In Vivo Characterization of the Role of Trpc1 Channel in Skeletal Muscle Function

Olivier Schakman[1] , Nadège Zanou[1], Georges Shapovalov[2], Chiara Gallo[2], Alexander Dietrich[3], Jean Lebacq[1], Urs Ruegg[2], Lutz Birnbaumer[4], Philippe Gailly[1]

[1]*University of Louvain, Louvain, Belgium. olivier.schakman@uclouvain.be*
[2]*University of Geneva, Geneva, Switzerland.* [3]*University of Marburg, Marburg, Germany.*
[4]*National Institute of Environmental Health Sciences, North Carolina, USA.*

Abstract

Skeletal muscle contraction is reputed not to depend on extracellular Ca^{2+}. Indeed, stricto sensu, excitation-contraction coupling does not necessitate entry of Ca^{2+}. However, we previously observed that, during sustained activity (repeated contractions), entry of Ca^{2+} is needed to maintain force production. The influx of Ca^{2+} through TRPC1 represents a minor part of the entry of Ca^{2+} into muscle fibers at rest, and the activity of the channel is not store dependent. The lack of TRPC1 does not affect intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) transients reached during a single isometric contraction. However, the involvement of TRPC1-related Ca^{2+} entry is clearly emphasized in muscle fatigue. Indeed, muscles from $TRPC1^{-/-}$ mice stimulated repeatedly progressively display lower $[Ca^{2+}](i)$ transients than those observed in $TRPC1^{+/+}$ fibers, and they also present an accentuated progressive loss of force. Interestingly, muscles from $TRPC1^{-/-}$ mice display a smaller fiber cross-sectional area. In the present study, we evaluated the involvement of the canonical transient receptor potential TRPC1 ion channel in muscle function, basal activity and paws coordination. To study the role of TRPC1 in skeletal muscle functions, we investigated in vivo the ability of the $TRPC1^{-/-}$ mice to perform voluntary (running wheel and escape test) or endurance exercises (treadmill and wire test). If $TRPC1^{-/-}$ mice do not exhibit any difficulty to perform voluntary exercise, they show a predisposition to muscle fatigue. Indeed, the performances of the $TRPC1^{-/-}$ mice in the treadmill and wire test were lower than those observed in control mice. We also compared basal activity (home cage activity) and coordination (rotarod, balance-beam test). We conclude that TRPC1 ion channels modulate the entry of Ca^{2+} during repeated contractions and help muscles to maintain their force during sustained repeated contractions.

Introduction

Skeletal muscle contraction is reputed not to depend on extracellular Ca^{2+}. Indeed, stricto sensu, excitation-contraction coupling does not necessitate entry of Ca^{2+}. However, we observed that, during sustained activity (repeated contractions), entry of Ca^{2+} is needed to maintain force production. We previously reported that in myoblasts, TRPC1 ion channel is by far the most widely expressed channel of the TRPC subfamily (at least 100 times more than other isoforms). In soleus, EDL and tibialis anterior muscles, TRPC1 is also one of the most expressed TRPC isoforms. In several investigations, TRPC1 has been reported to be responsible for store-operated Ca^{2+} entry. In a recent study, we indeed confirmed that repression of TRPC1 in C2C12 myoblasts reduced the store-operated Ca^{2+} entry and that involvement of TRPC1-related Ca^{2+} entry is clearly emphasized in muscle fatigue (2).

Aim of the study

In the present study, we evaluated in vivo the possible involvement of TRPC1 in muscle function, basal activity and paws coordination in $TRPC1^{+/+}$ and $TRPC1^{-/-}$ mice.

Materials and methods

Generation of TRPC1−/− mice has been described previously (1). TRPC1−/− and TRPC1+/+ were obtained from heterozygous animals. TRPC1−/− were compared with their TRPC1+/+ control sex-matched littermates. Mice were placed in individual “Physiocages” (Panlab-Bioseb, Vitrolle France) in which they were accustomed

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for at least 24 h before starting the measurements. They had free access to food and water. Food and drink consumption and rearing were measured during 48h. Open field test. Mice were placed in a square arena (60 x 60 cm) on one side of the arena facing the wall. Mice were video tracked (Ethovision, Noldus) during 20 min. Distance moved and speed were recorded. Rotarod test. Mice were tested for their ability to keep their balance on a rotating rod. The time (latency) taken to fall off the rod rotating under continuous acceleration (e.g. from 4 to 40 rpm) was measured. Wheel running test. Each mouse was individually housed in a standard cage containing a low inertia running wheel. Wheel-running distances were measured during the active part of the day (from 6 pm to 6 am). Escape test. Each mouse was placed in front of a tube and a cuff was wrapped around the tail and connected to a fixed force transducer. In response to gentle pinching of the tail, the mice tried to escape into the tube and a short peak of force was recorded. Results are presented as the mean of the five highest peaks of force recorded, related to body weight (mN.g-1). Treadmill test. The mice were placed on a treadmill with an uphill inclination of 30°, at a speed of 5m/min for 5 min, followed by a progressive increase in speed up to 17 m/min. The test was stopped when the mouse remained on the shocker plate (back of the treadmill) for 20 s without attempting to reengage the treadmill, and the time to exhaustion was determined. Wire test. Mice were suspended by their forelimbs to a 1.5 mm thick, 60 cm long metallic wire at 45 cm above soft ground. The time until the mouse fell down was recorded. Three trials were performed per session with a maximum time per trial was set to 180 s. Results are presented as the mean ± SEM (n=5-10 for each strain). The experiments were conducted and the animals were cared in accordance with the directives of the institutional animal care and use committee of the University of Louvain.

Results and conclusion

TRPC1 −/− mice Present In Vivo Signs of Fatigue. Indeed, TRPC1+/+ and TRPC1−/− mice performed very differently in the two endurance tests (treadmill test and wire test). TRPC1+/+ mice ran easily during the progressive increase of treadmill speed and resisted beyond 45 min of the test. Interestingly, TRPC1−/− mice presented difficulties when speed was progressively increased and ran only <20 min (n = 10) (P < 0.001), suggesting a predisposition to muscle fatigue. A similar result was obtained with the wire test, we observed that TRPC1+/+ mice rarely fell down before the end of each trial. However, TRPC1−/− mice were unable to resist so long [57 ± 13 s (n = 11) in TRPC1−/− vs. 174 ± 4s (n = 10) in TRPC1+/+ mice. In contrast, similar performances were observed in TRPC1+/+ and TRPC1−/− mice in the tests evaluating basal activity (open field test), paw coordination (rotarod test) and voluntary exercise (running wheel test and escape test). In conclusion, the lack of TRPC1 has a repercussion on adult muscles in vivo: TRPC1 KO mice show more susceptibility to fatigue. But the lack of TRPC1 impaired neither basal activity nor paws coordination.

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References

1. Dietrich, A., Kalwa, H., Storch, U., Schnitzler, M., Salanova, B., Pinkenburg, O., Dubrovska, G., Essin, K., Gollasch, M., Birnbaumer, L., Gudermann, T. (2007). Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflugers Arch* **455,** 465-477.

2. Zanou, N., Shapovalov, G., Louis, M., Tajeddine, N., Gallo, C., Van Schoor, M., Anguish, I., Cao, M.L., Schakman, O., Dietrich, A., Lebacq, J., Ruegg, U., Roulet, E., Birnbaumer, L., Gailly P. (2010) Role of TRPC1 channel in skeletal muscle function. *Am J Physiol Cell Physiol* **298**: C149-C162.