β_1-adrenergic receptors are more evenly distributed (Ostrom et al., 2001). Interestingly, M_2 muscarinic receptors, which can inhibit cAMP production by the Gi modulation of AC, are exluded from caveolae (Feron et al., 1997). This might contribute to the differential effect of M_2 muscarinic receptor activation on cAMP production from β_1-adrenergic receptors (decrease) and β_2-adrenergic receptors (no change) (Aprigliano et al., 1997). Collectively, these studies indicate that components of the β_2-adrenergic receptor signaling pathway lead to spatial compartmentalization of cAMP signaling by restriction of receptors to membrane microdomains and/or by dual stimulation of Gs/Gi.

The idea that the receptors might not be evenly distributed on the cell surface membrane is supported by results obtained in neurons in which it has been shown that β_2-adrenergic receptors co-assemble with the L type Ca channel, Gs, adenylyl cyclase, PKA and phosphatase 2A (Davare et al., 2001). Whether the same co-assembly exists in cardiac myocytes is currently unknown, however some pieces of information support this idea. L type Ca^{2+} channels are concentrated at the t-tubules in mammalians cardiac myocytes (see section 2.1.1) and immunohistochemical data shows that many of the proteins in the cAMP/PKA pathway are also located predominantly at the t-tubules (Gs protein, (Laflamme and Becker, 1999); AC (Gao et al., 1997a), PKA, (Santana et al., 2002)). Interestingly, the protein phosphatase calcineurin (enzyme counter-balancing the cAMP/PKA phosphorylation) is also concentrated at the t-tubules and co-localized with the L type Ca^{2+} channel (Santana et al., 2002).

Finally, PKA is spatially localized via binding to A-kinase anchoring proteins (AKAP). This might explain why most studies in mammalian expression systems have failed to reproduce the observation that I_{Ca} increases upon activation of the PKA pathway (see section 3.3.1). An early study showed that mAKAP is concentrated at the t-tubules (Yang et al., 1998), however this now appears to belong to the RyR macromolecular complex (Marks, 2001). Recently, it has been shown that AKAP15 is anchored to the cardiac L-type Ca^{2+} channel (Hulme et al., 2003). Both AKAP15 and L type Ca^{2+} channel co-localize at t-tubules (Hulme et al., 2003). In mammalian cardiac myocytes, the cAMP microdomain might be different at the cell surface and the t-tubules. Using the detubulation technique, a recent study showed that L-type Ca^{2+} channels are better coupled to the β-adrenergic pathway at the t-tubules than at the

surface membrane (Brette et al., 2004a). This indicates that β-adrenergic receptors are present on the surface membrane, however, to the best of our knowledge, no immunohistocemistry data are available concerning a possible β-adrenergic receptors sub-localization (t-tubules *vs* surface membrane) in cardiac myocytes.

In summary, modulation of I_{CaL} via cAMP appears to be highly regulated by compartmentalization, due to the existence of microdomains formed by PDE and/or receptor localization.

3.3.2   *cGMP*

cGMP is an important intracellular second messenger of various extracellular stimuli regulating L-type Ca current. cGMP is the product of guanylyl cyclase activity which is stimulated by nitric oxide (NO). In the heart, NO formation via NO synthase (NOS) can be activated via various factors (*e.g.* atrial natriuretic factor, cathecolamines acting on β3-adrenergic receptors, stretch; see for review Hare and Stamler 1999). It has also been proposed that acetylcholine acting via M2-receptors can induce NO synthesis, although this is controversial, in addition to activation of the Gi pathway (reviewed in Harvey and Belevych 2003, see also section 5.1.2). Classically, the regulation of L type Ca^{2+} channel activity in the heart is due to the activity of the NOS3 enzyme subtype (see for review Hare 2003), but a recent study using knockout mice also showed that NOS1 regulates L type Ca^{2+} channel activity (Ashley et al., 2002; Sears et al., 2003). In addition to modulation of Ca^{2+} channels by the signaling pathway downstream of cGMP (below) it has also been suggested that NO may have a direct effect (cGMP independent) via either nitrosylation of the L type Ca^{2+} channel (Campbell et al., 1996; Hu et al., 1997) or by modulating the activity of G proteins (Abi-Gerges et al., 2002).

In cardiomyocytes, most studies agree that L-type Ca^{2+} current is modulated by cGMP and it is generally believed that cGMP plays a role antagonistic to that of cAMP: the "yin-yang" hypothesis (Goldberg et al., 1975). However, both inhibition and stimulation of I_{CaL} by cGMP mediated mechanisms have been reported (see table 1). The variety of results may be explained by: (i) the balance between synthesis and degradation of cGMP and cAMP; (ii) modulation of I_{CaL} via PKG dependent phosphorylation or cross-talk with cAMP signaling via cGMP-sensitive PDE (PDE2 and PDE3); (iii) whether cGMP is able to modulate the three main target enzymes, namely PKG, PDE2 and PDE3 in a given species. The effects of each of these enzymes is discussed below and illustrated in figure 5.

PKG dependent modulation of I_{CaL}

The activation of cGMP dependent protein kinase (PKG) is a function of intracellular concentration of cGMP. Direct intracellular dialysis of PKG from a patch pipette decreases $I_{Ca,L}$ via phosphorylation of the L-type Ca^{2+} channel by PKG or an associated regulatory protein (Mery et al., 1991; Sumii and Sperelakis, 1995; Haddad et al., 1995) (see also table 1). The modulation of $I_{Ca,L}$ by PKG has been associated with phosphorylation at serine 533 of the α_{1C} subunit in *Xenopus* oocytes since replacement of serine by alanine at a consensus site (S533A) for PKG-dependent phosphorylation prevents inhibition by the PKG activator 8-Br-cGMP (Jiang et al., 2000). Phosphorylation at serine 533 of the α_{1C} subunit seems to be a sufficient condition since inhibition still occurs in the absence of either β- or $\alpha_2\delta$ subunits (Jiang et al., 2000, see also 4.1.1 and see Figure 6)

However, since Ca^{2+} channel phosphorylation is a balance between kinase and phosphatase activity, an alternative mechanism has been proposed in which PKG stimulates phosphatase activity, hence decreasing I_{CaL}. This hypothesis was proposed after it was shown that inhibition of I_{CaL} induced by 8-Br-cGMP in guinea pig ventricular cells was antagonized by dialysis with okadaic acid (inhibitor of phosphatases 1 and 2A) (Sakai et al., 1999). In a subsequent study, Shen and Pappano (2002) failed to observe an 8-Br-cGMP induced inhibition of I_{CaL} prestimulated by IBMX when ATPγS was included in the pipette. Since ATPγS is a kinase but not a phosphatase substrate, they concluded that, as the effect of thiophosphorylation by PKA on I_{CaL} cannot be reversed by activation of PKG, the effect occurs by dephosphorylation of the site phosphorylated by PKA (Shen and Pappano, 2002). However, using a similar approach in rat ventricular myocytes, Méry et al., (1991) observed an irreversible effect of cGMP in cells perfused with ATPγS and suggested that PKG phosphorylation was directed to the Ca^{2+} channel or an auxiliary protein than to phosphatase.

There is a third hypothesis to explain the effects of cGMP on I_{CaL}. The NO donor DEANO is able to antagonize the stimulation of I_{CaL} induced by low isoproterenol concentration, via PKG activation (Abi-Gerges et al., 2001). While these results can be interpreted as a PKG-dependent phosphorylation of the channel (above), surprisingly, the inhibitory effect of DEANO on I_{CaL} was prevented by PTX, which inactivates G_i/G_o proteins (Abi-Gerges et al., 2001). This suggests a PKG mediated phosphorylation of Gi protein, as reported in expression system (Pfeifer et al., 1995).

Although PKG activation normally inhibits I_{Ca}, a PKG mediated increase in basal I_{Ca} caused by NO donors and 8-Br-cGMP has been described in rabbit newborn and juvenile ventricular and adult atrial myocytes but not in rabbit adult ventricular cells (Kumar et al., 1997; Han et al., 1998; Wang et al., 2000). This PKG mediated increase of I_{CaL} has been correlated with higher PKG expression, which could be responsible for differences in the

significance of cGMP in this species (Kumar et al., 1999; Wang et al., 2000). To answer this (and other) question, an alternative approach was used to investigate the specific effect of PKG. In transgenic mice in which PKG I is overexpressed in myocardium (Schroder et al., 2003), basal Ca channel activity is decreased (table 1) by both the nitric oxide donor DEANO and 8-Br-cGMP in myocytes from transgenic mice but not from wild type mice. This supports the concept that the level of PKG expression is important for basal I_{CaL} modulation and further demonstrates the inhibitory effect on Ca^{2+} channel activity induced by PKG (Schroder et al., 2003).

cGMP sensitive PDEs modulation of I_{CaL}

I_{CaL} is stimulated by cAMP-dependent protein kinase (see above) and the cAMP level is regulated in part by the rate of degradation by PDEs. At least four PDE subtypes are present in cardiomyocytes (for review Maurice et al.,, 2003). Among them, PDE2 and PDE3 are stimulated and inhibited respectively by cGMP. This dual regulation of PDE by cGMP may lead to the different cGMP-dependent responses of I_{Ca} observed in different species (Ono and Trautwein, 1991; Mery et al., 1993; Kirstein et al., 1995; Shirayama and Pappano, 1996; Vandecasteele et al., 1998) (see also table 1). For example, in human atrial cells, nanomolar concentrations of NO donors (SIN-1 and SNAP) stimulate basal I_{CaL} (Kirstein et al., 1995; Vandecasteele et al., 1998), whereas at micromolar concentrations, the stimulatory effect of SIN-1 on I_{Ca} was strongly attenuated (Kirstein et al., 1995). Similar effects have been found with direct intracellular application of low and high cGMP concentrations (Rivet-Bastide et al., 1997; Vandecasteele et al., 2001). These results suggest the development of a secondary inhibitory effect at higher concentrations of NO donors and cGMP and thus with a lower sensitivity to cGMP. The stimulatory effect of low cGMP concentration by activation of PKA results from inhibition of PDE3 (Kirstein et al., 1995; Vandecasteele et al., 2001). The inhibition at higher cGMP concentration occurs via stimulation of PDE2, thus decreasing PKA activity (Vandecasteele et al., 2001). The interplay between mechanisms is species dependent: in frog and human, PDE2 and 3 activation/inhibition is the major mechanism for cGMP modulation of I_{CaL} (Mery et al., 1993; Vandecasteele et al., 2001). However in guinea-pig, PDE3 (but not PDE2) and PKG appear important. This explains the biphasic response of I_{CaL} to cGMP, first an increase due to an inhibition of PDE3, then a decrease due to PKG phosphorylation (Ono and Trautwein, 1991; Shirayama and Pappano, 1996).

In summary, cGMP appears to produce different effects upon I_{CaL} depending on: (i) the presence and relative activities of PKG, PDE2, PDE3 in a given species; (ii) whether adenylyl and/or guanylyl cyclases are constitutively active; (iii) experimental conditions (*i.e.* the level to which the NO/cGMP/PKG cascade is stimulated, the nature and concentration of the agent

used to elicit a response, and whether the effect of cGMP on I_{Ca} is examined under basal conditions or after prestimulation by the cAMP cascade). This modulation can vary with development, and under pathophysiological conditions.

Compared witth cAMP, little is known about compartmentalization of cGMP signaling. NOS3 localizes to caveolae (Feron et al., 1998), allowing spatial confinement of NO effects (Barouch et al., 2002). It is also tempting to speculate that if PDEs are localized with Ca^{2+} channels, cGMP might selectively activate PDE2 and thereby decrease cAMP modulation of I_{CaL}. Some evidence to support this hypothesis has been reported, such as significant local depletion of cAMP near the L-type Ca^{2+} channels due to activation of PDE2 by stimulation of guanylyl cyclase in frog ventricular myocytes (Dittrich et al., 2001).

4 BIOPHYSICAL STRUCTURE-FUNCTION OF CARDIAC Ca^{2+} CHANNELS

Pharmacological tools have led to the classification of L-type Ca^{2+} channels, which are highly sensitive to DHPs, expressed in different tissues and heart in particular (Nowycky et al., 1985). Their high affinity for DHP agonists and antagonists made possible their biochemical characterization first in skeletal muscle (Tanabe et al., 1987) and subsequently in cardiac cells (Mikami et al., 1989). Identification of the α_1 subunit which forms the pore of the DHP receptor and confers its sensitivity to voltage and pharmacological components on this Ca^{2+} channel was then possible (see for review Catterall 2000). Development of molecular biology over the last decades helped to clone these subunits and to start to elucidate how Ca^{2+} channels work and are regulated at a molecular level. Ten different α_1 subunits for Ca^{2+} channels have been cloned with specialized biophysical and pharmacological properties and different patterns of expression. A new nomenclature, proposed in 2000, divided the Ca^{2+} channels into three sub-families (Ertel et al., 2000). The first subfamily of high voltage activated channels (HVA), Ca$_V$1, consists of the DHP sensitive L-type Ca^{2+} channels. The second one, of HVA, DHP insensitive, Ca$_V$2 channels mainly expressed in brain and the last one, Ca$_V$3, corresponds to the T-type channels (low voltage activated channel, LVA).

HVA Ca^{2+} channels are multimers of proteins and can be composed of five subunits unlike LVA where the α_1 subunit would be enough to form a channel. HVA Ca^{2+} channels contain a principal transmembrane subunit α_1 of about 190 kDa which forms the pore of the channels and auxiliary subunits such as an intracellular β of 50-70 kDa depending on β subtype, a disulfide-linked $\alpha_2-\delta$ subunit complex of 170 kDa (Catterall 2000; Peres-Reyes 2003). Although, γ_1, a protein of 33 kDa is well known to co-purify with the L-type Ca^{2+} channels of skeletal muscle (Takahashi et al., 1987), it is still controversial whether recently cloned γ-like subunits (γ_{2-8}) are real auxiliary subunits for HVA Ca^{2+} channels (Black, III, 2003; Qiao and Meng, 2003).

4.1 Molecular Structure of the predominant L-type Ca^{2+} channel

4.1.1 Structure-function of α_{1C}

Using homology with the skeletal muscle Ca^{2+} channel, the cardiac L type Ca^{2+} channel has been identified and cloned (Mikami et al., 1989). It is encoded by *CACNA1C* (Soldatov, 1994) and has been mapped to the distal region of chromosome 12p13 (Schultz et al., 1993). α_{1C}, like other α_1 subunits of HVA and LVA Ca^{2+} channels, is a protein of about 190 kDa with

24 putative transmembrane segments according to its hydropathy profile. It is organized into four repeated domains (I to IV) of six transmembrane segments (S1 to S6) with intracellular N- and C- termini (Tanabe et al., 1987; Catterall, 2000) (see Figure 6). L type Ca^{2+} channels have complex permeation properties and exhibit various selectivities for divalent and monovalent cations (see Tsien et al., 1987 for review). Indeed, Ca^{2+} is both an effective permeator and a potent blocker of L type Ca^{2+} channel. A model for the mechanism of Ca^{2+} channel permeation has been proposed by Hess and Tsien (1984) and Almers and McCleskey (1984) which proposed that Ca^{2+} ions pass though two Ca^{2+} selective sites in single file pore. Divalent cations with low binding affinity can move quickly through the channel. Those with higher affinity will also move quickly but can stick in then interfere with the movement of less affinity divalent cations (anomalous mole fraction effect, Hess et al., 1986), and also block the permeation pathway for monovalent cations (Lansman et al., 1986). Analysis of the dependence of single channel conductance on divalent cations concentration has led Yue and Marban (1990) to propose that the pore contains at least three binding sites. Key determinant in the molecular site of the selectivity filter of these channels has been demonstrated later; the selectivity filter is formed by parts of the four S5-S6 linker segments (P loop). Four conserved glutamate residues in the putative pore-lining regions of repeats I-IV, form the selectivity filter that binds Ca^{2+} in the pore of α_{1C} (Tang et al., 1993; Yang et al., 1993; Cibulsky and Sather, 2000; Cibulsky and Sather, 2003). Recently, X-ray crystallographic analysis of the pore-forming portions of the bacterial K^{+} channels (Doyle et al., 1998; Jiang et al., 2002a; Jiang et al., 2003a) allowed a model of the Ca^{2+} channel outer vestibule formed by the P loops to be built (Lipkind and Fozzard, 2001) and provided evidences for the gating of voltage gated channels (Bezanilla, 2000; Jiang et al., 2002b; Jiang et al., 2003b). Hodgkin and Huxley, in 1952, hypothesized that a large flow of ions would be the result of displacement of a few charged particles whose distribution is modified by the membrane potential (Hodgkin and Huxley, 1952a). Development of electrophysiological techniques and cloning of voltage gated channels confirmed this hypothesis. The fourth transmembrane segment (S4) of voltage gated channels contains between four and eight basic residues (arginines or lysines) (see Figure 6). The S4 segments containing these positive charges were described as possibly involved in the gating structure of voltage gated channels in 1984 by Noda et al. and this has been confirmed by mutational analysis (Stuhmer et al., 1989; Papazian et al., 1991; Liman et al., 1991; Noceti et al., 1996). Because of their structural homologies, it is assumed that the gating of all voltage-gated channels, including Ca^{2+} channels, is similar (Bezanilla, 2000). When the membrane is hyperpolarized, the channel is closed and these segments lie deep in the membrane. During a depolarization, the electric field forces the positively charged S4

segments to move by a helical turn to an external position. Acidic residues in S2 and perhaps S3 seem to also be important in the change of protein conformation leading to the opening of the channel (Bezanilla, 2000; Jiang et al., 2003b). A recent study, using a mutational approach, suggests that the domains I, III and IV rather than domain II are critical for channel opening. By swapping $Ca_V1.2$ domains by similar $Ca_V3.1$ domains (LVA channel), the authors showed that these three domains contribute strongly to the voltage dependence of activation of Ca^{2+} channels but that the S4 segments did not account for their voltage dependence, suggesting roles for S1-S3 and S5-S6 segments to confer differences in voltage dependence of Ca^{2+} channels (Li et al., 2004).

The α_1 subunit of HVA Ca^{2+} channels presents, in its I-II linker, a highly conserved sequence that binds the β subunit named AID (Alpha Interacting Domain) (Dolphin, 2003a). The AID corresponds to the following consensus sequence **QQLEEDL-GY--WITQ-E** for L-type channels and is absolutely essential for the binding of the auxiliary subunit (Pragnell et al., 1994). Although, it seems possible that α_1 N- and C-termini of various Ca^{2+} channels also bind β (Dolphin, 2003a). Furthermore, it has been suggested that the I-II loop of Ca^{2+} channels contains an endoplasmic reticulum retention signal that restricts the plasma membrane incorporation of α_1 and that β subunits binding to the I-II loop favors its plasma membrane expression (Bichet et al., 2000). Nevertheless, this hypothesis is controversial, since it has been proposed that β subunits regulate the biophysical properties of the channel without affecting the expression of α_{1C} at the plasma membrane (Neely et al., 1993). A recent study shows that α_{1C} surface expression, evaluated by gating currents, is not affected by co-expression of β subunits, suggesting that β binding to the I-II loop of α_1 subunits only affects primarily channel activity (Neely et al., 2004).

Drug binding domains have been investigated and we have now more information about molecular pharmacology of Ca^{2+} channels (Doering and Zamponi, 2003). Various compounds bind L-type Ca^{2+} channels, among them the DHPs. Mutational studies and creation of chimerical channels allowed the S5 and S6 and pore lining region S5-S6 of domains III and IV of L-type channels to be identified as the important regions responsible for DHP binding (Ito et al., 1997; Sinnegger et al., 1997; Yamaguchi et al., 2000b; Wappl et al., 2001; Lipkind and Fozzard, 2003; Yamaguchi et al., 2003) (see Figure 6).

Phenylalkylamines (D888, verapamil, D600) also block $Ca_V1.2$ channels (as well as $Ca_V1.3$ and $Ca_V1.4$ channels). Specific binding sites have been identified on the α_{1C} IIIS6 and IVS6 regions. Mutations in these regions attenuate Ca^{2+} current block by phenylalkylamines (Hockerman et al., 1995; Johnson et al., 1996; Hockerman et al., 1997).

Benzothiazepines (*e.g.* diltiazem) are used clinically for their antihypertensive and antiarrhythmic effects. Binding to α_{1C} subunit of L-type Ca^{2+} channels involves domains III and IV (Cai et al., 1997) and more specifically the S6 segments of these domains (Kraus et al., 1996).

A depolarization from a negative to a more positive potential induces the opening of the Ca^{2+} channel. When the membrane is maintained depolarized, the channels enter a non-conductive state, an "inactivated" state. As previously described, $Ca_V1.2$ presents two types of inactivation: a voltage-dependent inactivation and a Ca^{2+}-dependent inactivation. Inactivation of HVA Ca^{2+} channel is not yet fully understood, but over the past years, some molecular determinants involved in this processes have been discovered. From studies of chimeras between the fast inactivating α_{1E} and α_{1C} and achieving point mutations in the S6 II, III, IV it was suggested that the S6 segments of each four domains of α_{1C} are crucial for voltage-dependent inactivation (Hering et al., 1996; Stotz et al., 2000; Berjukow et al., 2001; Stotz and Zamponi, 2001b) (see Figure 6). But transmembrane segments are not the only part of α_{1C} involved in voltage-dependent inactivation since intracellular regions also appear to be implicated. The I-II loop (Stotz and Zamponi, 2001b) and the C-terminal part of α_{1C} are also key determinants for voltage-dependent inactivation (Klockner et al., 1995; Soldatov et al., 1998).

Increase of intracellular Ca^{2+} concentration accelerates inactivation of most of the HVA Ca^{2+} channels (Zuhlke et al., 1999; Shirokov, 1999; Liang et al., 2003). The C-terminus of α_{1C} contains not only important determinants for voltage-dependent inactivation, but also for Ca^{2+}-dependent inactivation. It contains a putative Ca^{2+} binding site, an EF-hand region located in the proximal part of the tail, which was first described as the crucial determinant for Ca^{2+}-dependent inactivation (de Leon et al., 1995). Following this study, subsequent work has concentrated on the C-terminal part of α_{1C}. Zhou *et al.* (1997) identified a downstream region critical for Ca^{2+}-dependent inactivation which does not contain the EF-hand. The major component of the C-terminus of α_{1C} implicated in the Ca^{2+}-dependent inactivation is an IQ motif located downstream of the EF-hand, which is a calmodulin-binding site, the critical Ca^{2+} sensor for Ca^{2+}-dependent inactivation (Zuhlke and Reuter, 1998) (see Figure 6). Thereafter, it was suggested that the EF-hand region regulates the voltage-dependent inactivation (Bernatchez et al., 1998) and supports the transduction of Ca/Calmodulin binding into channel inactivation (Peterson et al., 2000). A region of more than 100 amino-acids starting from the EF-hand including the IQ motif is implicated in Ca^{2+}-dependent inactivation. Two more regions are involved: the first one named LA (Romanin et al., 2000) or peptide A (Pitt et al., 2001) can bind calmodulin in absence of Ca^{2+}. The preassociation of the calmodulin free of

Ca²⁺ (apocalmodulin) with the L type Ca²⁺ channel has been confirmed by fluorescence resonance energy transfer (FRET) (Erickson et al., 2001). Hence, apocalmodulin is tethered to the channel facilitating the rapid triggering of Ca²⁺-dependent inactivation (Erickson et al., 2003). A very recent report demonstrated that a single tethered calmodulin is both necessary and sufficient to produce Ca²⁺-dependent inactivation (Mori et al., 2004).

The second region, peptide CB (Pate et al., 2000) or peptide C (Pitt et al., 2001) at high Ca²⁺ concentration can form a complex with the calmodulin bound to the IQ motif, which would constitute a minimal switch to induce Ca²⁺-dependent inactivation (Mouton et al., 2001a).

Two models have been proposed for inactivation of Ca²⁺ channels according to these studies: (i) a constriction of the pore by the S6 segments, a C-type inactivation model (ii) and a *hinged-lid* mechanism involving intracellular gates.

Soldatov's group (Shi and Soldatov, 2002) proposed a model of a C-type/slow inactivation similar to the one proposed for K⁺ channels (Choi et al., 1991; Lopez et al., 1994) where a constriction of the pore occurs by the S6 segments lining the intracellular part of the pore. $Ca_V1.2$ channels reveals two inactivation components of the Ba²⁺ current decay. The authors showed that mutations in the S6 of the four domains I-IV impairs the slow voltage-dependent inactivation and accelerate the fast inactivation of Ba²⁺ currents, suggesting that the two mechanisms are linked. These residues at the C-terminal region of each S6 form an annular determinant which mediates the slow inactivation component of L-type Ca²⁺ channels. The mutated channel does not exhibit Ca²⁺-dependent inactivation or sensitivity to β-subunit induced modulation of inactivation. The voltage dependence of these channels remains identical to a non-mutated channel suggesting that the voltage sensors are not affected (Shi and Soldatov, 2002). This is consistent with a C-type inactivation, where a constriction of the pore then reached by the Ca²⁺ sensors of the C-terminus of $α_{1C}$ would be the mechanism (Shi and Soldatov, 2002; Soldatov, 2003).

In the second model, the conformational changes following a depolarization and channel opening would unmask docking sites and the C-terminus and the I-II loop would occlude the pore (Stotz et al., 2000). Two important facts are in favor of this hypothesis: the implication of the I-II loop of $α_{1C}$ which governs the voltage-dependent inactivation of $Ca_V1.2$ channels (Stotz and Zamponi, 2001b) and the modulation of the inactivation kinetics by β subunits bound to this intracellular loop (Dolphin, 2003a).

It has also been suggested that voltage-dependent inactivation and Ca²⁺-dependent inactivation use the same determinants and confirmed that the I-II loop and the C-terminus of $α_{1C}$ interact with the pore to induce in the inactivated state (Cens et al., 1998; Kim et al.,

2004). Furthermore, the gating currents recorded during depolarization (ON charges) are not impaired by intracellular Ca^{2+} while Ca^{2+}-dependent inactivation affects the return (OFF charges) of the voltage sensors to their initial state (Leroy et al., 2002; Isaev et al., 2004), confirming that the docking sites are located in the internal cavity of the channel or formed after rearrangement during a depolarization.

The molecular site responsible for the cardiac EC coupling in α_{1C} (CICR, see section 2.2, *vs* voltage activated coupling of α_{1S}) has been investigated using chimeric cDNA (Tanabe et al., 1990a; Tanabe et al., 1990b). Using a preparation lacking L-type Ca^{2+} channel (skeletal muscle from Murine Muscular Dysgenesis, *mdg*), a skeletal coupling can be restored when myotubes are injected with α_{1S} and a cardiac one when α_{1C} is injected. They found that replacing the cytoplasmic loop between domains II and III of the cardiac L type Ca^{2+} channel (see Figure 6) with the skeletal counterpart was sufficient to cause the EC coupling to be skeletal type (voltage-activated coupling). This suggests that contrary to the skeletal α_{1S}, the cardiac isoform does not interact directly with ryanodine receptors (RyRs) via the loop II-III.

However, a possible interaction between α_{1C} and RyRs has been suggested by a functional study showing that BayK 8644 (a DHPs agonist) increased the frequency of local Ca^{2+} release events (Ca^{2+} sparks, see section 2.2.1) in ferret ventricular myocytes (Katoh et al., 2000). These experiments were performed in the absence of external Ca^{2+}, ruling out a possible enhancement of Ca^{2+} sparks frequency due to Ca^{2+} entry. They proposed that binding of BayK 8644 to α_{1C} reveals a weak link with RyRs, which may be due to an unknown intermediate protein (Katoh et al., 2000). Interestingly, a possible interaction between the C-terminus of α_{1C} and particularly of the CB and IQ motifs, with ryanodine receptors has been described (Mouton et al., 2001b). Recently, Woo *et al.* (2003) showed a possible functional role for this peptide from the C-terminal tail of α_{1C} (LA peptide, 1571-1599, Romanin *et al.* 2000) using atrial myocytes. In these cells, Ca^{2+} spark frequency is high near the cell surface membrane and low in the cell center. Infusion of the peptide via a patch pipette increased four fold the Ca^{2+} spark frequency in the center (where RyRs are not in close contact with the L type Ca^{2+} channel, see section 2.2.1). They proposed that a possible interaction between the C-terminal tail of α_{1C} and RyRs might sensitize RyRs, thereby increasing their open probability; although further investigations are required to explore this novel finding.

$Ca_V1.2$ channels are modulated by phosphorylation by various kinases (see Figure 6). α_{1C} presents numerous putative phosphorylation sites for protein kinases in its N- and C-terminal regions and in the S4-S5 loop of domain II (Soldatov, 1994). Despite the fact that α_{1C} is a substrate for phosphorylation by PKA in vitro (De Jongh et al., 1996; Puri et al., 1997), several

attempts to mimic the adrenergic stimulation of $Ca_V1.2$ channels in expression systems have failed (Perez-Reyes et al., 1994; Singer-Lahat et al., 1994; Zong et al., 1995; Perets et al., 1996). Nevertheless, a few cases, the expected stimulation of Ca^{2+} currents after cAMP increases did occur (Yoshida et al., 1992; Yatani et al., 1995). A possible missing link has been then identified as AKAPs (A kinase–anchoring proteins), which bind to the C-terminus of α_{1C} (Gao et al., 1997b; Hulme et al., 2003) (see Figure 6). Furthermore, AKAPs bind PKA and would allow it to phosphorylate the ser1928 located in the C-terminus of α_{1C} and to enhance channel activity (De Jongh et al., 1996; Mitterdorfer et al., 1996; Perets et al., 1996; Gao et al., 1997a; Hulme et al., 2003).

Moreover, cardiac L-type channels can be modulated by other kinases and by PKC and PKG in particular. In heterologous systems, PKC has been reported to enhance currents induced by expression of $Ca_V1.2$ channels (Singer-Lahat et al., 1992; Bourinet et al., 1992; Shistik et al., 1998) but there are exceptions (McHugh et al., 2000). Nevertheless, the N-terminus of the α_{1C} subunit seems crucial for the modulation of $Ca_V1.2$ by this kinase (Shistik et al., 1998; McHugh et al., 2000).

A direct phosphorylation of α_{1C} by PKG has been demonstrated from a functional study performed in oocytes. In this study, activation of PKG by 8-Br-cGMP inhibits the Ca^{2+} currents and this effect does not depend on co-expression of β subunits. The authors showed that this effect is prevented by the PKG inhibitor KT5823 and they identified the serine 533 in the I-II intracellular linker as the phosphorylated amino acid (Jiang et al., 2000).

Alternative pre-RNA splicing generates functional and structural diversity of α_1 subunits of Ca^{2+} channels (Jurkat-Rott and Lehmann-Horn, 2004). To obtain an mRNA from the pre-RNA that can be translated into a protein, the non-coding introns and alternative exons must be removed. Different isoforms of α_{1C} subunits following this process have been identified. These display dissimilar biophysical and pharmacological properties. The IVS3 and the S3-S4 loop of domain IV of α_{1C} are subject to alternative splicing, resulting in different subunits whose expression is tissues dependent or regulated during development (Perez-Reyes et al., 1990; Diebold et al., 1992; Feron et al., 1994; Abernethy and Soldatov, 2002). The C-terminus and the S6 segment of domain I are also subject to alternative splicing affecting current density and inactivation (Soldatov et al., 1997; Klockner et al., 1997; Goodwin et al., 1999). Sensitivity to DHPs is also modified by alternative splicing of these regions (Welling et al., 1997) and some modifications occur in IIIS2 (Soldatov et al., 1995) and IVS3 (Safa et al., 2001). Understanding of the function and regulation of splicing isoforms would present opportunities for the design of more selective therapeutic agents in the future (Jurkat-Rott and Lehmann-Horn, 2004).

4.1.2   Structure-function of β subunit

Four intracellular β (β$_1$–β$_4$) subunits of about 50-70 kDa have been identified and diversity is extended by alternative splicing. The first one, β$_{1a}$ has been co-purified with the DHPs receptor in skeletal muscle (Tanabe et al., 1987) and then cloned (Ruth et al., 1989). Three more genes encoding for β$_2$, β$_3$, β$_4$ have been cloned (Perez-Reyes et al., 1992; Hullin et al., 1992; Castellano et al., 1993a; Castellano et al., 1993b), located in different chromosomes in humans: 17q21 for β$_1$ (Gregg et al., 1993), 10p12 for β$_2$ (Taviaux et al., 1997), 12q13 for β$_3$ (Collin et al., 1993) and 2q22 for β$_4$ (Taviaux et al., 1997). In human myocardium, the expression of three genes, β$_1$, β$_2$, β$_3$ has been demonstrated at the mRNA and protein level (Collin et al., 1993; Haase et al., 1996; Freise et al., 1999). Moreover, a more recent study shows that different splice variants for β$_2$ (β$_{2a}$-β$_{2e}$) and β$_3$ (full length and a truncated β$_3$) are also expressed in human heart cells (Takahashi et al., 2003; Hullin et al., 2003).

Co-expression of β subunits with α$_1$ subunits in heterologous expression systems, affects the HVA targeting to the plasma membrane as well as HVA biophysical properties (Walker and De Waard, 1998; Herlitze et al., 2003; Dolphin, 2003a). A model for the structure of β that describes the auxiliary subunits as composed of five domains (D$_1$-D$_5$) has been proposed (Dolphin, 2003a). D$_2$ and D$_4$ are highly conserved between the four β subunits but the three other domains are subject to alternative splicing. In many aspects, β subunits contain homologies with members of the MAGUK, a characteristic that has been confirmed by recent crystallographic analysis of the core domains of β$_2$ (Van Petegem et al., 2004; Opatowsky et al., 2004). D$_2$ is a Src Homology-3 (SH3) (Hanlon et al., 1999) frequently found to bind to proline-rich motifs(McPherson, 1999). This domain is followed by a linker corresponding to D$_3$. D$_4$ has been identified as a Guanylate Kinase (GK) domain (Hanlon et al., 1999) which was said to contain a very important molecular determinant for the binding of the β subunit to the I-II loop of α$_1$ subunits of HVA (Pragnell et al., 1994; De Waard and Campbell, 1995). This Beta Interaction Domain (BID) is composed of 41 amino acids highly conserved in the four β subunits. The consensus sequence for the BID is :

K - - E - - - - PYDVVPSMRP - - LVGPSLKGYEVTDMMQKALFDF (De Waard et al., 1994).

However, according to a recent crystallographic analysis of the core domains of β$_2$, this region is largely buried and therefore, is unavailable for protein-protein interactions (Van Petegem et al., 2004). Previous mutations in the BID might have disrupted the folded structure and abolished AID interaction.

The N-terminal part of β_{1b} also presents low homology with a PDZ (Post-Synaptic Density protein 95 (PSD-95) , Discs large protein (*dlg*) and Zona Occludens-1 (ZO-1)) domain present also in MAGUK (Hanlon et al., 1999) although it is not present in other β subunits . Proteins presenting a PDZ domain are known to traffic and cluster ion channels and receptors (Nourry et al., 2003). This suggests that the β subunits could be involved in trafficking of the α_1 subunit to the plasma membrane and would play a role in its localization with other intracellular proteins (Dolphin, 2003a). This requires further investigation since two recent studies present contradictory results. McGee *et al.* (2004) showed that the major property of the SH3-GK module of β subunits is to regulate their inactivation kinetics. On the contrary, Takahashi *et al.* (2004) showed, using point mutation in this module, the role in Ca^{2+} channels trafficking.

Nevertheless, their implication in Ca^{2+} channel targeting has been suggested by studies realized in polarized epithelial cells, showing a selective and differential trafficking of Ca^{2+} channels to basolateral or apical membranes allowed by different β subunits (Brice and Dolphin, 1999; Bogdanov et al., 2000). The location also depends on α_1 subunits co-expressed, since the same β_{1b} subunit traffics the neuronal Ca^{2+} channels, $Ca_V2.1$, to the apical membrane and $Ca_V1.2$ to the basolateral membrane (Brice and Dolphin, 1999). When expressed alone, β_{2a} and β_{1b} are associated with the membrane (Chien et al., 1995; Brice et al., 1997; Bogdanov et al., 2000), suggesting that trafficking Ca^{2+} channels to the membrane is a property of β subunits. However, this is not linked to its membrane association or its palmitoylation, since β_3 and β_4 which are not associated with the membrane are able to traffic channels (Takahashi et al., 2003). Furthermore, only β_{2a} is palmitoylated at its N-terminal part (Chien et al., 1996) and mutation of its palmitoylation sites does not prevent the trafficking of $Ca_V1.2$ to the membrane (Birnbaumer et al., 1998). Moreover, β_{2e}, a splice variant of β_2 which is not palmitoylated, is still associated to the plasma membrane (Takahashi et al., 2003).

Additionally, it has been proposed that the I-II linker of α_1 subunit contains an endoplasmic reticulum retention signal and that the binding of β to the AID would allow the traffic of α_1 from the reticulum endoplasmic to the plasma membrane (Bichet et al., 2000). Some electrophysiological studies agreed with this finding, showing that co-expression of β subunits increases gating currents of $Ca_V1.2$ channels (Josephson and Varadi, 1996; Colecraft et al., 2002). These conclusions are still controversial since this was not observed in *Xenopus* oocytes (Neely et al., 1993; Olcese et al., 1996; Neely et al., 2004), but it is unclear whether this difference is due to the expression of endogenous β subunits expressed by oocytes (Canti et al., 2001) which would be responsible for the trafficking of over-expressed Ca^{2+} channels.

Previously, it has been shown that a single point mutation in the β interaction domain of α_{1C} disrupts the co-localization and plasma membrane targeting of both subunits, without affecting modulation of single channel properties or whole cell currents (Gerster et al., 1999). This suggests two independent effects of β subunits: β subunits are involved in the trafficking of Ca^{2+} channels to the plasma membrane with the α_1 subunits and modulate their biophysical properties (Gerster et al., 1999).

The increase of current density can be attributed to the effect of β subunits on channel trafficking as well as to an increase in the open probability of the channels (Wakamori et al., 1993; Neely et al., 1995; Costantin et al., 1998; Dzhura et al., 2000). An extensive amount of work has been published concerning the effects of β on Ca^{2+} channel properties and several reviews summarize these studies (Walker and De Waard, 1998; Birnbaumer et al., 1998; Dolphin, 2003a). These effects are more or less pronounced according to the α_1 or the β subunits co-expressed. However, to summarize the effects of β subunits on Ca^{2+} channels, it is generally agreed that these auxiliary subunits affect their voltage dependence, hyperpolarizing their activation by increasing the coupling between the charge movement and the pore opening (Olcese et al., 1996) and hyperpolarize their steady-state inactivation. They accelerate channel activation, modify channels inactivation kinetics (either acceleration or slowing) (Walker and De Waard, 1998; Dolphin, 2003a). β_{1b}, and β_2 (especially β_{2a} and β_{2e}) decrease the inactivation rate (Cens et al., 1999; Takahashi et al., 2003) whereas β_3 accelerates the inactivation decay of $Ca_V1.2$ (Castellano et al., 1993b). β subunits modulate inactivation kinetics because they interact with I-II loop of the α_1 subunit, on a crucial part which could work as an inactivation ball (Cens et al., 1999). The retardation in inactivation kinetics of Ca^{2+} channels by β_{2a} has been shown to be due to its palmitoylation (Qin et al., 1998b). Palmitoylation induces immobilization of the channel inactivation gate constituted by the I-II loop of the α_1 subunit, through a membrane-anchoring site constituted of two palmitic acids bound to cysteines present in the N-terminus of the auxiliary subunit (Restituito et al., 2000). This work is in agreement with the model for voltage-dependent inactivation presenting the I-II loop as crucial for the voltage-dependent inactivation of Ca^{2+} channels.

Phosphorylation of β subunits has been suggested as a possible pathway for regulation of Ca^{2+} channels. β_{1b} contains a motif that can be phosphorylated by PKA (De Jongh et al., 1989) and that β_{2a} is phosphorylated by the same kinase (Gerhardstein et al., 1999). It has been also suggested that β-adrenergic stimulation of L-type Ca^{2+} current in cardiomyocytes was due to a phosphorylation of β subunits by PKA (Haase et al., 1993). Even if the mechanism for this regulation remains unclear, it seems more likely that the α_{1C} subunit is the target of this kinase

to modulate the channel (Gao et al., 1997b). β subunits in the BID also contain as well predicted phosphorylation sites by PKC whose functional role remains unknown (Walker and De Waard, 1998), but the structure recently proposed of β subunits suggests that they are buried within the molecule, so their phosphorylation is unlikely to occur (Van Petegem et al., 2004).

A role for β subunits in other types of regulation has recently been demonstrated. Members of Ras-related GTPase subfamily (RGK family), Kir/Gem, Rem, Rem2, Rad, expressed in striated muscle cells, such as cardiac cells have been identified as interacting proteins of Ca^{2+} channel β subunits (Beguin et al., 2001; Finlin et al., 2003). This interaction is stronger in the presence of GTPγs and antagonized by Ca^{2+}/Calmodulin (Beguin et al., 2001). Co-expression of Rad, Rem or Kir/Gem with L-type ($Ca_V1.2$ and $Ca_V1.3$) Ca^{2+} channels inhibits their plasma membrane functional expression (Beguin et al., 2001; Finlin et al., 2003). This property is inherent to β subunits and does not affect T-type Ca^{2+} channels (Finlin et al., 2003).

These ancillary subunits are very important because they not only regulate biophysical properties of HVA Ca^{2+} channels but also their expression at the membrane. A possible regulation of gene silencing by a truncated β subunit splice variant through nuclear relocalization has been recently proposed (Hibino et al., 2003), which suggests the possible involvement of these auxiliary subunits to a new pathway of transcription, adding one more fascinating property to these subunits.

According to all these studies, it is easy to speculate that new studies about β subunits will reveal a specific functional role of β subunits in cardiac cells. For example, using the technology of adenoviral transfection in cultured myocytes, Yue's laboratory showed that overexpression of exogenous β subunits ($β_{1b}$, $β_{2a}$, $β_3$ and $β_4$) enhance I_{Ca} density in adult rat ventricular myocytes. This suggests that β subunits are the limiting factor for expression of L-type Ca^{2+} channels in the heart (Wei et al., 2000). They also showed that $β_{2a}$ is not the main β subunit in ventricular rat myocytes since the reconstituted currents have markedly different inactivation kinetics than in control (Colecraft et al., 2002). Such diversity of β subunits in the rat myocytes has been confirmed by RT PCR recently (Chu et al., 2004) and might have clear impact on electrophysiological properties of I_{CaL}.

4.1.3 Structure-function of $α_2δ$

The $α_2δ$ subunit was initially co-purified with $α_1$ from skeletal muscle (Nakayama et al., 1987; Catterall et al., 1988), its cDNA sequence has been then described (Ellis et al., 1988).

Our knowledge about the function of these auxiliary subunits (~175 kDa), is not as extensive as for the β subunits. Structurally, they are post-translationaly cleaved to yield disulphide-linked α_2 and δ proteins (De Jongh et al., 1990; Jay et al., 1991) (see Figure 6). The transmembrane δ part anchors the α_2 protein to the membrane via a single putative transmembrane segment and both proteins are highly glycosylate (Klugbauer et al., 2003; Canti et al., 2003). Four genetically distinct $\alpha_2\delta$ subunits have been described so far. The gene encoding for the more characterized $\alpha_2\delta-1$ has been located to the human chromosomal region 7q21-q22 (Arikkath and Campbell, 2003). Three more genes have been described which encode for $\alpha_2\delta-2$, $\alpha_2\delta-3$, $\alpha_2\delta-4$ located respectively to the human chromosomal regions 3p21.3, 3p21.1 and 12p13.3 (Arikkath and Campbell, 2003). $\alpha_2\delta-1$ demonstrates alternative splicing, resulting in five isoforms, $\alpha_2\delta-1a$, b, c, d, e expressed in a tissue-specific manner (Angelotti and Hofmann, 1996). Similarly, three splice variants for $\alpha_2\delta-2$ (a, b, c) have been described (Hobom et al., 2000). $\alpha_2\delta-1$ is expressed in heart (Gong et al., 2001) and it has been shown that the five splice variants for $\alpha_2\delta-1$ are found in cardiovascular system, but $\alpha_2\delta-1c$ and $\alpha_2\delta-1d$ are the main isoforms expressed in mouse heart (Angelotti and Hofmann, 1996). While $\alpha_2\delta-2$ and $\alpha_2\delta-4$ are present in heart (Gao et al., 2000; Qin et al., 2002), $\alpha_2\delta-3$ is only found in brain (Klugbauer et al., 1999a; Gong et al., 2001). Like the β subunits, $\alpha_2\delta$ subunits are also known to exhibit two major regulatory roles. They modulate the targeting of Ca^{2+} channels to the plasma membrane as well as their biophysical properties (Klugbauer et al., 2003; Herlitze et al., 2003). The role of $\alpha_2\delta$ for Ca^{2+} channels targeting to the plasma membrane is still controversial. Gao *et al.* (1999) suggested that its co-expression did not target α_{1C} to the plasma membrane. But more studies in different heterologous expression systems demonstrated that $\alpha_2\delta$ increases the current density of HVA Ca^{2+} channels such as $Ca_V1.2$ channels (Singer et al., 1991; Bangalore et al., 1996; Felix et al., 1997; Klugbauer et al., 1999a) or $Ca_V2.3$ (Jones et al., 1998; Klugbauer et al., 1999a) and possibly of T-type channels (Dolphin et al., 1999; Dubel et al., 2004). This increase of the current density would be due to an increase of targeting of the channels to the plasma membrane (Shistik et al., 1995; Dolphin et al., 1999; Yamaguchi et al., 2000a) or to a stabilization of the channels in the membrane (Dolphin et al., 1999). Co-expression of $\alpha_2\delta$ also modulates the biophysical properties of HVA (Klugbauer et al., 2003). These auxiliary subunits shift their voltage dependence of activation and inactivation to more negative potentials (Felix et al., 1997; Shirokov et al., 1998; Hobom et al., 2000) and promote inactivation of gating currents (Shirokov et al., 1998). The region of the $\alpha_2\delta$ subunit involved in the modulation of the gating properties of the HVA Ca^{2+} channels is localized in the δ domain of the protein (Felix et al.,

1997). They increase the gating current size for $Ca_V1.2$ (Bangalore et al., 1996) and $Ca_V2.3$ (Jones et al., 1998). This increase is correlated to the enhancement of whole cell ionic current, suggesting that these subunits induce Ca^{2+} channel targeting to the plasma membrane. They also accelerate their activation and inactivation rate (Bangalore et al., 1996; Shirokov et al., 1998; Qin et al., 1998a). A possible role of modulation of T-type channels by $\alpha_2\delta$ has been also suggested. Its co-expression with α_{1G} hyperpolarizes its voltage dependence and accelerates the decay of the currents and shifts the steady-state inactivation curve (Hobom et al., 2000).

Even if its modulatory role is still controversial, $\alpha_2\delta$ (Klugbauer et al., 2003; Herlitze et al., 2003) might play a major role to lead to the correct targeting of Ca^{2+} channels to the plasma membrane and modulate, in cooperation with β subunits, the Ca^{2+} entry in cardiac cells.

4.1.4   *Structure-function of γ.*

γ subunit was originally thought only to be associated with L-type Ca^{2+} channels in skeletal muscle where it was first identified (Glossmann et al., 1987). Its primary structure was then described (Jay et al., 1990). This first member of γ subunits, $\gamma1$, due to low level of expression of α_{1S}, has been co-expressed with $Ca_V1.2$ channels in heterologous systems. It shifts the voltage dependence of inactivation to more negative potentials and also accelerates inactivation kinetics (Singer et al., 1991; Eberst et al., 1997). So far, 8 γ subunit ($\gamma1$-8) isoforms have been identified (Kang and Campbell, 2003). $\gamma1$ and $\gamma6$ are expressed in skeletal muscle and $\gamma2$-5 and $\gamma7$-8 are mainly expressed in brain (Burgess et al., 1999; Chu et al., 2001). The presence of $\gamma4$, $\gamma6$ and $\gamma7$ has been reported in heart cells (Chu et al., 2001). More recently, a new $\gamma7$ has been cloned, and found to exhibit a longer C-terminus than previously predicted and shows striking properties (Moss et al., 2002). Co-expression of this neuronal protein with Ca^{2+} channels reduces expression of channel proteins, specifically abolishes N-type currents but also affects $Ca_V1.2$ channels (Moss et al., 2002). This suggests that this γ-like protein would not be associated with a functional channel at the plasma membrane but regulates their expression by a mechanism which remains to be elucidated. Accordingly, a recent study of the three-dimensional structure of cardiac L-type channels determined by single particle electron microscopy reveals differences in the putative transmembrane region with the structure of skeletal L-type channels (Wang et al., 2004a). This is consistent with the lack of an associated γ subunit (Wang et al., 2004a). So far, the expression of γ subunits in heart cell is not well established and their possible physiological role is not clear.

4.2   T-type Ca^{2+} channels

To date, three T-type Ca^{2+} channels have been cloned (Perez-Reyes, 2003). After many unsuccessful attempts to clone T-type channels by PCR, progress in the sequencing of human, yeast and *C. elegans* genomes provided a novel library that could be screened with a computer (*i.e. in silico*). This led to the cloning of the first T-type channel, Ca$_V$3.1, by Perez-Reyes *et al.* (1998) which exhibited similar biophysical properties to T-type currents recorded in neurons. The full length human Ca$_V$3.1 was subsequently cloned (Monteil et al., 2000a). The gene encoding this channel, *CACNA1G* has been mapped in the human to chromosome 17q22 (Perez-Reyes, 1998). The screening of a human heart library allowed the cloning of Ca$_V$3.2, whose gene, *CACNA1H*, has been mapped to human chromosome 16p13.3 (Cribbs et al., 1998). Screening of rat brain led to identification of the rat version of Ca$_V$3.1, Ca$_V$3.2 and of Ca$_V$3.3 (Lee et al., 1999a). The gene encoding for Ca$_V$3.3, *CACNA1I,* has been located in the human on chromosome 22q12.3-13.2 (Mittman et al., 1999b). The structure of the α_1 subunit for T-type channels is similar to those of HVA Ca^{2+} channels. They are proteins of 24 transmembrane segments arranged in four domains of six segments with a positively charged S4. The S4 segments confer to the channels, their sensitivity to variation of the membrane potential (Perez-Reyes, 2003). In HVA Ca^{2+} channels, four glutamate residues in each P-region form the selectivity filter that binds Ca^{2+} (Tang et al., 1993; Yang et al., 1993; Cibulsky and Sather, 2000; Cibulsky and Sather, 2003). For LVA Ca^{2+} channels, the selectivity filter consists of an arrangement of two glutamate and two aspartate residues in the P-loops of domains I-IV of the α_1 subunit (Talavera et al., 2001). Point mutations of these divergent residues led to the conclusion that they are critical for the selectivity and the permeation properties of Ca$_V$3.1 (Talavera et al., 2001). The activation kinetics and their sensitivity to protonation is also dependent on these residues (Talavera et al., 2003a; Talavera et al., 2003b). Of course, as with Ca$_V$1.2, S4 segments in domains I, III, IV are critical for Ca$_V$3.1 opening (Li et al., 2004). Furthermore, S6 segments are implicated in the voltage-dependent inactivation (Marksteiner et al., 2001) as well as the carboxy-terminus (Staes et al., 2001; Park et al., 2004), the I-II and II-III intracellular loops (Park et al., 2004). A recent study on Ca$_V$3.2 Ca^{2+} channels underlined the importance of several domains, IS2-S3, IS5-S6 and IIS2, in the activation processes of these channels (Khosravani et al., 2004). Point mutations in the IS2-S3 and IS5-S6 hyperpolarized the activation and a mutation in the IIS2 slowed their activation. The S2 of domain II is also implicated in the voltage dependent inactivation of LVA Ca^{2+} channels (Khosravani et al., 2004).

The currents mediated by rat and human isoforms of the three $Ca_V3.x$ channels exhibit different sensitivity to nickel. Nickel is ten fold more potent to inhibit T-type currents conducted through $Ca_V3.2$ channels than currents conducted through $Ca_V3.1$ or $Ca_V3.3$ (Lee et al., 1999b). These three channels begin to activate at very negative potentials, around -70 mV (Perez-Reyes, 2003) (see Figure 1). Currents mediated by $Ca_V3.1$ show the fastest activation and inactivation kinetics (Klockner et al., 1999; Monteil et al., 2000a; Chemin et al., 2002). The three channels exhibit no differences in the voltage dependence of their steady-state inactivation or their activation (Klockner et al., 1999). Nevertheless, a study comparing the three human variants of T-type channels showed that these properties are slightly hyperpolarized for $Ca_V3.1$ and $Ca_V3.2$ in comparison to $Ca_V3.3$ (Chemin et al., 2002). $Ca_V3.3$ deactivates faster than the other two channels and recovery from short term inactivation is three-fold slower for currents mediated by $Ca_V3.3$ and $Ca_V3.2$ than for $Ca_V3.1$ (Klockner et al., 1999). In contrast, reactivation after a long prepulse is faster for $Ca_V3.3$ and slowest for $Ca_V3.2$ (Klockner et al., 1999; Chemin et al., 2002). Furthermore, $Ca_V3.3$ is the only channel exhibiting voltage-dependent facilitation (Klockner et al., 1999; Chemin et al., 2002). These LVA Ca^{2+} channels, unlike the L-type channels have been reported to lack Ca^{2+} binding domains in their C-terminal region so they do not exhibit Ca^{2+}-dependent inactivation (Perez-Reyes, 1998). Nevertheless, $Ca_V3.2$ but not $Ca_V3.1$ or $Ca_V3.3$ exhibits slower activation and inactivation kinetics when Ca^{2+} is the charge carrier, suggesting a possible modulation by Ca^{2+} of this channel (Klockner et al., 1999). They differ also from HVA Ca^{2+} channels in what their intracellular I-II loop does not contain an AID responsible for binding β subunits (Dolphin, 2003a). Co-expression of cloned β subunits has little or no effect on cloned T-type channel activity, unlike $\alpha_2\delta$ which can induce an increase of the current density (Dolphin et al., 1999; Hobom et al., 2000; Gao et al., 2000; Dubel et al., 2004). γ subunits have very little effect on T-type channels. γ_2 but not γ_3 or γ_4 slows the decay of the tail currents recorded after co-expression with $Ca_V3.3$ (Green et al., 2001). It has also been reported that γ_2, γ_4 and γ_5 accelerate inactivation of $Ca_V3.1$ and hyperpolarize its steady-state inactivation (Klugbauer et al., 2000). More recently, it has been proposed that $\alpha_2\delta-2a$ and γ_5 improve the coupling between the charge movement and the opening of $Ca_V3.1$ expressed in HEK293 cells, supporting the hypothesis that T-type channels may interact with auxiliary subunits (Lacinova and Klugbauer, 2004).

Heart cells express $Ca_V3.1$ channels as well as $Ca_V3.2$ (Cribbs et al., 1998; Monteil et al., 2000b), while $Ca_V3.3$ is mainly expressed in brain (Lee et al., 1999a). Based on nickel sensitivity, it has been proposed that the T-type Ca^{2+} current in cardiac cells is mainly carried through $Ca_V3.2$ channels (Hagiwara et al., 1988; Pascarel et al., 2001). However, this issue

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remains controversial and recent reports present evidence supporting $Ca_V3.1$ as the functional cardiac T-type Ca^{2+} channel during murine embryonic period (Cribbs et al., 2001) whereas another one suggests this role for $Ca_V3.2$ (Niwa et al., 2004). As for HVA Ca^{2+} channels, alternative splicing increases functional and structural diversity of LVA Ca^{2+} channels (Jurkat-Rott and Lehmann-Horn, 2004). Splice variants of $Ca_V3.1$ channels differ in their II-III loop which modifies their inactivation kinetics and steady-state inactivation (Mittman et al., 1999a; Chemin et al., 2001). Deletion of seven amino acids residues in the linker between domain III and IV of $Ca_V3.1$, by alternative splicing, induces a positive shift of its activation and inactivation and slows its activation kinetics (Monteil et al., 2000a; Chemin et al., 2002). The C-terminus of $Ca_V3.3$ is also modified by alternative splicing, leading to channels with truncated C-termini and with altered inactivation properties (Murbartian et al., 2002).

It is clear that endogenous T-type currents are subject to hormonal regulation and are modulated by guanine nucleotides and protein kinases in various type of cells (Perez-Reyes, 2003; Yunker, 2003). However, less is known about the molecular determinants involved in their modulation. Cloned T-type Ca^{2+} channels harbor many consensus phosphorylation sites for different kinases (Cribbs et al., 1998; Lee et al., 1999a; Mittman et al., 1999a; Klugbauer et al., 1999b; Mittman et al., 1999b; Chemin et al., 2001). So far, only the $Ca_V3.2$ channels have been identified as subject to modulation by intracellular messengers when expressed in heterologous systems. They are modulated by CAMKII which phosphorylates a serine present in their II-III intracellular loop, which is absent from unregulated $Ca_V3.1$ channels (Wolfe et al., 2002; Welsby et al., 2003). Phosphorylation by CAMKII increases activity of $Ca_V3.2$ channels at negative potentials, hyperpolarizing their activation (Wolfe et al., 2002; Welsby et al., 2003). These T-type channels are also modulated by PKC which leads to a three-fold increase in the amplitude of barium currents recorded in oocytes (Park et al., 2003). They are also inhibited by G-protein βγ subunits that bind to their II-III loop (Wolfe et al., 2003). Future studies on cloned LVA Ca^{2+} channels will certainly reveal more ways to regulate T-type channels and therefore, the activity of pace-maker cells and cardiac function.

4.3 Molecular structure of others Ca^{2+} channels (α_{1D}, α_{1E})

$Ca_V1.2$ channels are known to be predominantly expressed in heart cells, although, α_{1C} is not the only α_1 subunit present in cardiomyocytes, since two more subunits, α_{1D} ($Ca_V1.3$) and α_{1E} ($Ca_V2.3$) have been detected in heart tissues. RNA encoding α_{1D} has been detected in these cells and expression of $Ca_V1.3$ channels at the plasma membrane was confirmed using antibodies (Takimoto et al., 1997; Wyatt et al., 1997; Xu et al., 2003). It has been suggested that they play a role in cardiac pacemaker activity (see section 2.1.2) and during development

of embryonic hearts (Xu et al., 2003). Ca$_V$2.3 channels, have also been detected at RNA and protein levels by RT-PCR and immunodetection in rat atrial and ventricular myocytes (Weiergraber et al., 2000; Mitchell et al., 2002). The functional significance in cardiac myocytes (if any) of this Ca^{2+} channels remains undetermined. Transgenic mice lacking Ca$_V$2.3 (-/-) did not show heart defects at the adult stage (*e.g.* bradychardia), although an increase in the variation of beating frequency lead to the suggestion that Ca$_V$2.3 might be implicated in stabilizing the heart beat of prenatal murine hearts (Lu et al., 2004).

Like α_1 subunits from other Ca^{2+} channels, α_{1D} and α_{1E} are proteins of about 2000 amino acid residues organized into four domains of six transmembrane segments, with the positively charged S4 conferring sensitivity to potential changes.

Only a few studies describing the biophysical properties and modulation of Ca$_V$1.3 channels have been published. These channels activates at relatively hyperpolarized potentials, depending on the studies. The use of different extracellular concentrations of divalent ions, the expression of different cloned channels from different types of cells could explain these differences as well as the co-expression of different or no auxiliary subunits (Xu and Lipscombe, 2001; Koschak et al., 2001; Bell et al., 2001; Scholze et al., 2001; Xu et al., 2003). But several groups confirmed that they activate at more negative potentials than Ca$_V$1.2 (Xu and Lipscombe, 2001; Koschak et al., 2001; Zhang et al., 2002b; Kasielke et al., 2003) (see Figure 1). A hyperpolarizing shift of the steady-state inactivation curve for Ca$_v$1.3 compared with Ca$_v$1.2 currents has also been observed (Koschak et al., 2001; Scholze et al., 2001). The inactivation kinetics of barium currents induced by expression of Ca$_V$1.3 channels are slower when Ca^{2+} was used as charge carrier, so these channels exhibit a Ca^{2+} dependent inactivation (Xu and Lipscombe, 2001; Bell et al., 2001; Koschak et al., 2003). Not much is known about the molecular determinants implicated in inactivation of these channels. Nevertheless, it is easy to speculate that the mechanism leading to Ca^{2+}-dependent inactivation is similar to that for Ca$_V$1.2 channels since the α_{1D} C-terminus is highly homologous to the one for Ca$_V$1.2 channels (Koschak et al., 2001).

Like other L-type channels, α_{1D} has an AID in its I-II loop (Dolphin, 2003a) and co-expression of a β and α_2-δ subunits increases the current density but does not modify the biophysical properties of the channel (Williams et al., 1992; Xu and Lipscombe, 2001).

These L-type channels exhibit sensitivity to DHPs antagonists. They are blocked by nimodipine (Safa et al., 2001) and nitrendipine, but the neuronal isoform is less sensitive than Ca$_V$1.2 channels (Xu and Lipscombe, 2001). They are also blocked by nifedipine and activated by the DHPs agonist BayK8644 (Koschak et al., 2001; Bell et al., 2001). Isradipine block of a pancreatic human Ca$_V$1.3 is 8.5-fold lower than that for Ca$_V$1.2, which can be attributed to

differences in the voltage-dependent interaction of DHPs antagonists (Koschak et al., 2001). Different sensitivity to DHPs has also been described for two splice variants cloned from embryonic cardiomyocytes (Xu et al., 2003).

Expression of $Ca_V2.3$ in heterologous systems produces current activating and inactivating at more negative potentials than $Ca_V1.2$ channels (Berrou et al., 2001). The biophysical properties of $Ca_V2.3$ channels are intermediate between those of T-type and L-type channels. They activate at more positive potentials than T-type channels and their steady-state inactivation occurs at more positive potentials than for $Ca_V3.2$ but more negative than for $Ca_V3.1$ and $Ca_V3.3$ (Lee et al., 1999a). Inactivation kinetics of barium currents induced by $Ca_V2.3$ expression is faster than for $Ca_V1.2$ or $Ca_V3.1$ but slower than for $Ca_V3.2$ and $Ca_V3.3$ (Lee et al., 1999a; Berrou et al., 2001). As for α_{1C}-containing channels, multiple sites within α_{1E} are implicated in the voltage-dependent inactivation of $Ca_V2.3$ channels. The voltage-dependent inactivation involves the I-II loop of α_{1E} (Stotz et al., 2000; Berrou et al., 2001) as well as the S6 segments of the domain II and III suggesting that as for L-type currents, the I-II linker forms a hinged lid that may dock to the S6 segments of the pore forming subunit (Stotz et al., 2000; Stotz and Zamponi, 2001a). Co-expression of auxiliary subunits modulates $Ca_V2.3$ channels. Like other HVA Ca^{2+} channels, they contain an AID which binds β subunits (Dolphin, 2003a). The main effect of $\alpha_2\delta$ subunit is to increase current density and β subunits also increase the number of channels expressed at the plasma membrane, hyperpolarize the voltage-dependence of their activation and inactivation and slows their inactivation kinetics (Olcese et al., 1996; Parent et al., 1997; Jones et al., 1998). Mutations of the AID of α_{1E} affects the kinetics and the voltage-dependence of inactivation of $Ca_V2.3$, reinforcing the hypothesis of involvement of the I-II loop and beta subunits as crucial determinants for inactivation of Ca^{2+} channels (Berrou et al., 2001; Berrou et al., 2002). Since it was first cloned (Schneider et al., 1994; Williams et al., 1994), it has been suggested that the inactivation of $Ca_V2.3$ is not modulated by intracellular Ca^{2+}. Therefore, α_{1E} has been used to generate α_{1C}-α_{1E} chimera to study the Ca^{2+}-dependent inactivation of L-type channels (de Leon et al., 1995; Parent et al., 1997; Zhou et al., 1997). However, more recently, different studies describe opposite effects of Ca^{2+} on $Ca_V2.3$ activity. At concentrations between 0.1 and 1 μmol/L, Ca^{2+} increases the current density and slows inactivation kinetics by activating a PKC (Leroy et al., 2003; Klockner et al., 2004). This modulation occurs only for neuronal splice variants that contain an arginine-rich region in their intracellular II-III loop, while neuroendocrine/heart isoforms are not regulated by Ca^{2+} or PKC (Pereverzev et al., 2002; Klockner et al., 2004). Another group showed that calmodulin binds to the IQ motif present in the C-terminus of $Ca_V2.3$ (Erickson et al., 2001) and that these channels undergo Ca^{2+}-dependent inactivation, which is mediated by

the calmodulin N-terminal lobe, when a low concentration of Ca^{2+} chelator is present (Liang et al., 2003). Activity of $Ca_V2.3$ channels, at low Ca^{2+} concentration (between 0.1 and 1 μmol/L) are potentiated by activation of a PKC by Ca^{2+} while Ca^{2+}-dependent inactivation occurs at higher internal Ca^{2+} concentrations.

Unlike the other HVA Ca^{2+} channels, no pharmacological compounds have been identified as specific blockers of the $Ca_V2.3$ channels. Expression of α_{1E} appears to correlate in part to that which has been described as R-type currents ("R" for resistant to known pharmacological tools) (Soong et al., 1993; Schneider et al., 1994; Williams et al., 1994). More recently, a toxin has been isolated from the tarantula *Hysterocrates gigas*, named SNX482, described as a selective blocker for α_{1E} containing Ca^{2+} channels (Newcomb et al., 1998). This toxin blocks the channel by interacting with the domains III and IV of α_{1E} but seems to block L-type channels as well (Bourinet et al., 2001).

5 CARDIAC Ca^{2+} CHANNELS AND PHYSIO-PATHOLOGICAL CONDITIONS

Although several of human genetic disease associated with mutations in ionic channels (channelopathies) exist in the heart and has been linked to the long Q-T syndrome (mutations in Na$^+$ or K$^+$ channels, see for review (Marban, 2002)) and inherited channelopathies exist for Ca^{2+} channels (*e.g.* hemiplegic migraine, point mutation in α_{1A}, hypokalaemic periodic paralysis mutation in α_{1S}, see for review (Lorenzon and Beam, 2000)), so far no cardiac disease is known to be linked to mutations in the cardiac α_1 or β subunits. However, change of I_{Ca} expression and function has been described in disease and I_{Ca} can be linked to cardiac pathology. For example, it is well kwown that arrhythmogenic EADs are potentiated by I_{CaL} agonist (Bay K8644) whereas I_{CaL} blocker (Nitrendipine) abolished EADs in ferret ventricular muscle (Marban et al., 1986). We will now describe several cardiac diseases where I_{Ca} has been implicated.

5.1 Diseases with auto-immune components

5.1.1 *Congenital heart block*

Congenital heart block in a structurally normal heart is strongly associated with auto-antibodies reactive with ribonucleoproteins SSA/Ro and SSB/La. This is presumed to be due to the trans-placental passage of auto-antibodies from the mother into the fetal circulation, which can be asymptomatic or exhibit rheumatologic disease such as systemic lupus erythematosus (for review see Boutjdir 2000).

An early study demonstrated that these antibodies inhibit I_{CaL} in rabbit ventricular myocytes (Garcia et al., 1994). Subsequently, Boutjdir and colleagues provided compelling evidence that these autoimmune antibodies can block I_{CaL}. They found that positive IgG fraction (whole IgG containing anti-SSA/Ro and SSB/La antibodies purified from sera) and/or affinity purified anti-52 kD SSA/Ro antibodies, from mothers with CHB children, were able to decrease I_{CaL} amplitude in human fetal myocytes (Boutjdir et al., 1997). At the single channel level, the open probability of L-type Ca^{2+} channels was decreased (Boutjdir et al., 1997). These auto-antibodies produced a similar decrease in I_{CaL} amplitude in rat ventricular cardiomyocytes (Boutjdir et al., 1998), canine Purkinje myocytes (Qu et al., 2001), and rabbit sinoatrial cells (Hu et al., 2004). Interestingly, I_{CaT} from rabbit sinoatrial cells is also blocked by these antibodies (Hu et al., 2004). Inhibition of I_{Ca} was confirmed in expression system (*Xenopus* oocytes expressing α_{1C} or α_{1H}, (Xiao et al., 2001a)). These data provide compelling

evidence that auto-antibodies can block I_{Ca}, and may thus play a role in the pathogenesis of bradycardia and atrioventricular block in congenital heart block.

An intriguing question is how intracellular antibodies can recognize an extracellular Ca^{2+} channel epitope. Numerous homologous epitopes were found in 3D representations of human α_{1C}, especially with the S5-S6 linker of domain VI (Qu et al., 2001). Furthermore, α_{1C} binding by positive maternal IgG has been confirmed by immunoprecipitation and immunohistochemical study in human fetal cardiomyocytes (Qu et al., 2001). However, the exact site(s) of interaction is not yet determined.

Although an acute effect of these antibodies has been shown on I_{CaL}, a chronic effect is also suspected. I_{CaL} density is decreased in ventricular cells from mice and rabbit pups born from mothers immunized with recombinant 52kD-SSA/Ro protein suggesting that prolonged exposure of pups' heart to maternal antibodies during pregnancy could lead to reduced channel expression (Qu et al., 2001; Xiao et al., 2001b). This hypothesis is supported by a lower amount of L-type Ca^{2+} channel protein assessed by ELISA and Western blot in pup hearts from immunized mothers. A down-regulation by internalization of L-type Ca^{2+} channel induced by anti-52kD-SSA/Ro antibodies has also been suggested (Xiao et al., 2001b).

There is striking evidence that auto-antibodies can directly affect I_{Ca}, but an alternative mechanism has also been described. The L-type Ca^{2+} channel may not be the only target for maternal anti-SSA/Ro and/or SSB/La antibodies, because a specific cross-reaction between the recombinant 52kDa SSA/Ro protein and the second extracellular loop of serotoninergic 5-HT$_4$ receptor expressed in human atrial cells has been identified (sequence G21V) (Eftekhari et al., 2000). In contrast to the results describe above, anti-G21V affinity-purified autoantibodies from lupus patients or from rabbits immunized with the G21V peptide do not inhibit basal I_{CaL} in adult human atrial myocytes. However, these antibodies antagonize serotonin-induced activation of I_{CaL} (Eftekhari et al., 2000; Salle et al., 2001); the authors hypothesized that during the early phase of fetal development the serotoninic pathway is more prominent than the β-adrenergic pathway so that 5-HT$_4$ receptor block leads to a reduction of I_{Ca} which could in turn induce bradycardia and atrioventricular block (Eftekhari et al., 2001).

Taken together, these results suggest that a mother's antibodies can either directly interact with Ca^{2+} channels, or can alter channel function indirectly via receptor modulation, and thus contribute to sinus bradycardia and atrioventricular block of their children. But an intriguing question remains: why do autoantibodies have deleterious effects on children while most mothers do not exhibit cardiac symptoms?

5.1.2 Cardiomyopathies

A number of antibodies have been identified in sera from patients with myocarditis/dilated cardiomyopathy (for reviews see Malkiel et al., 1996; Caforio et al., 2002) and myocarditis/Chagas' cardiomyopathy (reviewed by Kierszenbaum, 2003).

Myocarditis is an inflammatory disease of the myocardium, and idiopathic, autoimmune, and infectious forms of inflammatory cardiomyopathy are recognized. Inflammatory myocardial disease is involved in the pathogenesis of dilated cardiomyopathy and Chagas' cardiomyopathy (Richardson et al., 1996). Thus, myocarditis and dilated cardiomyopathy DCM and Chagas' cardiomyopathy are thought to represent the acute and chronic phases of an inflammatory process of the myocardium that can be viral, post-infectious immune or organ-specific autoimmune. The infectious agent in Chagas' disease is the protozoan *Trypanosoma cruzi*. According to the Word Health Organization, 16–18 million people are infected by the parasite in South America, and another 90 million are at risk of becoming infected. Chagas' disease is a complex, multifaceted and widespread disease in which, during the chronic phase, a slowly evolving cardiomyopathy affecting about a third of infected people can be observed that leads to severe cardiac dilatation, congestive heart failure, arrhythmias, and death (Higuchi et al., 2003).

The sera of patients with myocarditis and dilated cardiomyopathy shows high frequencies of antibodies directed against the adenine nucleotide translocator (ANT) of the inner mitochondrial membrane (Kuhl et al., 1991), and specific binding by cross-reaction to the L type Ca^{2+} channel has been demonstrated (Schultheiss et al., 1988). Furthermore, it has been shown that anti-ANT antibody increases I_{Ca} amplitude in frog and rat myocytes (Morad et al., 1988; Schultheiss et al., 1988), which will contribute to increased intracellular Ca^{2+} and thus could impair cardiac function (see section 5.2.2).

The sera of patients with idiopathic dilated cardiomyopathy and Chagas' disease also contains autoantibodies directed against the β_1-, β_2-adrenoceptor (Borda et al., 1984; Wallukat and Wollenberger, 1987; Limas et al., 1989; Magnusson et al., 1994; Wallukat et al., 1995; Elies et al., 1996) and/or the muscarinic M_2-receptor (Fu et al., 1993; Goin et al., 1994; Elies et al., 1996). Immunoglobulins purified from sera of mice experimentally infected with *T. cruzi* during the acute or chronic phase of chagasic cardiopathy can respectively enhance or reduce L-type Ca^{2+} current in myocytes isolated from guinea-pig control hearts (Mijares et al., 1996a). These effects were inhibited by propranolol and atropine respectively, suggesting a preferential activation of β-adrenoceptors by antibodies during the acute phase and of M_2-receptors during the chronic one (Mijares et al., 1996a). These experiments functionally demonstrated the agonist-like activity of Chagasic antibodies on ß-adrenergic and muscarinic receptors.

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The second extracellular loop of cardiovascular G-protein coupled receptor (GPCR) appears to be one of the main targets of these antibodies (see for review see Hoebeke 2001). Hence an alternative approach to investigate the functional role of these anti-GPCR antibodies is to investigate the effect of antibodies raised against a peptide corresponding to this loop (Magnusson et al., 1994; Fu, 1995; Mijares et al., 1996b; Matsui et al., 1997; Elies et al., 1998; Lebesgue et al., 1998).

Monoclonal anti-β_2 adrenoceptor antibodies raised against the second extracellular loop increase I_{CaL} in guinea-pig ventricular cardiomyocytes (Mijares et al., 1996b; Lebesgue et al., 1998). Monoclonal or polyclonal antibodies generated against the second extracellular loop of the human muscarinic receptor mimic those found in Chagas' disease, decreasing the amplitude of I_{CaL} that has been prestimulated by isoproterenol (Zhao et al., 1996; Nascimento et al., 2001; Hernandez et al., 2003). In addition to β-adrenergic and muscarinic stimulation acting via the cAMP pathway (Wallukat et al., 1995; Fu, 1995; Borda and Sterin-Borda, 1996; Wallukat et al., 1999b; Chiale et al., 2001), it has been shown that antibodies directed against the second extracellular loop of the M_2-muscarinic receptor are also able to activate the NO/cGMP pathway (Sterin-Borda et al., 1997; Nascimento et al., 2001; Sterin-Borda et al., 2003) (see also section 3.3.2).

Importantly, in contrast to the classical agonists isoproterenol and carbachol, these antibodies fail to produce desensitization of the reaction cascade within 6 hours in cultured neonatal myocytes, inducing chronic stimulation of the GPCR (Magnusson et al., 1994; Wallukat et al., 1995; Wallukat et al., 1999a; Wallukat et al., 1999b). Such chronic stimulation of β-adrenergic and/or muscarinic cascades may have profound consequence on Ca^{2+} signaling, which may impair cardiac function (see section 5.2.2) and be pro-arrhythmic. For example, it has been shown that overexpression of ß$_2$-adrenergic receptors in mice accelerates the progression of myocardial fibrosis and heart failure (Liggett et al., 2000). More recently, Jahns et al.,, (2004) have demonstrated that agonistic anti-β_1-adrenoceptor antibodies alone are sufficient to engender myocardial dysfunction characteristic of heart failure.

Thus data are emerging that suggest physiopathologic roles for antibodies, mediated by direct and/or indirect modulation of I_{Ca}. However it is unclear whether autoreactivity to cardiac antigens initiates heart injury, amplifies ongoing disease, or is merely a sign of prior myocyte damage.

5.2 Cardiac remodeling

The diseased heart undergoes biochemical, electrophysiological and structural changes globally defined as remodeling (Swynghedauw, 1999). In this section we will review three types of remodeling in which modification of I_{Ca} has been implicated.

5.2.1 *Cardiac memory*

Memory is a form of remodeling common to many organ and cell systems. In the heart, cardiac memory is characterized by an altered T-wave on the electrocardiogram during sinus rhythm, induced by a preceding period of abnormal ventricular activation (Chatterjee et al., 1969). The direction of the change in the T-wave is in that of the vector of the inciting and abnormally activated QRS complex, hence the term "cardiac memory" was suggested: the T-wave "remembered" the ectopic QRS (Rosenbaum et al., 1982). Importantly, this occurs in hearts that have no demonstrable hemodynamic or structural abnormalities or ischemia. A variety of pathologies that alter ventricular activation pattern induce cardiac memory in humans including intermittent left bundle branch block, post-tachycardia syndrome, ventricular pre-excitation and extrasystoles (reviewed in (Patberg and Rosen, 2004)). Rosen's laboratory developed a canine model of ventricular pacing to induce cardiac memory and showed that cardiac memory was associated with altered action potential characteristics (smaller notch and longer action potential). They first showed that the transient outward current density was reduced and that this was associated a decrease at the mRNA level, possibly accounting for the change observed in the action potential (Yu et al., 1999). Further investigation showed that cardiac memory was attenuated by I_{CaL} blockade (nifidipine), suggesting that I_{CaL} is important in the development of cardiac memory (Plotnikov et al., 2003). Indeed, a short-term memory protocol (2 hours ventricular pacing) showed a significant decrease in nuclear CREB, that did not occur in control dogs or dogs treated with nifidipine (Patberg et al., 2003). These changes implicate effects of I_{CaL} at the transcription level (see section 2.3). At the electrophysiological level, no difference was found in the peak density of I_{CaL} in cardiac memory myocytes compared to control, although activation was more positive and I_{CaL} inactivation slower (Plotnikov et al., 2003), which may contribute to the longer action potential. Although the process leading to cardiac memory is far from being completely understood, there is a clear implication of I_{CaL} at the electrophysiological and transcriptional levels.

5.2.2 *Heart failure*

Heart failure is the leading heart disease in the Occidental world. It is characterized by a decrease in ejection fraction, and can result in death in two main ways: a progressive decline in contractile function or sudden death due to arrhythmias. At the cellular level, ventricular myocytes from failing hearts are hypertrophied and show an increase in action potential duration, a decrease in contractility and a blunted β–adrenergic responsiveness (for review see (Tomaselli and Marban, 1999; Towbin and Bowles, 2002)). The mechanisms behind these observations are still unclear although the decrease in contractility might be linked to a decrease in the amplitude and the rate of decline of the systolic Ca^{2+} transient (Houser and Margulies, 2003).

Animal models provide useful tools to gain insight into the mechanisms of heart failure, which can be induced by a variety of means (*e.g.* rapid pacing, aortic constriction, infusion of angiotensin II or catecholamines). Over the past 15 years, investigations of I_{CaL} in animal models of hypertrophy or heart failure have led to a profusion of data with either decreased, increased, or unchanged I_{CaL}. This variety of results might reflect the different degree of heart failure, species, and experimental conditions. However, in the majority of studies I_{CaL} amplitude, expressed as density, is unchanged (see (Tomaselli and Marban, 1999) for extensive review); this has been shown in ventricular myocytes from failing rat (Gomez et al., 2001), dog (O'Rourke et al., 1999), rabbit (Pogwizd et al., 1999) and guinea pig (Ahmmed et al., 2000) hearts: although cell surface area approximately doubles, I_{Ca} density is unchanged

Less data are available from human ventricular myocytes because of the obvious problem of obtaining "healthy" controls. However, several groups have studied myocytes from donor hearts unsuitable for transplantation, and in human studies peak I_{Ca} density is also unchanged in myocytes from failing heart (Beuckelmann et al., 1991; Schroder et al., 1998; Chen et al., 2002; Piacentino, III et al., 2003). At the single channel level, failing human myocytes show increased open probability and availability of L-type Ca^{2+} channels compared to non-failing myocytes (Schroder et al., 1998). This resembles the effect of β-adrenergic stimulation on the L-type Ca^{2+} channel (see section 3.3.1), and it has been proposed that channel phosphorylation is increased in the failing heart because of impaired dephosphorylation (Schroder et al., 1998). This idea has recently been supported by the observation that although global I_{Ca} amplitude is the same in failing and non-failing heart, the activation curve of I_{Ca} is shifted to the left, suggesting β–adrenergic stimulation (Chen et al., 2002), and I_{Ca} from failing heart shows less response to isoproterenol (β–adrenergic agonist). Conversely, PP2A decreases I_{Ca} density in the failing heart but not in normal heart, suggesting an increase in basal phosphorylation. Interestingly, these authors also showed that support using a left ventricular assist device

restored Ca^{2+} channel density and regulation (Chen et al., 2002), consistent with a recent clinical study showing that such support also induces clinical recovery of heart failure by modification of sarcoplasmic reticulum Ca^{2+} handling (Terracciano et al., 2004). Ca^{2+} channel density, assessed using Bay K8644, is decreased in myocytes from failing heart (Chen et al., 2002); this agrees with a recent study in an animal model, which showed a reduction in Ca^{2+} channel density (assessed by recording charge movement, Balijepalli et al., 2003) in failure, although the density of I$_{Ca}$ was unaltered, suggesting upregulation of existing channel function. Studies at the protein expression level show conflicting results, one showing unchanged mRNA levels for α$_{1C}$ and α$_2$-δ in heart failure, but with β subunit expression reduced by ~75% (Hullin et al., 1999), and another showing that α$_{1C}$ and β subunit expression are unchanged (Schroder et al., 1998).

These alterations in the density and regulation of the L type Ca^{2+} channel might contribute to the abnormal contractility and blunted adrenergic responsiveness of the failing heart. The mechanisms underlying the altered density and regulation of the L type Ca^{2+} channel are not yet known. Intriguingly, 4 isoforms of α$_{1C}$ of the L-type Ca^{2+} channel are expressed in the human heart, and isoform switching occurs in failing human myocytes (Yang et al., 2000). This may also contribute to the observed change in L type Ca^{2+} channel density during heart failure. An alternative possibility to explain the reduction in L type Ca^{2+} channel density is a reduction in the density of the t-tubules, where the L-type Ca^{2+} channel and current are concentrated (see section 2.1.1). It has been shown in the canine pacing model of heart failure that t-tubule density decreases, leading to a decrease in peak I$_{Ca}$ density (Balijepalli et al., 2003). A similar decrease in t-tubule density has been observed in failing rabbit heart (Quinn et al., 2003). To date, only preliminary reports of t-tubule structure in failing human myocytes have appeared, and show conflicting results, one study showing a decrease in density (Wong et al., 2002) and the other no change (Ohler et al., 2002). Clearly, this issue requires a full investigation.

In summary, in ventricular myocytes from failing heart, L type Ca^{2+} channel density appears to be reduced, although other changes, for example increased basal phosphorylation and isoform switching, may help to maintain I$_{Ca}$ density.

Given the absence of changes in I$_{CaL}$ amplitude but a decrease in sarcoplasmic reticulum Ca^{2+} release, it is tempting to speculate that a decrease in EC coupling gain (see section 2.2.1) can explain Ca^{2+} dysfunction in heart failure. However, no changes in EC coupling gain have been shown in heart failure in rabbit (Pogwizd et al., 2001), dog (Hobai and O'Rourke, 2001) or human (Piacentino, III et al., 2003). In these cases, a decrease in sarcoplasmic reticulum Ca^{2+} content appears to be responsible for defective EC coupling. In contrast, in rat a reduction

in the ability of I_{CaL} to trigger sarcoplasmic reticulum Ca^{2+} release was found (reduced EC coupling gain), without altered sarcoplasmic reticulum Ca^{2+} load (Gomez et al., 1997; Gomez et al., 2001). Three hypothesis has been proposed to explain this altered EC coupling gain during heart failure: (i) a change in the co-localization of L type Ca^{2+} channels and RyRs; (ii) an increase in the gap between the t-tubule and sarcoplasmic reticulum membranes; (iii) t-tubule remodeling (Gomez et al., 2001).

Given the reduction in sarcoplasmic reticulum Ca^{2+} release (either by reduced sarcoplasmic reticulum Ca^{2+} load or change in EC coupling), it might be expected that I_{CaL} inactivation would be slowed because of less Ca^{2+}-dependent inactivation. This has been observed in some (*e.g.* (Ahmmed et al., 2000; Gomez et al., 2001)), but not all, studies, although contaminating currents (*e.g.* Na/Ca exchanger) might explain this discrepancy. Such slowing of I_{CaL} inactivation will lead to greater Ca^{2+} entry during the action potential (see section 2.1.1) and will promote the occurrence of arrhythmias (EADs).

It is also noteworthy that frequency-dependent facilitation of I_{Ca} is blunted in myocytes from failing human heart (Piot et al., 1996; Sipido et al., 1998b; Barrere-Lemaire et al., 2000). Thus increasing stimulation rate induces a decrease of I_{CaL} which is associated with a decrease in action potential duration (Sipido et al., 1998b; Li et al., 1999). This might help explain the negative staircase (decrease of contraction with increase in rate stimulation) and the beneficial effects of slowing heart rate observed in heart failure.

Although I_{CaL} amplitude is unchanged in ventricular myocytes from failing hearts (above), a decrease has been described in atrial myocytes from two animal models (Li et al., 2000; Boixel et al., 2001), although heart failure induces a smaller decrease of I_{Ca} amplitude (~30%) than atrial fibrillation (~65%) and does not change action potential duration (Li et al., 2000) (see also section 5.2.3). However, in human failing atrial myocytes, I_{CaL} density appears unchanged (Mewes and Ravens, 1994).

No change in I_{CaL} and I_{CaT} density has been observed in sinoatrial node myocytes from a rabbit model of heart failure (Verkerk et al., 2003). However, I_{CaT} density has been reported to be increased in ventricular cells isolated from animal models of heart failure (e.g. cat (Nuss and Houser, 1993); hamster (Sen and Smith, 1994), rat (Martinez et al., 1999; Izumi et al., 2003; Ferron et al., 2003)) This increase is reminiscent of the electrophysiological phenotype observed in fetal cells and may therefore represent a reversion to the fetal phenotype: the balance between I_{CaL} and I_{CaT} density changes during development, with a progressive decrease of I_{CaT} density which is not detectable in most adult ventricular myocytes (*e.g.* ventricle (Cohen and Lederer, 1988); rabbit (Wetzel et al., 1993)). Interestingly, I_{CaT} can be

reexpressed when ventricular adult rat myocytes dedifferentiate during cell culture (Fares et al., 1996).

In contrast with animal models, no significant I_{CaT} has been detected in atrial or ventricular myocytes isolated from human hearts showing hypertrophy or dilated cardiomyopathy; *e.g.* (Beuckelmann et al., 1991; Le Grand et al., 1991; Chen et al., 2002; Piacentino, III et al., 2003). Therefore, a role for I_{CaT} in human heart failure and associated arrhythmogenesis appears unlikely.

The development of heart failure is poorly understood. It arises after cardiac hypertrophy, which is an adaptive response that helps the heart deal with an increase in workload. If this increase in workload has a pathophysiological origin (hemodynamic load, neurohormonal stimulus *e.g.* renin-angiotensin II, endothelin1, catecholamines), the hypertrophic response may progress from compensated (heart meets the need of the body) to decompensated hypertrophy, which leads to cardiac dysfunction and overt failure.

Work in transgenic mice suggests that Ca^{2+} signaling is a likely contributor to this process. Transgenic mice overexpressing the L type Ca^{2+} channel develop cardiac hypertrophy and cardiomyopathy and die of congestive heart failure (Muth et al., 2001). In contrast, the L type Ca^{2+} channel inhibitor diltiazem has been reported to blunt cardiac hypertrophy in a mouse model of familial hypertrophic cardiomyopathy (Semsarian et al., 2002). Ca^{2+} signaling can occur at different levels, and transgenic mice overexpressing calmodulin also show severe cardiac hypertrophy (Gruver et al., 1993). Similarly, transgenic mice overexpressing nuclear CaMKII-δ_B show cardiac hypertrophy and dilatation (Zhang et al., 2002a), and cardiac specific activation of calcineurin in transgenic mice induces a robust hypertrophic response that progresses to dilated heart failure (Molkentin et al., 1998). Conversely, calcineurin null mice fail to undergo cardiac hypertrophy in response to pressure overload, isoproterenol infusion or angiotensin II infusion (Bueno et al., 2002). Interestingly, NFAT3 null mice showed similar resistance to hypertrophy, establishing the critical downstream role of NFAT3 in mediating calcineurin-regulated hypertrophy (Wilkins et al., 2002) (see also section 2.3 and Figure 4).

In summary, it seems unlikely that I_{CaL} contributes to the defects in EC coupling observed in heart failure in large mammals (rabbit, dog, human). However, it may contribute to the development of arrhythmias (EADs) and to the negative staircase observed in heart failure. The role of I_{CaL} in the processes leading to heart failure via ET coupling requires further investigation.

5.2.3 Atrial fibrillation

Atrial fibrillation is the most frequently encountered arrhythmia in clinical practice (Kannel et al., 1982). Chronic atrial fibrillation is characterized by a disorganized high rate (atrial cells fire at 400-600 times per minutes) instead of the normal heart rate (~60 beats per minutes in human). An important advance in the understanding of atrial fibrillation was made with the recognition that atrial fibrillation, once initiated, alters electrophysiological properties in a manner that favours the ease of inducing and maintaining the arrhythmia: "atrial fibrillation begets atrial fibrillation" (Wijffels et al., 1995). This process is called "electrical remodeling". Sustained atrial fibrillation can cause heart failure after several weeks or months (Fenelon et al., 1996). Conversely, heart failure promotes atrial fibrillation and produces atrial ionic remodeling (Li et al., 2000).

At the cellular level, atrial fibrillation is characterized by a depolarized resting potential, a decrease of the action potential plateau, decreased action potential duration and loss of effective atrial contraction (see for review (Nattel, 2002)). Involvement of Ca^{2+} channels was suggested by an early study showing that the Ca^{2+} channel antagonist verapamil depresses the AP plateau of normal atria but not in atria from patients with atrial fibrillation (Hordof et al., 1976).

Animal models for atrial fibrillation have confirmed this suggestion. In a rapid pacing dog model of atrial fibrillation, I_{CaL} density was reduced without concomitant change in its kinetics or voltage dependence, compared with sham-operated dogs (Yue et al., 1997). In contrast, I_{CaT} density was not altered in this model. Rapid pacing also decreased action potential duration and action potential adaptation to rate. In contrast to to sham operated animals, nifedipine did not alter the action potential duration in the paced dog model suggesting that I_{CaL} depression was responsible for the action potential shortening in these dogs (Yue et al., 1997). Recognition that reduction of I_{CaL} is an important factor in atrial fibrillation was strengthened by the observation that I_{CaL} current density is decreased by ~ 65% in myocytes from patients with chronic atrial fibrillation (Van Wagoner et al., 1999). In contrast to heart failure, β–adrenergic responsiveness of atrial myocytes from patients with chronic atrial fibrillation is not impaired. Indeed, the relative response to a maximal dose of isoproterenol was greater in myocytes from patients with chronic atrial fibrillation (~4 fold) compared with patients with normal sinus rhythm (~3 fold) (Van Wagoner et al., 1999).

Although a decrease in I_{CaL} amplitude is assumed to be the most important factor in altering the duration of the action potential and its loss of adaptation to rate in atrial fibrillation, a decrease in the amplitude of the transient outward potassium current (I_{to}) has also been observed (Van Wagoner et al., 1999). The decrease in density of both currents has been

linked to transcriptional down regulation of α and β subunits of these channels (Bosch et al., 2003), although the molecular events leading to such ionic remodeling remain incompletely understood.

Thus although it is clear that the properties of I_{CaL} change during atrial fibrillation, it remains to determine whether I_{CaL} change is a cause or consequence of this pathology.

6 CONCLUSIONS AND FUTURES DIRECTIONS

The story of Ca^{2+} currents in cardiac myocytes is not over yet. The translation research, where the use of transgenic mice addresses integrative normal and pathological questions provides a helpful tool for a better understanding of Ca^{2+} current's function but also leads to conflicting results. The observation that knock out mice for $Ca_V1.3$, $Ca_V2.3$, $Ca_V3.1$, $Ca_V3.2$ are healthy, however $Ca_V1.2$ -/- transgenic mice die at the embryonic stage clearly emphasizes the crucial role of I_{CaL} in the heart. This is consistent with the observation that no cardiac diseases are linked to mutations of Ca^{2+} channels. It appears clear that we have to think in terms of microdomains for studying Ca^{2+} channels. This concept of microdomain is clear in neurons, and requires further investigation in cardiac cells. We described that ET coupling due to Ca^{2+} microdomain is important in neurons, however in cardiac myocytes it is still unclear how cells distinguish between regular global dynamic Ca^{2+} signals involved in EC coupling and Ca^{2+} involved in ET coupling. Furthermore, it is well established that I_{Ca} participates in the excitation-secretion coupling in neuronal cells, *e.g.* the release mechanism of neurotransmitter vesicles at the synapse, due to Ca^{2+} microdomains (See for review Neher, 1998). In cardiac myocytes excitation-secretion coupling concept has not yet emerged and to date only one report shows the importance of I_{CaT} in the secretion of atrial natriuretic factor by atrial cells (Leuranguer et al., 2000). The development of new microscopic techniques such as FRET, should provide new information regarding inter- and intra-molecular arrangements and compartmentalization. Hence, it appears that the term "Ca^{2+} currents story" in the heart is not so appropriate and perhaps should be renamed "the Ca^{2+} currents saga".

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Table 1: Nomenclature of Ca^{2+} channels. This table shows all Ca^{2+} channels described to date, for further information about non-cardiac Ca^{2+} channels see Catterall, 2000.
L: long lasting; T: tiny and Transient; N: Neither T nor L and Neuron; P: Purkinje; Q: After P; R: Resistant. HVA: High Voltage Activated; LVA: Low Voltage Activated. DHP: dihydropyridine.

Ca^{2+} channel	Ca^{2+} current	Previous nomenclature	Localization	Electrophysiological and pharmacological phenotype
$Ca_V1.1$	L	α_{1S}	Skeletal muscle	
$Ca_V1.2$	L	α_{1C}	**Heart**, Endocrine cells, Neurons, smooth muscle	HVA DHP sensitive
$Ca_V1.3$	L	α_{1D}	Endocrine cells, Neurons, **Heart**	
$Ca_V1.4$	L	α_{1F}	Retina	
$Ca_V2.1$	P/Q	α_{1A}	Neurons	
$Ca_V2.2$	N	α_{1B}	Neurons	HVA DHP insensitive
$Ca_V2.3$	R	α_{1E}	Neurons, **Heart**	
$Ca_V3.1$	T	α_{1G}	**Heart**, Neurons	
$Ca_V3.2$	T	α_{1H}	**Heart**, Neurons	LVA DHP insensitive
$Ca_V3.3$	T	α_{1I}	Neurons	

Table 2: Summary of the effects of NO/cGMP/PKG cascade modulation on basal I_{Ca} and prestimulated I_{Ca}. All these studies were performed with the whole cell configuration of the patch-clamp technique (otherwise stated). (WT: wild-type; TG: transgenic).

Modultion of cGMP dpt-pathway	Preparations	Tissu	Basal ΔI_{Ca}	ΔI_{Ca} prestimulated	cAMP-elevating agents	Refs
NOS inhibitors/NO scavengers						
L-NMMA 1 mM L-NNA 1 mM	Guinea pig	ventricle	↗			1
PTIO 0.5 mM	Guinea pig	ventricle	↗			2
L-NMMA 1mM	Human	atrium	↔			3
NO donors						
SIN-1 0.1-10 nM SIN-1 0.1-100 μM	Frog	ventricle	↔ ↔	↗ ↘ ↔	Fsk 0.3 μM / ISO 0.1-1 μM / cAMP 10 μM Fsk 0.3 μM / ISO 0.1-1 μM / cAMP 10 μM 8-Br-cAMP 10 μM	4
nitroprusside 10 μM	Guinea pig	ventricle	↔	↘	IBMX 0.1 mM	5
SIN-1 10 μM	Guinea pig	ventricle	↔	↘	IBMX 0.1 mM	6
SIN-1 10-100μM SIN-1 10μM SIN-1 100μM SIN-1 1-100 μM	Guinea pig	ventricle	± ↔	↗ or ↘ ↘ ↘	ISO 10 nM ISO 10 nM ISO 1μM / IBMX 0.1 mM / cAMP 10 μM or 8-Br-cAMP 10 μM	7
DEANO 100 μM GSNO 1 mM SNAP 1μM-1 mM SNP 5-500 μM SIN-1 0.1-1 mM SPNO 0.1 μM DEANO 10-100 μM DEANO 100 μM	Rat	ventricle	↔ ↔ ↔ ↔ ↔	↘ ↔ ↔ ↔ ↔ ↔ ↘	ISO 1-10 nM ISO 1-10 nM / cAMP 10-100 μM cAMP 10-100-μM ISO 1-10 nM ISO 1-10 nM cAMP 10-100 μM IBMX 10 80 μM	8
SNAP 1 pM-1μM DEANO 1 pM-1 nM SNAP 1 pM-1μM	Rat	ventricle	↔	↗ ↗ ↔	ISO 0.1-0.5 nM ISO 0.01pM-0.5 nM L858051 0.1-0.3 μM	9
DEANO 0.1 μM overexpressed PKG 1	TG vs WT mice	ventricle (cell-attached)	↘	↘	ISO 1μM	10
GSNO 10 μM	Newborn Rabbit	ventricle	↗	↗	IBMX 50μM	11
GSNO 10 μM	Adult Rabbit	ventricle	↔			11
SIN-1 1 pM-10 nM SIN-1-100 μM SIN-1 0.1-1 nM	Human	atrium	↗ moderate↗	↔	milrinone 10 μM	12
SNAP 100 mM	Human	atrium	↗			3
GSNO 10-100 μM	Rabbit	atrium	↗	↗ ↔	ISO 1 nM / IBMX 10 μM ISO 100 nM	13
cGMP intrapipette						
0.3-20 μM	Frog	ventricle	↔	↘ moderate ↘ ↔	ISO 0.3 μM cAMP intra 5-20 μM IBMX 0.1 mM 8-Br-cAMP 1μM	14
0.1-20μM	Frog	ventricle	↔	↘ ↔ ± ↔	ISO 0.3-2 μM/ cAMP intra 5-30 μM 8-Br-cAMP 5 μM cAMP 5 μM + IBMX 100 μM	15
5 μM	Guinea-pig	ventricle	↔	↘	cAMP intra 50 μM / 8-Br-cAMP 50 μM IBMX 0.1 mM	16
1-10 μM 1 mM	Guinea pig	ventricle	↔	↗ ↔ ↘ ↘	ISO 1-100nM / Fsk 0.5-1μM / cAMP 50-100 μM 8-CPT-cAMP / 8-Br-cAMP (both 50μM) IBMX 40 μM ISO 30 nM	17
30 μM 30-300 μM 1 mM 1 mM 100 μM	Guinea pig	ventricle	↗	↗ ↘ ↔ ↘	ISO 3-nM ISO 3-nM ISO 30 nM IBMX 0.1 mM	18
10 μM	Guinea pig	ventricle	↔	↘	IBMX 0.1 mM	5
0.1-100μM	Rat	ventricle	↔	↘	cAMP intra / 8-Br-cAMP intra / IBMX (all at 0.1mM)	19
100 μM	Young rabbit	ventricle	↗	↗	cAMP 100 μM	20

Modulation of cGMP dpt-pathway	Preparations	Tissu	Basal ΔI_{Ca}	ΔI_{Ca} prestimulated	cAMP-elevating agents	Refs
0.5 µM 50 µM	Human	atrium	↗ moderate↗			22
0.5 µM	Human	atrium	↗			
PKG activators						
8-Br-cGMP 20 µM	Frog	ventricle		↔	cAMP intra 5 µM	14/15
8-Br-cGMP 100 µM	Guinea-pig	ventricle		↘	IBMX 0.1 mM	16
8-Br-cGMP 10 µM	Guinea pig	ventricle	↔	±↘	cAMP intra 50µM	17
8-Br-cGMP 0.1mM	Guinea pig	ventricle	↔	↘	ISO 10 nM / IBMX 0.1 mM	6
8-Br-cGMP 1mM	Guinea pig	ventricle		↔ (ATPγSpip)	IBMX 0.1 mM	23
8-Br-cGMP 10-30 µM	Guinea pig	ventricle		± ↘	ISO 30 nM	18
8-Br-cGMP 100 µM	Guinea pig	ventricle		↔ ↘	cAMP 100µM IBMX 100 µM	24
8-Br-cGMP 0.1-10 µM	Young Rat	ventricle	↘			25
8-Br-cGMP 0.1-10µM	Rat	ventricle		↘	cAMP intra / 8-Br-cAMP intra (all at 0.1mM)	19
8-p-CPT-cGMP 100 µM	Rat	ventricle		↘	ISO 1 nM	8
8-Br-cGMP 30-100 µM 8-p-CPT-cGMP 30 µM	Newborn Rabbit	ventricle	↗ ↗			11
8-Br-cGMP 100 µM 8-p-CPT-cGMP 400 µM 8-p-CPT-cGMP 400 µM	Young rabbit	ventricle	↗ ↗	↗	IBMX 0.1 mM	20
8-Br-cGMP 30-100 µM	Adult Rabbit	ventricle	↔			11
8-Br-cGMP 30 µM	Rabbit	atrium	↗			13
8-Br-cGMP 300 µM	Rabbit	ventricle	↘	↘	IBMX 0.1 mM	26
8-bromo-cGMP 0.5-5 µM 8-bromo-cGMP 100 µM	Human	atrium	↔ ↗			22
8-Br-cGMP 0.1-1 mM	Cultured chick embryonic cells (cell-attached)		↘			27
8-Br-cGMP 1 mM	Cultured chick embryonic cells		↘	↘	PKA 1.5 µM	28
8-Br-cGMP 1 mM	Xenopus oocyte	α_{1c} (double microelectrode)	↘			29
8-Br-cGMP 1 mM overexpressed PKG 1	TG vs WT mice	ventricle (cell-attached)	↘	↘	ISO 1µM	10
8-Br-cGMP 1 mM	Mice	Ventricle (cell-attached)	↔	↘	ISO 1µM / 8-Br-cAMP 1 mM	30
PKG						
Active fragment 0.8 µM	Guinea pig	ventricle		± ↔	ISO 0.1 µM	17
Pre-activated PKG 50 nM	Young Rat	ventricle	± ↘	↘ ↘ ↘ ↗	ISO 2 µM Bay K 8644 1 µM IBMX 0.1-0.3 mM subsequent ISO 2 µM	25
Active fragment 10 nM-1µM	Rat	ventricle	↔	↘	cAMP intra / 8-Br-cAMP intra (all at 0.1mM)	19
Pre-activated PKG 25 nM	Cultured chick embryonic cells		↘	↔	Subsequent ISO 1-10 µM	28
Overexpressed PKG 1	TG vs WT mice	ventricle (cell-attached)	↔			10

References: (1) Gallo et al., 1998; (2) Gallo et al., 2001; (3) Vandecasteele et al., 1998; (4) Méry et al., 1993; (5) Levi et al., 1994; (6) Imai et al., 2001; (7) Wahler and Dollinger 1995; (8) Abi-Gerges et al., 2001; (9) Abi-Gerges et al., 2002; (10) Schröder et al., 2003; (11) Kumar et al., 1997; (12) Kirstein et al., 1995; (13) Wang et al., 2000; (14) Hartzell and Fischmeister, 1986; (15) Fischmeister and Hartzell, 1987; (16) Levi et al., 1989; (17) Ono and Trauwein 1991; (18) Shirayama and Pappano 1996; (19) Méry et al., 1991; (20) Han et al., 1998; (21) Rivet-Bastide et al., 1997; (22) Vandecasteele et al., 2001; (23) Shen and Pappano, 2002; (24) Sakai et al., 1999; (25) Sumii and Sperelakis, 1995; (26) Tohse et al., 1995; (27) Tohse and Sperelakis, 1991; (28) Haddad et al., 1995; (29) Jiang et al., 2000; (30) Klein et al., 2000.

Figures legends:

Figure 1:

Comparison of cardiac Ca^{2+} channels electrophysiological properties. Peak current-voltage relationships for L-type $Ca_V1.2$ (α_{1C}) and $Ca_V1.3$ (α_{1D}), and T-type $Ca_V3.1$ (α_{1G}) channels are normalized. L-type $Ca_V1.3$ channels activate at negative membrane potentials similar to T-type $Ca_V3.1$ channels. Activation midpoints ($V_{1/2}$) are approximately -5 mV for L-type $Ca_V1.2$, -30 mV for L-type $Ca_V1.3$ and T-type $Ca_V3.1$. Curves were generated by a Boltzmann-GHK function using parameters obtained from recombinant channels expressed in *Xenopus* oocytes recorded under similar conditions (10 mmol/L extracellular Ba^{2+}). Reproduced with permission from "L-Type Calcium Channels Highs and New Lows, 2002, Lipscombe D, Circ Res, 90, 933-2002".

Figure 2:

Time course of I_{CaL} in guinea-pig, rabbit, and rat myocytes voltage-clamped with a guinea-pig action potential waveform. Top panel: command voltage. Bottom panel: recordings of I_{CaL} normalised to cell capacitance averaged from 12 (guinea-pig), 10 (rabbit) and 10 (rat) cells. Reproduced with permission from "Profile and kinetics of L-type calcium current during the cardiac ventricular action potential compared in guinea-pigs, rats and rabbits, 2000, Linz K.W., Meyer R., Pflügers Archiv, 439, 588-599".

Figure 3:

Sparklet-spark coupling in heart cells. Data were obtained in the loose-seal patch-clamp conditions. A. Triggering Ca^{2+} spark by single L-type Ca^{2+} channel opening. B. Representative example showing that a Ca^{2+} sparklet directly triggers a Ca^{2+} spark. Reproduced with permission from "Imaging Microdomain Ca^{2+} in Muscle, 2004, Cells Wang, SQ, Wei, C, Zhao, G, Brochet, DXP, Shen, J, Song, LS, Wang, W, Yang, D, Cheng, H. Circ Res, 2004, 94, 1011-1022".

Figure 4:

Possible excitation-transcription coupling in cardiac myocytes. CaM, calmodulin; CamKII, Ca^{2+}-calmodulin-dependent protein kinase II; NFAT, nuclear factor of activated T cells; DREAM, downstream regulatory element antagonistic modulator; CREB, cAMP-responsive element binding protein. Sources for the interaction between the molecules listed above are cited in the text.

Figure 5:

Schematic diagram of L type Ca^{2+} channel modulation by cGMP pathway in cardiac myocytes. NOS, nitric oxide synthase; NO, nitric oxide; GC, guanylyl cyclase; AC, Adenylyl Cyclase; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; PDE, phosphodiesterase; PKG, cGMP-dependent protein kinase; PKA, cAMP-dependent protein kinase; β_1R, type 1 β-adrenoceptor; AKAP, A-kinase anchoring protein; PPases, Protein phosphatases; $Ca_V1.2$, L type Ca^{2+} channel. Sources for the interaction between the proteins listed above are cited in the text.

Figure 6:

Schematic representation of cardiac L-type Ca^{2+} channel $Ca_V1.2$ (α_{1C}) subunit with accessory subunits (β and $\alpha_2\delta$). AID, Alpha Interacting Domain; PKA, cAMP-dependent protein kinase; PKC, protein kinase C, PKG, cGMP-dependent protein kinase; CaM, calmodulin, AKAP, A-kinase anchoring protein; see text for details.
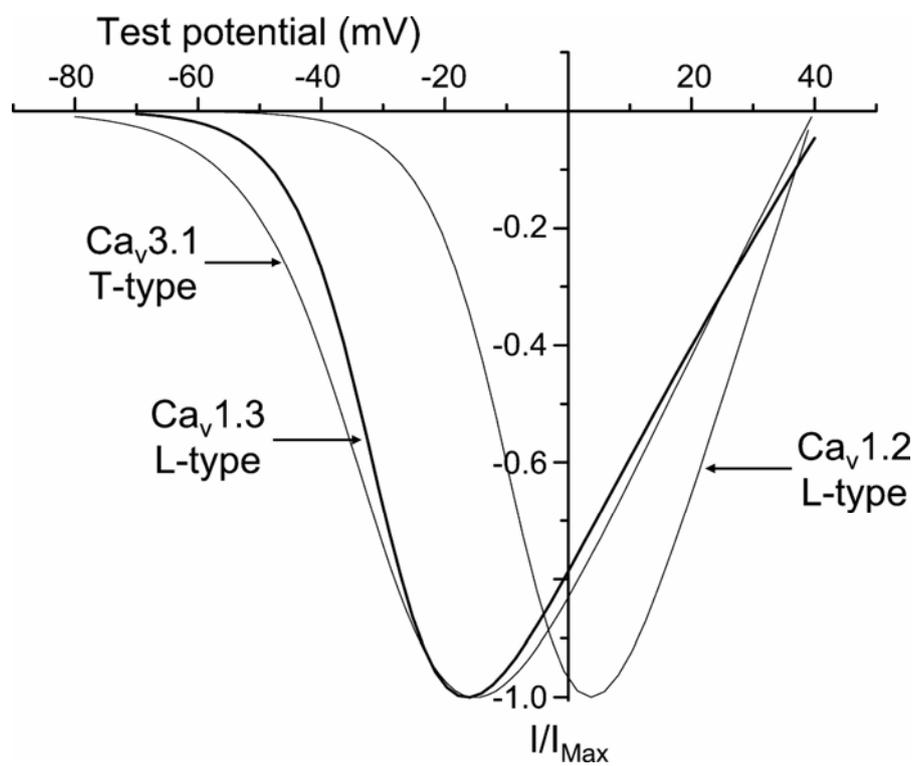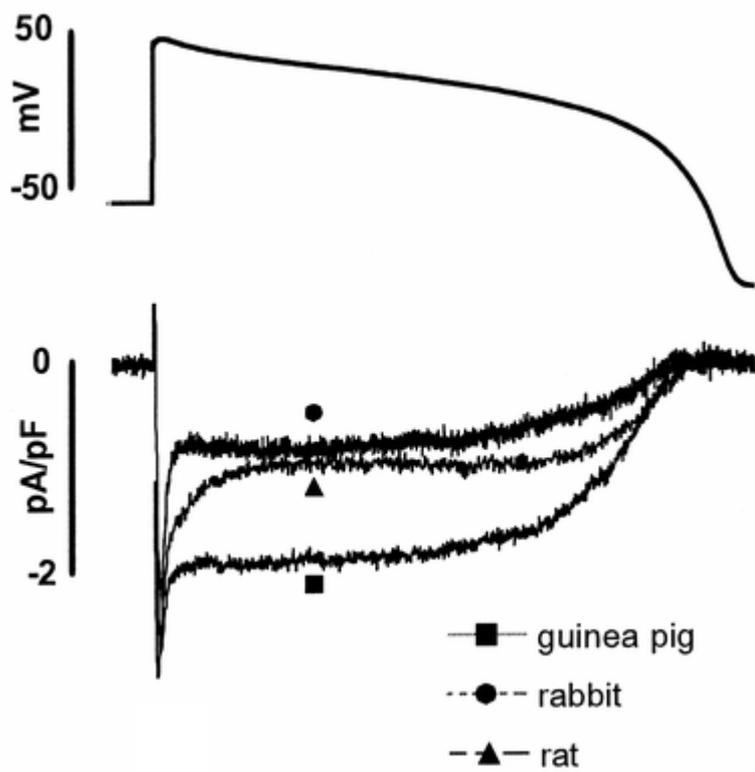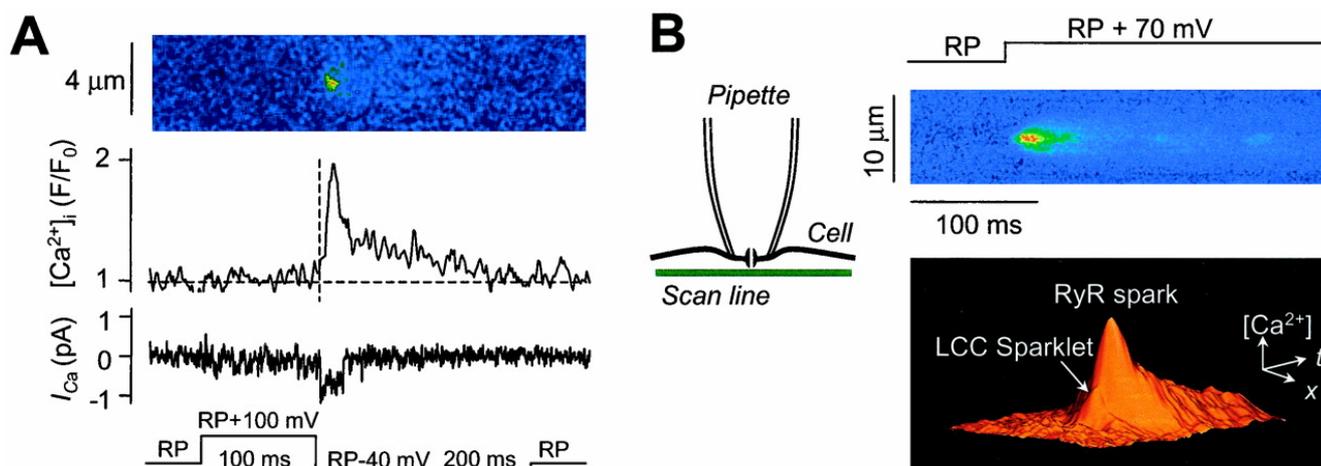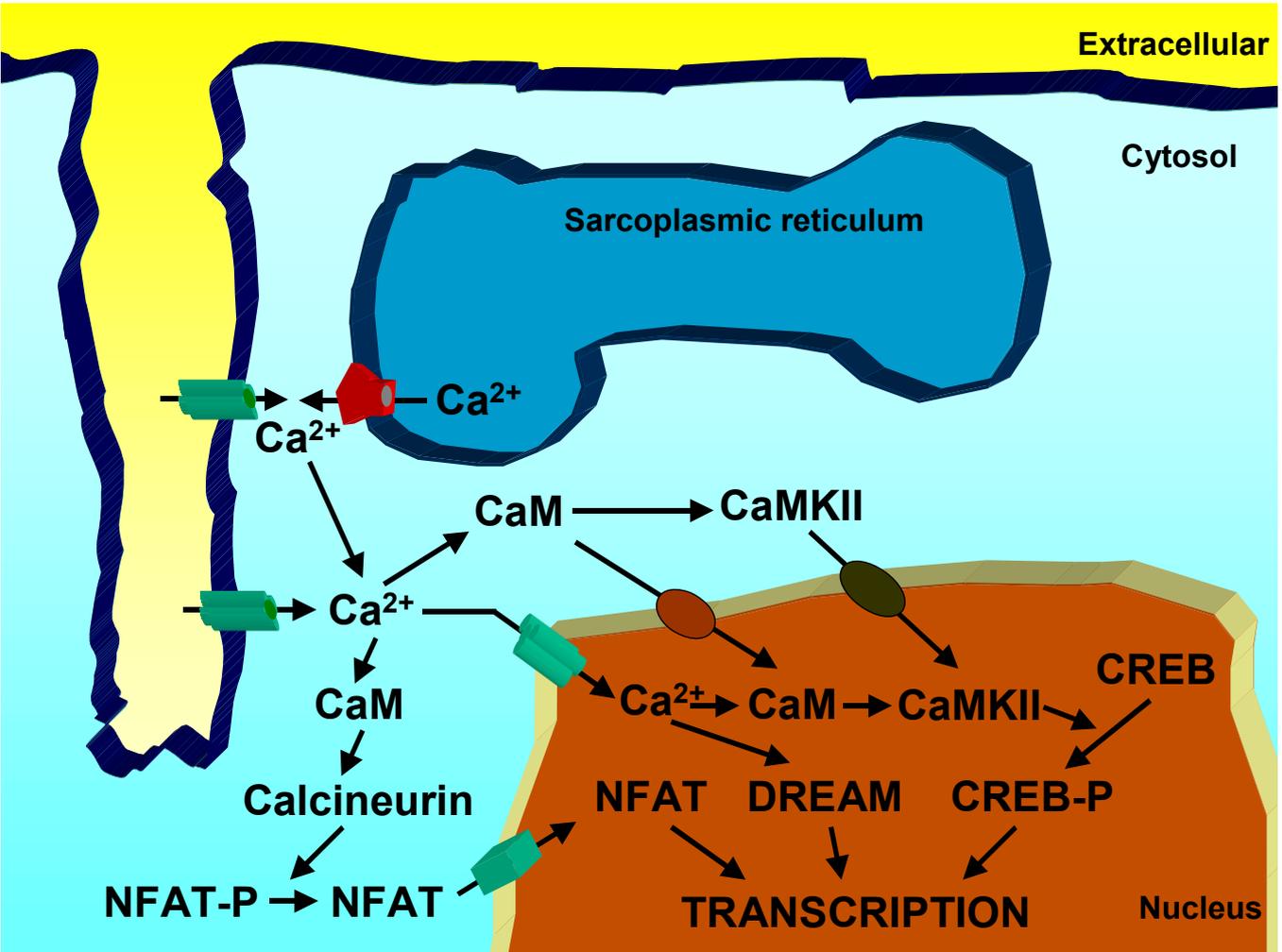
Figure 1

Figure 2

Figure 3

A

4 μm

$[Ca^{2+}]_i$ (F/F$_0$)

2

1

I_{Ca} (pA)

1

0

-1

RP | RP+100 mV 100 ms | RP-40 mV 200 ms | RP

B

Pipette

Cell

Scan line

RP | RP + 70 mV

10 μm

100 ms

RyR spark

LCC Sparklet

$[Ca^{2+}]$

t

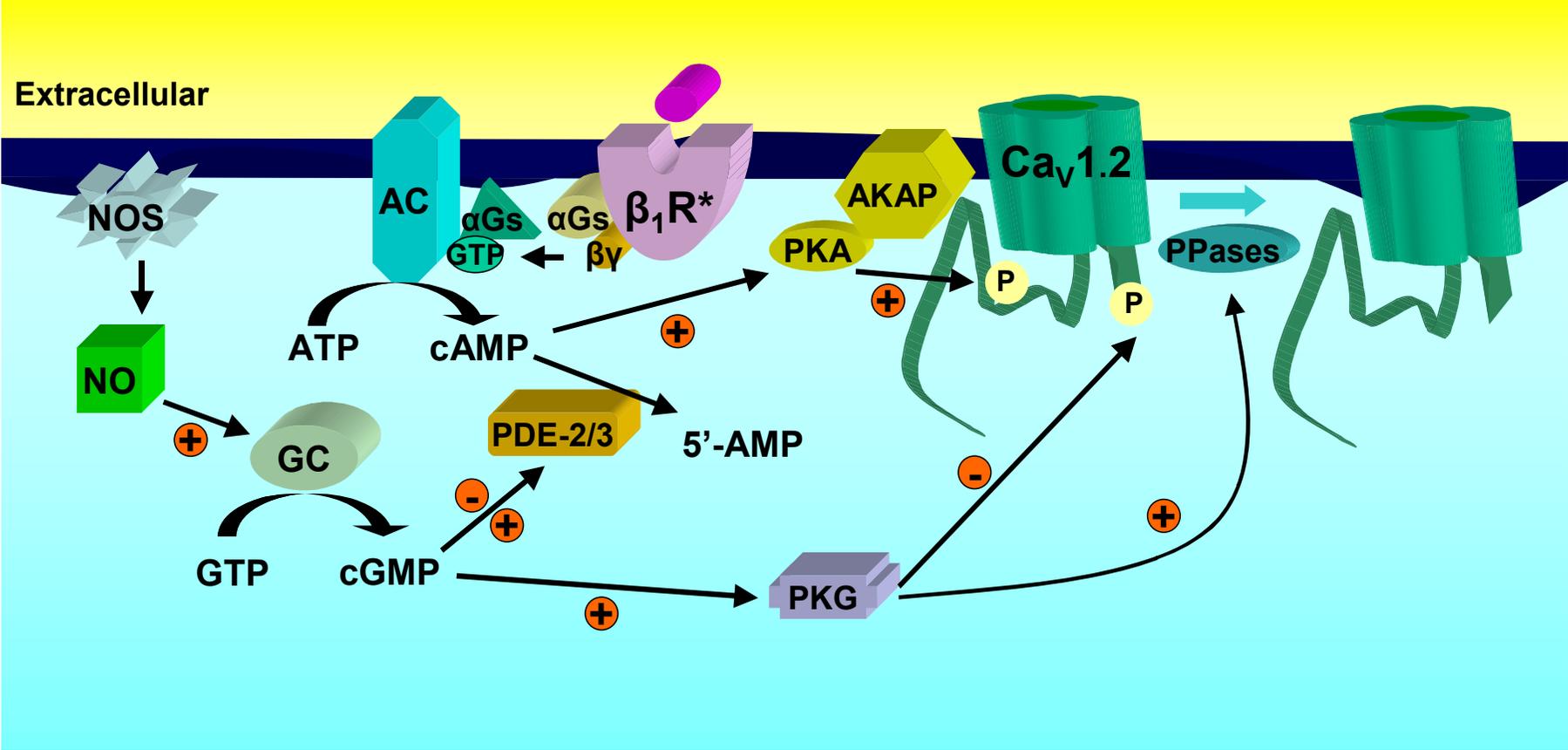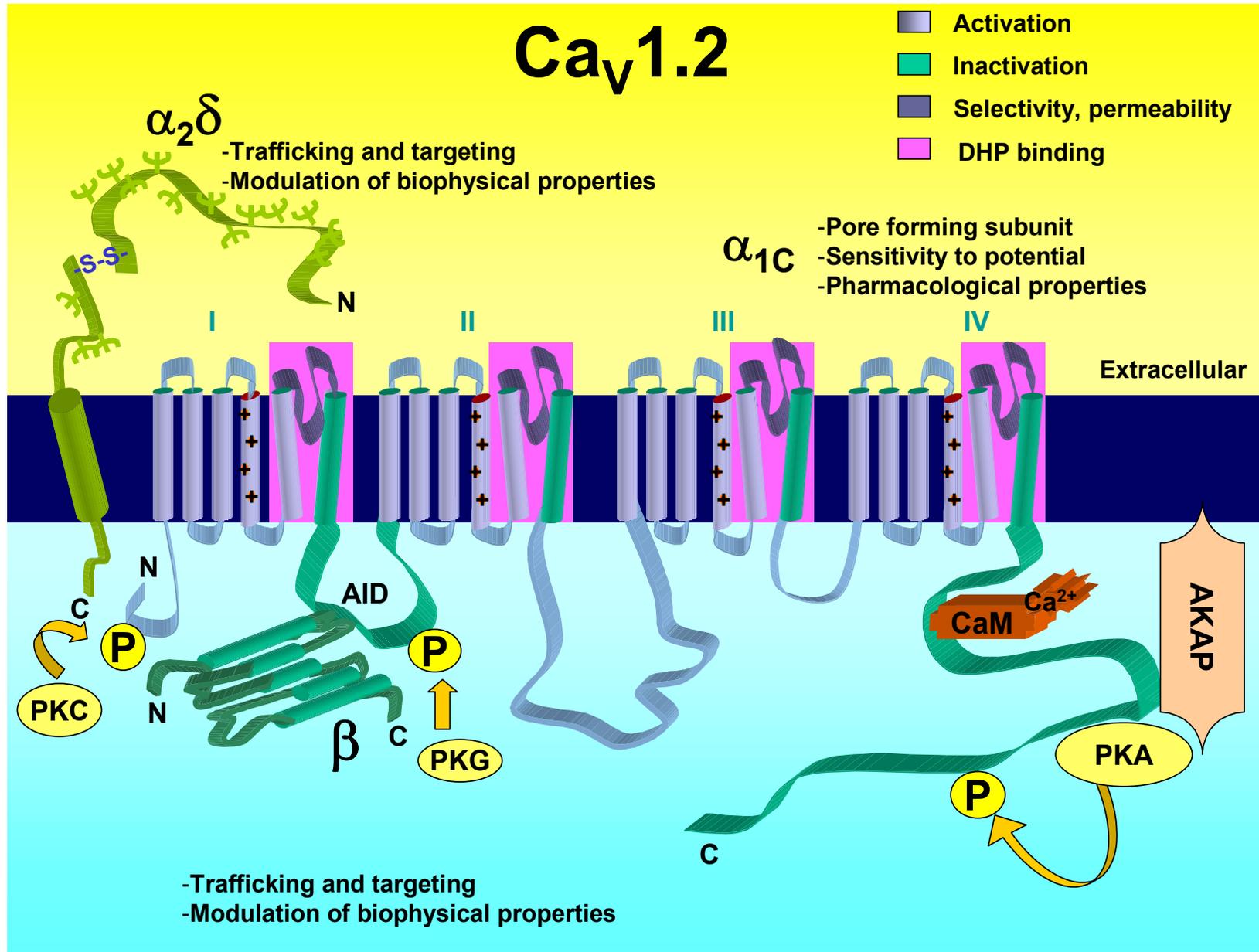x

Figure 4

Figure 5

Figure 6

(Isenberg and Klockner, 1982) (Rougier et al., 1969) (Lederer et al., 1990) (Fabiato, 1983) (Xiao, 2001) (Razani et al., 2002) (Harvey and Belevych, 2003) (Abi-Gerges et al., 2002) (Maurice et al., 2003) (Vandecasteele et al., 1998) (Razani et al., 2002) (Mery et al., 1993) (Imai et al., 2001) (Abi-Gerges et al., 2002) (Abi-Gerges et al., 2002) (Schroder et al., 2003)

(Kumar et al., 1997) (Wang et al., 2000) (Sumii and Sperelakis, 1995; Rivet-Bastide et al., 1997; Jiang et al., 2000; Klein et al., 2000; Shen and Pappano, 2002) (Malkiel et al., 1996; Brodde and Michel, 1999; Caforio et al., 2002; Kierszenbaum, 2003)

(Mijares et al., 1996b; Jahns et al., 2004)

(Hirano et al., 1999) (Brette and Orchard, 2003) (Lipsius et al., 2001) (Klockner et al., 1999) (Fabiato, 1983) (Trafford et al., 2002)

(Carl et al., 1995; Franzini-Armstrong et al., 1999; Scriven et al., 2000; Gathercole et al., 2000) (Ferrier and Howlett, 2001) (Dolmetsch, 2003) (Dolphin, 2003b) (Puglisi et al., 1999)) and rat (~ 40%, Takamatsu et al., 2003 (Takamatsu et al., 2003) (Hare and Stamler, 1999) (Harvey and Belevych, 2003) (Hare, 2003) (Eisner et al., 2000) (Levi et al., 1994; Gallo et al., 1998; Gallo et al., 2001) (Hartzell and Fischmeister, 1986; Fischmeister and Hartzell, 1987; Levi et al., 1989; Tohse and Sperelakis, 1991; Wahler and Dollinger, 1995; Tohse et al., 1995) (Hoebeke, 2001) (Magnusson et al., 1994; Catterall, 2000) (Perez-Reyes et al., 1998) (Woo et al., 2003b) (Romanin et al., 2000) (Zhou et al., 1997) (Gao et al., 1999) (Takahashi et al., 2004) (McGee et al., 2004) (Boutjdir, 2000) (Neher, 1998) (Lipscombe, 2002) (Linz and Meyer, 2000) (Balijepalli et al., 2003) (Bers, 2001).

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