Journal of Electromyography and Kinesiology 21 (2011) 525–532

Contents lists available at ScienceDirect

Journal of Electromyography and Kinesiology

journal homepage: www.elsevier.com/locate/jelekin

Effects of a single bout of isometric neuromuscular electrical stimulation on rat *gastrocnemius* muscle: A combined functional, biochemical and MRI investigation

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ARTICLE INFO

Article history:
Received 5 August 2010
Received in revised form 25 November 2010
Accepted 26 January 2011

Keywords:
Creatine kinase
Magnetic resonance imaging
Muscle injury
Skeletal muscle
Animal

ABSTRACT

While muscle damage resulting from electrically-induced muscle isometric contractions has been reported in humans, animal studies have failed to illustrate similar deleterious effects and it remains to be determined whether these conflicting results are related to differences regarding experimental procedures or to species. We have investigated *in vivo*, in rat *gastrocnemius* muscles, using experimental conditions as close as possible to those used in humans (i.e., muscle length, number of contractions, stimulated muscle), the effects of a single bout of neuromuscular electrical stimulation (NMES). Maximal tetanic force was measured before, immediately after and 1 h and 1, 2, 3, 7 and 14 days after NMES. Magnetic resonance imaging measurements, including volume of *gastrocnemius* muscles and proton transverse relaxation time (T_2) were performed at baseline and 3, 7, and 14 days after the NMES session. Control animals did not perform any exercise and measurements were recorded at the same time points. For both groups, blood creatine kinase (CK) activity was measured within the first 3 days that followed the initial evaluation. Maximal tetanic force decreased immediately after NMES whereas measurements performed 1 h and the days afterwards were similar to the baseline values. CK activity, muscle volume and T_2 values were similar throughout the experimental protocol between the two groups. Under carefully controlled experimental conditions, isometric NMES *per se* did not induce muscle damage in rat *gastrocnemius* muscles on the contrary to what has been repeatedly reported in humans. Further experiments would then be warranted in order to clearly delineate these differences and to better understand the physiological events associated with muscle damage resulting from NMES-induced isometric contractions.

1. Introduction

Neuromuscular electrical stimulation (NMES) is currently considered as a method of choice for increasing voluntary strength (Gondin et al., 2005), muscle mass (Gondin et al., 2005) and/or exercise capacity (Bourjeily-Habr et al., 2002; Maffiuletti et al., 2002). On that basis, NMES has been widely used as an adjunct to voluntary resistance training in highly-trained subjects (Delitto et al., 1989; Maffiuletti et al., 2002) and in patients in intensive care units (Gerovasili et al., 2009; Gruther et al., 2010) or affected by cardiac (Quittan et al., 2001) and respiratory chronic (Vivodtzev et al., 2006) diseases. However, growing evidence is emerging illustrating the potential damaging effects of electrically-induced isometric contractions in healthy humans, at least for the lower limbs (Aldayel et al., 2010a,b; Mackey et al., 2008; Maffiuletti et al., 2006). Indeed, three studies conducted in quadriceps muscles under isometric conditions (Aldayel et al., 2010a,b; Jubeau et al., 2008) have recently reported a 10–30-fold increase in creatine kinase (CK) activity coupled to significantly increased muscle soreness as a result of NMES. In the human *gastrocnemius* muscles, NMES resulted in a 5-fold CK activity increase, macrophage infiltration, *z*-lines disruption and a slightly modified desmin staining (Mackey et al., 2008), thereby clearly illustrating major muscle fibres alterations.

On the contrary to what has been reported in human lower limbs, no or minor muscle damage has been observed in rat hindlimb muscles after a single bout of isometric NMES (Geronilla et al., 2003; Hesselink et al., 1996; Kanzaki et al., 2010; Peters et al., 2003; van der Meulen et al., 1992). On that basis, one can wonder whether this discrepancy illustrates a reduced vulnerability of animals to NMES or a methodological issue regarding the lack of similarity between the corresponding protocols. In fact, while muscle length and the number of contractions (sometimes referred to exercise duration) have been largely acknowledged as the main determinants of muscle damage (Talbot and

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doi:10.1016/j.jelekin.2011.01.006

Morgan, 1998; Tiidus and Ianuzzo, 1983), they have never been carefully standardized between protocols conducted in animals and humans. Indeed, the number of contractions used in animals (ranging from 15 to 300) (Hesselink et al., 1996; Peters et al., 2003) was slightly higher than in humans (ranging from 40 to 180 contractions) whereas shorter stimulation trains were used in animals (0.3–2.8 s) as compared to humans (1–5 s). As a consequence, the whole exercise duration in most of the previous animal studies (i.e., 12–120 s) (Geronilla et al., 2003; Hesselink et al., 1996; Peters et al., 2003; van der Meulen et al., 1992) was largely shorter than what reported in humans (200–1080 s). In addition, it is noteworthy that rat dorsiflexor muscles have been stimulated at short muscle length (ankle joint fixed at 90°) whereas most of the human studies have been performed at long muscle length (Aldayel et al., 2010a,b; Jubeau et al., 2008). Considering that the magnitude of muscle damage is known to be directly related to muscle length (Talbot and Morgan, 1998) the protocol used in animals could have masked the potential damaging effects of NMES.

It is also of importance to underline that studies involving isometric muscle contractions in rats always require surgical procedures for motor nerve stimulation (van der Meulen et al., 1992) and/or force production measurements (Kanzaki et al., 2010) so that longitudinal studies are precluded. In animal studies, isolated investigations such as force production (Peters et al., 2003), histology (Hesselink et al., 1996) or protein content (Kanzaki et al., 2010) have been reported with a limited time-resolution ranging from a single to four consecutive measurements (Geronilla et al., 2003; Hesselink et al., 1996; Kanzaki et al., 2010; van der Meulen et al., 1992). On the contrary, humans have been investigated on the basis of multimodal approaches combining force, muscle soreness, biochemical (CK activity) and/or immunohistochemistry measurements with up to seven measurements performed after the NMES session. Given the wide range of NMES applications in the context of rehabilitation (Eriksson and Haggmark, 1979), especially in the human lower limb muscles (Bax et al., 2005), a straightforward methodological standardization between animal and human protocols is crucial in order to further clarify the interspecies differences reported so far regarding muscle damage resulting from isometric NMES.

Therefore, the aim of the present study was to investigate *in vivo* in rat *gastrocnemius* muscles, the effects of a single bout of NMES-induced isometric contractions on CK activity, maximal tetanic force, muscle volume and proton transverse relaxation time (T_2). In order to rule out any methodological bias, we chose to use experimental conditions i.e., muscle length, number of contractions, contraction duration, whole exercise duration (Aldayel et al., 2010a,b; Jubeau et al., 2008) and stimulated muscle (Mackey et al., 2008) as close as possible to those previously used in humans. Additionally, rat muscle function was originally assessed by a strictly non-invasive experimental setup (Giannesini et al., 2005) offering the possibility to get, under physiological conditions *in vivo*, information about muscle mechanical performance (i.e., maximal tetanic force), anatomy (i.e., muscle volume) and physiology (i.e., T_2 changes) in contracting muscle and to repeat these investigations several times in the same animal, thereby allowing the first multimodal follow-up study of muscle injury after NMES-induced isometric contractions in rats. We hypothesized that, under comparable experimental conditions and in the absence of species-related differences in the magnitude of muscle injury, NMES of rat *gastrocnemius* muscles should result in an increased CK activity and T_2 values and a decreased tetanic force as previously reported in humans (Aldayel et al., 2010a,b; Jubeau et al., 2008).

2. Methods

2.1. Experimental procedures

2.1.1. Experiment 1

Eight-week old male Wistar rats ($n = 18$; Janvier, Le Genest-Saint-Isle, France) were used for these experiments conducted according to the French guidelines for animal care and with the approval of the animal experiment committee of "Université de la Méditerranée, Marseille". Rats were housed in an environmentally controlled facility (12–12 h light–dark cycle, 22 °C), and received water and standard food *ad libitum* until the end of experiments. Animals were randomly assigned to NMES ($n = 6$) or to a control group (C, $n = 7$). For the NMES group, body weight, maximal tetanic force and magnetic resonance imaging (MRI) measurements were performed at baseline and 3, 7, and 14 days after the NMES session. Control animals did not perform any exercise and the whole set of measurements were recorded at the same time points. For both groups, CK activity was measured at baseline and 3 days afterwards. In another group of five rats, CK activity was measured at earlier time points, i.e., 1 and 2 days after NMES. The purpose of this additional experiment was to ensure that no change in CK values occurred during the first 3 days post-NMES (Warren et al., 1999).

2.1.2. Experiment 2

Maximal tetanic force was also measured in a group ($n = 6$) of older rats (ten-week old) before, immediately after and 1 h and 1, 2, and 3 days after NMES in order to determine whether the initially selected time-resolution (i.e., at 3, 7, and 14 days post stimulation) were sensitive enough to detect muscle damage and to obtain a time-resolution similar to that recently reported in humans (Aldayel et al., 2010a,b). Body weight was also measured within the first three days.

2.2. Animal preparation

Body weight was measured (Kern 440–49 N, Kern & Sohn GmbH, Germany) at the beginning of each testing session. Rats were initially anaesthetised in an induction chamber with 4% isoflurane (Forene; Abbott France, Rungis, France) mixed in 33% O_2 (0.5 L min^{-1}) and 66% N_2O (1 L min^{-1}). Once the right lower hindlimb was shaved, electrode cream was applied at the knee and heel levels in order to optimize electrical stimulation. Each anaesthetised rat was placed supine in a home-built cradle which has been specially designed for the strictly non-invasive functional investigation of the right *gastrocnemius* muscles (Giannesini et al., 2005). Due to the specific design of our experimental set-up (Giannesini et al., 2005), the contralateral leg could not be stimulated and was not used for control measurements. Throughout a typical experiment, anaesthesia was maintained by gas inhalation through a facemask continuously supplied with 2.5% isoflurane in 33% O_2 (0.4 L min^{-1}) and 66% N_2O (0.8 L min^{-1}). The facemask was connected to an open-circuit gas anaesthesia machine (Isotec. 3; Ohmeda Medical, Herts, UK). Corneas were protected from drying by applying ophthalmic cream (Lacrigel, Europhta, Monaco). Exhaled and excess gases were removed through a canister filled with activated charcoal (Smiths Industries Medical System, London, UK) mounted on an electrical pump extractor (Equipement Vétérinaire Minerve, France). In order to maintain the rat at a physiological temperature during anaesthesia, an electrical heating blanket (Prang + Partner AG, Pfungen, Switzerland) integrated in a feedback loop with a temperature control unit (Ref. No. 507137, Harvard Apparatus, Les Ulis, France) was connected to a rectal probe (Ref. No. 507145, Harvard Apparatus, Les Ulis, France).