Ca$^{2+}$ currents in compensated hypertrophy and heart failure

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Abstract

Transmembrane voltage-gated Ca$^{2+}$ channels play a central role in the development and control of heart contractility which is modulated by the concentration of free cytosolic calcium ions (Ca$^{2+}$). Ca$^{2+}$ channels are closed at the normal membrane resting potential of cardiac cells. During the fast upstroke of the action potential (AP), they are gated into an open state by membrane depolarisation and thereby transduce the electrical signal into a chemical signal. In addition to its contribution to the AP plateau, Ca$^{2+}$ influx through L-type Ca$^{2+}$ channels induces a release of Ca$^{2+}$ ions from the sarcoplasmatic reticulum (SR) which initiates contraction. Because of their central role in excitation-contraction (E-C) coupling, L-type Ca$^{2+}$ channels are a key target to regulate inotropy [1]. The role of T-type Ca$^{2+}$ channels is more obscure. In addition to a putative part in the rhythmic activity of the heart, they may be implicated at early stages of development and during pathology of contractile tissues [2]. Despite therapeutic advances improving exercise tolerance and survival, congestive heart failure (HF) remains a major problem in cardiovascular medicine. It is a highly lethal disease; half of the mortality being related to ventricular failure whereas sudden death of the other patients is unexpected [3]. Although HF has diverse aetiologies, common abnormalities include hypertrophy, contractile dysfunction and alteration of electrophysiological properties contributing to low cardiac output and sudden death. A significant prolongation of the AP duration with delayed repolarisation has been observed both during compensated hypertrophy (CH) and in end-stage HF caused by dilated cardiomyopathy (Fig. 1A) [4–8]. This lengthening can result from either an increase in inward currents or a decrease in outward currents or both. A reduction of K$^+$ currents has been demonstrated [6,9]. Prolonged Na$^+$/Ca$^{2+}$ exchange current may also be involved [9]. In contrast, there is a large variability in the results concerning Ca$^{2+}$ currents (I$_{Ca}$). The purpose of this paper is to review results obtained in various animal models of CH and HF with special emphasis on recent studies in human cells. We focus on: (i) the pathophysiological role of T-type Ca$^{2+}$ channels, present in some animal models of hypertrophy; (ii) the density and properties of L-type Ca$^{2+}$ channels and alteration of major physiological regulations of these channels by heart rate and β-adrenergic receptor stimulation; and (iii) recent advances in the molecular biology of the L-type Ca$^{2+}$ channel and future directions. © 1998 Published by Elsevier Science B.V.

1. Calcium, myocardial hypertrophy and heart failure

In addition to various structural, biochemical and energetic abnormalities, regulation of intracellular Ca$^{2+}$ ([Ca$^{2+}$]) is defective during HF. For full information, we recommend several papers and reviews that concentrate on specific aspects of the subject [10–13]. Briefly, acute and chronic forms of HF involve mechanical dysfunction during systole, diastole, or both phases of the cardiac cycle [11,13]. Reduced contraction and slowed relaxation can be observed in single ventricular myocytes isolated from failing human hearts [12]. The positive inotropy induced by accelerated heart rate is abolished in human HF [14]. This alteration, which is graded with the degree of ventricular dysfunction, [15] is associated with impaired [Ca$^{2+}$], handling [16]. In dilated human cardiomyopathy, there is a prolonged diastolic Ca$^{2+}$ transient (Fig. 1B) resulting from a diminished capacity to restore low resting Ca$^{2+}$ levels [7,16]. Increased resting or end-diastolic [Ca$^{2+}$], therefore results in Ca$^{2+}$ overload. Impaired Ca$^{2+}$ removal from the cytosol is an early manifestation of pressure overload hypertrophy which precludes impairment of myocardial relaxation.

The transition from compensated hypertrophy to cardiac dysfunction and overt failure is poorly understood. Decompensated eccentric hypertrophy defines end-stage HF,
whether ischemic or not. It is characterised by a large increase in cavity volume with respect to wall thickness. These alterations in cardiac size and shape reflect the ‘ventricular remodeling’ which may determine the occurrence of clinical signs of HF in dilated cardiomyopathy [17,18]. This change is associated with abnormalities of Ca\(^{2+}\) transport proteins and with quantitative changes in expression of the SR Ca\(^{2+}\) ATP-ase, the ryanodine receptor, and the Na\(^+\)/Ca\(^{2+}\) exchanger [13,19,20]. In animal models, depressed protein levels of phospholamban are also observed in overt HF but not in compensated hypertrophy [13,19].

### 2. T-type Ca\(^{2+}\) channels

In contrast to neurons that can express up to six types of I\(_{Ca}\) (I\(_L\), N, P, Q, R and T), cardiac cells express only L- and T-type I\(_{Ca}\). The L-type I\(_{Ca}\) (or I\(_{Ca,L}\); L for long lasting or large conductance) was first evidenced in multicellular cardiac preparations 30 years ago and initially termed ‘slow inward current’ [6,21,22]. The cardiac T-type I\(_{Ca}\) (I\(_{Ca,T}\); T for transient or tiny conductance) was discovered much more recently thanks to the development of the patch-clamp technique [23–26]. However, while our knowledge of the distribution, properties, functions and structure of L-type Ca\(^{2+}\) channels has increased extensively over this last period of time, much has still to be learnt about the T-type Ca\(^{2+}\) channels.

#### 2.1. Functional properties

T-type Ca\(^{2+}\) channels have been found in a wide variety of excitable and non-excitable cells [27]. They probably represent an heterogeneous subgroup of Ca\(^{2+}\) channels with significant differences in functional properties [28,29]. Their existence in the heart is well established [1,2,23–26]. The L- and T-type Ca\(^{2+}\) channels have distinct electrophysiological and pharmacological properties. During voltage-clamp depolarisation, I\(_{Ca,T}\) is characterised by a fast decay and slow rate of deactivation whereas I\(_{Ca,L}\) is more sustained and has faster deactivation. The L- and T-type Ca\(^{2+}\) channels are also distinguished by their threshold of activation and their voltage-dependence of availability for opening. I\(_{Ca,L}\) is activated by strong depolarisations whereas I\(_{Ca,T}\) is low-voltage-activated. At physiological concentration of extracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{o}\)), I\(_{Ca,T}\) activates at depolarisations ≥ −30 mV whereas I\(_{Ca,L}\) begins to activate at much more negative voltages (∼ −60 mV). I\(_{Ca,L}\) is fully available for activation at the resting membrane potentials of −50 mV whereas I\(_{Ca,T}\) requires more negative voltages (< −50 mV).

In addition to different unitary conductances (6 to 8 pS versus 21 pS, respectively, when using 110 mM Ba\(^{2+}\) ions as the charge carrier), T- and L-type Ca\(^{2+}\) channels have distinct regulatory and pharmacological properties [1,2,23–29]. For example, I\(_{Ca,T}\) is not stimulated by protein kinase A (PKA) activation which is a major regulation of I\(_{Ca,L}\). However, I\(_{Ca,T}\) is stimulated by growth hormones and by various agents releasing diacylglycerol (e.g. angiotensin II, endothelin-1, phospholipase C, phorbol esters) and is blocked by low concentrations of tetrodotoxin, U-88779E, Ni\(^{2+}\) ions and amiloride [1,2,25,26,30]. In contrast to I\(_{Ca,L}\), I\(_{Ca,T}\) is not the preferential target of those synthetic ligands widely referred to as ‘Ca\(^{2+}\) antagonists’ such as dihydropyridines (DHPs), phenylalkylamines and benzothiazepines (e.g. nifedipine, verapamil, diltiazem) despite potential blockade by these compounds [31]. The DHP agonist Bay K 8644 is probably the most selective L-type Ca\(^{2+}\) channel ligand (except in frog atrial cells) [2]. Of particular interest, the newly described compound Ro 40-5967 (mibefradil) is selective for I\(_{Ca,T}\) versus I\(_{Ca,L}\) [32]. In contrast to the L-type Ca\(^{2+}\) channel, the structure of the T-type Ca\(^{2+}\) channel family has not yet been identified.

#### 2.2. Physiological role in adult cardiac cells

I\(_{Ca,T}\) was first recorded in non-diseased cardiac cells from guinea pig ventricle and canine atrium and ventricle...
[23,24] and more recently in myocytes isolated from various species and tissues including canine Purkinje fibre, frog atrium and sinus venous, rabbit sinus arteriosus (SA) node and ventricle, chick embryonic ventricle and cat atrium [1,2,23–26,33,34]. When present, its amplitude ranges from only 10% in guinea pig ventricle to 100% of that of I_{CaT} in chick embryonic ventricle [33]. It is worth noting that I_{CaT} is scarce in ventricular cells from mature mammals (including rat, calf and rabbit), except perhaps in guinea pig, and that it has always been found concomitant with I_{Cal}. Because of its higher expression in sinus cells and its low threshold of activation, I_{CaT} has been suggested to play a role in the setting of the firing threshold in Purkinje fibres and nodal cells (pacemaking activity) [26] and ‘latent pacemaker’ activity in feline atrium [34]. I_{CaT} does not seem to play a role in triggering the SR Ca^{2+} release or in SR Ca^{2+} loading [1,2].

2.3. Re-expression in diseased cardiac cells

The physiological function(s) of T-type Ca^{2+} channels is (are) not clearly defined. In addition to a putative contribution to automaticity in adult cardiac tissues, I_{CaT} seems to be associated with rapid postnatal growth and hypertrophy. The presence of I_{CaT} during early development may be associated with the relatively undifferentiated (with respect to contractility) state of these tissues which also lack a well-developed T-tubular system [35]. For example, a robust I_{CaT} has been recorded in embryonic chick ventricular and freshly isolated neonatal rat ventricular cells [33,36]. I_{CaT} is apparently lost during maturation. However, mature atrial myocyte cells can re-express I_{CaT} under stimulation with growth hormones known to play significant roles in regulating cardiac growth during postnatal development and during hypertrophy. There is an increase in I_{CaT} in atrial myocytes from adult rats with Growth Hormone-secreting tumours [37]. A similar increase follows exposure of atrial cells to Insulin-Like Growth Factor 1 (IGF-1) in short term primary cultures of myocytes [38]. Re-expression of I_{CaT} has also been shown in adult rat ventricular myocytes grown in vitro during primary culture [39].

There is growing evidence that T-type Ca^{2+} channels mediate the responses to various hypertrophic signals. The expression of I_{CaT}, absent in myocytes isolated from normal adult feline left ventricle, is promoted by long-standing pressure-overload-induced hypertrophy [40]. Similarly, in a genetically determined cardiomyopathic Syrian hamster which develops a progressive and ultimately fatal congestive HF, I_{CaT} had a 2 to 3 fold higher density than in normal cells and displayed abnormal activation and inactivation kinetics whereas the density and the properties of I_{Cal} were not altered [41]. These alterations suggest a contribution of I_{CaT} to the pathogenesis of Ca^{2+} overload and to arrhythmogenic activity in this cardiomyopathy. Moreover, the Ca^{2+} antagonist Ro 40-5967, selective for I_{CaT} versus I_{Cal}, compared to the DHP amlodipine, improved survival in a rat model of chronic HF suggesting a possible involvement of T-type Ca^{2+} channels in this pathology [42].

In contrast with feline hypertrophied left ventricular myocytes and cardiomyopathic hamster hearts, in which I_{CaT} is overexpressed, no significant I_{CaT} has been detected in atrial or ventricular cells isolated from human tissues with various diseases including CH and dilated cardiomyopathy [6,43–47]. Although a low-voltage activated I_{Ca} has been detected in human atrial cells, this current is unrelated to T-type Ca^{2+} channels [48]. Rather, it seems to reflect the presence and activation of Na^{+} channels with an abnormal permeability for Ca^{2+} ions. In summary, the potential pathophysiological role of T-type Ca^{2+} channels in the development of hypertrophy and dilated cardiomyopathy has still to be demonstrated in humans. Furthermore, the presence of I_{CaT} in human cardiomyocytes and its potential role in pacemaking activity remains speculative since the tissues that are likely to express it (based on animal models studies) are not readily available for scientific studies.

3. L-type Ca^{2+} channels

3.1. Structure, functional properties and role

In contrast to I_{CaT}, I_{Cal} has been found in all cardiac cells that have been studied to date. I_{Cal} is both a trigger for Ca^{2+} release and a route to Ca^{2+} for refilling the SR and then plays an important role in E-C coupling. The cardiac L-type Ca^{2+} channel is a multimeric protein. The pore-forming subunit α1, which bears pharmacological binding sites for Ca^{2+} channel modulators, is associated with a transmembrane α2-δ subunit and a cytoplasmic β subunit (Fig. 2). A schematic model of the α1 subunit primary structure is presented in Fig. 3. Most of the important regions for Ca^{2+} channel activity and selectivity, such as pore localisation, are indicated as well as the drug binding regions of Ca^{2+} channel antagonists. The α1

![Fig. 2. Subunit composition of the cardiac L-type Ca^{2+} channel.](image-url)
Fig. 3. General structure of the α\textsubscript{1c} subunit with the functionally important regions.

subunit of the cardiac L-type Ca\textsuperscript{2+} channel is encoded by the class C gene (α\textsubscript{1C} subunit). Heterologous expression in Xenopus oocytes or fibroblastic cell lines allows recordings of functional expression of I\textsubscript{CaL} inhibited by DHP antagonists and activated by DHP agonists. The human α\textsubscript{1C} subunit has been cloned and sequenced and alternative splicing variants have been shown to display specific pharmacological profiles [49]. A second degree of diversity is brought about by the variety of combinations with different β subunits (four genes) [50]. All β subunits increase the level of expression of α\textsubscript{1c}-directed I\textsubscript{Ca} in expression systems [50]. The α\textsubscript{1c} subunit on its own induces only weak Ca\textsuperscript{2+} channel currents but when co-expressed with cardiac β subunits the current is increased several fold. Both activation and inactivation kinetics are also accelerated, as observed in animal cells [50]. Therefore, the β subunit is considered as a true endogenous modulator influencing the electrical activity as well as the pharmacological properties of Ca\textsuperscript{2+} channels [6,50]. One type of β subunit isoform and several spliced variants have been cloned in the human heart. Studies at the mRNA level suggest that cardiac L-type Ca\textsuperscript{2+} channels contain the α\textsubscript{1C} and the β\textsubscript{2a} subunit isoforms [51,52].

Biochemical studies have demonstrated that several kinases, including PKA, PKC, cGMP-dependent kinase and calmoduline kinase II, can phosphorylate cardiac Ca\textsuperscript{2+} channels [1]. The best known regulation of I\textsubscript{CaL} in cardiac cells is the regulation by β-adrenergic receptor stimulation which activates the intracellular cAMP cascade resulting in increased opening probability of Ca\textsuperscript{2+} channels [1]. Only the α and the β subunits of the Ca\textsuperscript{2+} channels possess consensus phosphorylation sites for PKA (Fig. 3) and have been reported to be phosphorylated in vitro. Despite extensive study of the pathway, the specific events that underlay protein phosphorylation in vivo are not clearly identified yet. There is controversy as to whether expressed cardiac L-type I\textsubscript{Ca} can reproduce or not the expected increase following activation of the PKA pathway [53] suggesting that the phosphorylation may also involve an unidentified protein expressed in cardiac tissue [54]. Interestingly, it has been recently found that the PKA-mediated regulation of L-type Ca\textsuperscript{2+} channels is critically dependent on a functional A-Kinase anchoring protein called AKAP79 and phosphorylation of the cardiac α\textsubscript{1c} subunit at the C-terminal residue Ser1928 (Fig. 3) in native cardiomyocytes [55]. Though the β\textsubscript{2a} subunit is also an excellent substrate of PKA in vivo, its phosphorylation is apparently not sufficient to significantly modulate channel function [55].

3.2. I\textsubscript{CaL} in animal models of CH and HF

The results obtained by many groups in various animal models are heterogeneous and puzzling [4,5,56–70]. Table 1 summarises these results. Besides species-dependence, there are many reasons for the apparent heterogeneity generated by the disparity of models, the importance of hypertrophy, the degree of hemodynamic stress adaptive mechanism limited to CH and the stage of HF. The experimental procedure, in particular when isolating the cells with enzymes, may also be selective and introduce variability. There may also be various degrees of hypertrophy or failure among cells in a same tissue heart. Nevertheless, the general trend is that the effects of CH range from no change to significant increases of I\textsubscript{CaL} (or number of DHP-receptors) density whereas the effects of HF range from no change to significant decrease. There is apparently no major effect on the electrophysiological properties of I\textsubscript{CaL} (see Table 1). Interestingly, a recent study performed
therefore possible that the nature isoform of the 
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ergic sensitivity of I has also been shown to be de-
release. Ca release.

Other animal models provide valuable information, studies in 
occurring during CH and overt HF. Although studies of 
alteration of E-C coupling.

channel is modified during pathology leading to functional 
subunit or subunit composition of the cardiac L-type Ca channel resides in the inability of the Ca2+ channel to activate SR 
channels to activate SR.

using confocal microscopy and patch-clamp methods 
showed that, though I_{cal} density and SR Ca2+-release 
channels are normal in experimental rat models of hyper-
tension-induced cardiac hypertrophy and HF, the defect 
resides in the inability of the Ca2+ channels to activate SR 
Ca2+ release. Beta-adrenergic stimulation overcame this 
defect in hypertrophic but not in HF myocytes. Beta-adren-
ergic sensitivity of I_{cal} has also been shown to be de-
creased in some studies. Interestingly, re-emergence of the fetal isoform of the \( \alpha_1 \) subunit of the cardiac L-type Ca\(^{2+} \) channel has been reported during left ventric-
ular remodeling in non infarcted rat myocardium. It is 
therefore possible that the nature (isoform) of the \( \alpha_1 \) subunit or subunit composition of the cardiac L-type Ca\(^{2+} \) channel is modified during pathology leading to functional 
alteration of E-C coupling.

3.3. I_{cal} in diseased human cardiomyocytes

One important objective in cellular electrophysiology 
applied to human cardiac tissue is to analyse the alterations 
occurring during CH and overt HF. Although studies of 
animal models provide valuable information, studies in 
human cells, though still limited, have a major interest 
because no single animal model adequately represents the 
wide variety of causes and manifestations of clinical syn-
dromes. Investigations of I_{cal} in human cardiomyocytes 
started only a decade ago with the development of the 
patch-clamp technique, enzymatic cell dissociation proce-
dures and surgical techniques. At present, experiments are 
carried out routinely on single myocytes isolated from 
small pieces of atrial or ventricular myocardium excised 
from hearts of patients undergoing corrective open heart 
surgery or cardiac transplantation. These studies have 
already provided valuable information concerning the 
nature, biophysics, pharmacology and regulation of ionic 
currents in normal and diseased tissues. However, potential 
modifications of I_{cal} or Ca\(^{2+} \) channel proteins during CH 
and HF are not well established in humans (see Table 2).

There is a general agreement that I_{cal}, but not I_{ca,t}, is 
commonly found in both right atrial and ventricular cells 
(6,36,43-48,72). Human I_{cal} exhibit properties similar to 
those of other mammalian species (6,36,43-48,72-74); 
e.g. it begins to activate at \(-40 \) to \(-30 \) mV, is maximal at 
\( \sim +10 \) mV with decay kinetics best described by a two 
exponential process (time constants \( \tau_1 \) and \( \tau_2 \) of approxi-
Table 2

<table>
<thead>
<tr>
<th>Model</th>
<th>$I_{CaL}$ / DHP-binding sites</th>
<th>Electrophysiology/Pharmacology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single ventricular myocytes end-stage HF</td>
<td>No change ($I_{CaL}$)</td>
<td>I/V curve and steady-state inactivation unchanged; Effect of forskolin unchanged</td>
<td>[76]</td>
</tr>
<tr>
<td>Left ventricular myocardium of end-stage HF</td>
<td>Decrease (DHP sites)</td>
<td>Decreased sensitivity of $I_{CaL}$ to $\beta$-adrenergic and serotoninergic stimulations</td>
<td>[75]</td>
</tr>
<tr>
<td>Atrial and ventricular myocytes</td>
<td>Decrease ($I_{CaL}$)</td>
<td>Upregulation of $I_{CaL}$ by high rates of stimulation is altered</td>
<td>[84]</td>
</tr>
<tr>
<td>Atrial and ventricular myocytes</td>
<td>Single ventricular myocytes end-stage HF</td>
<td>Left ventricular myocardium of end-stage HF</td>
<td>Atrial and ventricular myocytes</td>
</tr>
</tbody>
</table>

It is also generally agreed that there are no major functional abnormalities in the basic properties of $I_{CaL}$ in isolated myocytes from failing heart [45,46,75]. Recent data suggest that there is a decrease of $I_{CaL}$ in diseased human atrial myocytes [6,43,44] and in atrial and ventricular cells from failing hearts [75]. In agreement, a decrease (40–50%) in the number of DHP binding sites in end-stage failing hearts has been shown [76] but some results suggest that there is no effect of HF on the density of $I_{CaL}$ in human ventricular myocytes [7]. Therefore, as observed in animal models, the effects of HF range from no change to a substantial decrease. It is interesting to note that the apparent difference which is observed for example between dilated and non-dilated atria disappears when $I_{CaL}$ is submitted to maximal stimulation by $\beta$-adrenergic agents or by the DHP agonist Bay K 8644. This may indicate that dilatation reduces the basal activity of Ca$^{2+}$ channels rather than the number of functional channels. However, an alteration of both the $\beta$-adrenergic (Fig. 4) and the serotoninergic potenations of $I_{CaL}$ has also been shown in cardiomyocytes isolated from human hearts with end stage failure [75,77] which may reflect a down-regulation of receptors as has been well described for $\beta$-adrenergic receptors (mainly $\beta_1$) [78].

3.4. Frequency-dependent regulation of $I_{CaL}$ in HF

Modulation of $I_{CaL}$ by frequency of activation is probably important for heart physiology. Heart rate has long been known as a determinant of cardiac performance in the normal heart in vivo. In many animal species, including human, it has been shown that increasing the cardiac frequency induces a positive inotropic effect [79,80], known as the force-frequency relation or Bowditch `staircase' [81]. In physiological conditions, increased reflex norepinephrine release and circulating catecholamines during exercise induce both positive inotropic and chronotropic effects, so that an increase in heart rate is always coupled with an augmentation of contractility. In addition to the direct effect on cardiac contraction, amplification of the resting force-frequency relation during $\beta$-adrenergic stimulation is an important indirect mechanism regulating myocardial contractility in vivo [82]. Recent experiments showing amplification of the force-frequency relation by $\beta$-adrenergic receptor stimulation and by exercise in vivo have re-emphasised the importance of this potent inotropic mechanism in the physiology of the normal heart [82]. Various subcellular mechanisms have been proposed to be involved in the force-frequency relation. Though a role of L-type Ca$^{2+}$ channels, which constitute a major target of the $\beta$-adrenergic receptor stimulation, has been ignored or minimised, early studies suggested that an increase in the rate of cell stimulation can up-regulate Ca$^{2+}$ channel activity in mammalian cardiomyocytes [36,83]. Similar
Fig. 5. High frequency-induced upregulation of I_{Ca,L} and enhancement by isoproterenol in a human ventricular cell. The cell was isolated from the left ventricle of a transplanted heart obtained from a patient with terminal HF (ischemic cardiopathy; EF < 40%) and no drug treatment. Rates of stimulation were applied as shown in inset using the whole-cell patch-clamp technique (for details see [44] and [84]). This effect was rarely observed in cells isolated from failing hearts. It was more consistent in atrial myocytes isolated from patients with EF > 40% and no drug treatment (see Fig. 6). Currents were recorded as described in Fig. 4 (for further details and information see [84]).

frequency-dependent regulation of I_{Ca,L} can also be observed in myocytes from both right and left human atria and ventricles (Fig. 5) [36,84]. It occurs over a range of frequencies corresponding to heart rates frequently encountered in human pathophysiology [84]. This effect, related to changes in the gating properties rather than an increase in the number of active Ca^{2+} channels, is augmented by β-adrenergic receptors stimulation (Fig. 5) (and by other cAMP promoting agents) [36] and is probably involved in the amplification of the force-frequency effect by β-adrenergic stimulation. This regulation of transmembrane Ca^{2+} influx may be crucial in the adaptation of the normal human heart to stress and exercise [36,84].

Experiments in isolated myocardium from patients with moderate or end-stage HF suggest that the high rate-induced potentiation of cardiac contraction is impaired or absent [14,85,86]. Similar conclusions have been reached by increasing pacing rates during atrial or ventricular stimulation in patients with low left ventricular function [87]. In addition to abnormal intracellular [Ca^{2+}], handling [7,16], an alteration of transarcolemmal Ca^{2+} signalling via L-type voltage-gated Ca^{2+} channels may partly explain the impaired force-frequency relation observed in the failing myocardium. In particular, the high frequency-induced upregulation of I_{Ca,L} observed in atrial cells enzymatically isolated from hearts with a high ejection fraction (EF), is significantly altered in cardiomyocytes from end-stage failing hearts with low EF (Fig. 6). Stimulation by isoprenaline can preserve or enhance this upregulation in atrial cells taken from hearts with a high EF but not in those originating from hearts with low EF (Fig. 6) [84]. Consistently, stimulation by isoprenaline preserves the positive force-frequency in healthy, but not in failing, human hearts [88]. Since cAMP-dependent phosphorylation, resulting for example from β-adrenergic receptors stimulation, is important for the frequency-dependent facilitation of I_{Ca,L} in human cardiomyocytes, it is possible that alteration of this mechanism simply reflects the down regulation of β-adrenergic receptors in failing hearts [78] because no evidence for impairment of the signal transduction cascade beyond the level of GTP binding proteins has been found.

Fig. 6. Bar graphs showing the effect of HF on the potentiation of I_{Ca,L} by high rates of stimulation in human atrial and ventricular cells. Atrial cells were obtained during open heart surgery from non-failing hearts (EF > 40%) with various diseases such as aortic or mitral disease stenosis or insufficiency) or coronary artery disease. These patients were not treated with Ca^{2+} antagonists or β-blockers. Both atrial and ventricular cells were also obtained from explanted hearts of patients with terminal HF (ischemic or dilated cardiopathy; EF < 40%; no drug treatment). Experiments were performed as described in Fig. 4 (for details see [84]). Ca^{2+} entry was quantified by integrating I_{Ca,L} during the test pulse rather than by measuring peak current (see inset). Values (means ± SD) reflect comparisons between groups of patients (data for each patient were averaged from 2 to 6 cells). Currents were recorded as described in Fig. 4 (for further details and information see [84]).
4. Ca\textsuperscript{2+} channels and therapy of heart failure

4.1. Agents with positive inotropic activity

The \(\beta\)-adrenergic receptor agonists, such as dobutamine and norepinephrine, promote increased Ca\textsuperscript{2+} influx through Ca\textsuperscript{2+} channels via increased intracellular cAMP. They have a positive inotropic effect and improve the diastolic relaxation by enhancing Ca\textsuperscript{2+} re-uptake by the SR. Dobutamine is a synthetic catecholamine which is very effective acutely and widely used for short term therapy. However, both down-regulation and desensitisation of the \(\beta\)-adrenergic receptors rapidly induce intolerance and inefficacy of this drug. Furthermore, long term oral therapy with catecholamines is not only ineffective but accelerates mortality, in part because of increased arrhythmogenesis but also because it causes progression of cardiac dysfunction [89–91]. The use of phosphodiesterase inhibitors is also disappointing. These drugs have favorable acute hemodynamic effects without inducing short-term tolerance but, similarly to the \(\beta\)-adrenergic receptor agonists, they increase mortality during chronic therapy, apparently owing to ventricular arrhythmia and accelerated progression of the left ventricular dysfunction [92]. Therefore, cAMP-promoting agents produce little clinical benefit and only during short term therapy [88,93].

4.2. Rationale and beneficial effect of \(\beta\)-blocker therapy

The rationale for using \(\beta\)-blockers in congestive HF secondary to idiopathic dilated cardiomyopathy is based on the hypothesis that the disease is caused and/or worsened by abnormal activity of the sympathetic nervous system [90,91]. Although the mechanisms of benefit of \(\beta\)-blockade in patients with HF is probably multifactorial, both ‘up-regulation’ of \(\beta\)-receptors and prevention of Ca\textsuperscript{2+} overload may be involved in improvement of systolic and diastolic functions observed with this therapy in cardiomyopathy [94,95]. Furthermore, because the shape of the force-frequency relationship is inverted in human cardiac disease, reducing heart rate can improve contractility of the failing myocardium [12,15,16]. Numerous placebo-controlled studies have shown improvement of NYHA functional class and quality of life with \(\beta\)-blockers therapy in patients with idiopathic dilated cardiomyopathy. The long term hemodynamic effects include a consistent increase in ejection fraction and variable effects on exercise tolerance [94,95]. Two large prospective studies have demonstrated a beneficial effect of metoprolol and bisoprolol, respectively [94,95], concerning the number of readmissions to hospital due to worsening of HF but with no significant effect on survival on the total patient group. Therefore, the effect of \(\beta\)-blocker therapy on mortality in cardiomyopathies is uncertain. Large prospective studies are in progress (CIBIS II, Bisoprolol, BEST, Bucindolol).

4.3. Ca\textsuperscript{2+} channels blockers

By blocking the inward transmembrane I\textsubscript{CaL} and opposing the effects of increased [Ca\textsuperscript{2+}], Ca\textsuperscript{2+} antagonists induce coronary and peripheral vascular dilatation which results in increased coronary flow, reduced afterload and, therefore, reduced myocardial oxygen consumption. However, data accumulated from clinical trials conducted over the last decade have raised serious concerns regarding the safety of Ca\textsuperscript{2+} antagonists, in particular the short-acting Ca\textsuperscript{2+} channel blocker nifedipine, whether these drugs are used for hypertension, instable angina or recent myocardial infarction [96,97]. Concerning HF due to ischemic cardiomyopathy, the metanalysis of Furberg points out that Ca\textsuperscript{2+} channels blockers may exacerbate HF and that nifedipine provokes a dose-related increase in mortality in patients with coronary artery disease [96]. Ca\textsuperscript{2+} antagonists have a negative inotropic effect but their deleterious long term effects are mainly due to increased sympathetic activity and neurohormonal activation secondary to acute vasodilatation [96–98]. However, new Ca\textsuperscript{2+} antagonists such as amiodipine and felodipine (long-acting DHPs) are devoid of the problems linked to negative inotropy, positive chronotropy and neurohormonal activation. The PRAISE trial, a mortality and morbidity evaluation of amiodipine, showed significant benefit in patients whose heart failure was not due to coronary disease [99]. However, probably because Ca\textsuperscript{2+} antagonists lead to hemodynamic improvement without affecting other aspects of the pathophysiology of HF, they are unlikely to have an important effect on survival and progression of left ventricular dysfunction even if they do improve symptoms and exercise tolerance.

5. Concluding remarks and perspectives

Whether there is a change in L-type Ca\textsuperscript{2+} channel proteins or activity during CH and HF is not firmly established at the moment. Concerning animals, some heterogeneity probably arises from differences among species and models (degree of hypertrophy, stage of HF). Concerning humans, age, sex, pathology and drug treatment of patients certainly introduce variability in studies. In addition, studies are hampered by difficulties relating to regular obtention of homogeneous and abundant sources of tissues and to differences between experimental protocols. For example, most patients receive medication prior to and
during surgery. The interpretations may also be complicated when there is a lack of ‘normal’ tissue. However, despite the difficulties and limitations mentioned, it seems clear that L-type Ca$^{2+}$ channels are not involved in the prolongation of AP duration in HF because $I_{\text{cal}}$ is either decreased or unchanged. They have also probably no (or only a minor) role in CH. Nevertheless, a possible scenario could be that $I_{\text{cal}}$: (i) increases during CH to help maintain cardiac performance; and, then (ii) decreases during development of chronic hypertrophy and HF.

Impairment of Ca$^{2+}$ channel function in E-C coupling and of its major physiological regulations responsible for positive inotropy are more clearly evident than dramatic changes in Ca$^{2+}$ channel density and biophysical properties during HF. Increases of $I_{\text{cal}}$ by high heart rates and by $\beta$-adrenergic stimulation (and other camp-promoting agents), probably crucial for adaptation of the beating heart to exercise, are altered in human (Fig. 7). In addition to possible partial electromechanical uncoupling, these alterations may also contribute to prevent Ca$^{2+}$ overload, contractility and $O_2$ consumption and, thereby, decrease metabolic expenses of the working myocardium. Future studies should provide more information at the molecular level. Much remains to be studied to precise whether the Ca$^{2+}$ channel protein undergoes some change, in terms of $\alpha_1$ isoforms with distinct properties and/or subunit composition, that could account in part for some alterations of time- and voltage-dependent properties and of sensitivity to phosphorylation.

Finally, the cardiac T-type Ca$^{2+}$ channel has unique properties. Possibly involved in the pacemaker activity of the heart, its contribution and other function(s) remain to be clarified. Its expression (or re-expression) during early stages of development and during various hypertrophic states of the heart has been well established in animal models. For what role? The development of $I_{\text{cat}}$-selective Ca$^{2+}$ antagonists and the expected molecular cloning should add to our understanding of the pathophysiology of T-type Ca$^{2+}$ channels. These approaches are fundamental to developing new therapeutical strategies provided that $I_{\text{cat}}$ is present in the human heart and involved in pathophysiology.

Although less obvious than with animal models, working with human tissue provides valuable information. However, this approach cannot be exclusive and therefore should be used in parallel with animal models. The experience gained on human cells studies can help select animal models more closely related to the human pathology studied. Future studies will probably be based on techniques combining both molecular and electrophysiological approaches and on the forthcoming use of genetically engineered mice. Transgenic mice models of cardiac hypertrophy and dilated cardiomyopathy have been created [100]. The transgenic model of hypertrophy expresses oncogenic human H-ras, known to activate several features of hypertrophy, in a ventricular-specific manner. The model of dilated cardiomyopathy and heart failure reproduces the morphological and clinical pictures of pathology including.

![Diagram](image.png)

Fig. 7. Hypothetical schematic of the effect of HF on the human cardiac L-type Ca$^{2+}$ channel and regulation of $I_{\text{cal}}$ by the $\beta$-adrenergic pathway and rate of heart beating.
for example, cardiac dilatation accompanied by wall thickening and eccentric hypertrophy, impairment of both contractility and relaxation, decreased sensitivity to the β-adrenergic stimulation and myocardial fibrosis. Clearly these existing models and the creation of new models will be of great help for the development of new strategies to understand more precisely the role of Ca^{2+} (and other ion) channels.

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