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IP_3 receptors and Ca^{2+} signals in adult skeletal muscle satellite cells *in situ**

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ABSTRACT

In this short article we review muscle satellite cell characteristics and our studies in adult rodent muscle satellite cells *in situ*. Using confocal laser scanning microscopy and immunocytochemistry, a high level of IP_3 receptor (IP_3R) immunostaining was detected in satellite cells. These cells were identified by their peripheral position, their size, the shape of their nucleus, the paucity of the apparent cytoplasm, and the immunostaining with specific molecular markers such as α-actinin, the neural cell adhesion molecule (N-CAM) and desmin. High extracellular K^+ (60 mM) induced long-lasting Ca^{2+} signals in satellite cells *in situ*. We suggest that electrical activity stimulates IP_3-associated Ca^{2+} signals that could act in concert with signaling pathways triggered by growth factors and/or hormones.

Key words: Muscle satellite cells, Inositol 1,4,5-trisphosphate receptors, intracellular Ca^{2+}, Signal transduction

INTRODUCTION

In adult skeletal muscles, there is a population of small fusiform mononucleated cells, usually oriented parallel to the axis of the fibers, which may protrude above its surface as seen by electron microscopy. These cells, which are closely associated with myofibers, were first identified under the electron microscope and were called satellite cells (Katz, 1961; Mauro, 1961). A characteristic property of these cells is that they lie between the plasma membrane of the muscle fiber and the basal lamina (Muir, 1970). Muscle satellite cells have been identified in amphibian (Mauro, 1961; Katz, 1961; Popiela, 1976), reptilian (Kahn and Simpson, 1974), avian (Hartley et al, 1992) and mammalian skeletal muscles including muscle spindles (Gamble et al., 1978; Campion et al, 1981). The number of satellite cells in mammalian skeletal muscles depends on the muscle fiber type. Thus, in the adult *extensor digitorum longus,* which primarily contains glycolytic fibers, the percentage of satellite cells is lower than in the soleus muscle that

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contains slow oxidative fibers (Gibson and Schultz, 1982; Snow, 1983; Schultz and McCormick, 1994). Age-related differences in absolute numbers of skeletal muscle satellite cells have been reported in various animal species (Gibson and Schultz, 1983; Nnodim, 2000). In general, the overall number of satellite cells appears higher at birth (Bischoff, 1994; Hawke and Garry, 2001), and decreases during aging (Renault et al., 2002). This reduction in satellite cell number contributes to a diminution of the regenerative capacity of skeletal muscle (Grounds, 1998; Jejurikar and Kuzon, 2003). However, the impaired regenerative response observed with aging appears to be much more complex than just satellite cell senescence (see Bortoli et al., 2003; Conboy et al., 2003).

Morphological characteristics of satellite cells in mature muscles include a high nuclear-to-cytoplasmic ratio, a small organelle content, a smaller nuclear size compared with neighboring myonuclei, and an abundant heterochromatin in their nuclei (Muir et al., 1965; Muir, 1970; Cull-Candy et al., 1980) reflecting their mitotic quiescence (Schultz et al., 1978).

For many years the laborious identification of satellite cells *in situ* by light microscopy limited their study. However, the use of immunohistochemical techniques with antibodies directed to proteins expressed either in the basal lamina, such as laminin, or in the sarcolemma, like dystrophin, greatly facilitated their identification in fixed muscles. Muscle satellite cells are also usually difficult to identify *in vivo* under the light microscope, due to their close apposition to the muscle fiber sarcolemma (cleft width about 20 nm), and because they are usually covered by connective tissue overlying the muscle. However, the use of mild enzymatic digestion with collagenase (Cull-Candy et al, 1980), to remove the connective tissue, together with improved Normarski's or Hoffman's optic-additions to the light microscope, and the current use of confocal laser scanning microscopy greatly refined their visualization in single living muscle fibers, or bundles consisting of a few fibers of skeletal muscle.

The adult skeletal muscle demonstrates a remarkable capacity to adapt to strenuous activity and/or tissue damage, and satellite cells have been implicated as the major source of myogenic cells involved in growth, and repair of myofibers. The processes by which these physiologic adaptations occur are attributed to the activation, multiplication and fusion of satellite cells with myofibers (for a review see Hawke and Garry, 2001). Activation of satellite cells also plays a role in muscle growth following minimal activity (Kadi and Thornell, 2000) probably without muscle damage. The reaction of satellite cells to such muscle activity would probably not involve factors triggered by damage (e.g. growth factors), which are the major substances regulating the activation, proliferation, and differentiation of satellite cells (McFarland, 1999; Hawke and Garry, 2001), but could relate to muscle depolarization. Little is known concerning signal transduction pathways involved in muscle growth in response to activity (however, see Dunn et al., 2000; Pallafacchina et al., 2002). Understanding the signaling systems involved in directing satellite cells between proliferation and differentiation is of great importance. Many factors, including fibroblast growth factors, insulin-like growth factors, and interleukin-6 cytokines, have already been implicated in the control of satellite cell activity (for reviews see Hawke and Garry, 2001; Charge and Rudnicki, 2004). In the present article we review the evidence that inositol 1,4,5-trisphosphate receptors (IP_3Rs) are localized in satellite cells of mature muscle, and that depolarization triggers a long-lasting calcium signaling in those cells (Powell et al., 2003).

ADULT SATELLITE CELLS IN SITU EXPRESS IP_3 RECEPTORS

In adult mouse skeletal muscle, satellite cells can be identified *in situ* by their location on the periphery of myofibers, by their morphology and characteristic nucleus, and because they are covered by

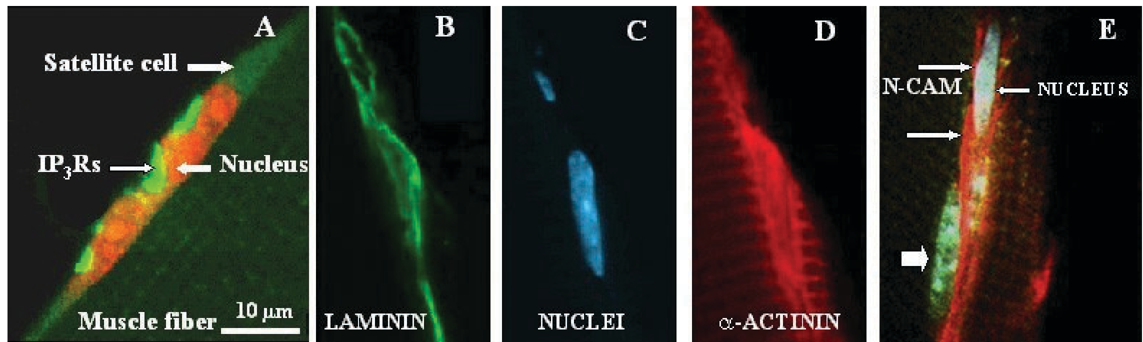

Figure 1. Confocal images of satellite cell immunostained with various protein markers in adult mouse skeletal muscles. A, projected image (12 sections, spaced by 0.12 µm) showing a duet of satellite cells *in situ* at the periphery of a myofiber (*Levator auris longus* muscle) immunostained for IP_3Rs (green), and stained for nuclei with propidium red (orange). Note the distinct intensity of the IP_3R-immunostaining in the cell's cytoplasm, and the IP_3R cross-striation pattern in the myofiber. B, C and D, single optical sections (0.12 µm thickness) of a cryostat section (10 µm) from an *Extensor digitorum longus* muscle revealing the presence of laminin in the basal lamina surrounding a duet of satellite cells (B), nuclei, stained with TOTO-3, (C) and α-actinin in the cytoplasm of the satellite cells, and in a cross-striated pattern in the myofiber (D). N-CAM immunostaining is shown in another satellite cell (E); this marker is found in quiescent, activated and proliferating cells, but not in fibroblasts.

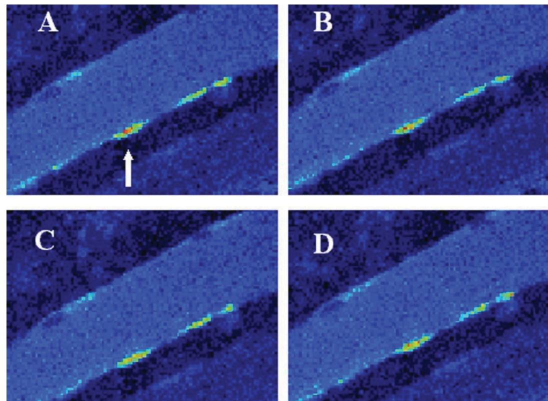

Figure 2. Calcium signals induced in satellite cells *in situ* by a high K^+ isotonic solution. A bundle of the hemidiaphragm was carefully dissected and loaded with fluo-3 for 30 min at room temperature and maintained in oxygenated Krebs-Ringer solution. Intact muscle fiber bundles were placed in a special chamber designed to fit on an upright confocal microscope. The fluorescence image was obtained 180 s (A), 190 s (B), 200 s (C) and 210 s (D) after substitution of the incubation saline by one containing 60 mM K^+ (replacing Na^+). High fluorescence can be seen in both the cytosol and nuclei of satellite cells (*arrows*), while the putative myonuclei (*arrowheads*) exhibit basal fluorescence. The thickness of the optical section was 1.0 µm.

laminin of the basal lamina. A positive immunostaining for IP_3Rs was obtained in the cytoplasm of satellite cells *in situ*. In the projected confocal image shown in Figure 1A, a high concentration of IP_3Rs is suggested by the intensity of the immunostaining in specialized regions of the satellite cell cytoplasm. These cells express the neural cell adhesion molecule (N-CAM) that is found in quiescent, activated and proliferating satellite cells (Hawke and Garry, 2001), and at the neuromuscular junction of adult fibers (Covault and Sanes, 1986), but not in fibroblasts or vascular tissue. Some of the satellite cells contain desmin, an intermediate cytoskeletal filament protein, which is considered a molecular marker of activated cells (Bockhold et al., 1998). Furthermore, satellite cells also contain α-actinin (Powell et al., 2003). Proliferating satellite cells in culture have been also shown to express α-actinin, as well as vimentin and desmin (Van der Ven et al, 1992). In double-immunolabeled muscle fibers, IP_3Rs were also found in cells identified as satellite cells by the presence of N-CAM, or desmin (Powell et al., 2003).

MUSCLE SATELLITE CELLS RESPOND TO K^+-INDUCED MEMBRANE DEPOLARIZATION

In myofibers loaded with fluo3-AM and stimulated by a high K^+ (60 mM) isotonic medium, an increase in cytoplasmic Ca^{2+} in satellite cells was observed (Powell et al., 2003). This calcium does not appear to come from the myofiber, but to be confined to each satellite cell. The Ca^{2+} transients in these cells were long lasting (200-300 sec) with respect to the transients observed in the myofiber. These data, and the fact that IP_3Rs are expressed in satellite cells suggest an IP_3-associated Ca^{2+} signaling in those cells.

Although the results with K^+-induced depolarization do not reveal how satellite cells can be depolarized *in situ*, it is conceivable that the K^+ concentration around the satellite cells could increase during trains of action potentials in the muscle fiber. This elevated level of K^+ could then depolarize the satellite cells. The dihydropyridine receptor (α_1-subunit) voltage sensor has been shown to trigger a depolarization induced IP_3 cascade in cultured muscle (Araya et al., 2003). It is likely that satellite cells may contain low levels of dihydropyridine receptors, since mRNA encoding skeletal muscle isoforms of the dihydropyridine receptor-α_1-subunit have been reported in human myoblasts (Tanaka et al., 2000). If the signal transduction model for muscle in culture (Powell et al., 2001; Araya et al., 2003) holds true for satellite cells, then an IP_3 cascade would end in Ca^{2+}-dependent satellite cell's nuclear activation. The more the particular muscle fiber contracts, the stronger the signal to the satellite cell, and the more likely it would be recruited for proliferation and fusion with the muscle fiber. This mechanism of recruitment might be important in mild exercise (Kadi and Thornell, 2000), in muscle growth after atrophy (Mitchell and Pavlath, 2001) and in muscle regeneration. Alternatively, the depolarization-induced Ca^{2+} signal could act in concert with signaling pathways triggered by growth factors and/or hormones (see Jaimovich and Espinosa, this issue).

We hope that the data presented here will stimulate further investigation on IP_3 signaling in satellite cells, which may be important for understanding the mechanisms involved in muscle growth and repair.

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