Cardiac calcium currents at the level of single channels

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Summary. Properties of cardiac Ca channels have come into sharper focus with the advent of single cell preparations and suction pipette recording methods. We briefly summarize our present picture of the gating and permeation properties of the conventional, dihydrophyridine-sensitive type of Ca channel (L-type). Distinctive features of a second type of voltage-gated Ca channel (T-type) are discussed.
Key words. Heart cells; calcium channels; single channels.

Silvio Weidmann has often pointed out how the development of the microelectrode in the late 1940's was crucial to his scientific path[39]. Many of us who follow in his footsteps would do well to reflect on our own debts to analogous advances in biological techniques. Although none of us actually worked directly with Silvio himself, we have been privileged to collaborate with other members of his scientific family, including Robert Weingart, Harald Reuter, and John McGuigan. During various stays in Bern, we have enjoyed the hospitality of the department and felt the positive influence of Weidmann's elegant work. It is a great pleasure, therefore, to submit this brief paper as part of a general review of progress in the field of cardiac electrophysiology. Early experiments by Giebisch and Weidmann[11], Beeler and Reuter[5], Rougier, Vassort et al.[36] and Trautwein and collaborators[29], relied on sucrose gap methods to characterize Ca channels in working myocardial preparations. These studies led to a relatively simple description of cardiac Ca conductance as a single population of channels with a linear current-voltage relationship and first-order activation and inactivation kinetics[1]. As table 1 indicates, the picture has changed substantially over the last half-dozen years, thanks to the development of single cell preparations[32,33] and better methods for isolating Ca currents in multicellular preparations[17,26]. Among their other advantages, single cells allow the application of the suction pipette methods of Hamill et al.[13] or Lee et al.[20], thus permitting a) recordings at the level of single ion channels, b) improved control of the potential across the surface membrane, c) improved control of internal and external ion concentrations, better separation of ionic currents, and d) studies of effects of intracellular messengers or enzymes. These new approaches have been particularly helpful in the study of cardiac calcium channels. Nowadays, investigators interested in the analysis of Ca channel physiology and pharmacology often turn to cardiac cells as a system of choice.

Changing views of cardiac Ca channels

The combination of multicellular, single cell and single channel recordings have given mutually consistent information about the gating, selectivity, ion permeation and modulation of the predominant kind of cardiac Ca channel (L-type). *Voltage-dependent activation* is a multi-step process with a sigmoid time course[8,23,34] rather than a first-order reaction as originally believed. *Inactivation* of L-type Ca channels depends jointly on intracellular Ca and membrane depolarization per se[16,21]. *Selectivity* of L-type cardiac Ca channels is extremely high, with selective ratios for Ca over monovalent ions like Na or K of $> 10^3$ [14,25]. The selectivity is achieved through high affinity Ca binding to multiple binding sites arranged single-file within the pore[15]. The pore must be at least 5 A in diameter since ions as large as tetramethylammonium are permeant[27]. *Block of L-type Ca channels by multivalent ions* like Cd, Mg, Co takes place when blocking ions lodging within the pore, most probably at the Ca binding sites[19]. *Ca channel block by organic Ca antagonists* like nifedipine, verapamil or diltiazem involves a different mechanism[24], that includes stabilization of the channel in an inactivated state[2,37]. *Modulation* of L-type Ca channels by agents like epinephrine and Bay K 8644 takes place through different, non-occlusive changes in the pattern of channel gating[38].

Two types of cardiac Ca channel

For many years it had been believed that heart cells possessed a single class of Ca channels but recent evidence has raised new questions[22]. Among other evidence, whole cell recordings by Bean[3] and Mitra and Morad[28], and single channel recordings by Nilius et al.[30] have pointed to the existence of a new type of cardiac Ca channel, distinguished by its rapidly inactivating time course, its activation at relatively negative potentials, and its relative insensitivity to dihydropyridines

Table 1. Properties of cardiac calcium channels

	Old view (1981)	New view (1986)
Activation	First-order (exponential on, exponential off)	Multi-step (sigmoid on, multi-exponential off)
Inactivation	Purely voltage-dependent	Both voltage- and Ca_i-dependent
Selectivity	Comparable fluxes of Na and Ca	Ca flux exceeds flux of all monovalents
Open channel conductance	Ohmic	Rectifying (conductance increases on either side of E_{rev})
Channel types	One type	Two types: DHP-sensitive (L) and DHP-resistant (T)

Table 2. Properties of T- and L-type Ca channels in heart cells

	T	L
*Activation range (for ~ 5 Ca)	Positive to −60 mV	Positive to −30 mV
Inactivation range (for 10 Ca)	Negative to −50 mV	Negative to −20 mV
Inactivation rate, mechanism	Rapid, purely V-dependent	Slow without Ca, Ca_i, V-dependent
Unitary conductance (110 Ca/110 Ba)	8 pS/8pS	8 pS/25 pS
Single-channel kinetics (110 Ba)	Late opening, brief burst, inactivation	Hardly any inactivation
Rundown after excision	Slow	Fast
Cd block	Less sensitive	Sensitive
Ni block	Sensitive	Resistant
ω-CgTx via block	Weak, reversible	Insensitive
Enhancement by Bay K 8644, inhibition by nifedipine	No	Yes

* Based on whole cell recordings in dog atrial cells[3], guinea pig atrial cells (unpublished experiments) and guinea pig ventricular cells[28].

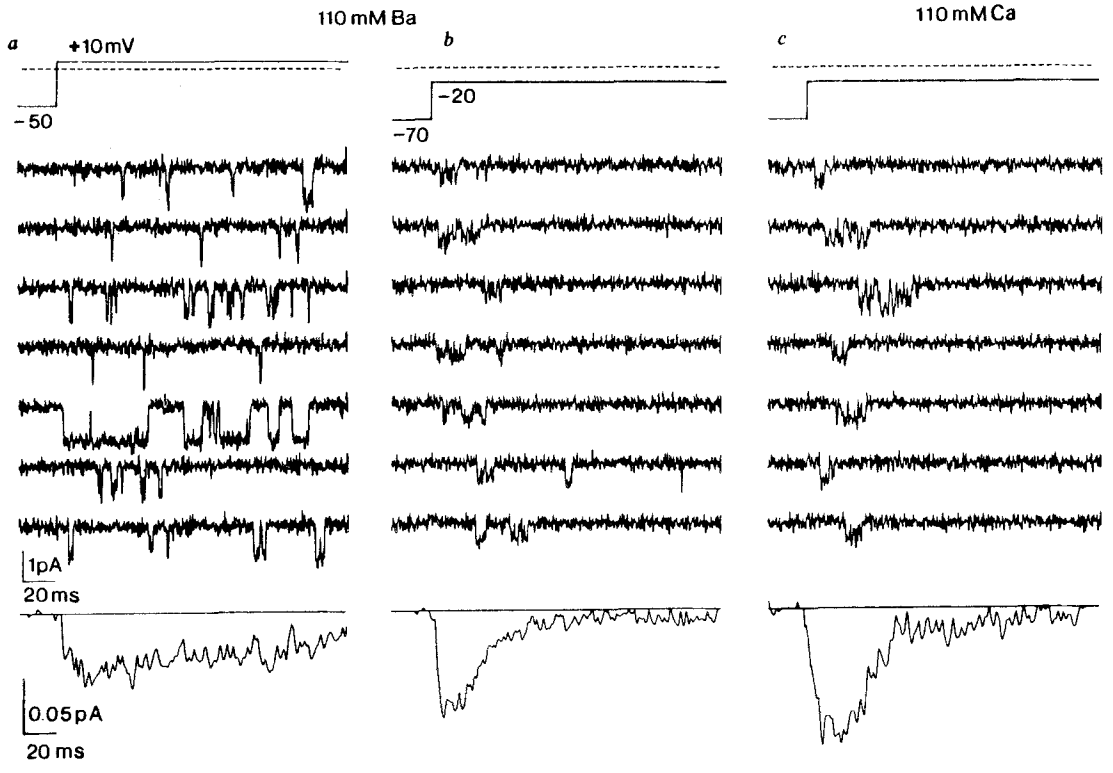

Figure 1. Evidence for the coexistence of two distinct types of Ca channel in guinea pig ventricular cells. *a, b* Cell-attached patch recordings with 110 mM Ba in the pipette. Activity of previously described type of Ca channel (*a*) or novel type of Ca channel (*b*) was evoked by appropriate voltage clamp protocols (top row). Zero membrane potential is indicated by the dotted line. A voltage step from a holding potential of −70 mV to a test potential of +10 mV produced a mixture of both types of activity (traces not shown). Selected sweeps with clearly detectable channel activity are illustrated. Averaged currents (bottom row) were obtained from all sweeps in each run (294 sweeps in *a*, 270 sweeps in *b*). The amplitude of the averaged currents is not meant to represent the overall balance between the two types of activity. Cell B2E. *c* Current traces recorded with 110 mM Ca in the pipette with the same voltage protocol as in *b*. The average current (bottom) was obtained from 179 sweeps. Note that the unitary current amplitude and average current time course are very similar with either 110 mM Ca (*b*) or 110 mM Ba (*c*) as the charge carrier. Cell B2C. Methods: Single guinea pig ventricular myocytes were freshly dissociated by enzymatic dispersion. Cell-attached patch clamp recordings[13] were made at room temperature (21 °C) with depolarizing pulses applied every 3 s. Patch pipettes contained 110 mM $BaCl_2$ or $CaCl_2$ plus 10 mM HEPES (pH 7.5 with TEA-OH). The membrane potential outside the patch was zeroed by an external solution containing (in mM): K-Aspartate 140, EGTA 10, Hepes 10 (titrated to pH 7.5 with KOH). The current traces were filtered (−3 db at 1 kHz, 8-pole Bessel filter), digitized at 5 kHz and stored and analyzed on a laboratory computer. Capacity and leak currents were subtracted digitally. From Nilius et al.[30].

like nifedipine and Bay K 8644. This novel kind of Ca channel has been labeled 'T-type' because of its tiny single channel conductance for Ba ions and its transient time course at most potentials. These properties are in contrast to the conventional, dihydropyridine-sensitive cardiac Ca channel, designated 'L-type' for its large unitary BA conductance and its relatively long-lasting time course at most potentials, and whose properties were briefly summarized above.

Figure 1 illustrates some of the features that distinguish T and L-type Ca channels. Activity of L-type channels (fig. 1a) was evoked by depolarizing pulses from a holding potential (HP) of −50 mV to a test level of +10 mV; with 110 mM Ba in the pipette, channel openings produce current pulses ~ 1.2 pA in amplitude. The averaged current is fairly well-maintained. Figure 1b shows strikingly different channel activity, recorded from the same patch with pulses from HP = −70 mV to −20 mV, a test potential too negative to activate L-type channels with 110 mM Ba in the pipette (external) solution. Here, openings appear as pulses of ~ 0.5 pA, and are generally grouped as a burst of activity near the beginning of each pulse. The averaged current peaks at ~ 10 ms and then decays rapidly. As the unitary current is larger in figure 1a than in b, despite the smaller driving force for Ba entry at the more positive test potential, there can be little doubt of the existence of two different types of channel.

The T-type Ca channel displays very similar unitary amplitude and slope conductance with either ~ 100 mM external Ba or Ca (8 pS). The time course of the averaged currents is also very much the same, regardless of the identity of the permeant divalent cation (fig. 1b, c). Both of these features are in contrast with the behavior of the L-type Ca channel. The slope conductance of the L-type Ca channel is about 2.5–3-fold larger with ~ 100 mM external Ba (18–25 pS)[8, 14, 38] than with ~ 100 mM Ca (8 pS). As already mentioned above, inactivation of L-type Ca channels is much faster with Ca rather than Ba.

Along with differences in Ca and Ba permeation, the two types of Ca channels also showed different sensitivities to block by Cd ions (fig. 2). The presence of micromolar Cd in the patch pipette inhibited L-type Ca channel activity, promoting a rapid flickering block of the long-lasting openings promoted by Bay K 8644[37]. In contrast this clear response, T-type Ca channel openings were not detectably changed in either their magnitude or duration. The difference in the Cd responsiveness of T and L-type channels was seen consistently in other experiments, and fits with previous results in neuronal preparations[10, 31].

The two types of Ca channels differ in their response to dihydropyridines. Nifedipine decreases and Bay K 8644 increases activity of L-type channels, neither agent has signi-

ficant effects on T-type channel activity at concentrations up to 10 μM. Another striking difference between T-type and L-type Ca channels is seen when patches are excised (fig. 3). As little as 1 min after manual excision of the patch from the cell-attached to the inside-out patch configuration, the activity of the L-type Ca channel disappeared irreversibly (fig. 3a, b), whereas T-type openings found in cell-attached patches (c) could still be recorded many minutes after excision without a clear sign of decreasing activity (d).

Analysis of whole cell recordings of T and L currents indicates that their relative size varies considerably from one part of the heart to another. The relative magnitude of T current is smallest in ventricular cells, larger in atrial cells[3], and largest in natural pacemaker cells (H. Irisawa, pers. comm.; B. P. Bean, pers. comm.). Using Ni as a relatively selective inhibitor of T-type channels, Irisawa and colleagues[12] have provided compelling evidence that activation of T-type Ca channels contributes significantly to the last phase of pacemaker depolarization in sinoatrial node cells. This leaves L-type Ca channels as the predominant pathway for Ca entry for excitation-contraction coupling and maintenance of the action potential plateau.

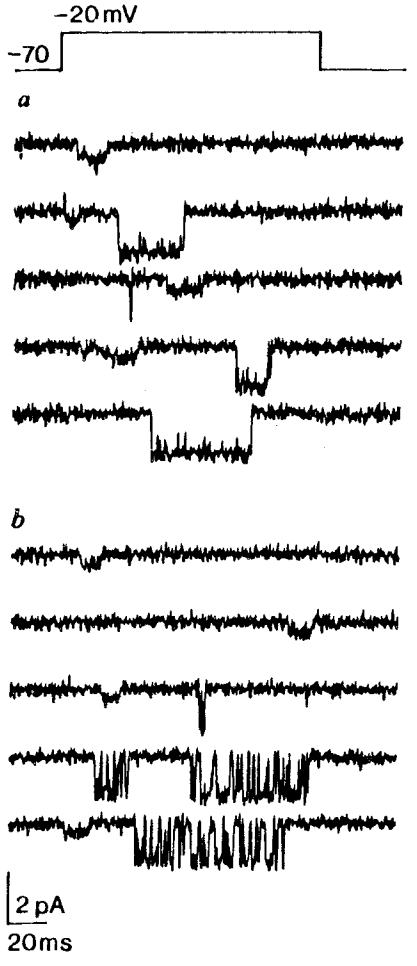

Figure 2. Different effects of Cd on T-type and L-type Ca channels. Selected current traces from two cell-attached patches. Pipettes contained 50 mM Ba as the charge carrier with no Cd (*a*) or 10 μM Cd (*b*). Bath solution contained 5 μM Bay K 8644. Upper trace shows voltage clamp protocol. Cells G34C, G34K. From Nilius et al.[30].

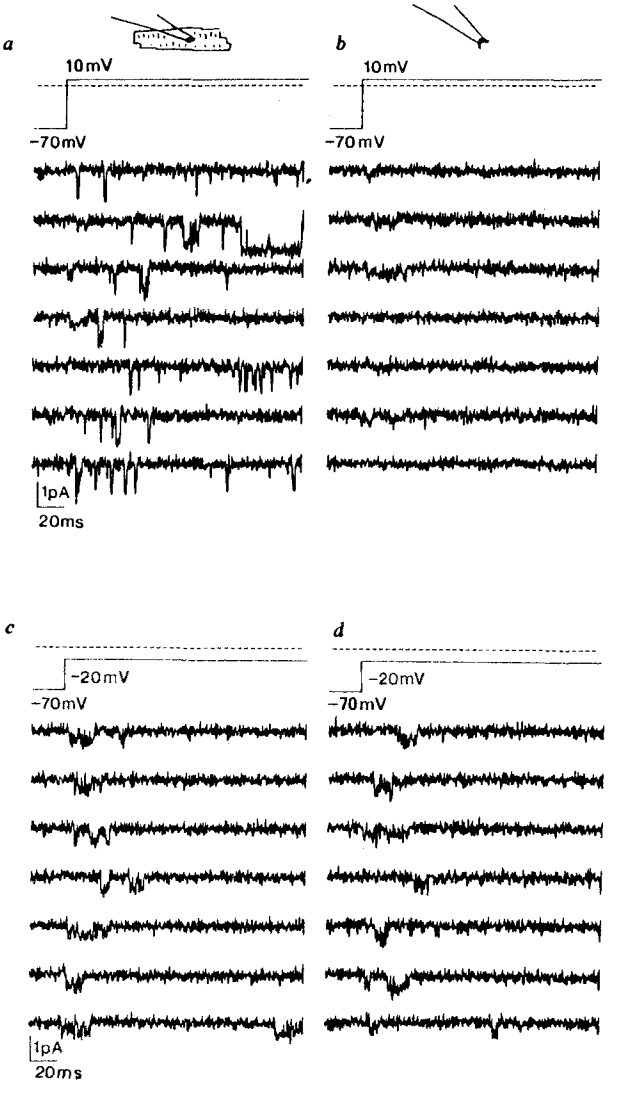

Figure 3. Differential effect of patch excision on the two types of Ca channels in a single patch. *a*, *c* cell-attached patch recordings of combined L-type and T-type Ca channel activity 10 min before excision (*a*) and exclusively T-type Ca channel activity 6 min before excision (*c*). *b*, *d* excised patch recordings 1 min after excision (*b*) and 6 min after excision (*d*). Activity of the small conductance T-type Ca channel remains unchanged (*b*, *d*), while activity of the large-conductance L-type channel could no longer be detected (*b*). Cell B2E. From Nilius et al.[30].

Future prospects

Some of the most exciting developments in the study of cardiac Ca channels still lie ahead of us. The recent description of two types of voltage-gated Ca channels has been closely followed by evidence for another Ca channel in cardiac sarcolemmal membranes that opens even at the normal resting potential[35]. Finding even a small density of such channels intact channels might carry important implications for the regulation of resting Ca levels. Also, with recordings of Lee et al.[22] in mind, one should be alert to the possibility of additional types of Ca channels.

Further experiments may clarify the functional roles of the various types of Ca channels, their distribution within a given cell (surface membrane, transverse tubules) and within different regions of the heart. It is widely assumed that L-type Ca channels in ventricular muscle tend to be localized

within the T-system, as in skeletal muscle, but this needs quantitative analysis. Last, but far from least, a host of questions can be asked about the structure of Ca channels. How closely related are various types of cardiac Ca channels to each other, to functionally similar Ca channels in other tissues, and to other types of channels? With efforts to apply biochemical and molecular genetic methods for channel purification and reconstitution, cloning and expression, answers to such questions may not be long in coming.

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