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L-type and T-type Ca^{2+} Current in Cultured Ventricular Guinea Pig Myocytes

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Summary

The aim of this investigation was to study L-type and T-type Ca^{2+} current (I_{CaL} and I_{CaT}) in short-term cultured adult guinea pig ventricular myocytes. The isolated myocytes were suspended in serum-supplemented medium up to 5 days. Using whole-cell patch clamp techniques I_{CaL} and I_{CaT} were studied by applying voltage protocols from different holding potentials (–40 and –90 mV). After 5 days in culture the myocytes still showed their typical rod shaped morphology but a decline in cell membrane capacitance (26 %). The peak density of I_{CaT} was reduced significantly between day 0 (–1.6±0.37 pA/pF, n=9) and day 5 (–0.4±0.13 pA/pF, n=11), whereas peak I_{CaL} density revealed no significant differences during culturing. The I_{CaT}/I_{CaL} ratio dropped from 0.13 at day 0 to 0.05 at day 5. Compared with day 0 I_{CaL} the steady state inactivation curve of day 1, day 3 and day 5 myocytes was slightly shifted to more negative potentials. Our data indicate that guinea pig ventricular L-type and T-type Ca^{2+} channels are differently regulated in culture.

Key words

Ventricular myocytes • Short-term culture • L-type Ca^{2+} channel • T-type Ca^{2+} channel

Introduction

Isolated myocytes from hearts of small mammals play an important role in exploring the properties and biological function of the two types of Ca^{2+} channels expressed in the sarcolemma of cardiomyocytes: the long-lasting L-type or high-voltage-activated channel and the low-voltage-activated transient T-type channel (Bean 1985, Nilius *et al.* 1985). L-type Ca^{2+} current (I_{CaL}) is essential in maintaining the plateau of the action potential, and provides influx of Ca^{2+} ions for initiation of cardiac contraction (McDonald *et al.* 1994). T-type Ca^{2+} current (I_{CaT}) is supposed to play a role in spontaneous electrical activity of nodal cells and embryonic cardiomyocytes and has also been associated with cell growth (Vassort and Alvarez 1994). Besides freshly isolated myocytes, cultured myocytes are an attractive complement for electrophysiological and molecular biological experiments (Clark *et al.* 1998, Mitcheson *et al.* 1998). Two basic methods are used for culturing adult ventricular myocytes: 1) the "redifferentiation" method (serum supplemented medium and usually myocytes in suspension) and 2) the "rapid attachment" method with serum-free medium and cell attachment to the surface of culture dish (Mitcheson *et al.* 1998). Application of the first method may result in morphological alterations after several days in culture in terms of rounding of cardiomyocytes, loss of cross-

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striation, appearance of lamellipodia (Claycomb *et al.* 1984, Stimers and Dobretsov 1998), and occurrence of spontaneous activity (Jacobson 1977, Jacobson and Piper 1986, Stimers and Dobretsov 1998). Investigations of possible modulations of Ca^{2+} currents in cultured myocytes have only focused on I_{CaL} and on species like cat (Schackow *et al.* 1995), rabbit (Mitcheson *et al.* 1996), and rat (Berger *et al.* 1994, Ellingsen *et al.* 1993). Although guinea pig hearts are widely used for studying both types of Ca^{2+} currents (Mitra and Morad 1986), comprehensive data on Ca^{2+} currents in culture are not available. Regarding the occurrence of spontaneous activity of cultured myocytes, alterations in I_{CaT} may particularly be interesting since I_{CaT} is supposed to play a role in pacemaker function (Vassort and Alvarez 1994). In this study, using the whole cell patch-clamp technique, we investigated I_{CaL} and I_{CaT} in cultured ventricular myocytes from adult guinea pigs. We demonstrate that under our culture conditions (serum-supplemented and cells in suspension) guinea pig myocytes preserve their typical morphology and I_{CaT} shows a significant reduction in current density during 5 days of culture, whereas I_{CaL} stays stable.

Methods

Myocyte isolation and short-term culture

Single cardiomyocytes from both ventricles of adult guinea pigs were prepared as described by Piper *et al.* (1982). The coronary system of the heart was perfused in a Langendorff apparatus with a solution containing 100 IU/ml collagenase (Worthington CLS 2, Worthington Biochemical Corporation, New Jersey, USA). Isolated myocytes were suspended in non-adhesive plastic Petri dishes with vents (35.0/10 mm, Greiner; Austria) at a final density of approximately $2x10^5$ cells ml^{-1} in 2 ml cell culture medium M-199 (Sigma) containing fetal bovine serum (5 %, Sigma), penicillin (50 IU/ml, Sigma) and streptomycin (50 µg/ml, Sigma). The cells were maintained at 37 °C in a 5 % CO_2/95 % air incubator up to 5 days (day of myocyte isolation: d_0, days 1 to 5 after isolation: d_1, d_3 and d_5, respectively).

Electrophysiological recordings

Membrane currents were recorded in the whole-cell single-electrode voltage-clamp configuration of the patch-clamp technique (Hamill *et al.* 1981), using a List L/M-EPC 7 amplifier (List, Darmstadt, Germany) and a Digidata 1200 Interface (Axon Instruments, Foster City). A personal computer equipped with pClamp 5.7.1 software (Axon) was used for generation of voltage clamp protocols, data storage and evaluation. The myocytes were placed in an experimental chamber and continuously superfused with a Na^+ and K^+ free extracellular solution (composition in mM: Tris 137, $MgCl_2$ 1, $CaCl_2$ 5.4, CsCl 20, glucose 5 adjusted to a pH of 7.4 with HCl) at a temperature of 36-37 °C. When filled with pipette solution (composition in mM: CsCl 125, MgATP 5, EGTA 15, TEA-Cl 20 HEPES/Cs^+ 10, adjusted with CsOH to pH 7.2) patch electrodes had a tip-resistance in the range of 2 to 4 MΩ. After seal formation and membrane rupture, a single 10 mV hyperpolarizing pulse (from -50 mV) was applied. The resulting capacitive current transient was integrated and divided by the voltage step to determine the cell membrane capacitance (C_m). C_m was compensated up to 100 pF. Series resistance (R_s) was calculated by dividing the time constant of the capacitive transient by C_m. R_s prior to compensation was 5.54±0.23 MΩ (n=53). R_s was usually compensated to more than 50 %. Ca^{2+} current amplitudes were measured as the difference between peak inward and the current at the end of the pulse. To take into account the variations in cell size, current amplitudes were normalized to cell capacitance and are given as current density. I_{CaL} and I_{CaT} were separated by using holding potentials of -90 or -40 mV (see results for further details). For estimation of the cell surface membrane area, myocyte length and width were measured and the "visible membrane area" (length multiplied by width) was calculated.

Statistical analysis

All data are expressed as means ± S.E.M. Statistical significance was evaluated by the two-tailed Student's t-test or, if more than two conditions were compared, by one-way analysis of variance (ANOVA) with the Scheffé *post hoc* test. Differences with $P<0.05$ were considered significant.

Results

Cell morphology and membrane capacitance during culture

Freshly isolated adult guinea pig ventricular myocytes (d_0 cells) and short-term cultured myocytes (d_1 to d_5) were studied in these experiments. All cells used showed clear cross-striation and a rod-shaped form during the culturing. On d_0 and d_1 the typical rod shape of

adult ventricular myocytes with rectangular "stepped" ends was clearly visible. During the following days the edges of the cells became rounded, but the myocytes never showed pseudopodia-like outgrowth. In two myocyte preparations the percentage of rod-shaped myocytes, as a measure of living cells was determined (approximately 400 cells for each culture day). The ratio of rod-shaped to rounded cells declined from d_0 to d_5 by only 20 % (data not shown). Thus, no prominent changes in the viability of myocytes could be observed. To address a possible change in myocyte dimensions during culture, the length and width of d_0 and d_5 myocytes (n=200 for each day) were measured in two myocyte preparations. Mean length (136±1.9 µm and 124±1.6 µm, for d_0 and d_5, respectively) and width (27±0.5 µm and 22±0.4 µm, for d_0 and d_5, respectively) showed a significant ($P<0.005$) decrease. The frequency distributions of the two parameters (Figs 1A and 1B) suggest a shift in myocyte dimensions towards smaller cells. Note that the number of large cells (length ≥ 150 µm and width ≥ 30 µm) is higher for d_0 (white columns) than for d_5 myocytes (black columns) as well as that very short cells (length < 90 µm) are missing at d_0. This decrease in myocyte dimensions is also reflected by a significant decrease of membrane capacitance ($P=0.015$ between d_0 and d_5) (C_m, Fig. 1C). The average C_m of freshly isolated cells was 124±6.3 pF (n=24), cells studied on d_1, d_3 and d_5 had a C_m of 106±7.9 pF (n=14), 97±5.5 pF (n=13) and 92±8.5 pF (n=16), respectively. Thus, the decrease of C_m in d_5 myocytes amounted to about 26 % compared to d_0 cells.

Voltage dependence of I_{CaL} and I_{CaT} during culture

For each cell Ca^{2+} currents were elicited by depolarizing the membrane (400 ms) to various test potentials from a holding potential (HP) of –90 and –40 mV. Figure 2 shows typical current recordings (the first 150 ms) in a d_0 myocyte and in a d_5 myocyte. Voltage steps from a HP of –40 mV mainly activated I_{CaL}. Ca^{2+} currents activated by depolarization to –20 and +20 mV were larger when HP was –90 mV. The difference current (a result of the subtraction of currents at the two different HPs) was taken as a measure of I_{CaT}. The current traces indicate that mainly I_{CaT} is reduced during culture: at a test potential (TP) of + 20 mV (the potential where I_{CaL} is maximal) I_{CaL} was –1 nA and –1.2 nA (in a d_0 and a d_5 cell, respectively), whereas at TP of –20 mV (where I_{CaT} is maximal) I_{CaT} was -0.25 nA (d_0) and –0.04 nA (d_5).

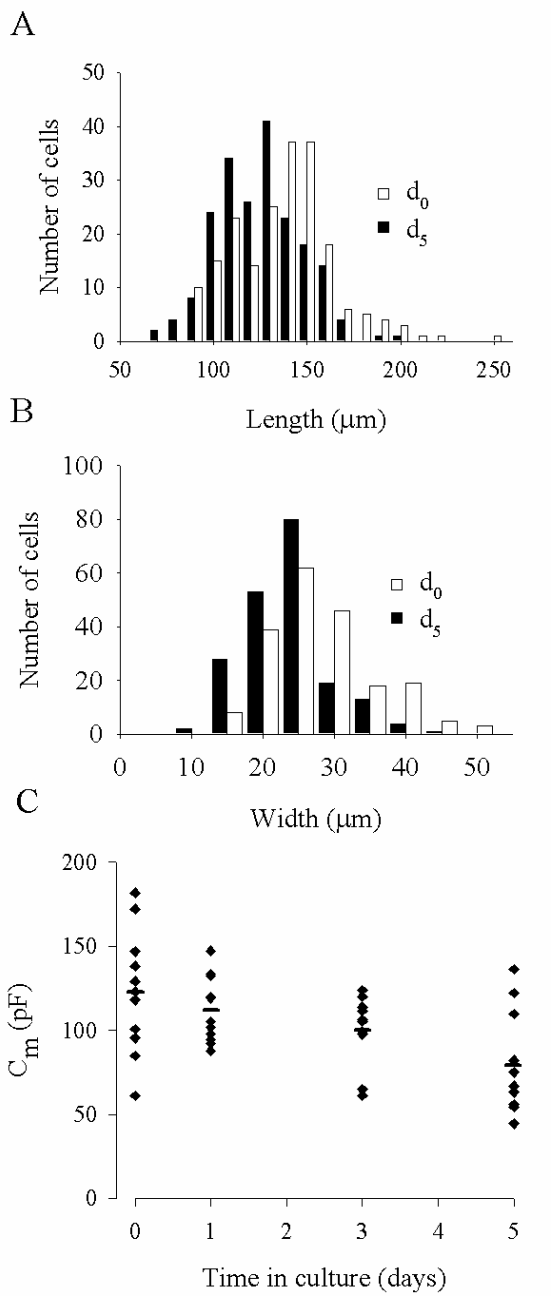

Fig. 1. Frequency distributions of length (A) and width (B) of d_0 myocytes (white columns) and d_5 myocytes (black columns; n=200). Panel C shows the membrane capacitance (C_m) during culture.

Figure 3 shows mean current density-voltage relations of Ca^{2+} currents in d_0 (Panel A, n=9) and d_5 (Panel B, n=11) myocytes. Using a HP of –90 mV (closed circles), depolarizing steps positive to –50 mV induced an inward maximal current at +20 mV and reversing near +80 mV. At a HP of –40 mV (open circles) the activation threshold was at –30 mV. The difference between currents elicited from HP of –90 mV and –40 mV reveals the existence of I_{CaT} in ventricular d_0

cells. I_{CaT} was activated near –40 mV and peaked in magnitude at –20 mV with a maximal current density of -1.6±0.4 pA/pF. I_{CaL} also showed a typical bell shaped pattern with maximal current density of –11.5±1 pA/pF at + 20 mV. On d_5 I_{CaL} had a maximal current density of -8.8±0.8 pA/pF at +20 mV whereas I_{CaT} had a maximal value of –0.4±0.1 pA/pF at –10 mV.

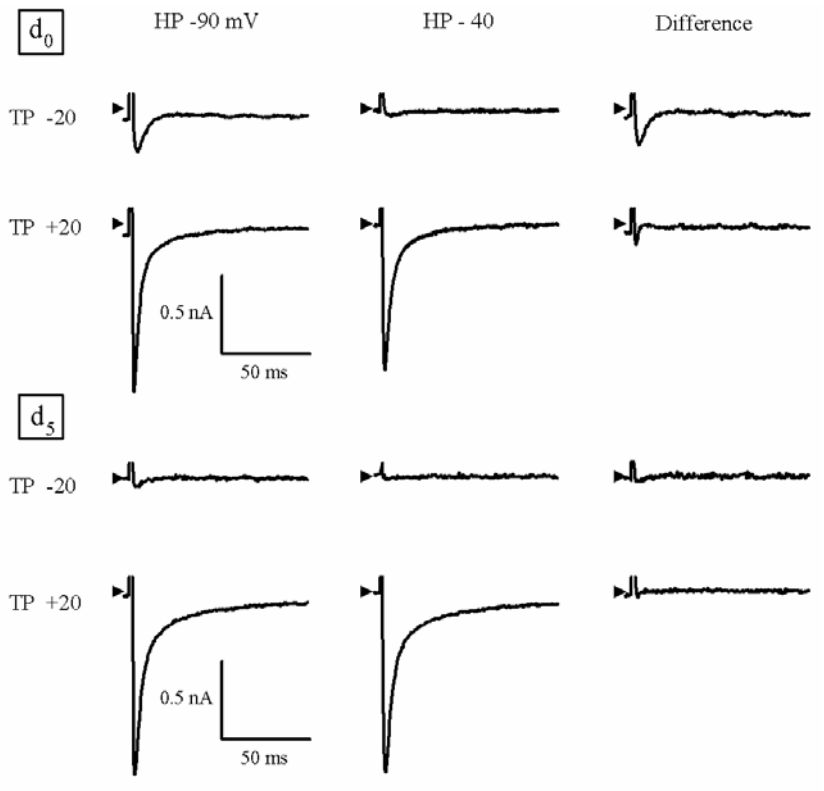

Fig. 2. Representative traces of Ca^{2+} currents (150 ms) elicited by 400 ms depolarizing test pulses to -20 and +20 mV from HPs of –90 and –40 mV in a d_0 and in a d_5 cell. Subtraction of the current at a different HP yielded the difference current, which is mainly I_{CaT}. ‣ indicates zero current.

Peak current densities of $I_{CaL(max)}$ (A) and $I_{CaT(max)}$ (B) of individual myocytes are plotted as a function of culture time in Figure 4. Mean $I_{CaL(max)}$ (Fig. 4A) did not show significant differences between d_0, d_1 and d_3 cells in culture (–12.5±1.0 pA/pF, –12.9±1.1 pA/pF and –11.9±1.1 pA/pF), but declined in d_5 myocytes to –9.6±0.8 pA/pF. However the reduction was not statistically significant (p=0.1). In contrast, $I_{CaT(max)}$ (Fig. 4B) shows a reduction of current density with time in culture (d_0: –1.6±0.4 pA/pF, d_1: –1.2±0.2 pA/pF, d_3: –0.7±0.3 pA/pF, and d_5: –0.4±0.1 pA/pF). The difference between d_0 and d_5 was significant (p=0.023) and the I_{CaT}/I_{CaL} ratio dropped from 0.13 to 0.05 after 3 days in culture. For a better comparison the time course of I_{CaL}, I_{CaT} amplitudes (current amplitude not current density) and C_m alterations are expressed relative to d_0 (given in %) in Figure 4C. As can be seen, the percentage decrease in I_{CaL} and C_m (same myocytes as shown in Figs 4A and 4B) can almost be superimposed, whereas I_{CaT} shows a more prominent reduction.

Voltage dependence of activation and inactivation of I_{CaL} and I_{CaT}

In order to determine the voltage dependence of activation, peak values of I_{CaL} and I_{CaT} were divided by the driving force, normalized and then plotted against the potential of the test pulse (Fig. 5). The apparent reversal of L-type Ca^{2+} current (near +70 mV) was also used for calculation of activation curves of T-type Ca^{2+} current (Hirano *et al.* 1989). The data from different culture days were fitted with a Boltzmann function

$$d_\infty(V_m) = 1/(1 + \exp[-(V_m - V_{half})/k])$$

where V_m is the membrane potential, V_{half} the membrane potential at half maximal activation and k is the slope factor. A double-pulse protocol was used to determine steady-state inactivation. A 400 ms conditioning pulse to various voltages was followed by a test pulse for 400 ms to elicit I_{CaL} or I_{CaT}. For I_{CaL} the conditioning pulses varied from –40 to +50 mV and the test pulse was +20 mV. For I_{CaT} the conditioning pulses varied from –90

to –30 mV and the test pulse was –20 mV. Data were normalized by dividing the test current by maximal current. The following form of the Boltzmann function was used for data fitting:

$$f_\infty(V_c) = 1/(1 + \exp[(V_c - V_{half})/k])$$

where V_c is the voltage of the conditioning pulse, V_{half} the potential of half-maximal inactivation and k is the slope factor.

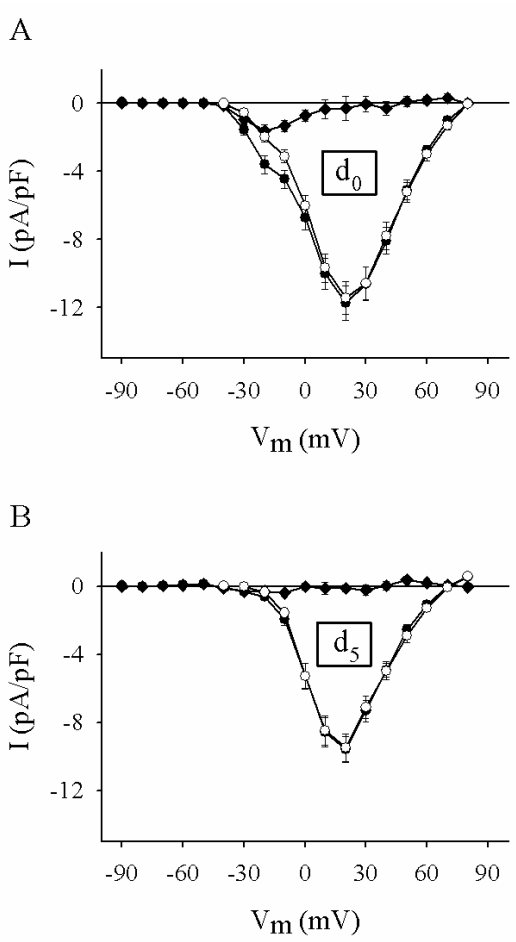

Fig. 3. Current density-voltage relations of Ca^{2+} currents recorded from a HP of –90 (●) and –40 mV (○) for freshly isolated myocytes (**A**) and cells after 5 days in culture (**B**). Notice that the difference current (◆) is markedly reduced in d_5 myocytes.

Figure 5A shows the voltage dependence of activation and inactivation for I_{CaL} in d_0 (open circles) and d_5 myocytes (closed circles), whereas Table 1 summarizes the activation and inactivation parameter values for all culture days. The activation curves showed no significant differences in their slope factor or V_{half} between the different days in culture. However, steady-state inactivation showed a slight but significant parallel shift between d_0 and d_1, d_3 and d_5, which is shown in Figure 5A for d_5 cells (V_{half} was shifted by –4.6 mV). For I_{CaT} no significant differences in parameters of voltage dependence of d_∞ and f_∞ could be observed in d_0 and d_1 cells (Fig. 5B, for numeric values see Table 1). Because of the low I_{CaT} amplitude at d_3 and d_5 d_∞ and f_∞ were not calculated for those days.

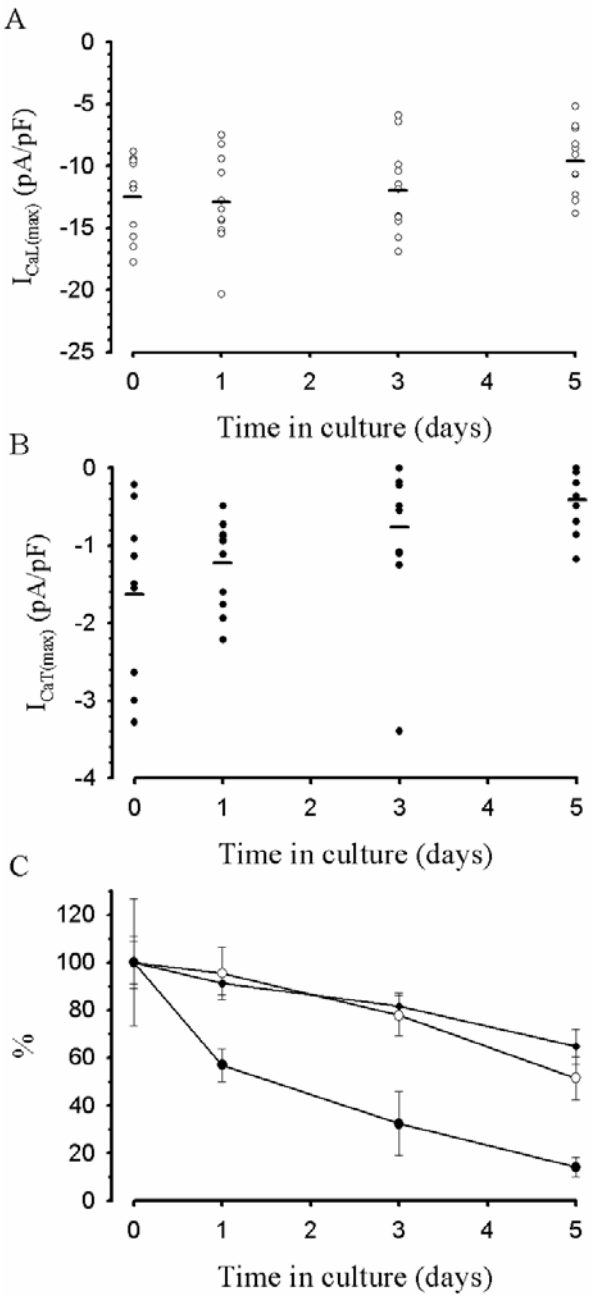

Fig. 4. Peak current densities of I_{CaL} (**A**) and I_{CaT} (**B**) during culture. Panel **C** shows the percentage change of I_{CaL} (○), I_{CaT} (●) (note that current but not current density is shown in this panel) and C_m (◆) during culture. The horizontal bars in panels A, B, and C indicate the mean value of each culture day.

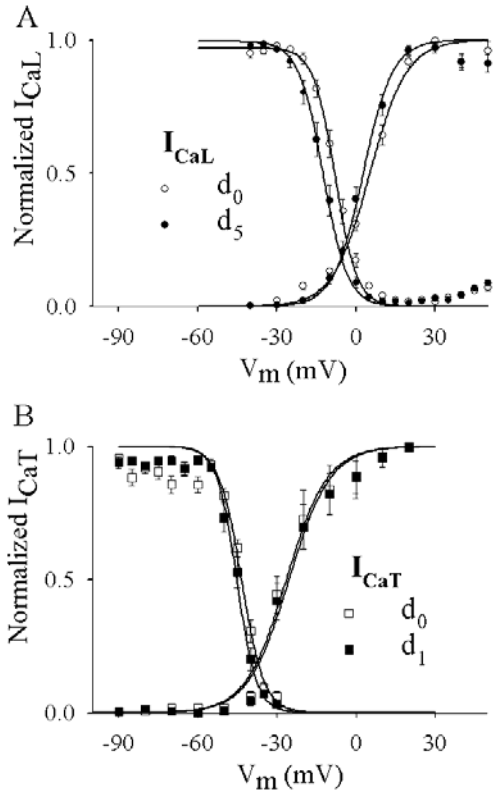

Fig. 5. Voltage dependence of steady-state activation and inactivation for Ca^{2+} currents. (**A**) Voltage dependence of steady-state activation and inactivation of I_{CaL} in d_0 (○) and d_5 (●) ventricular cells. The continuous curves were obtained by fitting the data with the Boltzmann equation. (**B**) Voltage dependence of steady-state activation and inactivation of I_{CaT} in d_0 (□) and d_1 (■) ventricular cells. The continuous curves were obtained by fitting the data with the Boltzmann equation. See Table 1 for numeric values.

Discussion

Cell culture and cell morphology

In the present study we kept adult guinea pig ventricular myocytes for 5 days under culture conditions without loss of rod-shaped morphology, although the cells were in suspension and serum was added to the medium. Contrary data are available from adult rat ventricular myocytes, which became rounded and started to send out pseudopodia-like projections after 2-4 days in culture (Claycomb *et al.* 1984, Stimers and Dobretsov 1998). Besides dedifferentiation of the cells a further common feature of short-term culture is a prominent reduction of myocyte membrane capacitance (C_m). C_m loss has been reported for adult guinea pig ventricular myocytes (65 % after 2 days, Lipp *et al.* 1996), for adult rabbit ventricular myocytes (54 % after 6 days, Mitcheson *et al.* 1996; 50 % after 4 days, Christé 1999) and for adult rat ventricular myocytes (35 % after 4 days, Stimers *et al.* 1998). Compared with these results the loss in C_m in our study was rather small (26 % after 5 days in culture). The nature of the observed changes in myocyte dimensions is not clear at present. However, Figures 1A and 1B suggest a general shift of the length and width distribution towards smaller cell dimensions. It has been shown that a lack of contractile activity in cultured feline cardiomyocytes (cells attached, serum supplemented) led to a decrease in cell size and loss of myosin heavy chain protein (Clark *et al.* 1993). Since the myocytes in our study did not contract spontaneously during culture, a subsequent slight remodeling of the contractile apparatus may account for these changes. In general, myocyte isolation and culturing consist of a complex series of steps, in which the nature and quality of the myocyte isolation procedure, used species and various other factors (e.g. culture conditions) contribute to the outcome.

Table 1. Activation and inactivation parameters of I_{CaL} and I_{CaT}.

Ca^{2+}-channel	Days in culture	Activation-parameters		n	Inactivation-parameters		n
		V_{half} (mV)	k		V_{half} (mV)	k	
L-Type	0	5.2±1.0	7.0±0.5	11	–7.9±0.9	4.1±0.1	12
	1	1.7±0.8	5.8±0.3	11	–12.6±1.3*	4.5±0.1	12
	3	2.1±1.5	5.2±0.2	11	–12.5±0.9*	4.4±0.1	13
	5	2.7±1.2	5.6±0.2	11	–12.5±1.3#	4.6±0.1	11
T-Type	0	–26.4±3.6	7.7±1.4	9	–43.4±0.6	4.3±0.3	12
	1	–25.4±2.7	7.9±1.4	11	–45.2±0.9	3.8±0.2	11

The parameter values are means ± SEM and were calculated after fitting the data of each experiment with the Boltzmann funtion as described in the text. V_{half}: potential of half-maximal activation or inactivation; k: slope factor of activation or inactivation. * Indicates $P<0.05$ when the parameters were compared with the values of d_0. # Indicates borderline situation ($P= 0.06$) when the parameters are compared with the values of d_0.

Cell culture and Ca^{2+} currents

The properties of Ca^{2+} currents were investigated in several animals like rabbit, rat or cat using acutely isolated as well as cultured myocytes. Nevertheless, to the best of our knowledge, guinea pig ventricular myocyte Ca^{2+} currents have not been characterized comprehensively during short-term culture, although these myocytes are frequently used for studying I_{CaL} and I_{CaT}. Only one study has shown that I_{CaL} in short-term cultured adult ventricular myocytes from guinea pigs on day 0 and day 4 responded similarly to β-adrenoreceptor activation by isoproterenol (Pabbathi *et al.* 2002).

In d_0 cells we found maximum densities of I_{CaL} and I_{CaT} of –12.5±0.95 pA/pF and –1.6±0.4 pA/pF (5.4 mM Ca^{2+} as charge carrier). Thus, the I_{CaT}/I_{CaL} ratio (0.13) is identical to the value found by Balke *et al.* (1992ab) and is in the range (~0.1) reported for guinea pig ventricular myocytes by McDonald *et al.* (1994).

L-type Ca^{2+} current in other culture studies

I_{CaL} has already been investigated in cultured rabbit ventricular myocytes, where I_{CaL} density was reduced significantly after one day in culture but showed a partial recovery after 4 to 6 days (Mitcheson *et al.* 1996). In rat ventricular myocytes peak I_{CaL} density increased by 55 % after 24 h in culture and was still larger after 48 h than in d_0 cells, before returning to control levels after 72 h (Ellingsen *et al.* 1993). In cat ventricular myocytes (plated on laminin) I_{CaL} was significantly larger after 14 days in culture than I_{CaL} measured in d_0 myocytes (Schackow *et al.* 1995). In our investigation I_{CaL} density remained almost constant, suggesting that the initial density of functional L-type Ca^{2+} channels could be maintained. Speculations about the differences between the reported studies are limited by the fact that different species, different culture techniques and isolation procedures (e.g. enzyme) were used. Statistical analysis of I_{CaL} activation and inactivation variables (d_∞ and f_∞) confirmed a slight shift of the f_∞ parameter V_{half} between d_0 and d_5 cells to more negative potentials (–4.6 mV) in our study. The mechanisms underlying this shift are presently unknown but it is noteworthy that similar shifts in V_{half} of steady-state inactivation (without reaching statistical significance) were reported for cultured human atrial (–2.8 mV between d_0 and d_{21}, Benardeau *et al.* 1997) and rabbit ventricular myocytes (–8.1 mV between d_0 and d_1, Mitcheson *et al.* 1996).

T-type Ca^{2+} current in other culture studies

T-type Ca^{2+} current showed a different behavior during culturing in our study. In d_5 cells peak I_{CaT} density declined to 25 % compared to d_0 myocytes. The I_{CaT}/I_{CaL} ratio dropped from 0.13 to 0.05 after 3 days in culture. Studies examining I_{CaT} in cultured cardiomyocytes are very scarce in the literature and are limited to rats (Fares *et al.* 1996, Izumi *et al.* 2003). It has to be mentioned that the expression of ventricular I_{CaT} channels differs strongly between rats and guinea pigs. In rat ventricles I_{CaT} is known to be present in the neonatal stage but absent in adult ventricular cells. Thus, a direct comparison with our data on guinea pigs is rather limited. In adult rat ventricular myocytes a re-expression of T-type Ca^{2+} channels was observed after 8 days in culture whereby the myocyte dedifferentiation was characterized by myocyte rounding, cell spreading and development of cell-cell contacts (Fares *et al.* 1996), suggesting the activation of a "fetal" gene-expression profile involving re-expression of T-type Ca^{2+}-channels. Our observation of a decrease in I_{CaT} without signs of dedifferentiation suggests that a "fetal" gene program was not activated under our experimental conditions.

Possible mechanisms underlying the decline of Ca^{2+} currents

The loss of ion-channels may be due to a reduction of membrane area (Christé 1999) or to an independent membrane area decrease (e.g. alterations in channel turnover). Since the decline of I_{CaL} amplitude overlaps with the decline in C_m (Fig. 4C), it may be simply explained by the observed reduction of membrane area (and L-type channels within), whereas different (additional) mechanisms are likely to play a role for I_{CaT}. A comparison of I_{CaT} density distribution at d_0 (ranging from >3 to 0.2 pA/pF) and d_5 (1.5 to 0.1 pA/pF; Fig. 4B) suggests that the decrease in I_{CaT} may not only reflect changes in channel density of individual myocytes but also "cell-selection-phenomenon" because myocytes expressing a large number of T-channels may have a lower survival rate under our culture conditions. However, further studies are warranted to elucidate the mechanisms underlying the observed current alterations.

Conclusions

Taken together, the present study is the first description of I_{CaL} and I_{CaT} in cultured guinea pig ventricular myocytes. Our data suggest a different

regulation of L-type and T-type Ca^{2+} channels during short-term culture and provide basic information for future studies exploring the effects of putative modulators on the expression of L-type and T-type Ca^{2+} channels.

Acknowledgements

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References

BALKE CW, ROSE WC, MARBAN E, WIER WG: Macroscopic and unitary properties of physiological ion flux through T-type Ca^{2+} channels in guinea pig heart cells. *J Physiol Lond* **456**: 247-265, 1992a.

BALKE CW, ROSE WC, MARBAN E, WIER WG: Macroscopic and unitary properties of physiological ion flux through L-type Ca^{2+} channels in guinea pig heart cells. *J Physiol Lond* **456**: 267-284, 1992b.

BEAN BP: Two kinds of calcium channels in canine atrial cells. Differences in kinetics, selectivity and pharmacology. *J Gen Physiol* **86**: 1-30, 1985.

BENARDEAU A, HATEM SN, RUCKER MARTIN C, TESSIER S, DINANIAN S, SAMUEL JL, CORABOEUF E, MERCADIER JJ: Primary culture of human atrial myocytes is associated with the appearance of structural and functional characteristics of immature myocardium. *J Mol Cell Cardiol* **29**: 1307-1320, 1997.

BERGER HJ, PRASAD SK, DAVIDOFF AJ, PIMENTAL D, ELLINGSEN O, MARSH JD, SMITH TW, KELLY RA: Continual electric field stimulation preserves contractile function of adult ventricular myocytes in primary culture. *Am J Physiol* **266**: H341-H349, 1994.

CHRISTÉ G: Localization of K^+ channels in the T-tubules of cardiomyocytes as suggested by the parallel decay of membrane capacitance, IK_1 and IK_{ATP} during culture and by delayed IK_1 response to barium. *J Mol Cell Cardiol* **31**: 2207-2213, 1999.

CLARK WA, RUDNICK SJ, LAPRES JJ, ANDERSEN LC, LAPOINTE MC: Regulation of hypertrophy and atrophy in cultured adult heart cells. *Circ Res* **73**: 1163-1176, 1993.

CLARK WA, DECKER ML, BEHNKE-BARCLAY M, JANES DM, DECKER RS: Cell contact as an independent factor modulating cardiac myocyte hypertrophy and survival in long term primary culture. *J Mol Cell Cardiol* **30**: 139-155, 1998.

CLAYCOMB WC, BURNS AH, SHEPHERD RE: Culture of the terminally differentiated ventricular cardiac muscle cell. Characterization of exogenous substrate oxidation and the adenylate cyclase system. *FEBS Lett* **9**: 261-266, 1984.

ELLINGSEN O, DAVIDOFF AJ, PRASAD SK, BERGER HJ, SPRINGHORN JP, MARSH JD, KELLY RA, SMITH TW: Adult rat ventricular myocytes cultured in defined medium: phenotype and electromechanical function. *Am J Physiol* **265**: H747-H754, 1993.

FARES N, GOMEZ JP, POTREAU D: T-type calcium current is expressed in dedifferentiated adult rat ventricular cells in primary culture. *C R Acad Sci III* **319**: 569-576, 1996.

HAMILL OP, MARTY A, NEHER E, SAKMANN B, SIGWORTH FJ: Improved patch clamp-techniques for high resolution current recordings from cell and cell-free membrane patches. *Pflügers Arch* **391**: 85-100, 1981.

HIRANO Y, FOZZARD HA, JANUARY CT Characteristics of L- and T-type Ca^{2+} currents in canine cardiac Purkinje cells. *Am J Physiol* **256**: H1478-H1492, 1989.

IZUMI T, KIHARA Y, SARAI N, YONEDA T, IWANAGA Y, INAGAKI K, ONOZAWA Y, TAKENAKA H, KITA T, NOMA A. Reinduction of T-type calcium channels by endothelin-1 in failing hearts in vivo and in adult rat ventricular myocytes in vitro. *Circulation* **108**: 2530-2535, 2003.

JACOBSON SL: Culture of spontaneously contracting myocardial cells from adult rats. *Cell Struct Funct* **2**: 1-9, 1977.

JACOBSON SL, PIPER HM: Cell cultures of adult cardiomyocytes as models of the myocardium. *J Mol Cell Cardiol* **18**: 661-678, 1986.

LIPP P, HÜSER J, POTT L, NIGGLI E: Spatially non-uniform Ca^{2+} signals induced by reduction of transverse tubules in citrate loaded guinea-pig ventricular myocytes in culture. *J Physiol Lond* **497**: 589-597, 1996.

McDONALD TF, PELZER S, TRAUTWEIN W, PELZER DJ: Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells. *Physiol Rev* **74**: 365-507, 1994.

MITCHESON JS, HANCOX J, LEVI AJ: Action potentials, ion channel currents and transverse tubule density in adult rabbit ventricular myocytes maintained for 6 days in cell culture. *Pflügers Arch* **431**: 814-827, 1996.

MITCHESON JS, HANCOX J, LEVI AJ: Cultured adult cardiac myocytes: future applications, culture methods, morphological and electrophysiological properties. *Cardiovasc Res* **39**: 280-300, 1998.

MITRA R, MORAD M: Two types of calcium channels in guinea pig ventricular myocytes. *Proc Natl Acad Sci USA* **83**: 5340-5344, 1986.

NILIUS B, HESS P, LANSMAN JB, TSIEN RW: A novel type of cardiac calcium channel in ventricular cells. *Nature* **316**: 443-446, 1985.

PABBATHI VK, ZHANG YH, MITCHESON JS, HINDE AK, PERCHENET L, ARBERRY LA, LEVI AJ, HANCOX JC: Comparison of Na^+/Ca^{2+} exchanger current and of its response to isoproterenol between acutely isolated and short-term cultured adult ventricular myocytes. *Biochem Biophys Res Commun* **297**: 302-308, 2002.

PIPER HM, PROBST I, SCHWARTZ P, HÜTTER FJ, SPIECKERMANN PG: Culturing of calcium stable adult cardiac myocytes. *J Mol Cell Cardiol* **14**: 397-412, 1982.

SCHACKOW TE, DECKER RS, TEN-EICK RE: Electrophysiology of adult cat cardiac ventricular myocytes: changes during primary culture. *Am J Physiol* **268**: C1002-C1017, 1995.

STIMERS JR, DOBRETSOV M: Adrenergic stimulation of Na/K pump current in adult rat cardiac myocytes in short-term culture. *J Membrane Biol* **16**: 205-216, 1998.

VASSORT G, ALVAREZ J: Cardiac T-type calcium current: pharmacology and roles in cardiac tissues. *J Cardiovasc Electrophysiol* **5**: 376-393, 1994.

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