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ERG1A POTASSIUM CHANNEL ABUNDANCE VARIES WITHIN SPECIFIC SKELETAL MUSCLE AND FIBER TYPE

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A Thesis Submitted to the

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THESIS APPROVAL

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A thesis submitted to the SIU Honors Program

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ABSTRACT

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TITLE: ERG1A POTASSIUM CHANNEL ABUNDANCE VARIES WITHIN SPECIFIC SKELETAL MUSCLE AND FIBER TYPE

MAJOR PROFESSOR: Amber Pond, Ph. D.

Skeletal muscle atrophy is defined as a 5% or greater loss of muscle mass that occurs with muscle disuse, muscle and neural injuries, disease (e.g., cancer, diabetes), and also happens naturally with aging. Atrophy results from an imbalance of protein degradation and protein synthesis which produces a net loss of protein. The protein loss occurs mostly through three proteolytic systems: Calpain enzymes, cathespin enzymes, and the largest contributor, the ubiquitin proteasome pathway (UPP). The ether-a-go-go related gene (ERG1A) is a voltage gated K^+ channel that is upregulated in atrophying skeletal muscle. Expression of ERG1A in skeletal muscle increases UPP activity and induces atrophy. Initial immunohistochemistry suggested that ERG1A might be more abundant in certain muscle fibers. Indeed, skeletal muscle fibers vary in their composition of the myosin heavy chain (MyHC) proteins which participate in muscle contraction. MyHC composition determines muscle fiber contraction speed, producing a slow-twitch fiber type and a number of fast-twitch fiber types. Different muscles are composed of varying numbers of fast and slow-twitch fibers which produce their specific contraction speed. Because ERG1A appears variably expressed in muscles composed of mixed fiber types and is a K^+ channel which has the potential to modulate contraction speed by enhancing repolarization, we hypothesized that ERG1A would be more abundant in slow-twitch fibers than in fast-twitch fibers in skeletal muscle. To test this hypothesis, we cryo-sectioned *Soleus* (SOL), *Extensor Digitorum Longus* (EDL), and *Gastrocnemius* muscles of rats. These muscles were chosen because the SOL and EDL have an abundance of slow and fast-twitch fibers, respectively, while the *Gastrocnemius* contains a more heterogeneous composition. The sections were co-immunostained for the ERG1A protein and either the fast or slow-twitch MyHC. ERG1A fluorescence was measured in the sarcolemma of each fiber type and compared. The data reveal that the ERG1A protein is more abundant in muscle fibers of the Sol than in the EDL; however, these muscles are very homogeneous in terms of fiber type so it was not possible to get enough data from both fiber types within a muscle to compare ERG1A composition in each. Therefore, we immunostained fibers from the heterogeneous *Gastrocnemius* muscle. The data reveal that ERG1A is 3.2-fold greater in slow than in fast fibers in this muscle. This knowledge will allow us to concentrate our investigations into the role of ERG1A in the development of atrophy on muscles in which ERG1A is most highly abundant. Indeed, atrophy is a potentially debilitating condition for which there is no truly effective pharmacological treatment. Currently, exercise and diet are the best treatments to combat atrophy; however, sick and elderly individuals may not be able to participate in this effectively. Thus, further research into the mechanism(s) that regulate muscle atrophy is necessary for developing new treatments or therapies to combat muscle atrophy for those sick or elderly individuals.

INTRODUCTION

Skeletal muscle

Skeletal muscle comprises 35-45% of the human body mass and is critical for movement, posture, and temperature regulation. Skeletal muscle atrophy is a debilitating condition that occurs with many different diseases (e.g., cancer cachexia, HIV/AIDS, and diabetes), injury (e.g. spinal cord damage or denervation), disuse, fasting, and glucocorticoid treatment. Skeletal muscle atrophy also occurs with normal aging and space flight (Ma, et al., 2017; Frontera and Ochala 2015; Bonaldo and Sandri 2013; Baoge et al., 2012; Smith et al., 2008). Muscle atrophy occurs because there is an imbalance in protein degradation and protein synthesis that results in a net loss of muscle protein. The majority of muscle degradation results from the activity of three proteolytic systems: calpains, cathespins, and the ubiquitin proteasome pathway (UPP, also known as the ubiquitin proteolysis pathway). It has been shown that the UPP is the primary proteolytic system involved in skeletal muscle atrophy and is suggested to be responsible for as much as 75% of protein breakdown (Kandarian et al. 2006; Glass 2003; Franch et al. 2005). Skeletal muscle atrophy has been linked with increased human morbidity and mortality, however, there are no reliable pharmaceutical therapies to combat this problem. Indeed, the best treatment for atrophy is good diet and exercise; however, not all people are able to be active (Bonaldo and Sandri 2013; Baoge et al., 2012; Lynch and Ryall 2008; Derbre et al., 2012; Fareed, et al., 2006; Guasconi and Puri 2008; Han and Mitch 2011; Smith and Lin 2013). Therefore, new therapies for atrophy would greatly benefit medical practice and this can only happen if research reveals new targets for treatment.

Skeletal Muscle Fiber Types

Skeletal muscle is composed of muscle fibers which are made up of contractile units called sarcomeres (Figure 1). Each sarcomere is bordered by a Z-disk on each end with a line of proteins in the

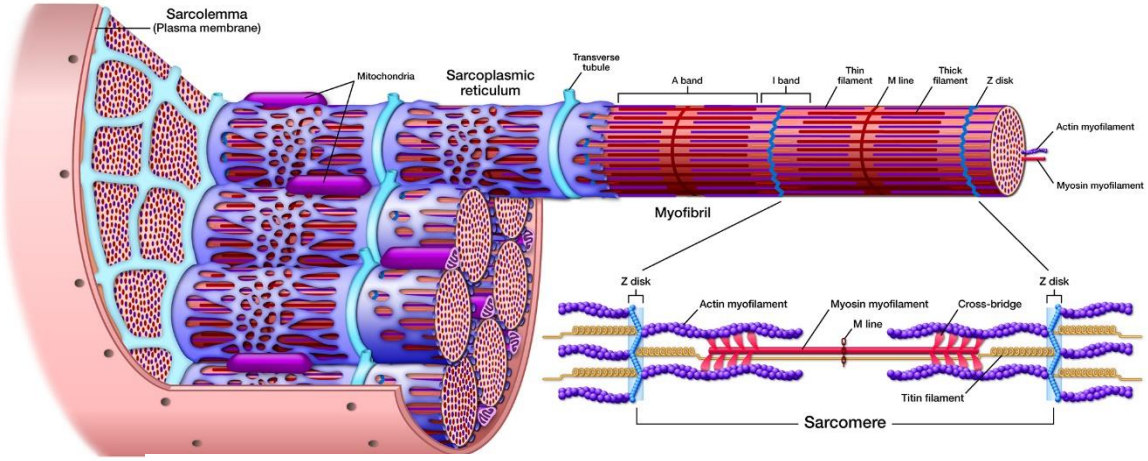


Figure 1. Sarcomere: The skeletal muscle contractile unit.[WW]

center which make up the M-line. The myosin heavy chain (MyHC) proteins, which are the main components of the thick filaments, are connected to the M-line and extend outward from there toward the Z disk. The thin filaments, which are connected to the Z-disk proteins by anchor proteins (e.g., titin), are mainly composed of actin proteins. Calcium ions bind to troponin proteins located on the thin filament proteins and move tropomyosin to expose sites on the actin to which the MyHCs can bind. The MyHC heads bind to the actin forming cross bridges which change conformation, bending like a ratchet

and causing an overlap of the thin and thick filaments. This interaction can be released and then repeated so that the thin and thick filaments interdigitate and become smaller, resulting in muscle contraction. ATP is used to promote the release of the filament interaction and resulting in muscle relaxation.

Skeletal muscle fibers vary in their speed of contraction (fast or slow), resistance to fatigability (high or low), and main metabolic pathway for energy production (aerobic or anaerobic). They are generally referred to in terms of speed of contraction and the main fibers described are slow-twitch (Type 1) or fast-twitch fibers (Type 2A, 2X, or 2B). Others have been described, but those are generally muscle specific and/or not abundant. The difference in the speed of contraction is determined by the myosin heavy chain (MyHC) proteins which compose the thick filaments of the skeletal muscle (Table 1).

Table 1. Myosin heavy chain proteins detected in rodents.

Genes	Proteins		Expression pattern
MYH2	MyHC-2A		Fast type 2A fibers
MYH1	MyHC-2X		Fast type 2X fibers
MYH4	MyHC-2B		Fast type 2B fibers
MYH7	MyHC-β		Heart and slow muscles
MYH3	MyHC-emb		Developing Muscle

Slow-twitch fibers generally contain the MyHC-β protein which is encoded by the MYH7 gene. Fibers with this protein produce ATP mainly by aerobic metabolism, being more resistant to fatigue. Fast contracting fibers mainly contain one or more of the fast MyHC proteins: MyHC-2A, MyHC-2B, or MyHC-2X. Fast fibers vary in speed of contraction from 2A, 2X, to 2B with the 2A being the slower and the 2B being the fastest. They also vary in terms of the main form of metabolism used to produce ATP, with 2A relying more on aerobic metabolism and 2X and 2B relying more upon the anaerobic pathway. Consequently, 2A is more fatigue resistant than 2X and both 2A and 2X are more fatigue resistant than 2B (Schiaffino 2018; Schiaffino and Reggiani 2011). Muscles may contain mainly one type of fiber or be composed of numerous types in various combinations. This variability gives muscle a great deal of flexibility in a muscle's ability to generate force and movement. It appears to be the impulse which determines the fiber type developmentally. Chronic, slow neural impulses produce slow-twitch fibers while faster impulses will result in development of fast-twitch fibers. However, muscle has a great deal of plasticity and fiber type can change even in adult muscle. For example, cross-reinnervation can change fiber type: fast muscles turn slow when reinnervated with a slow nerve and slow muscles turn fast when reinnervated by a fast nerve. With application of electrical stimulation, chronic slow frequency stimulation of denervated slow muscles can maintain a slow fiber phenotype. Chronic slow frequency stimulation of denervated muscles expressing mainly fast-twitch fibers can switch them to slow fiber type and vice versa. These data further demonstrate that neural input determines the fiber type (Schiaffino and Reggiani 2011; Liu et al., 2015). Human skeletal muscle contains three main types of fibers: Type 1, 2A and 2X. To date, only trace amounts of 2B are detected. The four main fiber types described above are found in rodents. In rats, the *Gastrocnemius* muscle contains a heterogeneous composition of fiber type, having both fast and slow-twitch fibers (Chabowski et al., 2006). The *Extensor digitorum longus* muscle in rats is composed mainly of fast-twitch fibers (Close 1967) while the *Soleus* muscle contains mainly slow-twitch fibers (Schiffiano et al., 1970).

ERG1A

The *ether-a-gogo related gene 1* (ERG1A) encodes a potassium channel that is partially responsible for repolarization of the cardiac action potential. The ERG1A protein has six membrane-spanning domains, a conserved pore domain, and an N-terminus with numerous potential signaling domains (Figure 2) [Vandenberg et al., 2012; Jones et al., 2004]. Numerous alternative splice variants have been reported, but it is the *ERG1A* and *ERG1B* gene products which are detected in cardiac tissue (Figure 3; London et al., 1997; Lees-Miller et al., 1997). Indeed, a heteromultimeric channel composed of these two variants is reported to contribute to cardiac repolarization; and mutations in the ERG1A gene are known to cause a heart arrhythmia known as Long QT Syndrome 2. The ERG1A protein is detected in heart, but also it has been reported in brain, smooth muscle of numerous mammalian species, and various cancer cells [London et al., 1997; Vandenberg, et al., 2012]. The alternative splice variant ERG1B subunit is essentially identical to the 1A variant except that is has a truncated N-terminus. The 1B is considered to be a cardiac specific isoform (Andersen et al.1997, Andersen et al. 2000), although there are reports of its detection in brain and cancer cells (Lees-Miller et al., 1997, London et al., 1997) Recently, it has been shown that in rodents the ERG1A K^+ channel variant is upregulated in skeletal muscle atrophying in response to either disuse or cancer cachexia (Wang et al., 2006). Additionally, when ERG1A is ectopically expressed in the *Gastrocnemius* muscles of mice, abundance of the UPP E3 ligase MuRF1 is increased, UPP activity is increased, and myofiber size decreases significantly (Wang et al., 2006). The data suggest that ERG1A plays a role in the mechanism(s) producing skeletal muscle atrophy.

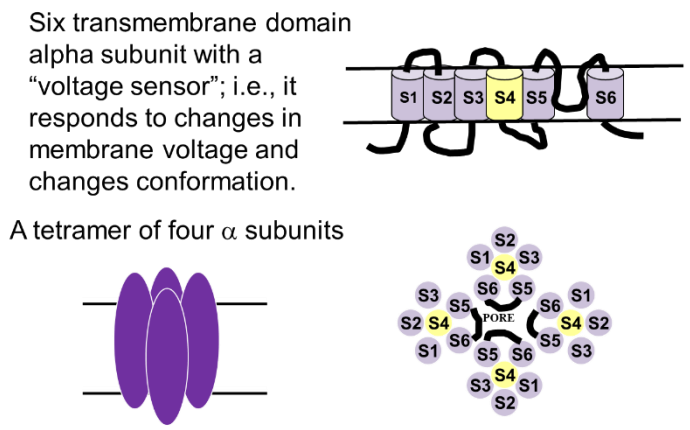


Figure 2. ERG1 potassium channel is composed of 4 alpha protein subunits.

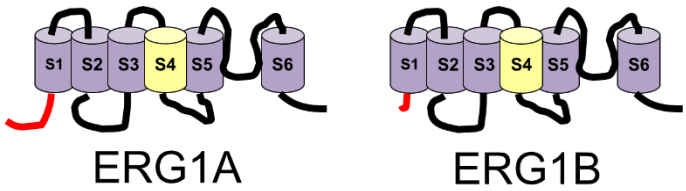


Figure 3. ERG1 has two prominent alternative splice variants, 1A and 1B.

Hypothesis

ERG1A is a potassium channel known to repolarize the cardiac action potential. It has been detected in atrophying skeletal muscle, however, it is not known if the ERG1A channel contributes to the atrophic process or is up-regulated to combat this process. The up-regulation of the MuRF1 E3 ligase and increased UPP activity strongly suggest that it contributes to atrophic mechanisms. Because it has the potential to repolarize membranes and, thereby, decrease action potential duration, *we hypothesized that ERG1A could slow muscle contraction and thus be more abundant in slow-twitch muscle fibers than in fast-twitch muscle fibers*.

To test this hypothesis, we used immunohistochemistry to measure ERG1A fluorescence in the muscle fibers of both the *Sol* and *EDL* muscles. We intended to compare ERG1A fluorescence in each fiber type within a muscle; however, because each muscle is truly nearly homogeneous in terms of fiber type composition, we were unable to compare ERG1A fluorescence per fiber type within muscle.

Nonetheless, we did discover that ERG1A is more abundant in *Soleus* muscle, where slow-type fibers are more abundant, than in the EDL muscle which is composed of mainly fast-type fibers. These results were confirmed with immunoblots of muscles from old and young rats. To look at specific fiber type composition, we needed to test ERG1A abundance in a muscle that had a more heterogeneous composition of fiber types. Thus, we used immunohistochemistry to co-immunostain *Gastrocnemius* muscle for ERG1A and a fiber type specific myosin heavy chain (MyHC) protein. We then measured ERG1A fluorescence in each fiber type to determine if there was a difference in abundance of the ERG1A K^+ channel between slow-twitch and fast-twitch muscle fibers.

Methods

Animals. All procedures were approved by the SIU SOM Animal Care and Use Committee. *Soleus*, *Extensor digitorum longus*, and *Gastrocnemius* muscles from FDB rats (NIH; Bethesda, MD) at both 3 and 30 months of age were generously provided by Dr. Don Torry (SIU SOM).

Cryo-sectioning. Muscles were embedded in OCT Compound and cross sectioned at 20 um using a Leica cryostat microtome.

Immunohistochemistry (IHC). Muscle sections were not fixed. They were incubated with 3% hydrogen peroxide (15 minutes) and then rinsed with PBS. Next they were dipped in 0.3% Sudan black in PBS containing 0.5% triton X-100 for 30 seconds and then rinsed with PBS. The sections were incubated in blocking buffer (5% NGS + 2% Tritonx-100 in PBS) for one hour and then incubated with either anti-slow or anti-fast MyHC mouse primary antibody at room temperature also for an hour. One section per muscle was not incubated with the primary antibody as control. The sections were then rinsed with PBS. Non-control sections were incubated in an in-house anti-ERG1A primary antibody (Pond et al., 1999) overnight. After incubation, the sections were rinsed and secondary antibodies (goat anti-mouse IgG labelled with Alexa-fluor 568 and goat anti-rabbit IgG conjugated with HRP-tyramide Alexa-fluor 488) were mixed and applied to all sections, including controls, at room temperature for 1 hour. Sections were then rinsed with PBS. The ERG1A signal was developed using a Tyramide kit (per manufacturer's instructions) for 5 min.

Imaging. Images were acquired using a Leica DM4500 microscope with a Leica DFC 340FX camera. Acquisition parameters were maintained identically across samples. ERG1A (green) fluorescence in muscle fibers stained for either slow or fast MyHC (red) was measured. At least two fields per section, with three sections per muscle were photographed.

ImageJ analysis. Using ImageJ software, single-pixel brightness measurements were taken from the sarcolemma of complete fibers that also were stained for the desired type of muscle fiber (fast or slow). Brightness values were recorded as integers ranging from 0 (no signal) to 256 (white) and the average brightness value (±SEM) for each section was determined.

Homogenization. All the procedures were carried out on ice and all solutions contained a mixture of protease inhibitors (1 mM PMSF, 2 μM pepstatin, 1 mM benzamidine, 8 μg/ml aprotinin, 1 mM iodoacetamide, 1 mM 1,10-phenanthroline, 1 μg/ml leupeptin). Approximately 1 g of muscle was homogenized in 10 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) on ice. Nuclei and cell debris were removed by centrifugation (1,000 × g, 10 min). The first supernatant was collected and the pellet was resuspended with 8 ml TE buffer and centrifuged (1,000 × g for 8 min). These supernatants were pooled and centrifuged together at 40,000 × g for 10 min. Supernatants were poured off and the pellets were resuspended in 15 ml of homogenization buffer (TE plus protease inhibitors as described above)

containing 0.6 M potassium iodide (a chaotropic agent), and incubated on ice for 30 min. The suspension was then centrifuged at 40,000 × g at 4°C for 10 min. This pellet was then resuspended by vortexing and recentrifuged at 40,000 × g at 4°C for 10 min three more times to remove the potassium iodide. Finally, the pellet was resuspended in an appropriate amount of solubilization buffer (TE buffer as described above plus with 2% Triton X-100), incubated on ice for 1 hour and centrifuged at 17,400 × g at 4ºC for 30 min to pellet insoluble materials. Protein assays (Bio-Rad DC kit; Hercules, CA) were performed according to manufactures directions, and the samples were aliquoted and frozen at -70°C (Wang et al., 2006).

Immunoblot. Polyacrylamide SDS gels (separating gel 7.5% and stacking gel 4% acrylamide) were used with each electrophoresis experiment. Aliquots of sample protein (indicated in each figure) were combined with sample diluting buffer (0.3M Tris, 50% glycerol, 5% SDS, 0.5M DTT, and 0.2% Bromophenol Blue) and boiled for five minutes. The gel was then loaded with standard (BioRad Kaleidoscope Precision Plus Protein Standards; BioRad, Hercules, CA; 12µl) and samples and electrophoresed at 200V until the dye front exited the gel. The proteins were then transferred to PVDF membrane (BioRad, Hercules, CA; 0.2µM pore size). Specifically, the membrane was initially soaked in methanol, rinsed in deionized water, and then placed in transfer buffer (192 mM glycine, 0.1% SDS, 25 mM Tris, pH 8.3 with 20% methanol). Once electrophoresis was complete, the gel was removed and transferred to PVDF membrane (Trans-Blot® Cell; BioRad). The membrane was rinsed in PBS 3 times for five minutes each rinse. The sample-containing membrane was then incubated in blocking buffer (0.2% I-Block [Applied Biosystems; Foster City, CA] and 0.1% Tween-20 in 1X PBS, pH 7.4) and rocked for one hour. The necessary primary antibody was diluted (per figure legends) in a second blocking buffer (5% NGS, 0.2% Triton X-100, 0.1% sodium azide in PBS) and the primary antibody was placed on top of the membrane and incubated at 4°C in a wet chamber overnight. The next day the membranes were removed from primary antibody and rocked for 10 minutes each in two changes of I-block buffer (0.2% I-Block and 0.1% Tween-20 in 1X PBS). It was then removed from the buffer and incubated in a goat anti-rabbit alkaline phosphatase (AP)-conjugated secondary antibody (diluted 1:1000 in I-block buffer) for 1 hour. The secondary antibody was removed and the membranes were washed three times for 15 minutes in I-block buffer. The membrane was then washed twice in assay buffer (0.1% Tween 20 in TBS). Membranes were then laid on a plastic block and Immun-Star AP substrate (BioRad, Hercules, CA) at 37°C was applied to the membranes for five minutes. The substrate solution was then removed and the membranes were blotted and wrapped in clear plastic wrap. These were developed using a SRX-101A film processor (Konica Minolta, Chiyoda, Tokyo, Japan). After films were developed, they were scanned into a computer and ImageJ was used to measure optical densities of each band.

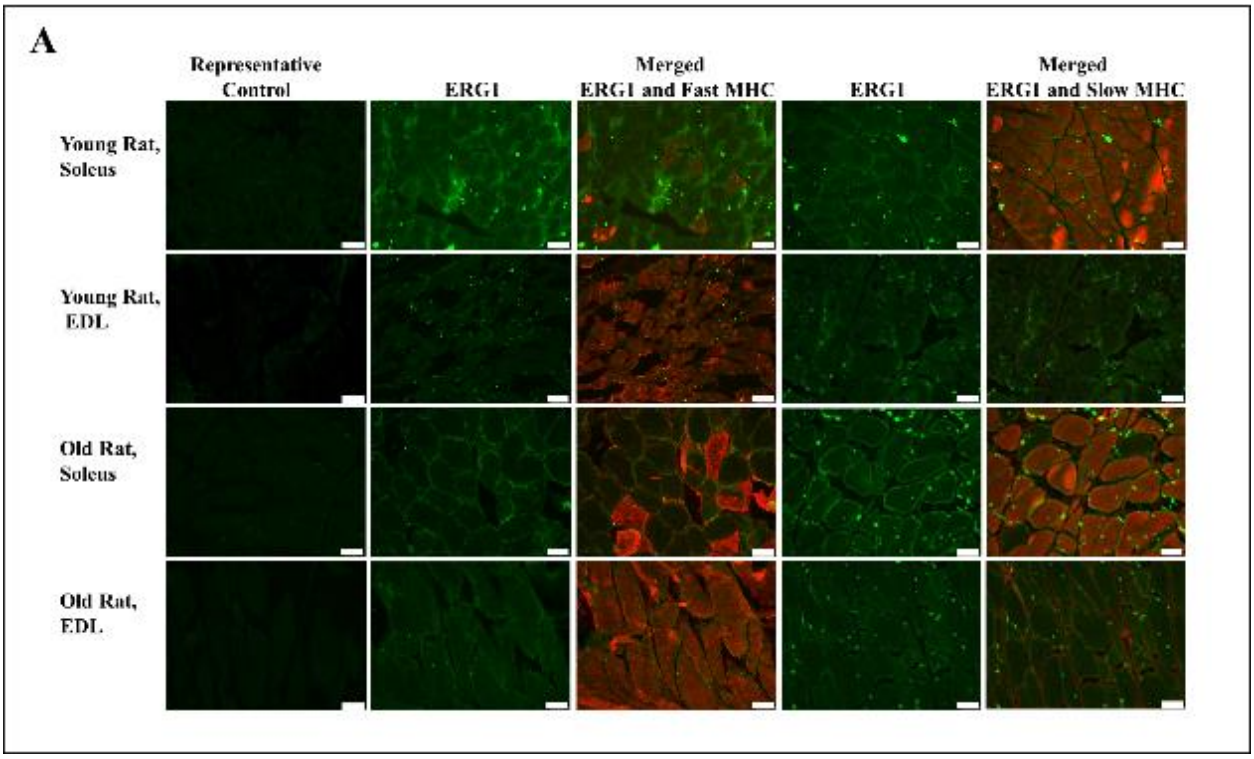
Statistics. Data from the *EDL* and *Sol* muscles were analyzed by ANOVA for a completely randomized design. When significant differences were found, means were separated by Fisher's Protected Least Significance Difference. Data of the *Gastrocnemius* muscle were analyzed by a paired Students t-test. All data were analyzed using the General Linear Model Procedure of SAS.

Results

ERG1A Fluorescence is More Intense in *Soleus* than *Extensor Digitorum Longus* Muscles of Rats. Immunohistochemistry (IHC) reveals that ERG1A fluorescence intensity, as detected in the muscle fiber sarcolemma, is significantly greater in the *Sol* than in the *EDL* muscles of both young (39.4%) and old (36.5%) rats (Fig. 4 A,B). There is no significant difference in ERG1A fluorescent intensity that can be attributed to age; however, there is a trend for intensity to be greater in the muscle from the older

animals. Immunoblots were also performed and the data show that, similar to the IHC data, ERG1A protein is a significant 48.3 % greater in *Soleus* than *EDL* muscles of old rats while it is 24.0% more abundant in *Soleus* than in *EDL* muscles of young rats (Fig. 5A-C). Although it is not statistically significant, there is a another trend for ERG1A to be more abundant in old rats than in young rats.

ERG1A Fluorescence is More Abundant in Slow-Twitch than Fast-Twitch Fibers of the Rat *Gastrocnemius* Muscle. Using IHC, we determined that ERG1A is more abundant in slow-twitch fibers than fast-twitch fibers of rat *Gastrocnemius* muscle (Fig. 6 A, B), demonstrating that trends suggested by the results of the *Sol* and *EDL* experiments were likely caused by differences in ERG1a expression within fiber type rather than in muscle type.



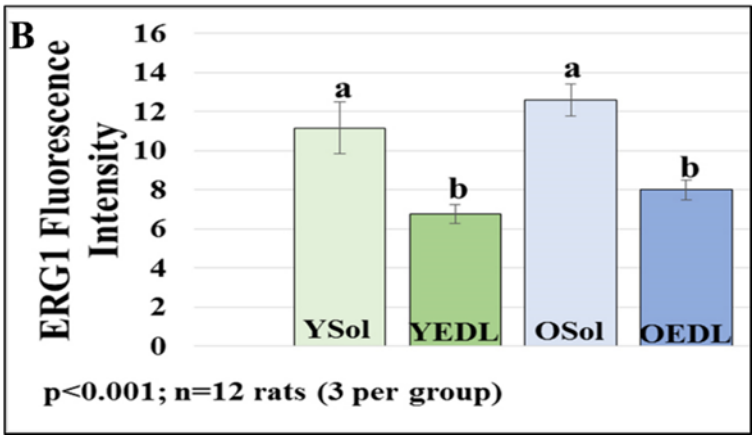


Figure 4. ERG1A fluorescence is more abundant in the *Soleus* muscle than in the *Extensor Digitorum Longus* muscle of young and old rats.

A. Representative fluorescent images of *Soleus* (Sol) and *Extensor Digitorum Longus* (EDL) muscle sections from Young (3 months old) and Old (30 months old) rats immunostained for ERG1A (green) and either fast or slow myosin heavy chain protein (red). Scale bars =50um

B. ERG1A fluorescence is significantly more abundant in the Sol than in the EDL muscles of both young and old rats. Each bar represents the mean fluorescent intensity ± the standard error of the mean.

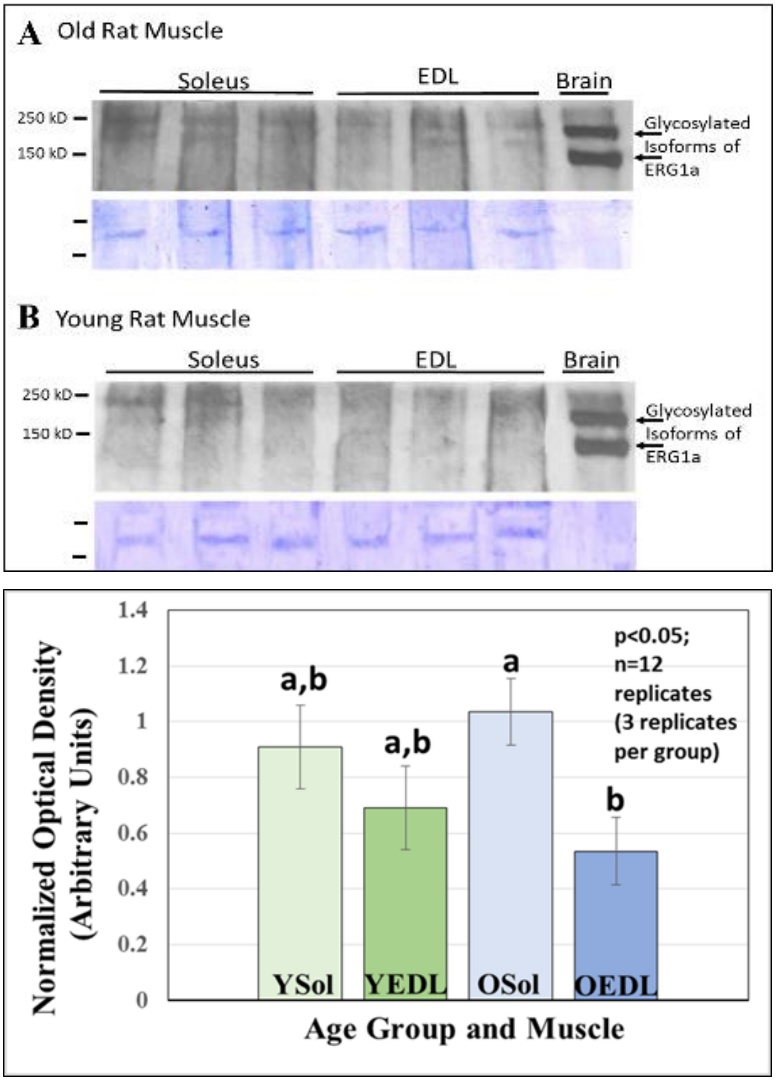
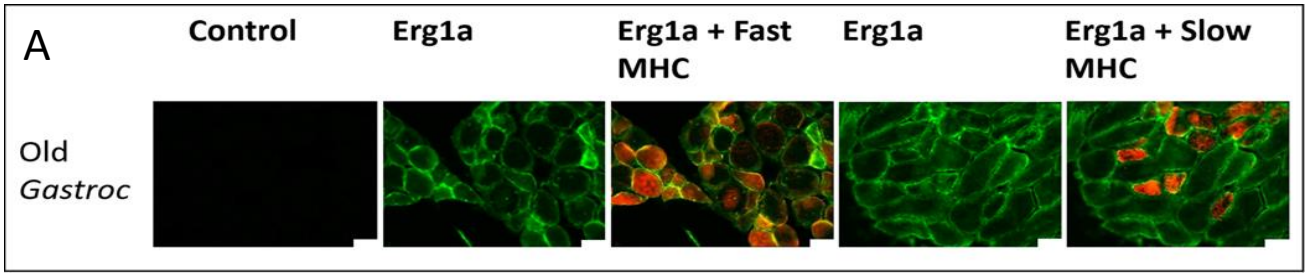


Figure 5. ERG1A protein is more abundant in the *Soleus* muscle than in the *Extensor Digitorum Longus* muscle of young and old rats.

A. Immunoblot of *Soleus* (Sol) and *Extensor Digitorum Longus* (EDL) muscles from 30 month old rats. Skeletal muscle homogenate (70 µg protein) was loaded into each lane. Coommassie stained membrane below blot (in blue) indicates that equal amount of skeletal muscle homogenate were loaded into each lane of the gel.

B. Immunoblot of Sol and EDL muscles from 3 month old rats. Skeletal muscle homogenate (70 µg protein) was loaded into each lane. Coommassie stained membrane below blot (in blue) indicates that equal amount of skeletal muscle homogenate were loaded into each lane of the gel.

C. ERG1A protein is more abundant in the Sol than in the EDL muscle and this difference is statistically significant in the old rat skeletal muscle. Bar graph depicts mean normalized optical densities ± standard error of the mean.



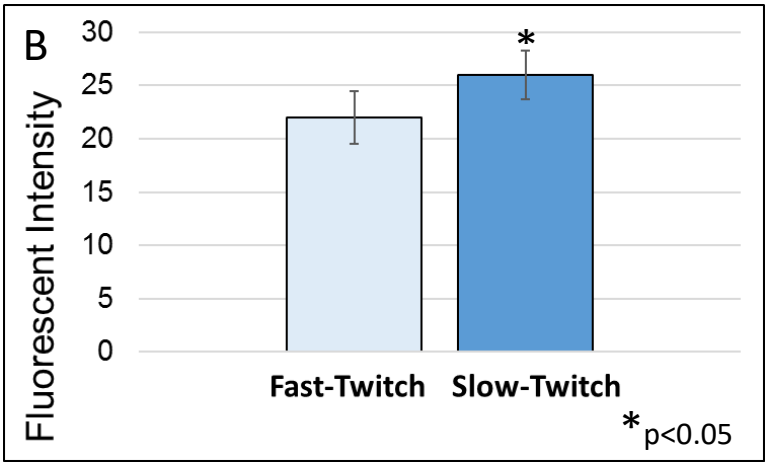


Figure 6. ERG1A fluorescence is more abundant in slow-twitch than fast-twitch fibers of the rat *Gastrocnemius* muscle.

A. representative images of rat *Gastrocnemius* muscle sections. Red represents MyHC fluorescence used to determine fiber type. Green represents ERG1A. Scale bars =50um.

B. Fluorescent intensities of fast and slow-twitch muscle fiber sarcolemma in *Gastrocnemius* muscle within muscle. Bars represent mean fluorescence ± SEM.

Discussion

The ERG1A channel is known to contribute to repolarization of the action potential in heart. It was not detected in skeletal muscle until the Pond and Hannon labs reported finding it in atrophying skeletal muscle (Wang et al., 2006). These laboratories also revealed that ectopic expression of *Merg1A* would increase UPP activity in skeletal muscle while both genetic and pharmacological block of MERG1A would inhibit the loss of muscle in hind limb suspended mice (Wang et al., 2006). Further, they reported that ectopic expression of *Merg1A* would enhance the abundance of the UPP E3 ligase, MuRF1 (Hockerman et al., 2014), suggesting that the increase in this protein contributes to the enhanced UPP activity. Interestingly, the expression of *Merg1a* did not affect expression or abundance of the UPP E3 ligase atrophy marker, *Mafbx*/ATROGIN1 (Hockerman et al., 2014). More work is needed to determine the role that the ERG1A K^+ channel plays in skeletal muscle

In a conventional action potential, a threshold potential is reached by small depolarizing signals causing a committed depolarizing chain of events beginning with the opening of voltage gated sodium channels. These channels allow sodium to rush into the cell and greatly depolarize it. Once the cell depolarizes to some critical point, a second (and then perhaps even a third) set of voltage gated channels open and these may allow calcium to rush in to depolarize the cell further or may allow potassium to rush out of the cell, repolarizing it. ERG1A is a voltage gated potassium channel that aids in the repolarization of cells. An upregulation of ERG1a would increase the number of total potassium channels in the cell membrane, allowing it to repolarize faster, leading to a shorter overall action potential duration which may speed up the impulse generated.

It is known that muscle fibers can remodel themselves is response to different neural stimuli. As previously stated, a fast-twitch fiber receives a faster pattern of neural impulses than a slow-twitch fiber. If a slow-twitch fiber is reinnervated to receive the neural impulse that would normally go to a fast-twitch fiber, the slow-twitch fiber will remodel and become a fast twitch fiber (Schiaffino et al. 2011). In the aforementioned cases, the neural input affects the protein expression. It is also possible that in cases where the actual neural input may not be changing, that how the cell perceives and responds to the input may change. With an increase in the expression of *Erg1a*, an increase in potassium channels could decrease skeletal muscle action potential duration in response to neural stimulation and thus decrease the speed of muscle contraction. It is well-known that skeletal muscle speed of contraction gets slower as animals age. Indeed, we saw a trend for more ERG1A to be expressed in both old *Soleus* and *EDL* than in young *Sol* and *EDL* muscles, however these values were not significant. Skeletal muscle naturally atrophies with normal aging so it was expected that we would find a greater abundance of ERG1A within the older rats. With a larger sample size of rats, it is possible that this difference would become significant. Indeed, it has been shown in humans that, even in healthy aging, skeletal muscle that is retained by exercise becomes slower with an increase in slow-type fibers which appear in clusters within skeletal muscle sections (Mosole et al., 2014). We see a trend here for there to be a greater abundance of ERG1A in the skeletal muscle of the older rats. Indeed, the ERG1A channel could be contributing to this remodeling. It is also possible that ERG1 could be contributing to the changes in metabolism that occur with the fiber-type switching. Indeed, slow-twitch fibers prioritize the use of aerobic respiration while fast-twitch fiber variants 2X and 2B prioritize anaerobic metabolism. This switch to more anaerobic metabolism may be beneficial in an atrophying muscle as it may promote the breakdown of internal energy storages. Further research is needed to determine the reason for this switching in atrophying muscle and for the potential affect ERG1A may have on metabolism.

Conclusion

Using IHC, we determined that in the young rats (3 months) there was a 39.4% difference in ERG1A expression with more in the *Soleus* than in the *EDL*. In the old rats (30 months) there was 36.5% more ERG1a protein in the *Soleus* than in the *EDL* muscle. Both differences were significant ($p<0.001$). We confirmed these finding in immunoblot showing a 48.3% difference in ERG expression in old rats and a 24% difference in expression in the young rats. Only the difference observed in old rats was significant ($p< 0.05$). Because *Soleus* is very homogeneous for slow-type fibers, we hypothesized that this fiber type would contain more ERG1A protein. Indeed, using IHC we revealed that there is more ERG1A protein in slow-type than in fast-type fibers. It is possible that this membrane bound voltage-gated channel is affecting the duration of the action potential generated and, thus, slowing the speed of contraction. Indeed, both aged and atrophic skeletal muscle exhibit slower rates of contraction (Lecker et al. 1999). We know that ERG1a also affects UPP activity in response to atrophic stimuli, however, little is known about the mechanism(s) by which it has its effect. Future studies would explore these facets of the ERG1a channel detected I skeletal muscle membrane. Indeed, there is a need for improved therapies to combat this debilitating condition.

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