Calcium currents and transients of native and heterologously expressed mutant skeletal muscle DHP receptor α1 subunits (R528H)

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Abstract Rabbit cDNA of the α1 subunit of the skeletal muscle dihydropyridine (DHP) receptor was functionally expressed in a muscular dysgenesis mouse (mdg) cell line, GLT. L-type calcium currents and transients were recorded for the wild type and a mutant α1 subunit carrying an R528H substitution in the supposed voltage sensor of the second channel domain that is linked to a human disease, hypokalemic periodic paralysis. L-type channels expressed in GLT myotubes exhibited currents similar to those described for primary cultured mdg cells injected with rabbit wild type cDNA, indicating this system to be useful for functional studies of heterologous DHP receptors. Voltage dependence and kinetics of activation and inactivation of L-type calcium currents from mutant and wild type channels did not differ significantly. Intracellular calcium release activation measured by fura-2 microfluorimetry was not grossly altered by the mutation either. Analogous measurements on myotubes of three human R528H carriers revealed calcium transients comparable to controls while the voltage dependence of both activation and inactivation of the L-type current showed a shift to more negative potentials of approximately 6 mV. Similar effects on the voltage dependence of the fast T-type current and changes in the expression level of the third-type calcium current point to factors not primarily associated with the mutation perhaps participating in disease pathogenesis.

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Key words: Dihydropyridine receptor; L-type calcium current; Calcium transient; Muscular dysgenesis mouse; Human myotube; Hypokalemic periodic paralysis

1. Introduction

Dihydropyridine (DHP) receptors of skeletal muscle function as both voltage-gated L-type calcium channels and voltage sensors of intracellular calcium release-initiating contraction [1]. They are composed of five tightly associated proteins, α1, α2/δ, β1, and γ, encoded by four different genes [2]. The α1 subunit is not only the largest of these, but also contains the ion-conducting pore, DHP binding sites, and putative voltage-sensitive structures in four transmembrane helices of four analogous domains, the S4 segments, with an unusually high density of positively charged residues. While the charges of domains I and III seem to be important for channel activation [3], the function of the arginines in repeat II has not been clarified sufficiently. At least mutations of inner arginines in repeat II do not mediate any detectable parameter change [3].

The outermost charge in repeat II, R528, is mutated to histidine in the human disease hypokalemic periodic paralysis [4], suggesting an important role of this residue for channel function. While the first report on DHP receptor-mediated L-type current in myotubes of an affected patient expressing both wild type and mutant channels revealed a 36 mV left shift in steady-state inactivation [5], expression of the rabbit channel in a fibroblast cell line showed a reduction of current density to be the only detectable effect of the amino acid exchange [6]. Introduction of an R528H equivalent, R650H, into the human cardiac α1 subunit and expression in human embryonic kidney cells displayed only barely significant changes in voltage dependence of activation and inactivation of the L-type current [7], leading the authors to conclude that the cardiac channel may not be an adequate model for effects of the skeletal DHP receptor mutant.

Proper functional expression of the skeletal muscle α1 subunit demands the co-expression of the other DHP receptor subunits, the ryanodine receptor and perhaps additional not yet identified triad proteins. Therefore, exclusive expression of R528H in a muscular environment might give a clue to the real disease pathogenesis. Because they provide the necessary environment, primary cultured myotubes from mice homozygous for the muscular dysgenesis (mdg) mutation and thus lacking functional α1 subunits [8] have been frequently used as expression system for recombinant α1 subunit cDNA. Instead of primary cell cultures which require animal maintenance, we successfully employed myotubes derived from a recently developed mdg cell line, GLT [9], and functionally characterized this expression system for the first time. Using whole cell tight seal recordings and fura-2 microfluorimetry, we were able to study not only the L-type current as in the previous reports on the R528H mutant, but also the voltage dependence of intracellular calcium release activation, neither of which has been described for the new cell line or for human myotubes.

2. Materials and methods

2.1. Mutagenesis

As rabbit and human α1 subunit proteins are 95.5% identical [10,11] in the physiologically expressed functionable isoform [12] and functional equivalence may be expected, rabbit cDNA in a PSG5 vector (Stratagene) was employed for site-directed mutagenesis. The G1583A cDNA mutation which results in the R528H amino acid change was introduced using the reverse oligonucleotide 5′-CGGATGCAGTGCAACACGG-3′ and verified by sequencing [6].

2.2. Cell culture

Myoblasts of an immortalized mdg mouse muscle cell line, GLT [9], were cultured in a medium consisting of 80% Dulbecco's modified Eagle medium (1 g glucose/l), 10% fetal calf serum, and 10% horse

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serum (all Gibco) in gelatin-coated bottles or dishes at 37°C and a CO_2 atmosphere of 5%. Serum content was reduced to 2% horse serum on day 2 after the last passing procedure to induce maturation and fusion of cells. To optimize the transfection method, we initially performed all experiments using both LacZ gene and the rabbit cDNA thus checking efficiency both quantitatively and functionally. Calcium phosphate precipitation yielded the best transfection rates. Lipofectamine treatment lead to vacuolization of the cells making patch-clamping unnecessarily difficult. Functional expression of L-type currents could be achieved by transfecting on days 6–8, with the best results on culture day 7, shortly after fusion commenced. Transfections on days 2–5 before fusion did not allow differentiation, later myotube fusion or detectable channel expression. To enable detection of cells which had taken up $\alpha 1$ subunit cDNA, cells were co-transfected with both the PSG5 vector and EBO-pcD-CD8 (Leu2) containing cDNA of the lymphocyte surface antigen CD8 (T-lymphocyte cytotoxic suppressor gene, gift of R.F. Margolskee) in a ratio of 5:1 using the calcium phosphate precipitation method at day 7 after the last passing step. Successful expression of the marker gene detected by polystyrene microspheres precoated with antibodies to CD8 [13] indicated candidate cells for patch-clamp experiments.

Human HypoPP myotubes were derived from satellite cells of biopsies from the lateral vastus muscle of patients carrying the R528H mutation. Control myotubes were obtained from subjects biopsied for malignant hyperthermia diagnosis and found to be negative in the in vitro contracture test. All procedures involving human material were performed in accordance with the Helsinki convention and approved by the Ethics Commission of the University of Ulm.

Biopsy material was enzymatically dissociated at 37°C in Hanks' solution (Biochrom) containing collagenase (250 U/ml, type II, Sigma) and bactotrypsin (Biochrom). The cells were grown in a 1:1 mixture of Ham's F12 and CMRL medium containing 5% horse serum and 5% fetal calf serum (both Biochrom) in a 5% CO_2 atmosphere at 37°C [5].

2.3. Electrophysiology

Standard whole cell recordings were performed on 8–15 day old myotubes after partial (30–60%) series resistance compensation using an Axopatch 200A patch-clamp amplifier (Axon Instruments). Voltage errors due to series resistances were less than 5 mV for GLT myotubes and less than 9 mV for human myotubes. The bathing solution contained (in mM): TEA-Cl 120, $CaCl_2$ 10, $MgCl_2$ 1, HEPES 10, glucose 5, 4-aminopyridine (4-AP) 1, EGTA 0.1, tetrodotoxin 0.02 (pH 7.4). Pipettes were filled with (in mM): CsCl 130, $MgCl_2$ 0.5, HEPES 10, EGTA 1, Mg-ATP 5, phosphocreatine 5

(pH 7.2). All measurements were performed at room temperature. Data were filtered at 2 kHz and analyzed using pClamp (Axon Instruments) and Excel (Microsoft).

L-type current activation parameters were obtained from the peak current-voltage relations I(V) by least square fitting of Eq. 1 which includes a correction term for a linear leak with conductance g_{leak} and reversal potential V_{leak}.

$$I(V) = g_{leak} \times (V - V_{leak}) + g_{max} \times (V - V_{Ca})/(1 + \exp((V_{0.5} - V)/k)) \tag{1}$$

V_{Ca} and g_{max} are reversal potential and maximum conductance respectively.

For steady-state inactivation, prepulses of 20 s duration to different voltages V_{pre} were applied and the current $I(V_{pre})$ which remained activatable by a subsequent test pulse to 20 mV was normalized by the current I_{max} obtained in the absence of a prepulse. $I(V_{pre})/I_{max}$ was described as Y(V) by Boltzmann functions of the form:

$$Y(V) = 1/(1 + \exp((V_{0.5} - V)/k)) \tag{2}$$

2.4. Calcium transients

Intracellular calcium transient measurements were performed using a modified pipette solution containing 100 μM of the penta-potassium salt of fura-2. Loading times of 5 min were required for the resting fluorescence of the indicator dye to reach a steady level. Calcium signals were elicited by step depolarizations. Fluorescence records were expressed as $\Delta F/F$ (F = fluorescence intensity at 380 nm excitation, $\Delta F = -(F_{transient} - F_{baseline})$). Recordings were made by means of an inverted microscope equipped for epi-illumination (Axiovert 100, Zeiss) employing a set of fura-2 filters (emission 510 nm, excitation 380 and 360 nm; Zeiss). The isosbestic wavelength 360 nm was used for control dye loading. A variable rectangular slit in the emission pathway mounted in front of the photomultiplier (valve R269, Hamamatsu; gauge MEA1530FK-XV2DN, Seefelder) excluded fluorescence from the lumen of the patch pipette. Fluorescence signals were recorded at a sampling rate of 1 kHz and later digitally filtered with a Gaussian algorithm at a cut off frequency of 100 Hz. The voltage dependence of activation A(V) of the calcium signals was also described as Y(V) with Eq. 2.

Inactivation of calcium transients was not studied, as it represents a combination of fura-2 and endogenous calcium buffer binding kinetics, calcium re-uptake into the sarcoplasmic reticulum, calcium transport into the extracellular space, and kinetics of ryanodine receptor inactivation.

Table 1
Parameters of L-type calcium currents and intracellular calcium transients

	WT rabbit cDNA	Mutant rabbit cDNA	Human control	Human HypoPP
Current activation				
$V_{0.5}$	23.1 ± 2.3 mV	20.9 ± 3.4 mV	14.3 ± 0.8 mV	8.6 ± 1.8 mV*
k	-6.8 ± 0.5 mV	-7.6 ± 0.6 mV	-5.9 ± 0.4 mV	-6.2 ± 0.4 mV
V_{Ca}	70.4 ± 6.9 mV	73.7 ± 6.6 mV	71.0 ± 4.4 mV	66.6 ± 4.4 mV
U_{max}	33.7 ± 3.2 mV	33.7 ± 3.2 mV	24.3 ± 1.1 mV	18.4 ± 1.8 mV*
I_{max}	-0.9 ± 0.2 pA/pF	-1.20 ± 0.3 pA/pF	-1.2 ± 0.1 pA/pF	-1.6 ± 0.1 pA/pF
Capacitance	315 ± 53 pF	216 ± 64 pF	474 ± 50 pF	433 ± 90 pF
Current density	-0.89 ± 0.2 pA/pF	-1.2 ± 0.3 pA/pF	-1.24 ± 0.1 pA/pF	-1.60 ± 0.2 pA/pF
n	7	7	24	26
Inactivation				
$V_{0.5}$	4.2 ± 1.0 mV	3.5 ± 2.7 mV	31.1 ± 1.2 mV	-9.4 ± 0.8 mV*
k	6.7 ± 0.6 mV	6.9 ± 0.5 mV	8.1 ± 0.5 mV	9.4 ± 1.1 mV
n	4	4	32	21
Kinetics				
τ_{m-slow}	61.9 ± 17.6 ms	51.8 ± 9.3 ms	77.2 ± 9.3 ms	73.3 ± 8.2 ms
τ_h	5.1 ± 1.1 s	4.1 ± 1.0 s	2.7 ± 0.4 s	2.3 ± 0.4 s
n	4	4	8	12
Ca transient				
$V_{0.5}$	-10.5 ± 3.3 mV	-15.7 ± 3.5 mV	-23.1 ± 1.5 mV	-22.0 ± 2.5 mV
k	-8.0 ± 1.3 mV	-8.1 ± 1.4 mV	-8.5 ± 1.1 mV	-8.3 ± 0.9 mV
n	13	7	7	6

Significant differences are marked with an asterisk. Abbreviations: U_{max} = voltage at current maximum, I_{max} = current maximum. For further abbreviations see text.
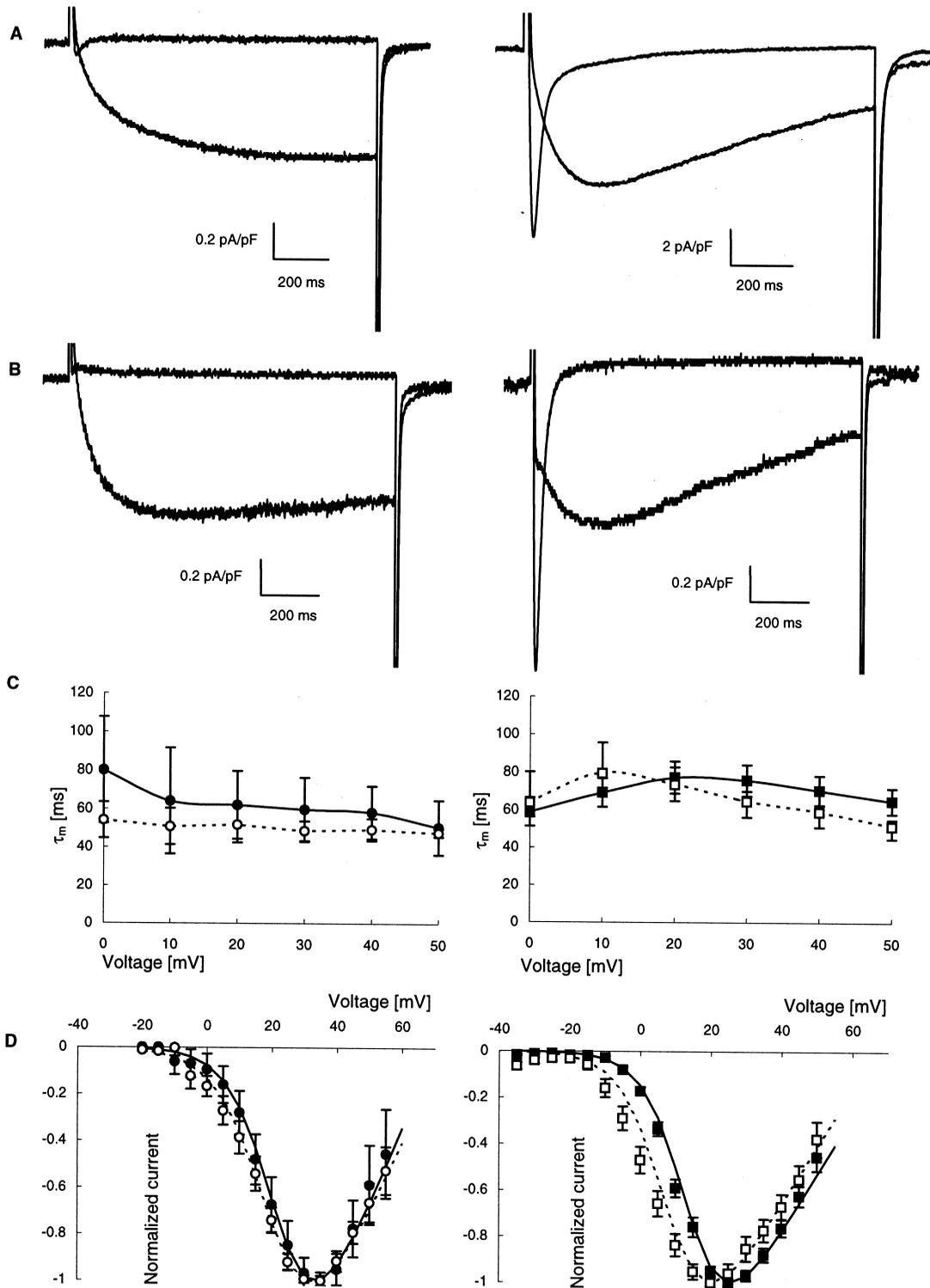
Fig. 1. Activation of calcium currents in immortalized mdg (GLT) myotubes (circles) and human myotubes (squares) expressing wild type (filled symbols) or mutant (open) α1 subunits. A: Representative current traces measured in a GLT myotube expressing wild type rabbit α1 subunits (left) and a normal human myotube (right). Voltage steps were applied from a holding potential of −90 mV to −20 mV and +25 mV respectively. Note the fast low-threshold T-type calcium current and the slow high-threshold L-type current in both myotubes. B: Current traces as in A, however for mutant (R528H) wild type rabbit α1 subunits (left) and a myotube of a HypoPP patient heterozygous for the R528H mutation (right) showing an incision in the L-type current trace suggesting a third-type current. C: Voltage dependence of activation time constants τ_{m-slow} for all four cell types: GLT cells on the left ($n=4$ each) and human myotubes on the right (control $n=8$ and HypoPP $n=12$). D: Average L-type current-voltage relation (means ± S.E.M.) in the GLT myotubes for wild type (filled circles) and mutant for $n=7$ each on the left, HypoPP myotubes ($n=26$) and human control myotubes ($n=24$) on the right. Symbols represent mean of data points, curves are defined by the mean of the fitted parameters (Eq. 1).
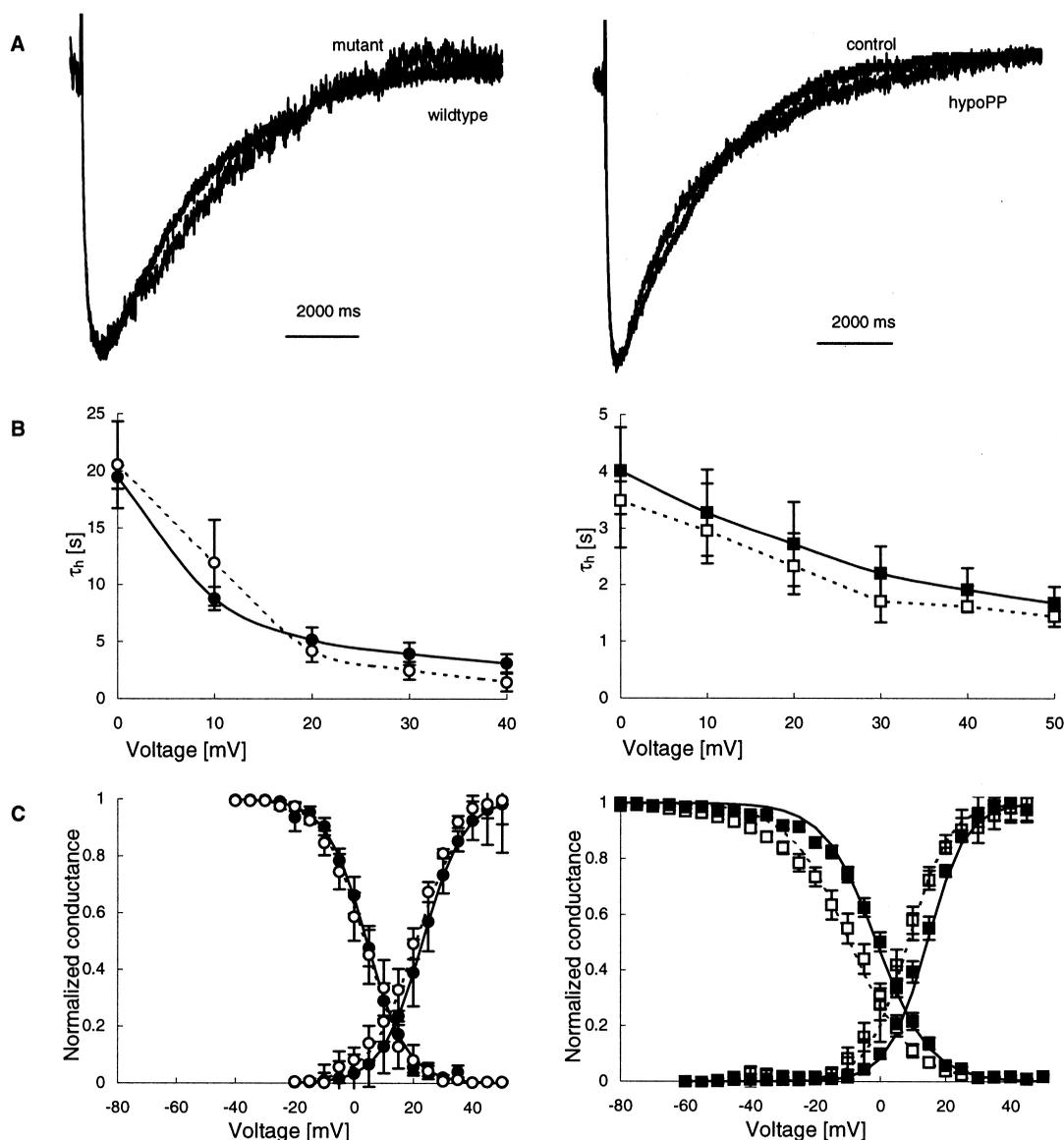
Fig. 2. Inactivation of calcium currents in GLT (circles) and human myotubes (squares) expressing wild type (filled symbols) or mutant (open) α1 subunits. A: Examples of superimposed current traces at 20 mV normalized to 1 measured in GLT myotubes with wild type and mutant rabbit α1 subunits (left) as well as in human control and HypoPP myotubes (right) elicited from a holding potential of −90 mV. B: Voltage dependence of inactivation time constants τ_h for all four cell types: GLT cells on the left ($n = 4$ each) and human myotubes on the right (control $n = 8$ and HypoPP $n = 12$). C: Average fractional steady-state inactivation (means ± S.E.M.) in GLT myotubes for wild type and mutant for $n = 4$ each on the left, human control ($n = 32$) and HypoPP myotubes ($n = 21$) on the right. Curves were calculated with the means of Boltzmann fitted parameters (Eq. 2). In addition, the corresponding steady-state activation curves calculated from the data given in Fig. 1C are shown.

2.5. Statistics

Data are expressed as mean ± S.E.M. Statistical evaluation of differences between wild type and mutant was done using Student's double-sided t-test, with $P \leq 0.05$ considered significant.

3. Results

3.1. GLT myotubes expressing normal and mutant rabbit DHP receptor α1 subunit

The present study is the first one to make use of the immortalized mdg cell line GLT [9] for functional expression of recombinant and wild type α1 subunits. Untransfected GLT myotubes ($n = 10$) showed no trace of slow high-voltage-activated calcium inward currents. These, however, were detect-

able after transfection and are therefore considered to be mediated by the exogenous α1 subunit (Fig. 1A, left). GLT transfected with R528H α1 cDNA revealed non-significantly higher current densities (Table 1) at similar transfection rates (25% for the mutant and 32% for wild type in a CD8^{+} cell population) and comparable maximal current amplitudes (−260 vs. −280 pA; Fig. 1B, left).

Activation was fitted by two exponentials starting 6 ms after onset of the depolarization to minimize the influence of the fast current components. The time constant for L-type current activation, τ_{m-slow}, showed considerable variability although overall little voltage dependence (Fig. 1C, left). Even though activation of the mutant channels was slightly faster on average, values for τ_{m-slow} did not differ significantly
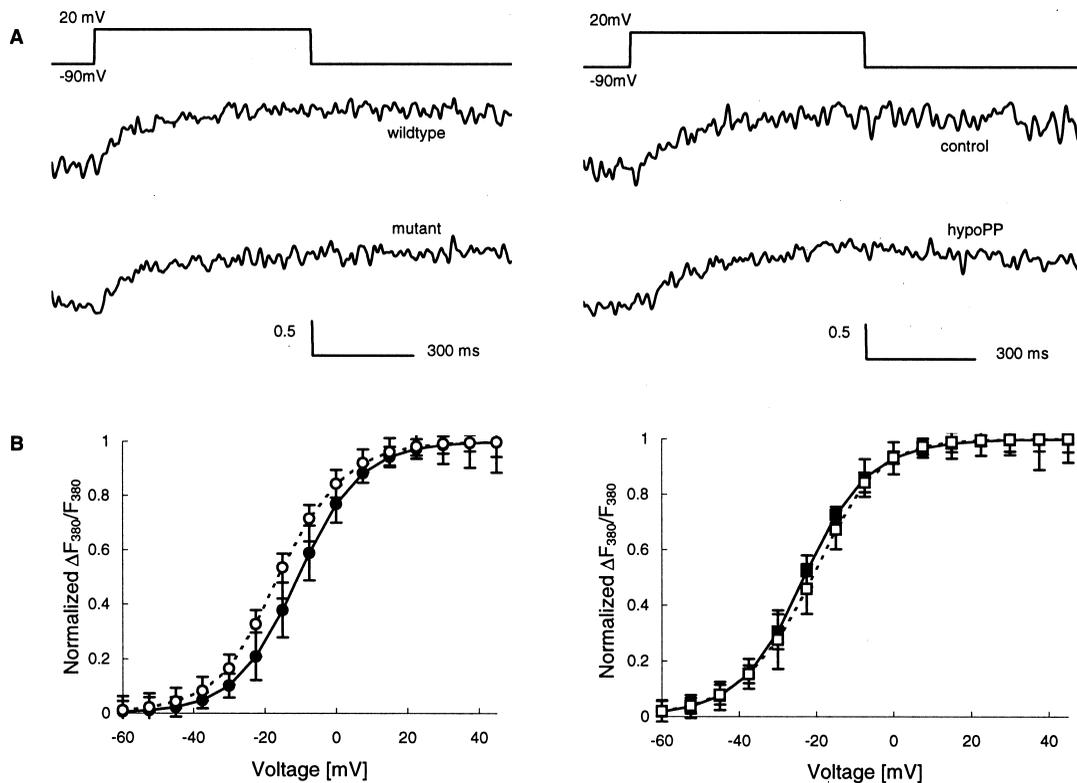
Fig. 3. Calcium transients in GLT and human myotubes. A: Average traces ($n = 8$) of calcium transients at 20 mV elicited from a holding potential of -90 mV and normalized to 1 for wild type and mutant rabbit channels expressed in GLT myotubes (on the left) as well as for normal control and HypoPP myotubes (on the right). B: Means of the transient steady-state ratios (means ± S.E.M.) in the GLT myotubes for wild type (filled circles, $n = 13$) and mutant (open circles, $n = 7$) on the left, hypokalemic periodic paralysis myotubes (open squares, $n = 6$) and unaffected controls (filled squares, $n = 7$) on the right. Curves represent means of the Boltzmann fitted parameters.

for depolarizing steps to 20 mV, i.e. near the current maximum (Table 1). Analysis of I-V data (Figs. 1D and 2C, left) according to Eq. 1 yielded similar best fit parameter values for the two groups (Table 1). Capacitances of cells expressing R528H mutant channels were by about 30% smaller on average.

The decay of calcium currents in GLT myotubes was evaluated by determining inactivation time constants, τ_h, in current traces of 20 s (Fig. 2A, left). No significant difference in kinetics between mutant and wild type could be seen (Fig. 2B, see also Table 1). Steady-state inactivation curves (Fig. 2C, left) were essentially identical.

3.2. Human myotubes expressing wild type and mutant DHP receptor α1 subunit

The similar results on GLT cells for wild type and mutant channels prompted us to reinvestigate myotubes from healthy controls and HypoPP patients. Three definitely clinically affected persons were biopsied, two members of family HypoPP19 described in [4], and one of family HypoPP11 described in [5]. Myotubes of nine unaffected individuals were cultured as controls.

Patients' myotubes required on average four culture days longer to obtain currents sufficient for measurement (Fig. 1A,B, right). Kinetics of activation and inactivation fitted as described above were not significantly different for mutant and normal L-type currents (Figs. 1B and 2A,B, right; see also Table 1). Comparing current-voltage relations (Fig. 1D), peak current density did not differ significantly, but the

voltage at which maximum current was reached marginally did (18.4 vs. 24.3 mV). Of the fitted parameters for steady-state activation and inactivation, the $V_{0.5}$ values were both shifted to the left by 6–8 mV (8.6 vs. 14.3 mV, $n = 26$ and 24, and -9.4 vs. -1.1 mV; Fig. 2C, right). The corresponding change in reversal potential was not statistically significant (Table 1).

In order to determine whether the displacements of the voltage dependencies were specific for the L-type current, additional parameters independent of the DHP receptor were examined. The percentage of cells expressing both L-type and fast transient (T-type) calcium currents was similar for patient and control myotubes (68.8% vs. 67.3%), while a third-type current first described by Rivet et al. [14] was distinguishable in 31.4% of cells of R528H carriers compared to 16.4% in controls. Voltage at current maximum and steady-state inactivation of the T-type current were significantly altered in a similar way as the L-type currents: voltage at current maximum: -29.0 ± 6.8 mV vs. -25.1 ± 5.8 mV; steady-state inactivation: $V_{0.5} = -55.9 \pm 2.4$ mV vs. -48.4 ± 2.5 mV, despite the steepness of the inactivation curve remaining unchanged: $k = 5.1 \pm 0.4$ mV vs. 4.7 ± 0.7 mV ($n = 16$ and 14, respectively).

3.3. Calcium transients in GLT and human myotubes

L-type current amplitude was reduced by the dye-containing pipette solution so that a second set of cells was required for calcium transient measurements. The voltage protocol consisted of rectangular pulses from the holding potential of

−90 mV to the test potentials from −60 mV to +40 mV. Neither depolarizing prepulses nor addition of heavy metal ions were applied to block T-type current influence in an effort to minimize possible effects on the dihydropyridine receptor by partial inactivation and change of superficial charge density. At membrane potentials where T currents were maximal, the fluorescence signal was small and no correlation could be seen between the size of the T current and the size of the fluorescence signal, thus justifying this procedure.

Fig. 3A shows the ratio $-\Delta F/F$ at 380 nm excitation (see Section 2) resulting from calcium release for GLT (left) and human myotubes (right) for depolarizing voltage steps from −90 mV to +20 mV. Maxima (average of last 20 points) for each depolarizing step were plotted against voltage and fitted with a Boltzmann relation (Eq. 2). When comparing the activation parameters for calcium transients (Fig. 3B) with those of calcium conductance (Table 1) it becomes obvious that calcium release is activated at about 35 mV more negative potentials ($V_{0.5}$). However, a significant difference between wild type and mutant could be detected neither in $V_{0.5}$ nor in k Boltzmann fit (Table 1).

4. Discussion

4.1. Calcium transients and currents of heterologously expressed DHP receptors

Attempts to utilize an immortalized mdg cell line for functional expression of skeletal muscle DHP receptors have failed in the past, presumably due to lack of fusion of precursor cells to myotubes [15]. Transfection had previously been performed directly before myotube fusion corresponding to culture days 4 and 5 in our cell system, a time point at which GLT, too, did not display the ability to fuse and to express detectable L-type currents. The decisive criterion for expression therefore may well be the time point of transfection, which was most successful on day 7 of culture, i.e. shortly after fusion commences.

Whole cell L-type currents in transfected GLT cells resembled those in primary dysgenic myotubes injected with cDNA of the rabbit channel showing similar voltage dependence of gating (activation: $V_{0.5} = 24$ mV, $k = -8.2$ mV according to [16]. Also, our average current density and thus channel density for wild type channels was within the range described for injected primary mdg cultures and our rather high $\tau_{\mathrm{m-slow}}$ corresponded to the negative linear relationship between these two parameters described by [17]. Judging by this, the structural and functional equivalence of our system to primary mdg seems confirmed, although the expression levels clearly ranged at the lower end of those for the primary myotubes.

Calcium transients in transfected GLT activated at 33 mV more negative potentials than in injected primary mdg cultures (18 mV [16]) while the voltage dependence of current conductance was similar. This may be due to an unusually high intracellular concentration of 5 mM free Mg^{2+} (as 5 mM $MgCl_2$) and absence of ATP in the solution used by [16] possibly rendering the ryanodine receptor less sensitive to voltage changes. Our solution contained 0.5 mM $MgCl_2$ and 5 mM MgATP resulting in approximately 1 mM free Mg^{2+}, i.e. near physiological values.

4.2. Mutant DHP receptors

Residue R528 of the α1 subunit, mutated in the human disease hypokalemic periodic paralysis [4], would be expected to play an important role in channel function. Because of the location of the mutation in one of the putative voltage-sensing regions (S4), we focused on three voltage-dependent gating processes attributed to the DHP receptor, i.e. activation and inactivation of the slow (L-type) calcium conductance and the activation of calcium release. Surprisingly, no clear effect on gating by the R528H mutation could be detected in the GLT cells. Also, expression of both mutant and wild type rabbit channels seemed comparable as judged by current densities and transfection rates. The fact that Lapie et al. [6] found a current density reduction of almost 70% using the same construct in a fibroblast cell line may well be dependent on the expression system employed, as other characteristics such as the voltage dependence of gating also deviated clearly from the results of Garcia et al. [16] and our own. It seems unlikely that reduction of current density should be the pathogenetic effect in hypokalemic periodic paralysis, as strength is not reduced in mice heterozygous for the mdg frame shift mutation nor do elderly human beings show paralytic attacks despite the reduction of DHP receptor expression down to 50% [18]. Also, the dominant mode of inheritance of the disease suggests a change of function by the mutation rather than a loss of function.

In human myotubes, a slight but systematic voltage shift of about −6 mV in activation and inactivation of both L-type and T-type currents was found. The twofold overexpression of the third-type calcium current, perhaps compensating a very slight L-type channel deficiency, implies a change in protein expression patterns. If such changes affected for example auxiliary α2/δ or β1 subunit expression crucial for voltage dependence of gating, they could well account for the above observations [19]. As the time point of introduction of the α1 subunit affects differentiation and fusion behavior of the myotubes, demonstrated by our experiments on GLT, the presence of the mutation in early stages of development in human cells may well change differentiation processes and protein expression patterns from the start.

Sipos et al. [5] reported a left shift of over 30 mV in L-type current steady-state inactivation in four myotubes of a single R528H carrier. Our present results in 26 steady-state inactivation measurements in myotubes of three affected carriers (including the father of the patient examined in [5]) using identical culture conditions, pipette and bath solutions fail to confirm this. Re-evaluation of the data given in [5] revealed the apparent employment of an incorrect calculation algorithm and the true parameters of steady-state inactivation of the mutant vs. wild type to be -5.5 ± 11.2 mV vs. -4.9 ± 4.3 mV and $k = 8.3 \pm 2.0$ mV vs. 8.8 ± 1.3 mV. Due to the large standard deviation and so few measurements ($V_{0.5}$ ranged from −21.55 mV to +2.75 mV for measurements of the mutant), the slight negative shift of 6 mV is not detectable in that data set (Sipos et al., in preparation).

Voltage-sensing S4 segments are thought to move outward during membrane depolarization. While single deletions of positively charged residues of IS4 and IIIS4 are sufficient to significantly affect voltage dependence of L-type current activation, this is not the case for inner charge deletions of IIS4 [3]. Stühmer et al. [20] introduced double deletions of these charges in IIS4 before achieving a detectable effect in the analogous sodium channel α subunit. Perhaps the R528H mutation indeed is not sufficient to disturb current activation

decisively, leaving a possible influence on calcium release to account for the pathogenesis of the weakness attacks observed in hypokalemic periodic paralysis patients. This would agree with Lerche et al. [7] who found no appreciable change in gating of an analogous mutation, R650H, in the cardiac channel. As calcium transients had not been measured before, the present study is the first to describe these. Lack of a clear effect in our measurements may indicate that more subtle functional changes account for disease pathogenesis. Secondary to the mutation, changes in expression patterns of channel subunits or regulatory proteins as suggested by the alterations in T-type and third-type calcium currents may be an important factor leading to the muscle cell membrane depolarization associated with the paralytic attacks in patients.

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