L-Type Calcium Channels, Potassium Channels, and Novel Nonspecific Cation Channels in a Clonal Muscle Cell Line Derived From Embryonic Rat Ventricle

Karin R. Sipido and Eduardo Marban

We have characterized the membrane currents in the H9c2 clonal muscle cell line derived from embryonic rat ventricle. These cells, established by selective serial passage and clonal proliferation, have been found by Hescheler and coworkers to express dihydropyridine-sensitive calcium channels that respond to \( \beta \)-adrenergic stimulation. We have investigated the macroscopic and elementary currents in these cells by using standard patch-clamp methods. In cells that are kept confluent for 3–4 weeks, we have confirmed the expression of L-type calcium channels and additionally established that the unitary conductance of many, but not all, of these channels (25 pS in 70 mM barium) is equal to that of cardiac rather than skeletal muscle. When the cells are proliferating rapidly, calcium channels are sparse or absent, but at least two distinct potassium channels and a nonspecific cation channel are observed. The nonspecific channel exhibits a conductance of 30 pS in physiological saline and conducts sodium, potassium, and calcium with nearly equal efficacy. Several unusual properties distinguish this nonspecific channel from others described previously. Gating is voltage dependent, with slow activation and marked increases in open probability at positive potentials. Unlike voltage, changes in \([Ca^{2+}]_i\) or in membrane stretch do not noticeably influence activity. In conclusion, our work and that of Hescheler et al indicate that H9c2 cells are potentially valuable surrogates for the investigation of ion channel regulation and muscular gene expression. (Circulation Research 1991;69:1487–1499)

S
ince the advent of enzymatic techniques for the dissociation of primary cells, much has been learned regarding the fundamental electrical properties of cells from a variety of cardiac tissues.\(^1\) Nevertheless, the use of primary cells entails a number of important limitations that are intrinsic to the approach: erratic technical success resulting from variable enzyme purity and other less tangible factors, genetic differences among individual source animals, and most importantly, the finite lifetime of such cells in primary culture. The latter feature makes primary myocytes unsuitable targets for some of the most powerful approaches in molecular biology, particularly stable transfection with foreign DNA.\(^2\) Unfortunately, immortal cardiac cell lines have proven notoriously difficult to establish. The cell line H9c2, initiated by Kimes and Brandt,\(^3\) was derived from embryonic rat heart. Cells were cultured and myoblasts selected by serial passage, to create a myoblast population from which the clonal H9c2 line was established. The initial characterization of this cell line\(^3\) confirmed that it was myocytic, as evidenced by expression of myofilament proteins and the M isozyme of creatine kinase, as well as by the cells’ ability to support action potentials and to contract in response to electrical stimulation. Although muscular in type, the depolarizing response of these cells to acetylcholine, as well as several other features, indicated a stronger homology to skeletal than to cardiac muscle. Kimes and Brandt argued that this represented a change in phenotype induced by long-term culture rather than spurious contamination with cells of skeletal muscle origin.

Since then, H9c2 has been passaged further and has become available commercially,\(^4\) but it has been largely neglected as a potential surrogate for primary
cardiac cells. Our interest in these cells was stimulated by a personal communication in 1988 from Jürgen Hescheler, who had made the intriguing observation that these cells develop L-type calcium channels with kinetics and $\beta$-adrenergic sensitivity reminiscent of those in primary cardiac ventricular myocytes (see Hescheler et al for a complete treatment). We confirm here that these cells do indeed exhibit L-type calcium channels and additionally establish that many of these channels have unitary conductance properties identical to those of cardiac (but not skeletal) muscle. The main focus of our work, however, has been on the characterization of a nonspecific channel that we discovered in the course of our initial survey of unitary currents in these cells. This channel carries sodium, potassium, or calcium and exhibits voltage-dependent gating but is neither activated nor inactivated by stretch or by cytoplasmic $[\text{Ca}^{2+}]$. These elementary properties of the nonspecific channel in H9c2 set it apart from all others that have been described to date.

FIGURE 1. Single-channel barium currents in an H9c2 cell. Panel A: Unitary currents evoked by depolarization (protocol as in top row) recorded from a cell-attached patch 5 weeks after plating. The pipette contained high-barium solution, and cells were depolarized in a high-potassium solution (see "Materials and Methods"). Panel B: After 5 $\mu$M Bay K 8644 was added to the bath, long openings were observed. Tracings at the bottom are ensemble averages from 150 sweeps (panel A) and 200 sweeps (panel B), excluding tail currents for clarity. Data were sampled at 10 kHz and filtered at 2 kHz.

Materials and Methods

Cell Culture

H9c2 cells were purchased from the American Type Culture Collection, Rockville, Md., at passage number 12 and grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO BRL, Gaithersburg, Md.) supplemented with 10% fetal calf serum (FCS, Sigma Chemical Co., St. Louis, Mo.). A stock of cells was grown in a culture flask and split every week. From this stock, cells were plated onto the polystyrene culture dishes (35 mm, Corning Glass Co., Corning, N.Y.) on which they were ultimately studied, then grown for up to 6 weeks in DMEM with 10% FCS (changed every 2–3 days) without further splitting. Cells used in this study had undergone 10–20 additional passages since purchase.

Patch-Clamp Recordings

Membrane currents were recorded at 22°C with an Axopatch 1-D amplifier (CV-4 or IHS-1 headstage, Axon Instruments, Foster City, Calif.) using either
the whole-cell or single-channel (generally cell-attached, although the excised inside-out configuration was used occasionally when indicated) variants of the patch-clamp technique. All the data shown are representative of results obtained in at least five cells, from at least three separate culture dishes. Whole-cell data were digitized at 1 kHz, filtered with a two-pole Bessel filter at 2 kHz, and stored on a PDP 1173 computer (Digital Equipment Corp., Brattleboro, Mass.) for later analysis. Single-channel data sampling rate varied between 10 and 2.5 kHz, with filtering at a frequency one fifth the corresponding sampling frequency. Unitary current records, with leak and capacity currents eliminated by subtraction of smooth functions fitted to blank sweeps, were converted into idealized form by half-height criteria. The idealized sweeps were used to construct ensemble averages or various types of histograms.

Pipettes were made from borosilicate glass (WPI, New Haven, Conn.). For single-channel recordings pipettes were coated with Sylgard (3M Co., Minneapolis, Minn.). Tip resistance in the high-sodium solution (see below) was 3–5 MΩ. For whole-cell recording, tip resistance was ~2 MΩ in pipettes filled with internal solution, and the series resistance (6–10 MΩ) was compensated as much as possible without ringing (typically ~50%).

**Solutions**

For recording unitary currents, cells were generally kept in a high-potassium solution to effectively zero the membrane potential: (mM) KCl 20, potassium glutamate 120, HEPES 10, MgCl₂ 1, dextrose 10 (pH 7.35 adjusted with KOH). Four different pipette solutions were used. The first, referred to as physiological solution or high-sodium solution, contained

---

**Figure 2. Evidence for L-type channels of two conductance levels.** Panel A: Selected sweeps showing two different unitary current levels in L-type channels in the presence of Bay K 8644 (5 μM) from a cell-attached patch with the pipette containing high-barium solution. The dashed line shows the open-channel current amplitude of the larger events. The holding potential was ~60 mV in the upper two sweeps and ~90 mV in the lowest sweep. Data were sampled at 10 kHz, filtered on-line at 2 kHz, and subsequently filtered digitally at 1.2 kHz. The same culture conditions existed as described in the Figure 1 legend. Panel B: Pooled data for unitary current amplitudes (in high-barium solution) recorded both during depolarizing steps and during repolarization from positive potentials in the presence of Bay K 8644 (5 μM). The filled symbols represent data from the larger conductance openings such as those in Figure 1, while the open symbols show the smaller conductance level. Each point represents the average of three or four patches (mean±SD), except the filled points at ~30 and +30 mV, which are single observations.
(mM) NaCl 135, KCl 4, dextrose 10, MgCl₂ 1, CaCl₂ 2, HEPES 10 (pH 7.35 adjusted with NaOH). The second was a high-potassium solution with the same composition as the depolarizing bath solution described above. The third was a high-calcium solution containing (mM) CaCl₂ 70, HEPES 10 (pH 7.35 adjusted with CsOH). The fourth was a barium solution containing (mM) BaCl₂ 70, HEPES 10 (pH 7.35 adjusted with tetraethylammonium hydroxide).

For recording whole-cell currents, cells were suspended in the standard physiological solution. Pipettes for internal perfusion were filled with (mM) KCl 140, MgCl₂ 1, MgATP 4, HEPES 10, EGTA 0.1 (pH 7.20 adjusted with KOH).

Results

Calcium Channels

When the cells are allowed to become confluent and are maintained in culture for 3–4 weeks, they exhibit barium currents whose macroscopic properties are L-type (maintained component during depolarization and enhancement by the dihydropyridine agonist Bay K 8644)⁷ and, even more specifically, cardiac rather than skeletal in subtype. The latter claim is based on the observations of Hescheler et al⁸ that activation is fast, as it is in heart (but not skeletal) muscle, and that cAMP-dependent processes enhance the current in a manner typical of heart cells. Nevertheless, another, more molecular, marker to distinguish cardiac from skeletal muscle channel isoforms would be useful given the initial claim that H9c2 cells express a skeletal muscle phenotype. One such marker is the single-channel conductance, which is considerably greater in cardiac than in skeletal muscle.⁸–¹⁰ To confirm or reject the cardiac identity of these channels, we performed cell-attached single-channel recordings. Figure 1 shows single-channel currents carried by 70 mM barium and evoked by depolarization from −90 to +20 mV. In the absence of drug (panel A), openings are sparsely distributed and brief (mean open time <1 msec), indeed, too brief to allow a reliable estimate of unitary conductance. If these channels are in fact L-type, Bay K 8644 should induce long openings,⁷ which would enable quantitation of single-channel current amplitudes.⁸–¹⁰ Panel B shows that this is indeed the case. Soon after 5 μM Bay K 8644 was added to the bath, channel activity was dramatically altered so that long openings (>10 msec) were often observed. The value of single-channel conductance obtained in this patch equals 25 pS, identical to that of the cardiac L-type calcium channel in 70 mM barium.¹⁰ The filled circles in Figure 2B show pooled data for unitary current–voltage relations from this and two other patches. The overall conductance value determined from a least-squares fit to the filled symbols is 24 pS, distinct from the 12–15 pS values that are characteristic of skeletal muscle channels in 70–100 mM barium.⁸,⁹

![Figure 3](http://circres.ahajournals.org/lookup/suppl/doi:10.1161/01.RES.69.6.1490/-/DC1/fig3.png)

**Figure 3.** Single-channel currents obtained from two cell-attached patches during voltage ramps from +60 to −80 mV, elicited from a holding potential of −60 mV. The pipettes contained standard high-sodium solution, while the bath contained high-potassium solution. Panel A: Low-conductance channel with extrapolated reversal potential of about −80 mV. Panel B: Patch containing two different channels, a high-conductance channel with no apparent reversal during the ramp, most obvious in the first two sweeps, and a channel that reverses at +10 mV. Data were sampled at 2.5 kHz and filtered at 0.5 kHz.
Although these results establish that dihydroxyridine-sensitive calcium channels in H9c2 cells can exhibit cardiac-type permeation properties, the single-channel currents in Figure 2A suggest that skeletal-type channels may also be expressed. Here, openings of two different amplitudes are apparent during steps to +10 mV. The smaller openings arise from channels with a 13 pS conductance in 70 mM barium, as revealed by the pooled data from three patches in Figure 2B (open symbols). As mentioned above, this value is virtually identical to that of the skeletal muscle L-type channel isoform exposed to Bay K 8644 and reconstituted in bilayers.8,9 We cannot exclude the possibility that the smaller conductance events are subconductances of the 24 pS channel, particularly since such subconductances have been described in Bay K 8644-modified cardiac L-type channels,11 but the frequent observation of summated stacking events (e.g., Figure 2A, sweeps 1 and 2) and the absence of any clear direct interconversions between the two conductances here and in two other patches make us favor the notion that the openings arise from two distinct isoforms of L-type calcium channels. Also in support of the separate identity of the two channel types is the fact that, of seven single-channel patches examined with barium and Bay K 8644, five exhibited channels of only one or the other conductance level.

Thus, our observations confirm the expression of L-type calcium channels in H9c2 cells. Some, but perhaps not all, of these channels resemble those in primary cardiac myocytes in terms of their unitary permeation properties.

**Single-Channel Recordings in Cells 10–20 Days After Plating**

In contrast to the densely confluent culture conditions that appear to maximize calcium channel expression, the majority of our experiments were performed...
in cells at an earlier stage in culture. The major difference electrophysiologically between the two culture conditions was in the expression of calcium channels; the channels described below were also seen in older cultures but were studied more extensively 10–20 days after plating. We will first survey the various single channels and the macroscopic currents observed in such cells, followed by an in-depth examination of a unique nonspecific channel that is commonly observed under these conditions.

Figure 3 shows results from two cell-attached patches stimulated by ramp depolarizations from a holding potential of −60 mV. The pipettes were filled with standard Tyrode's solution (see "Materials and Methods"). The patch on the left (panel A) contains a channel of 8 pS conductance that is most
active at the beginning of the ramp and remains outward at all potentials, with an extrapolated reversal of −75 to −80 mV. Step depolarizations revealed that this channel exhibits the kinetics of a “neuronal” delayed rectifier, as verified in Figure 4A by the individual current sweeps and the ensemble average current (bottom row) during depolarizations to +60 mV. Channels of similar kinetics but distinctly higher conductance (15 pS) have also been described in embryonic chick myocytes. Returning to Figure 3, the patch on the right (panel B) contains two other channel types. The first and second sweeps are dominated by a channel that again remains strictly outward at these potentials (consistent with potassium being the charge carrier) but that is more than twice as large (18 pS, mean of four patches) as that in panel A. Figure 4B shows currents during steps from −60 to +60 mV from this second type of channel. Activity during each sweep is greatest just after depolarization, giving rise to the transient ensemble average current in the bottom row. The channel types in Figures 4A and 4B share the characteristics that they open much more frequently at positive than at negative potentials. In physiological solution containing 4 mM potassium, inward currents were never observed even at potentials as negative as −120 mV, consistent with the idea that open probability is very low in this voltage range.

In contrast to the two presumed potassium currents, which are always outward in Figure 3, a different type of activity is clearly distinguishable in the fourth and fifth sweeps in panel B: here, several channels that reverse around 0 mV are apparent. Each exhibits a conductance of 30 pS. The reversal potential suggests that these openings may arise from nonspecific channels. The third sweep in panel B can be seen to contain currents from both the large potassium channel and one of the 30 pS channels. Figure 4C contrasts step analysis of this channel type with the two strictly outward channels under identical ionic conditions (high-calcium solution in the pipettes, cell-attached). The gating pattern as revealed by the ensemble average current (bottom row) is clearly different, with slow activation during the step depolarization and no apparent inactivation. Even more striking are the differences in permeation: current through the channel in panel C reverses during tails to −60 mV, unlike the unitary currents in panels A and B.
Macrosopic Currents

Are the various types of channels that give rise to outward currents distinguishable at the macroscopic level? Our examination of this question has been largely restricted to recordings in physiological solutions, in which potassium channels would also be active. In the 10–20-day postplating stage, whole-cell currents upon depolarization are dominated by outward currents that can assume several waveforms. Figure 5 shows three examples (panels A–C), each from a different cell. Panel A shows a family of superimposed current records during depolarizing pulses to test potentials ranging from −40 to 60 mV. This cell displayed a transient waveform with no hint of a slowly activating component, and presumably was richest in the type of channel highlighted in Figure 4B. Other cells, such as that in panel B, displayed a small early component but a sizable slowly activating outward current. Contrary to what might be expected if the cells in panels A or B had been rich in nonspecific channels, no tail currents were observed upon repolarization in either case (consistent with the very rapid deactivation of the single channels in Figures 4A and 4B). The presence of nonspecific channels might be suspected more strongly in Figure 5C. The direction of the time-dependent currents evoked by depolarization is noteworthy in this cell: The waveform (peak minus steady) is inward at negative test potentials but becomes outward with greater depolarization. The presence of inward tails upon repolarization to −80 mV also distinguishes this type of whole-cell record from those in panels A or B. Panel D shows instantaneous current–voltage analysis of tails from +60 mV elicited from the cell in panel C; the apparent reversal potential occurs at about −55 mV, a value between that expected for potassium channels in these solutions (−85 mV) and the near-zero value of the 30 pS channel. We interpret this intermediate reversal potential as reflective of opposing contributions from potassium channels and nonspecific channels.

Although in most cells the macroscopic membrane currents can probably be reproduced by a weighted average of the various general classes of single channels that we have catalogued in Figures 3 and 4, the pattern is quite variable from cell to cell, even within the same culture dish. Unitary recordings are clearly preferable if we are to investigate any one of these channels in detail. Because of its unusual combination of voltage-dependent gating and nonspecific permeation, we focused the rest of our investigation on unitary recordings of the 30 pS channel.

Permeation in the 30 pS Channel

The 30 pS channel was observed in most patches, including three in which high-sodium solution rather than high-potassium solution bathed the cell (confirming that the channel was not artifically produced by exposure to high-potassium solution). This type of channel supported current under a variety of ionic conditions. Figure 6 shows records from three different cell-attached patches in which the pipette contained either standard high-sodium Tyrode’s solution (panel A), high-potassium solution (panel B), or high-calcium solution (panel C). With all three external cations, the reversal potentials measured from ramp depolarizations occurred at weakly positive potentials. When sodium was the predominant external cation, the reversal potential equaled 12.3 ± 2.4 mV (mean ± SEM, n = 6), as compared with 3.1 ± 1.7 mV in potassium (n = 4) and 12.4 ± 6.8 mV in calcium (n = 5). Unitary conductance was roughly similar with all three solutions (as will be quantified further below), with the exception that the currents in calcium, particularly in the inward direction, are smaller and exhibit flickery block. Thus, in high calcium the channel displays an outwardly rectifying current trajectory, perhaps caused by partial block when calcium is the charge carrier.

These data suggest that this is a nonspecific channel capable of supporting current carried by sodium, potassium, or calcium, but we have also considered the possibility that the channel might be chloride selective. Two lines of evidence argue against this possibility. First, since external [Cl] equals 140 mM, internal [Cl] would have had to be implausibly high (≥200 mM) to
account for the slightly positive reversal potentials. Second, the reversal potential did not change significantly when patches were excised into a solution containing only 22 mM [Cl] (see Figure 10), in which the chloride equilibrium potential would have equalled −50 mV. We thus rejected the possibility that this is an anion channel and proceeded to characterize the relative permeabilities of various cations.

Figure 7 shows pooled data for unitary current amplitudes over a broad voltage range from a total of 14 cell-attached patches containing sodium, potassium, or calcium as the predominant extracellular cation. For maximal reliability, current amplitudes were determined from long (>10 msec) openings during steps rather than from ramps. Unitary conductance, from least-squares fits to the pooled outward currents, was virtually identical in sodium (30 pS) and potassium (31 pS), and the open-channel current trajectories displayed little rectification. Unitary conductance measured from outward currents was somewhat lower (25 pS) in high calcium (the value determined from inward currents would have been even smaller).

The inward currents in the 70 mM calcium solution must reflect net calcium entry, since calcium is the only significant external cation. In contrast, the outward currents are presumably carried predominantly by potassium, the richest intracellular cation, in all cases. From the perspective of Eyring-based permeation models, the rectification in calcium can be rationalized by supposing that calcium binds somewhat more avidly to the selectivity filter than either sodium or potassium, so that it acts as a partial blocker when it itself carries the charge. Indeed, the measured reversal potentials (from ramps such as those in Figure 6) can be readily fit by assuming that the relative permeabilities of Na:K:Ca in the Goldman-Hodgkin-Katz equation are 1.4:1.0:1.7 (assuming [K] = 125 mM and [Na]==[Ca]=0), consistent with the notion that calcium binds slightly more avidly to the channel than does either sodium or potassium (i.e., the channel is more “selective” for calcium).

**Gating of the Nonspecific Channel**

Unlike other nonspecific cation channels, the gating of the 30 pS channel is strikingly time and voltage dependent. Figure 4C has already shown that the channel activates slowly upon depolarization in 70 mM [Ca]o. Figure 8 verifies that this is also the
case in physiological saline. In this patch containing at least two channels, steps to +60 mV elicit long openings after variable latencies. After repolarization to −60 mV, the channel deactivates quickly. The ensemble average current reveals a progressive increase during the pulse, so slow that steady state is not reached even after 500 msec at +60 mV. Openings are much briefer at −80 mV (panel B); here the overall average current is considerably smaller despite the fact that the driving force is actually greater in absolute value than it had been at +60 mV. No clear time dependence is apparent in the ensemble current at −80 mV.

Figure 9A shows pooled results from four patches for average open probability during 650-msec steps over the voltage range from −80 to +60 mV. Open probability is less than 0.05 at negative potentials but increases by about an order of magnitude with extreme depolarization. Inspection of the individual records in Figure 8 leads to the distinct impression that an increase in open times with depolarization accounts for at least part of the enhancement in open probability. This idea is tested in Figure 9, which shows open time histograms from one patch at +60 mV (panel B) and −80 mV (panel C). Each histogram reports the events during 140 sweeps lasting 650 msec. At each voltage, open times are best described by one short and one long exponential term. Both open times are shorter at −80 mV, but the decrease of the long time constant from 33 to 12 msec is particularly marked. Nevertheless, the twofold to threefold decrease in open times cannot fully account for the overall 10-fold fall in open probability. An additional contribution is apparent from the reduction in the total number of openings at −80 mV (299 in 140 sweeps, down from 925 at +60 mV). We conclude that the channels not only dwell more briefly in open states at negative potentials, but also remain in nonconducting (closed or inactivated) states for longer periods. Both effects underlie the voltage dependence of the open probability.

While formulation and testing of an explicit kinetic model would ultimately be desirable, this cannot yet be achieved from our data: all but one patch contained several channels, and the overall activity in that patch was so dispersed that we could not be confident of its single-channel nature. Thus, closed times and first latencies, which are necessary to constrain a multistate model,23 could not be interpreted straightforwardly. The limited analysis presented here suggests that a realistic kinetic scheme will necessarily be complex; in particular, the observation of two very different exponential time constants in the open time histograms necessitates the inclusion of at least two distinct open states.24

Activation by Intracellular Calcium or by Stretch?

Since heart cells have been reported to contain three types of calcium-activated nonspecific channels,20–22 we determined whether or not the H9c2 channel is sensitive to cytoplasmic [Ca2+] by excising patches into solutions containing high-affinity calcium buffers and no added calcium. Figure 10 shows an example of the activity in a patch that had been excised 5 minutes earlier into 1 mM EGTA-containing solution ([Ca2+] <20 nM). Panel A shows repre-
A number of ion channels, including at least one that is nonspecific, 25 either open or close in response to membrane deformation. 26 To exclude these possibilities, we applied suction to the patch membrane during recording and gauged the effects on open probability. Figure 11 shows typical consecutive sweeps in a patch with no applied suction (panel A) and during the application of -30 cm H2O pressure to the pipette holder (panel B). Suction has little effect, as is apparent both from the selected records and from the diary of open probability in panel C, where the period of suction is indicated by a horizontal bracket. No effects were observed up to pressures of -40 cm H2O, the highest that could be applied without patch rupture. These results, which were confirmed in three other patches, indicate that the nonspecific channel in H9c2 cells is not stretch activated (or stretch inactivated).

Discussion

Nonspecific Channel

The nonspecific cation channel we have identified is biophysically novel since depolarization appears to be the primary stimulus for its activation; unlike most other nonspecific cation channels, 18-22,25 neither stretch nor intracellular Ca2+ acts as a regulator here. The fact that such a channel has not been described in intact primary cells may indicate that it represents an unusual gene product selected by long-term culture. Another, more provocative, possibility is that this channel might be normally expressed at an early stage of myocyte development but suppressed in adult tissue. During embryogenesis, the heart is not readily amenable to primary cell isolation, so the appropriate substrate for such channels might have escaped electrophysiological examination. Alternatively, the nonspecific channel might have been missed in previous work on primary cells even if it had been present; at the macroscopic level, we have
emphasized that the currents corresponding to the nonspecific channel overlap significantly with potassium currents. In single-channel recordings, long (>100 msec), strongly depolarizing voltage pulses, not ordinarily part of patch-clamp protocols for routine channels, are required to elicit robust activity. These very properties suggest that the possible physiological role of such a nonspecific channel, if any, would be greatest in situations in which depolarization is frequent. If the channels were appreciably activated by bursts of action potentials, for example, they might serve an integrative role by tending to bias the membrane potential toward plateau levels. The nonspecific nature of the channels also suggests they may dissipate ionic gradients when active and thus may play a role in the regulation of cell volume by depolarization. At present such conclusions must be qualified as speculative.

Changes in Membrane Properties in Culture

We have noted two peculiar features regarding the expression of calcium channels in H9c2 cells: first of all, calcium channel currents were not evident within the first 2 weeks after initial plating, consistent with the observations of Hescheler et al; even then, the density of channel expression (as gauged by the likelihood of finding single channels and by the amplitude of macroscopic currents) varies considerably from cell to cell, including those within the same culture dish. Although the origin of these features remains cryptic, they are shared by at least one other immortal cell line of muscular origin. Marks et al have found highly variable amplitudes of L-type calcium channel currents in cells from the smooth muscle–derived A7r5 line. These cells also fail to manifest any response to dihydropyridines until after they have been in culture for several weeks. At present, we can only acknowledge these puzzling phenomena; more work will be required to elucidate the origin of the temporal changes and heterogeneity of calcium channel activity in immortal muscle cell lines in culture.

Skeletal Versus Cardiac Muscle Phenotype

The initial characterization of H9c2 cells by Kimes and Brandt revealed closer homology to skeletal than to cardiac muscle. Of particular note was the depolarizing response to acetylcholine, suggesting that the cells they studied expressed nicotinic acetylcholine receptors characteristic of the skeletal muscle end plate. A number of lines of evidence suggest that the current phenotype of H9c2 cells, some 10 passages removed from those studied by Kimes and Brandt, is at least intermediate between cardiac and skeletal muscle. In preliminary experiments we have observed no change in whole-cell current during exposure to acetylcholine (5 μM, n=3, data not shown), contrary to expectations

![Figure 11](image-url)
from the results of Kimes and Brandt. The potassium channels we have identified have definite homologues in intact rat and mouse heart cells, but the great diversity of potassium channels precludes confident tissue-based classification.

Hescheler et al. have emphasized the similarity of the macroscopic barium currents in H9c2 to those in primary cardiac myocytes. At the unitary level we confirm the existence of a population of L-type calcium channels that exhibit heartlike permeation, but also a lower conductance form that may represent coexpression of the skeletal muscle α1 calcium channel isoform in these cells. The results give reason to wonder whether the cells might be capable of exhibiting either a cardiac or a skeletal muscle phenotype depending on culture conditions, possibly caused by differences in growth factor composition among sera. A multifaceted approach, spanning from investigation of transcriptional control to further characterization of the cells' physiology, will be necessary to define the precise mechanisms involved. If the proposed phenotypic plasticity of these cells can be firmly established, such a multifaceted approach can be expected to yield important insights into the processes that control muscular differentiation.

Acknowledgments

We thank Jürgen Hescheler for first bringing H9c2 cells to our attention, Kimberly Kluge and Katsuomi Iwakura for assistance with cell culture, William Rose for help with data acquisition and analysis, and Toren Finkel for critically reading the manuscript.

References

28. Marks TN, Dubayk GR, Jones SW: Calcium currents in the A7r5 smooth muscle-derived cell line. Pflugers Arch 1990;417:433–439

KEY WORDS: H9c2 cells • membrane currents • patch clamp • ion channels • cell culture
L-type calcium channels, potassium channels, and novel nonspecific cation channels in a clonal muscle cell line derived from embryonic rat ventricle.

K R Sipido and E Marban

Circ Res. 1991;69:1487-1499
doi: 10.1161/01.RES.69.6.1487

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/69/6/1487

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/