ELSEVIER

Journal of Neuroscience Methods 159 (2007) 244–251

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

A novel mouse skeletal muscle-nerve preparation and *in vitro* model of ischemia

Heather N. Wenk *, Edwin W. McCleskey

Vollum Institute, Oregon Health & Science University, 3181 S.W. Sam Jackson Park Road, Mail Code L474, Portland, OR 97206, United States

Received 17 May 2006; received in revised form 13 July 2006; accepted 17 July 2006

Abstract

The majority of patients in pain clinics are treated for muscle pain yet methods to study it in animals are relatively weak compared to methods to study skin pain. Here we describe an *in vitro* muscle-nerve preparation and model of muscle ischemia and contractile fatigue in mice. Timed muscle contraction is electrically evoked, while single unit activity of muscle sensory neurons and muscle contractile force are simultaneously recorded. The muscle is placed in a small (<1 mL) chamber where oxygen levels can be manipulated, drugs can be applied, and the extracellular milieu can be highly controlled. We demonstrate that we can record from sensory afferents that have the properties expected of ischemic nociceptors. This method serves for studying the neuronal and molecular mechanisms underlying ischemic pains such as angina, intermittent claudication, and sickle cell crisis.

Keywords: Muscle-nerve preparation; Sensory afferents; Muscle contraction; Ischemia

1. Introduction

Ischemic pain occurs when the local blood supply is insufficient for the metabolic needs of working muscle. The best known example is angina pectoris, the pain accompanying heart attack. In skeletal muscle, clinically relevant examples include intermittent claudication and the debilitating pain of sickle-cell anemia. Human psychophysical experiments involving ischemic pain have shown that temporarily restricting blood flow to an extremity is not painful unless the muscle is forced to contract (Lewis et al., 1931; Staunton et al., 1964). Early experiments suggested that ischemic pain occurs when an unknown metabolic product of muscle contraction accumulates in unperfused muscle (Lewis et al., 1931). However, the identity of this proposed metabolite and the mechanism underlying its ability to activate sensory receptors is still controversial.

Most of what we know about the properties of sensory afferents that innervate mammalian skeletal muscle was learned from *in vivo* experiments using anesthetized cats. Current interest in the molecular mechanisms of sensory transduction, together with increased availability of genetically altered mice, has fostered a need for new preparations to easily study muscle afferent function in smaller mammals.

In vitro tissue preparations allow experimental control over the extracellular milieu and the ability to apply drugs and chemical stimuli directly to the receptive field. The popular skin-nerve preparation, for example, has been used to study cutaneous sensations ranging from mechanical and temperature sensitivity to nociception (Kress et al., 1992; Reeh, 1986). Similar preparations have been used to record sensory activity from tissues such as viscera (Brierley et al., 2005) and the vibrissae of rats (Baumann et al., 1996). Recently a few muscle-nerve preparations have also been developed including a rat gracilis muscle-nerve preparation used to record mechanoreceptor activity in response to compressive stress (Ge and Khalsa, 2003; Khalsa and Ge, 2004).

Here we share our design and initial findings using a mouse plantar muscle-tibial nerve preparation that we have developed in conjunction with an *in vitro* model of muscle ischemia. Identified muscle afferents were classified according to axonal conduction velocity and pH-sensitivity. Muscle contractions were evoked by electrical stimulation of the plantar nerve and afferent neuron responses to the contractions were recorded under control and hypoxic conditions.

* Corresponding author. Tel.: +1 503 494 4321; fax: +1 503 494 6972.
E-mail address: hwenk@gustavus.edu (H.N. Wenk).

0165-0270/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2006.07.021

2. Materials and methods

2.1. Dissection

All procedures were approved by the OHSU IACUC committee and performed in accordance with their guidelines. Adult male C57BL6 mice were removed from their home cages and briefly anesthetized with halothane gas (2–4% in air). They were then fully anesthetized with an I.M. injection containing a mixture of ketamine (75 mg/kg), xylazine (5 mg/kg) and acepromazine (1 mg/kg).

The tibial nerve was cut at the branch point with the sciatic nerve and dissected free from the surrounding tissue down to the ankle joint. The skin was then removed from the plantar surface of the paw exposing the underlying muscle. The hind foot was removed from the animal at the ankle and placed in iced, oxygen-saturated SIF buffer. Both feet were used from each mouse, with one foot stored on ice before use. No difference in overall viability, electrically induced contraction strength, or sensory neuron response properties was detected between preparations used immediately after removal from the mouse and preparations stored in this manner for up to 6 h.

The preparation was further dissected in the recording chamber while constantly perfused with oxygen-saturated synthetic interstitial fluid (SIF; in mM: 123 NaCl, 3.5 KCl, 0.7 $MgSO_4$, 2.0 $CaCl_2$, 9.5 Na gluconate, 1.7 NaH_2PO_4, 5.5 glucose, 7.5 sucrose, 2.5 HEPES; pH 7.45 ± 0.05) (Koltzenburg et al., 1997). Skin and other extraneous tissue was dissected off the preparation leaving only the plantar muscles, the underlying mid-foot bones, and associated connective tissue. Since the tendons stay intact and attached to the bones the muscles maintain a physiological level of tension.

2.2. Extracellular recording

Fig. 1 shows a photograph and diagram of the preparation and recording chamber. The preparation was pinned to the sylgard bottom of the tissue chamber and the cut end of the tibial nerve was threaded through a small hole into a smaller recording chamber containing a layer of mineral oil floating on a bottom layer of SIF. The cut end of the nerve, or filaments dissected off of it, was lifted into the mineral oil layer and placed on an extracellular gold or platinum wire (0.01 in. diameter) recording electrode. Neural signals were amplified (DAM80, World Precision Instruments, Austin, TX), notch filtered (300 Hz, 10 kHz), run through a Humbug 60 Hz Noise Eliminator (Quest Scientific, North Vancouver, BC, Canada), broadcast over an audio speaker, displayed on an oscilloscope, and digitized by a DataPac PCI-

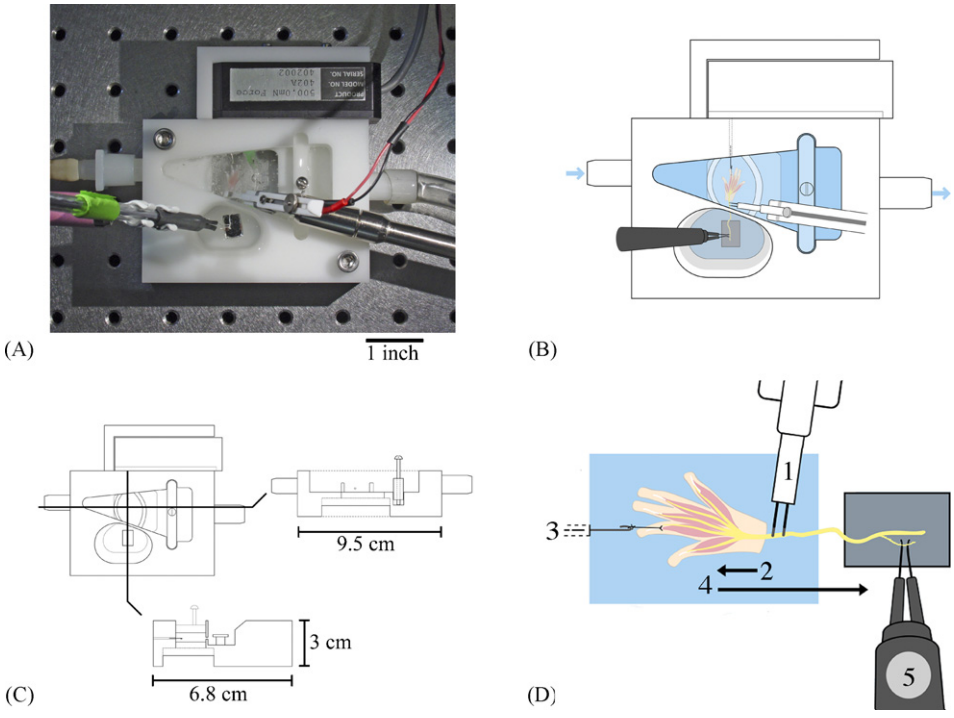

Fig. 1. Muscle-nerve preparation. (A and B) The photograph and drawing show the muscle-nerve preparation and basic electrophysiological recording set-up. The preparation consists of the metatarsal bones from the bottom of the mouse foot along with the attached plantar muscles and tibial nerve. The tissue is placed in the recording chamber pictured here and is constantly perfused with oxygenated synthetic interstitial fluid (SIF). The medial plantar nerve is run across a bipolar stimulating electrode and is insulated from the surrounding SIF with Vaseline or a similar grease. A force transducer built into the back of the chamber monitors the muscle contractions. The tibial nerve is threaded through a small hole into a separate recording chamber filled with mineral oil where an extracellular gold or platinum wire hook electrode is used to record single unit activity using standard teased fiber recording techniques. (C) The recording chamber design was based on an original skin-nerve preparation chamber designed by Peter Reeh. Major modifications to this design include the smaller size of the chamber and a built-in housing for the force transducer. Indicator lines mark positions corresponding to the two cross-sectional cutaway views. (D) The cartoon close-up of the preparation illustrates the following: (1) bipolar stimulating electrode is used to stimulate the tibial nerve at a level sufficient to activate efferent motor axons; (2) contraction is evoked in the plantar muscles; (3) timing and relative strength of contraction is recorded via a force transducer attached to one of the cuboid tendons; (4) sensory afferents respond to muscle contractions; (5) extracellular hook electrode is used to record afferent activity from a small filament teased from the cut end of the nerve.