

# The neonatal sarcoplasmic reticulum $\text{Ca}^{2+}$ -ATPase gives a clue to development and pathology in human muscles

Magdolna Kósa · Kitti Brinyiczki · Philip van Damme ·  
Nathalie Goemans · Károly Hancsák ·  
Luca Mendler · Ernő Zádor

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**Abstract** The sarcoplasmic/endoplasmic reticulum calcium ATPase 1 (SERCA1) has two muscle specific splice isoforms; SERCA1a in fast-type adult and SERCA1b in neonatal and regenerating skeletal muscles. At the protein level the only difference between these two isoforms is that SERCA1a has C-terminal glycine while SERCA1b has an octapeptide tail instead. This makes the generation of a SERCA1a specific antibody not feasible. The switch between the two isoforms is a hallmark of differentiation so we describe here a method based on the signal ratios of the SERCA1b specific and pan SERCA1 antibodies to estimate the SERCA1b/SERCA1a dominance on immunoblot of

human muscles. Using this method we showed that unlike in mouse and rat, SERCA1b was only expressed in pre-matured infant leg and arm muscles; it was replaced by SERCA1a in more matured neonatal muscles and was completely absent in human foetal and neonatal diaphragms. Interestingly, only SERCA1a and no SERCA1b were detected in muscles of 7–12 years old boys with Duchenne, a degenerative-regenerative muscular dystrophy. However, in adult patients with myotonic dystrophy type 2 (DM2), the SERCA1b dominated over SERCA1a. Thus the human SERCA1b has a different expression pattern from that of rodents and it is associated with DM2.

M. Kósa · L. Mendler · E. Zádor (✉)  
Department of Biochemistry, Faculty of General Medicine,  
University of Szeged, Dóm tér 9, H-6720 Szeged, Hungary  
e-mail: zador.erno@med.u-szeged.hu

K. Brinyiczki  
Department of Pathology, Faculty of General Medicine,  
University of Szeged, Szeged, Hungary

P. van Damme  
Neurology Department, University Hospital Leuven, Louvain,  
Belgium

P. van Damme  
Leuven Institute for Neurodegenerative Disorders (LIND), KU  
Leuven, Louvain, Belgium

P. van Damme  
Vesalius Research Center VIB, Louvain, Belgium

N. Goemans  
Department of Development and Regeneration, University  
Hospital Leuven, Herestraate 49, 3000 Louvain, Belgium

K. Hancsák  
Radnóti Miklós Experimental Grammar School, Szeged,  
Hungary

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## Introduction

The gene of the sarco/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase 1 (*ATP2a1*) encodes four isoforms resulted by development and tissue dependent splicing of the main transcript. Two of these isoforms are truncated because exon 11 and/or exon four are spliced out forming shorter proteins with no  $\text{Ca}^{2+}$ -pump activity and no expression in muscle (Chami et al. 2001). The non-truncated isoforms are expressed in adult fast (SERCA1a) and in neonatal/developing (SERCA1b) skeletal muscle (Brandl et al. 1986). The penultimate exon 22 containing a stop codon is preserved in SERCA1a mRNA and results in a 994 amino acid protein with a C-terminal Gly. In the SERCA1b mRNA exon 22 is excised and this allows the use of the next stop codon in

exon 23, hereby a DPEDERRK sequence is translated at the C-terminal instead of a Gly (MacLennan et al. 1985; Brandl et al. 1987; Zhang et al. 1995).

SERCA1a and SERCA1b are  $\text{Ca}^{2+}$ -pumps and have similar  $\text{Ca}^{2+}$ -transport rates and  $\text{Ca}^{2+}$ -dependency when expressed in COS-1 cells (Maruyama and MacLennan 1988). However this does not exclude in vivo functional differences i.e. in myotubes. Research on SERCA1b was hampered partly because only pan SERCA1-antibody existed for protein detection (Zubrzycka-Gaarn et al. 1984) and distinction between the two isoforms could be made only at the mRNA level (Zádor et al. 1996; Zádor et al. 1999).

The first SERCA1b specific antibody was generated against the C-terminal octapeptide and was found to be effective in rat, mouse and human (Zádor et al. 2007). It was shown that the SERCA1b protein is dominantly expressed in myogenic cells, myotubes (C2C12 and sol8), and in early regenerating rat muscle. Interestingly the neonatal mice diaphragm expressed practically only SERCA1b, not SERCA1a (Zádor et al. 2007) which was of special interest since the SERCA1-null mice (eliminating both SERCA1a and 1b expression) showed uneven diaphragm development and died in progressive respiratory failure soon after birth without compensatory up-regulation of other SERCA isoforms (Pan et al. 2003). Using SERCA1b specific antibody it was elicited that the SERCA1b protein itself is not expressed in passively stretched and denervated adult rat muscles in spite of the increased SERCA1b:SERCA1a mRNA ratio (Zádor et al. 1999, 2007). This suggested that there is a translational block of SERCA1b expression in adult rodent muscle.

In the meantime the pan SERCA1 antibodies have been misinterpreted as SERCA1a specific ones at least in four articles (Babu et al. 2007; Fajardo et al. 2013; Schneider et al. 2013; Lee et al. 2014). However none of these or any other work to our knowledge demonstrated the SERCA1a specificity for an antibody without reacting with SERCA1b. In fact, it is difficult to imagine that a SERCA1a specific antibody could have ever been made since it has to recognise only a single C-terminal glycine which is present in many other proteins not just in SERCA1a. Therefore the use of pan SERCA1 and SERCA1b specific antibodies was the only feasible tool to reveal the ratio between the SERCA1b and SERCA1a proteins in regenerating soleus muscle of rat (Zádor et al. 2007) and in infant muscle (Guglielmi et al. 2013a). In the present work this method is improved by evaluation of the signal ratios with appropriate statistics and by the extended evaluation of SERCA1a/SERCA1b protein ratio to fetal/neonatal human skeletal muscles, to the degenerating-regenerating Duchenne muscular dystrophy (DMD) and to myotonic dystrophy type 2 (DM2). The above illnesses have been reported

to have an altered  $\text{Ca}^{2+}$ -homeostasis (Kiviluoto et al. 2011; Santoro et al. 2014), DM2 even showed elevation of SERCA1b transcript (Vihola et al. 2013) but none of them have been analysed so far for the SERCA1b protein.

## Methods

### Animal treatment

Regeneration of soleus muscles in 300–330 g male Wistar rats was induced by notexin as described previously (Zádor et al. 1996). Muscles were removed after 5 days ( $n = 5$ ). Normal soleus was dissected from untreated leg of the animals ( $n = 5$ ). Animal experiments were approved by the Ethical Committee of Animal Treatment at the Medical Faculty of the University of Szeged, Hungary.

### Human samples

Permissions to collect human samples were received from the Ethical Committees of the University of Szeged and of the University Hospital Leuven, Belgium. Post mortem foetal and neonatal samples, altogether 19 muscles of five subjects who died of diseases not related to muscle disorders (labelled I–V. according to age) were analysed. Brief summaries of the medical histories are as follows: *I.* intrauterine death, gestational age not known, weight 1500 g; *II.* intrauterine death without known foetal or maternal cause, birth at the 30th gestational week; *III.* birth at 24th gestational week, death two months later, respiratory distress syndrome, necrotising enterocolitis, cerebral haemorrhage; *IV.* mature infant, death 2 and half weeks later, congenital heart disease followed by surgery; *V.* birth at 36th gestational week, death one month later, intrauterine infection and consequent sepsis. The investigated muscles were vastus lateralis, gluteus maximus, biceps brachii– (later referred to as vastus, gluteus, biceps) and diaphragm.

Biopsies of two DM2 patients and three DMD patients were investigated (with permission of the Ethical Committee of the University Hospital Leuven). DM2 patients were 42 and 43 years old, samples were taken from the quadriceps muscle. Patients suffering from DMD were 7, 8 and 12 years old, received corticosteroid (calcort or prednisone) treatment in history and biopsies were taken from tibialis anterior muscles.

### RNA detection

RNA was isolated from muscles and reverse transcription was made as described in (Zádor et al. 1996). The cycle number of PCR was adjusted to 35 that saturated

SERCA1a amplification but was linear for detection of SERCA1b mRNA (95–60–72 °C). Primers for human SERCA1 (fw: 5'CTCCATCTGCCTCTCCATGTC3' and rev: 5'ATGCTCACTTCCTTCTTTTCATCTT3') amplified a 248 and 206 bp fragment of SERCA1a and SERCA1b respectively as in (Zádor et al. 2007). Primers for human HPRT were 5'TGCTCGAGATGTGATGAAGG3' and 5'TC CCCTGTTGACTGGTCATT3' these amplified a 192 bp fragment in 33 cycles (95–60–72 °C). PCR fragments were separated on 6 % PAGE, stained with ethidium bromide and analysed by GEL DOC 2000 (Bio-Rad).

### Antibodies

The mouse anti-SERCA1 monoclonal antibody (A3, cell supernatant) that recognizes both SERCA1a and SERCA1b was received from prof. Frank Wuytack (Zubrzycka-Gaarn et al. 1984). Antisera raised in rabbit against the C-terminal octapeptide 994-DPEDERRK-1001 in human and rat SERCA1b enabled specific detection of SERCA1b (Zádor et al. 2007).

### Western-blot

Membrane microsomal fractions for gel loadings were prepared as described previously (Zádor et al. 1998). For total extracts muscles were homogenized in RIPA buffer (150 mM NaCl; 50 mM Tris-HCl (pH 7.5); 0.1 % Triton X-100; 0.1 % SDS; 25 mM sucrose) supplemented with protease inhibitor cocktail (Sigma Aldrich) and 1 mM PMSF. After centrifuging at 1,000×g homogenates were stored at –20 °C.

Gel loadings of human samples were normalized to 5.5 mg fresh weight (indicated later if different). Loading series were designed in order to stay within linear detection range. On the gels increasing loadings of one sample was followed by decreasing loadings of another sample to maximize protein transfer to the membranes. Immunoblots were developed as in (Zádor et al. 2007). The loading was checked by Ponceau S staining of the blots but this was informative only for the total extracts, since the SERCA band was not reliably visible in the membrane-microsomal fraction. Therefore loading was controlled by equivalence to fresh weight. Band quantities (intensity/mm<sup>2</sup>) were evaluated with Quantity One software (Bio-Rad). For estimation of SERCA1a/SERCA1b ratio increasing loading series of a certain muscle were evaluated in parallel with the loadings of the 5 days regenerating rat soleus muscle on the same blot. SERCA1b/SERCA1 signal ratios were calculated for each loading of a muscle. Since these ratios may show variations at each loading, the real value of the

SERCA1b/SERCA1 ratio was estimated from four increasing loadings of the investigated muscle by fitting a horizontal line with the method of least squares. The SERCA1b signal of a certain loading was always related to its SERCA1 signal and such ratios of the investigated muscles and the regenerating rat soleus (loaded on the same gel) were compared. Because of this no normalisation to internal control band was necessary. The ratios of different muscles (i.e. horizontal lines on graphs) were statistically compared by extra sum-of-squares F test (level of significance <0.05). If ratios of two muscles were not significantly different, the graph showed one common line instead of two (e.g. in Fig. 4). Graphs and statistical analysis were performed by GraphPad Prism.

Microsomal membrane fraction of a human muscle was also run on gradient gel (8–16 % acrylamide in Tris-glycine, Pierce) and developed by ECL (Amersham) using SERCA1 (1:100) and SERCA1b (1:2000) primary antibodies.

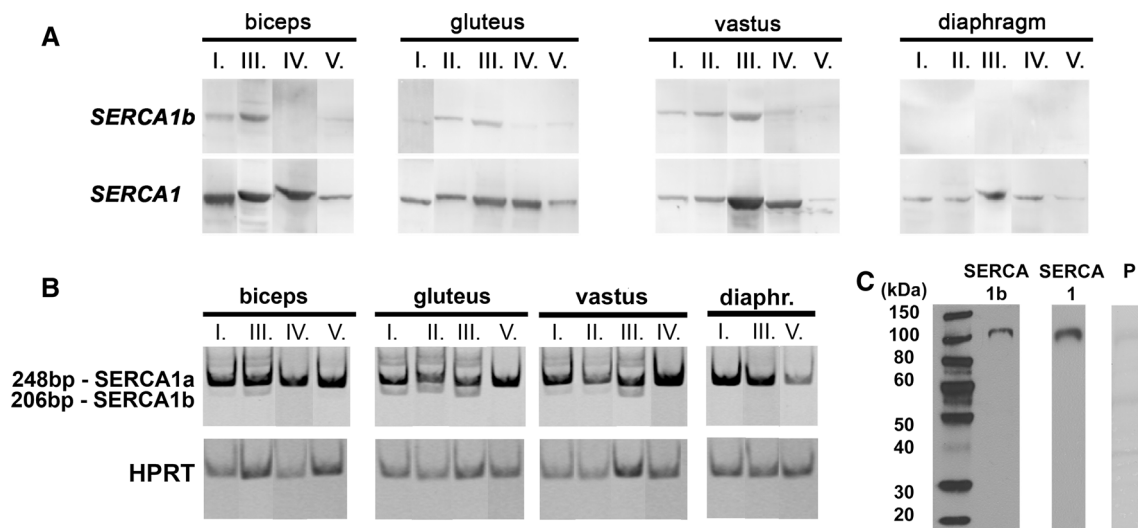
## Results

### SERCA1b protein and mRNA of foetal and infant muscles

SERCA1b was analysed with immunoblot and found to be present in leg, arm and gluteal muscles of the two feti (I., II.) and of the premature infant (III.), but it was absent in more matured infants (IV., V.) (Fig. 1a). All SERCA1b positive muscles had a prominent signal of SERCA1 but some mature infant muscles were negative for SERCA1b and positive for SERCA1 indicating the exclusive presence of SERCA1a. The diaphragm (an allotypical skeletal muscle) contained no SERCA1b, this was confirmed with increased amounts of loadings from younger (I.) and older (IV.) muscles and from whole diaphragm lysates (data not shown). If the muscle samples were put in order according to age the gradual loss of SERCA1b was always paralleled with relatively high level of SERCA1. This suggested a transition between the SERCA1b and SERCA1a isoforms in neonatal skeletal muscles but not in diaphragms where probably only SERCA1a was expressed in perinatal age.

A trace amount of SERCA1b mRNA was detected only in those muscles where SERCA1b protein was found. The cumulative levels of SERCA1a and SERCA1b mRNAs also correlated with the level of the pan SERCA1 protein in every investigated muscle (Fig. 1b).

Gradient gel immunoblot of neonatal human vastus muscle revealed a single band in the 110 kDa region with both SERCA1b and pan SERCA1 antibody, showing that



**Fig. 1** Expression of SERCA1b and SERCA1 in human muscles. **a** Immunoblots of muscle membrane-microsomal fractions. *Upper row* SERCA1b, *lower row* SERCA1. Roman numbers I–V. indicate foeti or infants in the order of age (I-youngest, V.-oldest). Each loading represents 5,5 mg fresh weight. **b** Detection of SERCA1a and

SERCA1b mRNAs by RT PCR of the investigated muscles. **c** Immunoblot of SERCA1b (*left lane*) and SERCA1 (*middle lane*) after gradient gel electrophoresis of membrane-microsomal fraction of vastus lateralis of infant III, P– Ponceau S staining of the membrane (*right lane*)

the seven amino acid difference between the two isoforms is not enough to separate them by this method (Fig. 1c).

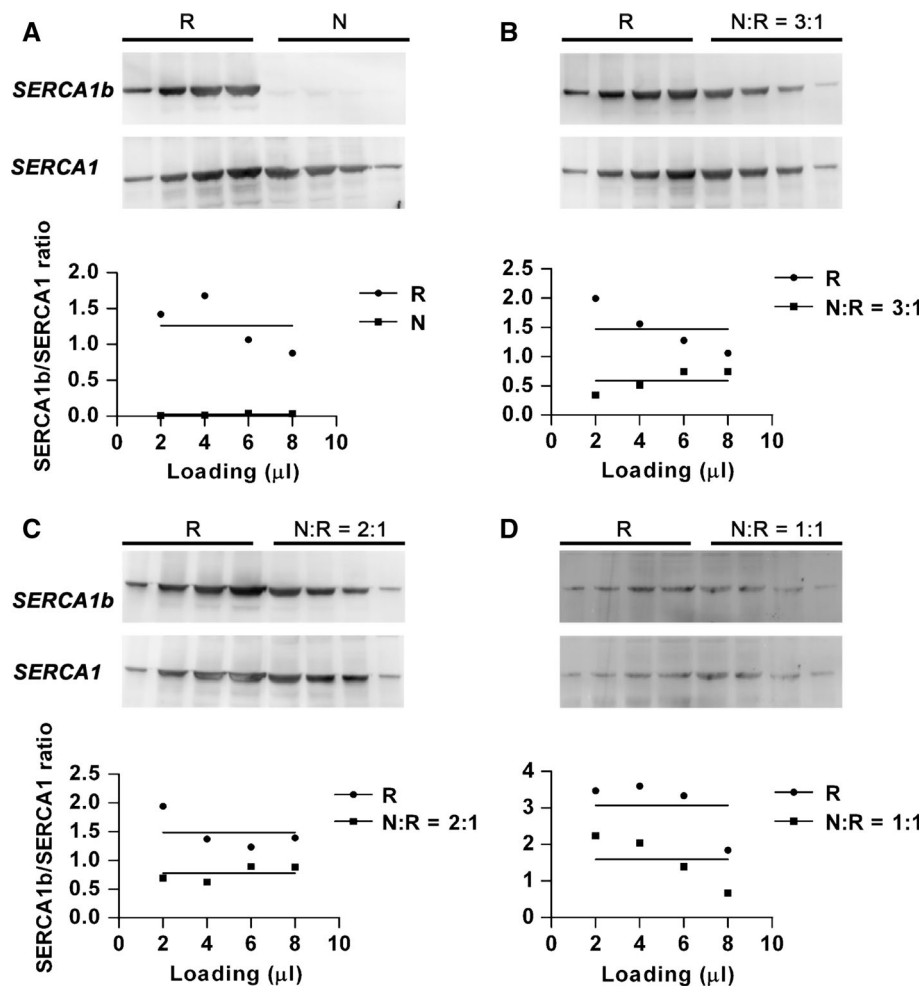
#### Estimation of SERCA1b/SERCA1a ratio

To estimate the SERCA1a/SERCA1b ratios SERCA1b and pan SERCA1 signals were compared on immunoblot (after being developed subsequently on the very same membrane). We applied four increasing loadings in order to avoid the effect of saturation. We first used 5 days regenerating rat soleus that expresses only SERCA1b and normal adult (non-regenerating) rat soleus that expresses only SERCA1a (Zádor et al. 1996, 2007) (Fig. 2a). The SERCA1b/SERCA1 ratio was very low in the normal soleus and showed the maximum possible value in the 5 days regenerating soleus (normal:  $0.025 \pm 0.007$ ; regen. soleus:  $1.261 \pm 0.178$ ;  $p = 0.0005$ ). The SERCA1b/SERCA1 ratios in three different mixes (1:3, 1:2 and 1:1 of 5 days regenerating and normal adult soleus) were each significantly lower than in 5 days regenerating soleus on the same blot. (Namely: 1:3 mix:  $0.587 \pm 0.098$  versus regen. soleus:  $1.472 \pm 0.202$   $p = 0.0078$ ; 1:2 mix:  $0.774 \pm 0.068$  versus regen. soleus:  $1.485 \pm 0.156$   $p = 0.0059$ ; 1:1 mix:  $1.588 \pm 0.355$  versus regen. soleus:  $3.065 \pm 0.409$   $p = 0.034$ ) (Fig. 2b–d). This shows that the presence of SERCA1a can be detected in this way if it makes 75–50 % ratio of total SERCA1 content. We assumed from the results that a lower SERCA1a ratio (with a higher SERCA1b/SERCA1 ratio) may not show a statistical difference from the pure SERCA1b due to the limited quantitative nature of the immunoblot.

#### SERCA1b/SERCA1a ratio in human muscles

Using the method above we estimated the SERCA1a content of perinatal human muscles, namely the biceps brachii of object III., the gluteus maximus of object II., and the vastus lateralis of object III., which apparently also contained SERCA1b (Fig. 1a). The SERCA1b/SERCA1 signal ratios were significantly less in each human sample than in the 5 days regenerating soleus (Fig. 3a–c) showing that a significant amount of SERCA1a was also present (biceps:  $0.234 \pm 0.044$  versus regen. soleus:  $0.714 \pm 0.153$   $p = 0.0238$ ; gluteus:  $0.059 \pm 0.010$  versus regen. soleus:  $0.464 \pm 0.135$   $p = 0.0246$ , vastus:  $0.269 \pm 0.041$  versus regen. soleus:  $1.317 \pm 0.138$   $p = 0.0004$ ). Comparing with the ratios of muscle mixes tested on Fig. 2 the biceps, the gluteus and the vastus appeared to contain more SERCA1a than 50 % of the total SERCA1 protein. This revealed that although these muscles showed high SERCA1b levels compared to the others they still had more SERCA1a than SERCA1b.

Homogenates of DM2 and DMD muscles were also analysed (Fig. 4). Both groups were SERCA1 positive but the DM2 patients appeared to express SERCA1b similarly as SERCA1 (Fig. 4a). The prominent expression of SERCA1 protein assured the presence of skeletal muscle in the samples of DMD patients, where adipose tissue takes over the place of skeletal muscle during the progression of the disease. The SERCA1b/SERCA1 signal ratio of one of the SERCA1b positive DM2 muscles was compared with that of pure regenerating soleus (Fig. 4b). There was no significant



**Fig. 2** Comparison of SERCA1b/SERCA1 signal ratios in normal and 5 days regenerating rat soleus muscles and in their different mixtures. *Panels* The first 4 lanes on each membrane show increased loadings of regenerating soleus membrane fractions (2–4–6–8 μl), the next 4 lanes are decreased loadings of the same scale from **a** normal soleus or **b–d** mixtures of normal and regenerating soleus **b** in 3:1, **c** in 2:1 and **d** in 1:1 ratio. Diagrams with horizontal lines on each panel represent the SERCA1b/SERCA1 signal ratios of different loadings of one muscle calculated with statistical methods (see

“Methods” section). *Note:* **a** There is practically only SERCA1 signal in the normal soleus indicating SERCA1a while the intense SERCA1b and SERCA1 signals in the 5 days regenerating soleus refers to the exclusive presence of SERCA1b. The SERCA1b/SERCA1 signal ratios are significantly higher in regenerating soleus than in mixtures of normal and 5 days regenerating soleus **b–d** indicating that the proportion of SERCA1a can be shown at least down to 50 % of total SERCA1. *R* regenerating rat soleus, *N* normal rat soleus; level of significance  $p < 0.05$

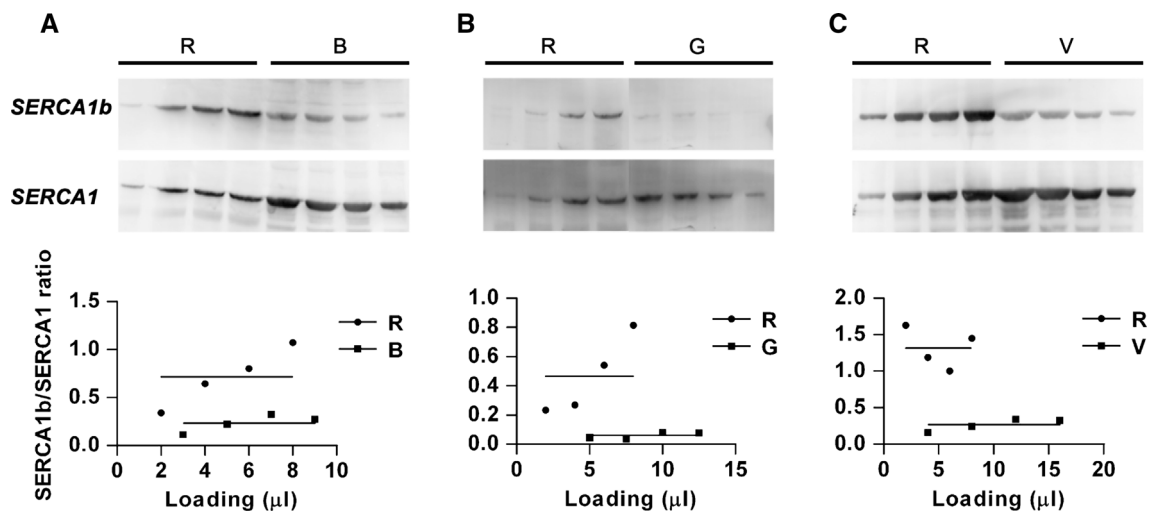
difference between the ratios of the DM2 muscle and the 5 days regenerating soleus (DM2:  $1.050 \pm 0.101$  vs. regen. soleus:  $2.634 \pm 0.947$   $p = 0.1473$ ), indicating that the SERCA1b is the predominant (>50 % of SERCA1) if not the exclusive isoform of SERCA1 in the DM2 muscle.

**Discussion**

SERCA1b is a dominant calcium pump in neonatal and regenerating muscle of rodents with a developmental role (Pan et al. 2003; Zádor et al. 2007, 2011). RNAi targeting of SERCA1b in regenerating soleus stimulated muscle growth in an autocrine/paracrine manner via calcineurin-

NFAT-IL4 pathway (Zádor et al. 2011). Despite the low and uneven efficiency of the applied in vivo transfection (Kósa and Zádor 2013) remarkably every fibre increased the cross sectional area, and in a week time the whole regenerating muscle gained weight about 30 % faster than the control transfected regenerating soleus. This finding further underlined the role of SERCA1b in muscle development. In case of store operated calcium entry (SOCE) the stromal interacting molecule 1 (STIM1) is sensing the  $Ca^{2+}$ -depletion in the SR (Stiber et al. 2008). STIM1 can bind to an internal peptide sequence present in SERCA1a (and also in SERCA1b) and stimulate the  $Ca^{2+}$  pump activity (Lee et al. 2014). Since SERCA1b is the dominant SERCA1 in myotubes (Zádor et al. 2007) it is also very





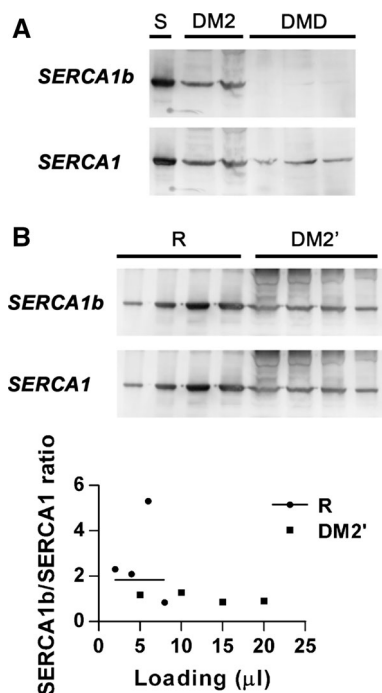
**Fig. 3** SERCA1b/SERCA1 signal ratios of human muscles compared to that of rat regenerating soleus. *Panels:* The first 4 lanes on each membrane are increased loadings of regenerating soleus (2–4–6–8  $\mu$ l); the next 4 lanes are decreased loadings of human muscles (adjusted to detection range). **a** biceps brachii of infant III. (loadings: 9–7–5–3  $\mu$ l; highest loading refers to 6 mg fresh weight); **b** gluteus maximus of fetus II. (loadings: 12.5–10–7.5–5  $\mu$ l; highest loading refers to 9 mg fresh weight); **c** vastus lateralis of infant III. (loadings: 16–12–8–4  $\mu$ l; highest loading refers to 8 mg fresh weight). Diagrams on each panel with horizontal lines show the SERCA1b/SERCA1

signal ratio of one muscle calculated with statistical methods (see Methods). *Note:* SERCA1b/SERCA1 ratio of each investigated human muscle is significantly ( $p < 0.05$ ) lower than that of the 5 days regenerating rat soleus. This indicates that each of them expresses both SERCA1a and SERCA1b. Particularly, the gluteus maximus of fetus II, whose SERCA1b/SERCA1 signal ratio is only 12.8 % of regenerating rat soleus has probably the highest SERCA1a proportion of the three muscles. *R* 5 days regenerating rat soleus, *B* human biceps brachii, *G* human gluteus maximus, *V* human vastus lateralis

likely that sarcolipin, a SERCA1 inhibitor is acting on SERCA1b when it opposes the effect of STIM1 in SOCE in myotubes (Seth et al. 2012). Unlike it is mentioned in otherwise standard reviews (Periasamy and Kalyanasundaram 2007; Schiaffino and Reggiani 2011), SERCA1b is not expressed exclusively in developing fast type fibres, but typically in any undifferentiated fibres developing either into fast or slow type (Zádor et al. 2007). The undifferentiated fibres may also express the slow muscle type SERCA2a and its inhibitor, the phospholamban (Vangheluwe et al. 2005) therefore the regulation of SERCA1b by phospholamban cannot be excluded (Fajardo et al. 2013). It is a question if the embryonic/neonatal fibres can be regarded as a real fibre type or being in a transition stage of development (Schiaffino and Reggiani 2011). The foetal, neonatal and the pathologic (DMD and DM2) muscles investigated here were all assumed to have developing fibres in a significant proportion. For this reason in these muscles it might have been difficult to relate the t SERCA1b/SERCA1a ratio to real fibre types. Our SERCA1b peptide antibody had limited use in immunohistochemistry of human muscle sections-unlike in 5 days regenerating rat soleus where the SERCA1 antibody also confirmed the expression of SERCA1b in each regenerating fibres (Zádor et al. 2007).

Therefore the switch between the neonatal SERCA1b and the adult fast SERCA1a isoforms is more important in earlier stages of muscle development than the differentiation of

fibre type (Pan et al. 2003; Zádor et al. 2007, 2011). Because the generation of SERCA1a specific antibody is not feasible (see Introduction), we designed a detection system in order to estimate the SERCA1a content based on the SERCA1b/SERCA1 ratio. This approach enabled us to verify the presence of SERCA1a when its ratio was between 50–100 % of total SERCA1. Our method therefore was not very accurate to quantitate the adult SERCA1 isoform but it gave a reliable estimation of which muscle specific SERCA1 isoform was dominating over the other one. In each foetal and neonatal muscle where SERCA1b was also present, the ratio of SERCA1a was more than 50 % therefore it was the dominant SERCA1. However, only SERCA1a but not SERCA1b was found in more matured infant muscles showing an earlier isoform switch in human than in rodents (Zádor et al. 2007). Because muscles with highest SERCA1b signal were picked for estimation of SERCA1a/1b ratio checking all our samples would not have given a different result. For some reason the expression of SERCA1b (mRNA and protein) was restricted to foeti but not to infants, therefore it appeared before intense muscle movements occurred. The mRNA level of SERCA1b has been reported to correlate with passive stretch (a condition of overload) and denervation (a condition of passive movement) in adult rat muscle (Zádor et al. 1999, 2007), but the SERCA1b protein never paralleled its mRNA level in such conditions, only in immature fibres (Zádor et al. 2007).



**Fig. 4** SERCA1b and SERCA1 expression in DMD and DM2 patients. **Panel a:** Control soleus at 5th day of regeneration (8  $\mu$ l) was loaded in the first lane, samples of DM2 muscles were loaded in the next two lanes, while DMD samples were loaded in the last three lanes. Loading of human samples was normalized to 100  $\mu$ g total protein. **Panel b:** First 4 lanes show increasing loadings of regenerating soleus (2–4–6–8  $\mu$ l); the second 4 lanes decreasing loadings of one DM2 muscles (DM2') (20–15–10–5  $\mu$ l; the highest loading was 100  $\mu$ g protein). Only the DM2 muscles contained SERCA1b. SERCA1b/SERCA1 signal ratios of regenerating rat soleus and DM2' muscle did not differ significantly, this means that SERCA1b is the dominant SERCA1 in these muscles. DM2 myotonic dystrophy type 2, DMD Duchenne muscular dystrophy, R regenerating rat soleus; level of significance:  $p < 0.05$

Although the rate of undifferentiated fibres has been found in a similar range (10–30 %) in diaphragm and limb muscles at foetal and neonatal age (Colling-Saltin 1978; Orliaguet et al. 2004) apparently the diaphragm is more matured for its appropriate function than the limb muscles. There are researchers who believe that other factors are more important for maturation of the diaphragm than the known fibre type differentiation markers (i.e. myosin heavy chains) (reviewed by Orliaguet et al. 2004). Since SERCA1b is allocated to developmental fibres the lack of SERCA1b in both foetal and neonatal diaphragms investigated here is consistent with this theory. The complete absence of SERCA1b in human foetal and infant diaphragms also hints to a difference between human and mice or rats. In correlation with this it is worth to note that the diaphragm of SERCA1 KO mice show functional and developmental defect in neonatal age (Pan et al. 2003) but apparently no similar symptoms seem to be present in Brody's diseased patients with SERCA1 mutation (Guglielmi et al. 2013b).

We could not detect SERCA1b in tibialis anterior muscle of DMD patients. This is curious because DMD is a degenerative-regenerative muscle disorder where one would expect the SERCA1b protein expressed. We did not have enough biopsies to test SERCA1b at the mRNA level but, anyway, up- or down regulation of SERCA1b splicing itself would not have been informative about the protein expression. The reason for this is that partial denervation and stretch may occur in DMD muscles and these conditions have been shown to upregulate SERCA1b splicing without expression of the SERCA1b protein (Zádor et al. 1999, 2007); on the other hand the entire lack of SERCA1b mRNA in DMD is also unlikely since some neonatal transcript is detectable even in adult muscles where its protein is not found (Zádor et al. 1999, 2007). A reduced SERCA1 expression has been shown in mdx mice and DMD patients (Kiviluoto et al. 2011; Zhao et al. 2012) but it is still a question why the adult fast and not the neonatal SERCA1 isoform remains expressed in our samples. It might be worth to consider that our samples were taken from DMD patients who received corticosteroid therapy, and corticosteroids are known to decrease SERCA1 transcript level (Gayan-Ramirez et al. 2000). Another, more feasible explanation is that children in DMD at ages 7–12 have already exhausted regenerative capacity and thereby had no cellular background for SERCA1b expression.

The other disease we investigated here, DM2 was especially interesting because it showed a higher SERCA1b mRNA level than the similar, type 1 myotonic dystrophy (DM1) (Kimura et al. 2005; Salvatori et al. 2009; Vihola et al. 2003, 2013). We found that SERCA1b also represented the dominant fraction of SERCA1 proteins in the investigated (quadriceps) muscle in DM2. This showed that our method might be used in clinical analysis. Santoro et al. (Santoro et al. 2014) found that the resting  $Ca^{2+}$  level was higher in DM1 and lower in DM2 compared to normal myotubes. Although a functional difference of SERCA1a and SERCA1b has not been found yet (Maruyama and MacLennan 1988), they suggested that the translated SERCA1 protein isoforms are partially accountable for the difference (Santoro et al. 2014). It is worth to mention that the presence of SERCA1b in DM2 is in line with the alteration of calcium handling compared to DM1 (Santoro et al. 2014), and it also suggests that analysis of SERCA1b/SERCA1a ratio at the protein level or the presumptive posttranslational modifications (Schöneich et al. 1999) at the different C-terminal of SERCA1b might be of interest to reveal differences in the mechanisms of diseases related to altered  $Ca^{2+}$  metabolism.

In summary, our work shows that the switch from SERCA1b to SERCA1a occurs in human muscle development somewhat earlier than in rodents and SERCA1b might be a pathological marker in DM2 patients.

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