Cell Biology International ISSN 1065-6995
doi: 10.1002/cbin.11190

RESEARCH ARTICLE

Isoproterenol induces an increase in muscle fiber size by the proliferation of Pax7-positive cells and in a mTOR-independent mechanism

Úrsula Maria C. Bastos, Ivone de Andrade Rosa, John D. Teixeira, Graciele Gonçalves, Manoel L. Costa, Luis Eduardo M. Quintas and Claudia Mermelstein*

Cell Differentiation Program, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 373, Rio de Janeiro, RJ, 21941-902, Brazil

Abstract

β-Adrenergic signaling regulates many physiological processes in skeletal muscles. A wealth of evidence has shown that β-agonists can increase skeletal muscle mass in vertebrates. Nevertheless, to date, the specific role of β-adrenergic receptors in different cell phenotypes (myoblasts, fibroblasts, and myotubes) and during the different steps of embryonic skeletal muscle differentiation has not been studied. Therefore, here we address this question through the analysis of embryonic chick primary cultures of skeletal muscle cells during the formation of multinucleated myotubes. We used isoproterenol (ISO), a β-adrenergic receptor agonist, to activate the β-adrenergic signaling and quantified several aspects of muscle differentiation. ISO induced an increase in myoblast proliferation, in the percentage of Pax7-positive myoblasts and in the size of skeletal muscle fibers, suggesting that ISO activates a hyperplasic and hypertrophic muscle response. Interestingly, treatment with ISO did not alter the number of fibroblast cells, suggesting that ISO effects are specific to muscle cells in the case of chick myogenic cell culture. We also show that rapamycin, an inhibitor of the mammalian target of rapamycin signaling pathway, did not prevent the effects of ISO on chick muscle fiber size. The collection of these results provides new insights into the role of β-adrenergic signaling during skeletal muscle proliferation and differentiation and specifically in the regulation of skeletal muscle hyperplasia and hypertrophy.

Keywords: β-adrenergic receptors; chick; isoproterenol; muscle differentiation; muscle hypertrophy; Pax7

Introduction

β-Adrenergic signaling is involved in the regulation of many physiological processes in skeletal muscles (Yang and McElligott, 1989; Lynch and Ryall, 2008). β-Adrenergic receptors (β-AR) agonists can increase skeletal muscle mass, protein gain, and decrease body fat. Although three β-AR exist (β1, β2, and β3), the β2-AR is the most abundant in skeletal muscle, with a minor β1-AR population. The endogenous catecholamines epinephrine and norepinephrine are the natural agonists of AR (α and β), and several exogenous compounds, such as isoproterenol (ISO), have been synthesized to selectively activate β-AR. To date, the specific roles are not known of β-AR during the different stages of embryonic skeletal myogenesis and in different myogenic cell phenotypes (muscle fibroblasts, myoblasts, and myotubes). Skeletal myogenesis is a multi-step process that involves the replication of presumptive myoblasts, the emergence of post-mitotic myoblasts, the elongation of post-mitotic myoblasts into bipolar cells, the intercellular recognition of bipolar myoblasts, myoblast fusion, the formation of multinucleated myotubes containing highly organized sarcomeric structures, and the continued growth of young myotubes into mature fibers (Choi et al., 1990). Only some of these complex and sequential series of events are observed when immortalized myogenic cell lines are induced to differentiate in vitro. In contrast, all these steps can be observed in detail and in a temporal sequence in

*Corresponding author: e-mail: mermelstein@ufrj.br

Abbreviations: ANOVA, analysis of variance; ATCC, American Type Culture Collection; cAMP, cyclic adenosine 3′,5-monophosphate; DAPI, 4,6-diamino-2-phenylindole dyhydrochloride; DMSO, dimethyl sulfoxide; ISO, isoproterenol; MEM, minimum essential medium; mTOR, mammalian target of rapamycin; MTT, 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2*H*-tetrazolium bromide; NP-40, nonyl-phenoxypolyethoxylethanol 40; Pax7, paired box protein 7; RAPA, rapamycin; β-AR, β-Adrenergic receptors

primary muscle cell cultures prepared from breast muscles from day-11 chick embryos (Ojima et al., 2016). The embryonic chick muscle culture is a robust model for studies of the molecular and cellular events that control skeletal muscle differentiation; for example, myotubes containing thousands of nuclei are easily observed in chick primary muscle cultures, compared to the few nuclei observed in myotubes obtained from immortalized muscle cell lines (Mermelstein et al., 2005).

It has been shown that β-AR first appear during the myoblast fusion stage of embryonic quail myogenesis and that mature myotubes contain ~80-fold more receptors per cell than proliferating myoblasts (Parent et al., 1980). This is consistent with the lesser response chick myoblasts have to ISO compared to myotubes (Curtis and Zalin, 1985). Interestingly, ISO provokes primary chick myoblasts to initiate precocious cell fusion (Curtis and Zalin, 1981), and this effect has been associated with the canonical rise in intracellular adenosine 3′,5-monophosphate (cyclic AMP) and the noncanonical Akt/mTOR pathway (Ryall et al., 2010). Importantly, no further study was dedicated to the analysis of the possible distinct role of β-AR signaling in the different cell phenotypes (fibroblasts, myoblasts and myotubes) that are present during embryonic skeletal muscle differentiation. Thus, the present work aimed to study the effect of the β-AR activity during in vitro chick skeletal myogenesis. We found that ISO, a potent β-AR agonist, induced an increase in myoblast proliferation, in the number of Pax7-positive myoblasts and in the size of myotubes. The collection of our data demonstrates the relevance of the use of β-AR agonists in muscle degeneration diseases.

Materials and methods

Antibodies and probes

DNA-binding probe 4,6-diamino-2-phenylindole dyhydrochloride (DAPI) was purchased from Molecular Probes (USA). The following antibodies were used in this study: anti-sarcomeric alpha-actinin (clone EA53; Sigma, USA), anti-desmin (Sigma), anti-Pax7 (Developmental Studies Hybridoma Bank, USA), Alexa Fluor 488-goat anti-rabbit IgG (Molecular Probes) and Alexa Fluor 546-goat anti-mouse IgG (Molecular Probes).

Cell cultures

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (USA). The protocol was approved by the Institutional Animal Care and Use Committee (Protocol Number: 055/16). Chick myogenic cultures were prepared as described previously (Ojima et al., 2016). Cells were plated at an initial density of 5×10^5 cells/35 mm dishes in 8–1–0.5 medium (minimum essential medium with 10% horse serum, 0.5% chick embryo extract, 1% L-glutamine, and 1% penicillin-streptomycin) and mantained at 37°C with 5% CO_2. Twenty-four-hour myogenic chick cultures were treated for 24 or 48 h with either: ISO (Sigma, USA) at 10, 50, and 100 nM; or with 0.1% dimethyl sulfoxide (DMSO), the same DMSO concentration used in 100 nM ISO, or with rapamycin (RAPA; Sigma) at 3 μM, or with Wnt5a-conditioned medium (10% v/v).

Mouse L-cell culture and Wnt5a-conditioned medium preparation

L-Wnt5a cells were obtained from American Type Culture Collection (ATCC, USA) and cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 0.4 mg/mL neomycin (Invitrogen) to maintain transgene expression during cell culture expansion. Conditioned medium from L-Wnt5a was collected according to the manufacturer's instructions. Activity of the conditioned medium was tested in previous studies of our group (Guerra et al., 2016). Wnt5a-conditioned medium (10% v/v) were added to 24-h myogenic chick cultures and cells were fixed with 4% paraformaldehyde (in phosphate-buffered saline) after 24 h.

Determination of protein concentration

Chick myogenic cells were plated at an initial density of 5×10^5 cells/35 mm culture dishes and incubated at 37°C and 5% CO_2. Total cell culture extracts were prepared from 48-h untreated and treated (with DMSO or ISO 100 nM) cells scraped off the dish in radioimmunoprecipitation assay buffer buffer (0.15 M NaCl, 1% NP-40, 0.25% sodium deoxicolate, 2 mM ethylenediaminetetraacetic acid, 0.05 M Tris-HCl, pH 67.4) with protease inhibitors. Protein concentration was estimated according to Bradford (1976). Protein concentration (mean ± standard deviation) was expressed as μg/μL. Experiments were performed in triplicate.

Cell viability assay

Cell viability was determined using 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) reagent (Sigma, USA) and as described in detail by Rosa de Andrade et al. (2018). Cells were plated at an initial density of 10^5 cells per well in 24-well plates and after 24 h some cultures were treated with ISO (10, 50, and 100 nM) for 48 h. Absorbance was measured in a microplate reader (FlexStation Reader; Molecular Devices, USA) at 570 nm.

Immunofluorescence and digital image acquisition

Myogenic cells were fixed and labeled with fluorescently labeled antibodies as described in detail by Possidonio et al. (2014). Cells were mounted in Prolong (Molecular Probes) and examined with an Axiovert fluorescence microscope (Carl Zeiss, Germany).

Quantification of chick cell cultures

Phase contrast microscopy images of live cultured cells were acquired with an Axiovert 100 microscope (Carl Zeiss). Myogenic cell cultures were quantified using a CellProfiler Analyst (http://cellprofiler.org; Jones et al., 2008). The DAPI labeling, together with the desmin labeling, enables the identification of myoblasts and fibroblasts by the presence of desmin and by the differences in their nuclear morphologies and fluorescence intensities. The muscle cell area was quantified using Fiji software. The percentage of myoblast cells was calculated by the number of myoblasts divided by the total number of muscle cells (myoblasts + nuclei within myotubes). All data was quantified from 50 randomly chosen microscopic fields collected from three independent experiments.

Statistical analysis

Statistical analysis of data related to the quantification of cell numbers was performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test; and the paired Student *t* test was used for the quantification of the percentage of Pax7-positive cells; and one-way ANOVA followed by Tukey's post-test for the quantification of the percentage of the area occupied by α-actinin in muscle cells (GraphPad Software, CA, USA). Statistical significance was defined as $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Results and discussion

ISO induces an increase in muscle fiber size acompanied by an increase in protein concentration

First, we analyzed the effects of ISO (10, 50, and 100 nM) in cell viability during chick in vitro myogenic differentiation. DMSO (0.1%) or ISO-treated cells did not exhibit loss of viability in all the concentrations tested using an MTT assay (Figure 1). To investigate the impact of ISO during skeletal muscle differentiation, cells were treated with ISO (50 and 100 nM) for 48 h and live cells analyzed using phase contrast microscopy (Figure 2A–D). Treatment of muscle cells with ISO for 48 h induced the formation of multinucleated myotubes with increased size compared with untreated and DMSO-treated cells (Figure 2A–D). Cells treated with ISO 100 nM for 48 h seem to have myotubes with increased width compared to those with ISO 50 nM (Figure 2A–D). The increase in the size of myotubes was not observed in cultures treated with ISO for 24 h (data not shown). We also analyzed whether the increase in myotube size was accompanied by an increase in protein synthesis. Protein concentration was determined in samples from 72-h control (CTRL) (untreated) cells and cells treated with DMSO or ISO 100 nM and showed an 20% increase in protein amount in ISO compared with untreated cells (5.0 ± 1.0 μg/μL in CTRL, 4.5 ± 0.5 μg/μL in DMSO and 6.0 ± 0.9 μg/μL in ISO 100 nM; mean ± standard deviation; $n = 3$).

ISO induces myoblast proliferation and muscle hypertrophy

Next, we decided to explore the effects of ISO in the different cell phenotypes (fibroblasts, myoblasts, and myotubes) that are present in embryonic chick muscle cultures. Twenty-four-hour Chick myogenic cells were treated with ISO (50 and 100 nM) for 48 h, labeled with an anti-desmin antibody (used as a muscle-specific cell marker) and the nuclear dye DAPI, and analyzed under a fluorescence microscope (Figure 3). Images were quantified in relation to: area of muscle cells (myoblasts and myotubes), number of myoblasts, number of fibroblasts, number of nuclei within myotubes, and percentage of myoblast cells (number of myoblasts divided by the number of nuclei in muscle cells); all in comparison with untreated cells (Figure 4). ISO treatment induced an

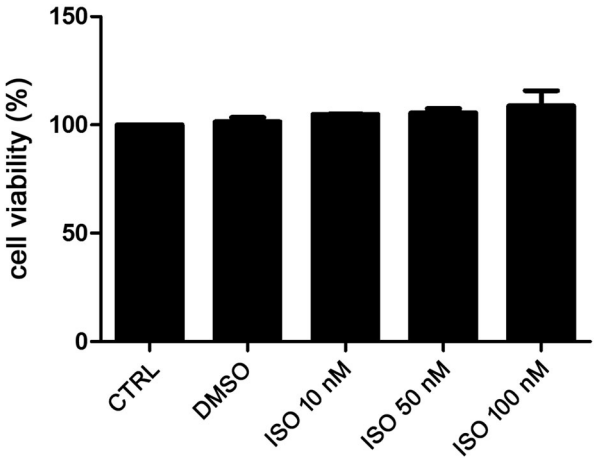


Figure 1 Isoproterenol does not affect the cell viability in chick myogenic cell cultures. Myogenic cells were incubated with different concentrations (10, 50, and 100 nM) of isoproterenol (ISO) for 48 h. Control cells (CTRL) were left untreated. Cell viability was analysed using a MTT (3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide)-based assay. Results are represented as mean ± standard deviation ($n = 3$) of independent experiments.

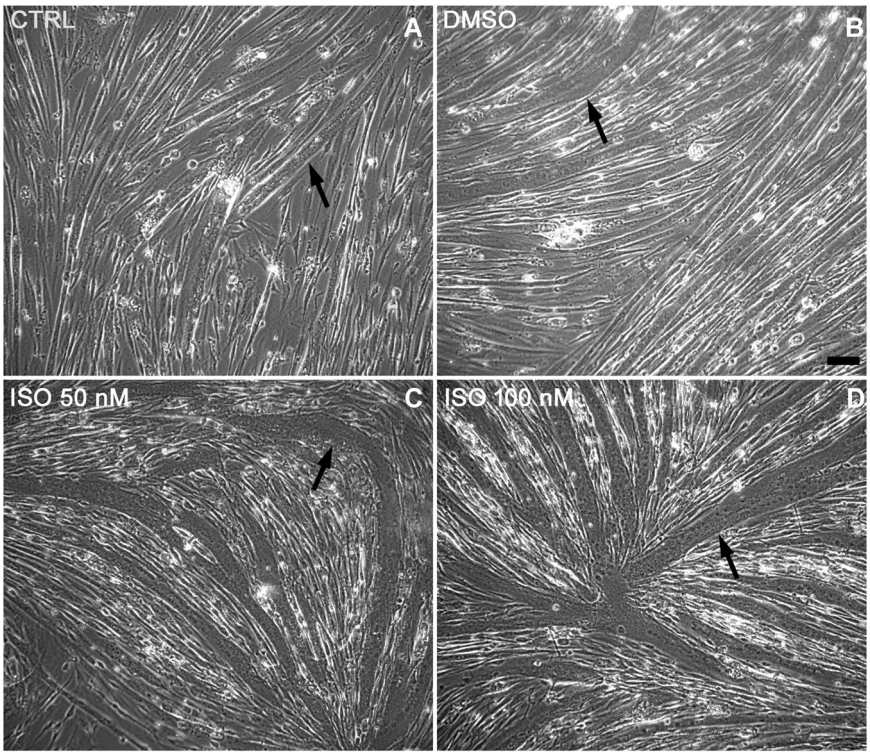


Figure 2 **Isoproterenol induces the formation of myotubes with increased size in chick myogenic cell cultures**. Seventy-two-hour myogenic cells were visualized under phase contrast microscopy (A–D). Arrows point to multinucleated myotubes in each condition (A–D). Note the increase in size of myotubes after treatment with 100 nM isoproterenol (ISO) (D). Scale bar in (B) represents 50 µm.

increase in the area of muscle cells (45% compared with CTRL), in the number of myoblasts (150% compared with CTRL), in the number of nuclei within myotubes (50% compared with CTRL) and in the percentage of myoblast cells (Figure 4A–E). The collection of these results suggest that ISO induces the proliferation of myoblasts and increase in the size of myotubes (Figures 2–4), suggesting the activation of a hypertrophic muscle phenotype. Some

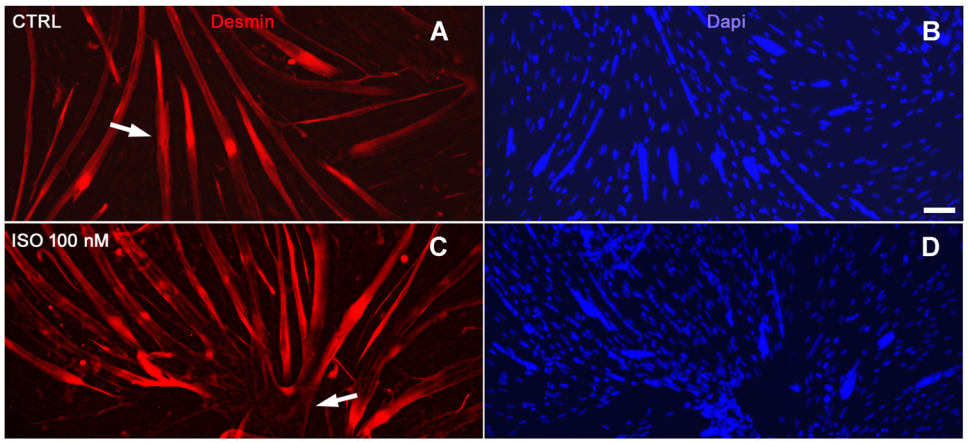


Figure 3 **Isoproterenol induces muscle hypertrophy**. Myogenic cells were grown for 24 h and treated with isoproterenol (ISO) 100 nM for the next 48 h (C–D). Control cells (CTRL) were left untreated (A–B). Seventy-two-hour cells were labeled with an anti-desmin antibody (red; A and C) and the nuclear dye 4,6-diamino-2-phenylindole dyhydrochloride (DAPI) (blue; B and D). Arrows point to multinucleated myotubes in each condition (A and C). Note that ISO 100 nM induces an increase in the size of multinucleated myotubes (C). Scale bar in (B) represents 50 µm.

Cell Biol Int **43** (2019) 1425–1434 © 2019 The Authors. *Cell Biology International* published by John Wiley & Sons Ltd on behalf of International Federation of Cell Biology

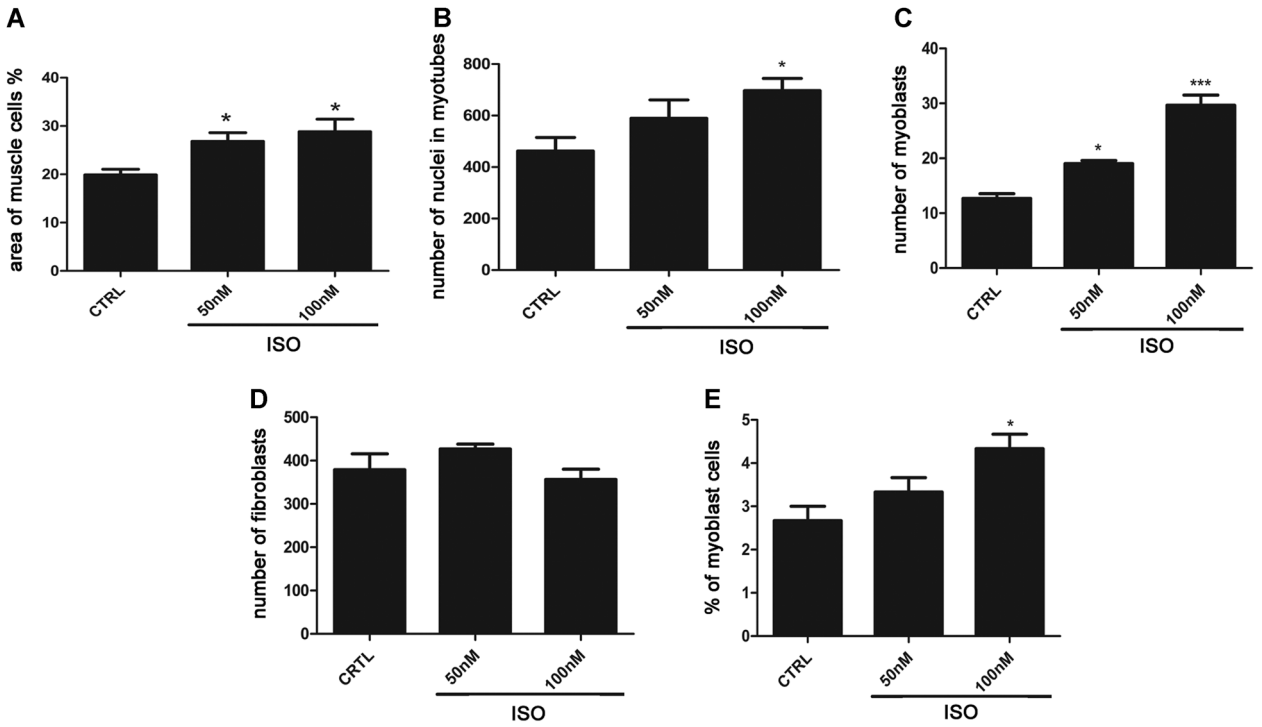


Figure 4 Isoproterenol induces an increase in the number of myoblasts and in the area of muscle cells. Myogenic cells were grown for 24 h and treated with isoproterenol (ISO) (50 or 100 nM) for the next 48 h. Control cells (CTRL) were left untreated. Seventy-two-hour cells were labeled with an anti-desmin antibody and the nuclear dye 4,6-diamino-2-phenylindole dyhydrochloride (DAPI). Images were quantified in relation to: the area of muscle cells (myoblasts and myotubes), number of myoblasts, number of fibroblasts, number of nuclei within myotubes, and percentage of myoblast cells (number of myoblasts divided by the number of nuclei in muscle cells). $*P < 0.05$, $***P < 0.001$; One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test; $n = 3$. At least 50 microscopic fields for each culture condition were scored in at least three independent experiments.

previous studies have shown that ISO and other β-AR agonists have a positive effect in protein accretion in chick muscle (Ji and Orcutt, 1991; Kumar et al., 2003), and in rat and mouse myogenic cell cultures (Harper et al., 1990). Also, our results are in accordance with the data reported by other researchers which showed that feeding of avians and mammalian livestock with β-AR agonists enhances muscle mass (Mersmann, 1998). In ruminants, the average increase of muscle cross-sectional area is around 11% and 39% (Johnson et al., 2014).

No significant differences were found in the number of muscle fibroblasts after exposure of cells to ISO (Figure 4D). Contrasting with our data, it has been shown that ISO induces an increase in rat and human cardiac fibroblast proliferation (Leicht et al., 2000; Turner et al., 2003), which was reported to be dependent on the presence of fetal calf serum (FCS). Interestingly, the authors describe that ISO did not induce cardiac fibroblast proliferation in serum-free medium (Leicht et al., 2000; Turner et al., 2003), similar to what was found for human Tenon's capsule fibroblasts (Cunliffe et al., 1995). We can hypothesize that the differences in proliferation response to ISO between cardiac and skeletal muscle fibroblasts could be due to the fact that primary cultures of chick skeletal muscle cells are grown in the presence of horse serum and without FCS. Indeed, it has been suggested that ISO acts synergistically with other serum-derived factors present in FCS to initiate a mitogenic response in cardiac fibroblasts (Leicht et al., 2000; Turner et al., 2003). Besides this, the fibroblast proliferative phenotype and response to stimuli have been shown to depend on the muscle type and, possibly, animal species (Archile-Contreras et al., 2010; Purslow et al., 2012). Further studies are necessary to unravel these questions.

ISO induces an increase in the number of Pax7-positive cells

Parent et al. (1980) have shown that β-AR appear during the myoblast fusion stage of myogenesis in primary cultures of skeletal muscle cells obtained from 10-day-old quail embryos. They also reported that quail muscle cells

become responsive to ISO following fusion; myotube membrane adenylate cyclase, but not myoblast membrane adenylate cyclase, was stimulated twofold to threefold by ISO (Parent et al., 1980). Our results are somehow in contrast with these previous data since we show that 24-h chick myogenic cultures respond to ISO by inducing the proliferation of myoblasts. Primary cultures of chick muscle cells grown for 24-h display different cell phenotypes: fibroblasts, myoblasts, and young myotubes; and more than one population of myoblasts can be found in these cultures, such as quiescent, proliferative, and post-mitotic myoblasts. To better understand which myoblast population is responding to ISO, we decided to analyze the effects of ISO in the percentage of Pax7-positive cells (Figure 5). It has been shown that Pax7 is expressed in both quiescent and proliferating myoblasts, but not in differentiated myoblasts (Zammit et al., 2006). Chick muscle cultures treated with ISO 100 nM showed a threefold increase in the number of Pax7-positive cells (Figure 5I). Interestingly, all Pax7-positive cells were found adjacent to the sarcolemma of myotube cells, suggesting that these Pax7-positive cells are in the process of cell fusion (Figure 5E-H). These results are in accordance with the increase in the number of nuclei within myotubes and in myoblast fusion and indicate a hyperplasic response evoked by ISO (Figure 4).

Rapamycin cannot inhibit ISO-induced effects on muscle fiber size

We also decided to test whether the ISO-induced effects on muscle fiber size were mediated by the mammalian target of rapamycin (mTOR) signaling pathway. mTOR is an evolutionarily conserved serine/threonine kinase which plays a vital role in the control of skeletal muscle mass (Yoon, 2017). Here, we used RAPA, a highly specific inhibitor of mTOR signaling, to test the involvement of the mTOR signaling in chick muscle cell cultures. Twenty-four-hour myogenic cells were treated with ISO 100 nM, or RAPA 3 μM, or with ISO and RAPA concomitantly. Immunofluorescence against sarcomeric-α-actinin together with the nuclear labeling showed that RAPA alone induced a decrease in myotube size, whereas ISO alone induced an increase in myotube size (Figure 6). Interestingly, when both reagents (ISO and RAPA) were added together, we could observe a similar size of myotubes as compared to ISO alone (Figure 6). These results show that RAPA did not inhibit the increase in myotube size induced by ISO (Figure 6I). The reduction in myotube size induced by RAPA alone is in accordance with previous data from different groups and can be explained by the inhibition of the mTOR pathway (Cuenda and Cohen, 1999). Our results strongly suggest that the ISO-induced effects on chick muscle fiber size are not mediated by the hypertrophic related-mTOR pathway.

ISO can rescue the Wnt5a-induced effects on muscle fiber size

Finally, we decided to test whether the Wnt5a-mediated signaling pathway could be involved in the increase in myofiber size induced by ISO. Wnt5a is a noncanonical Wnt ligand that is evolutionarily conserved and plays an important role in the early phase of muscle regeneration

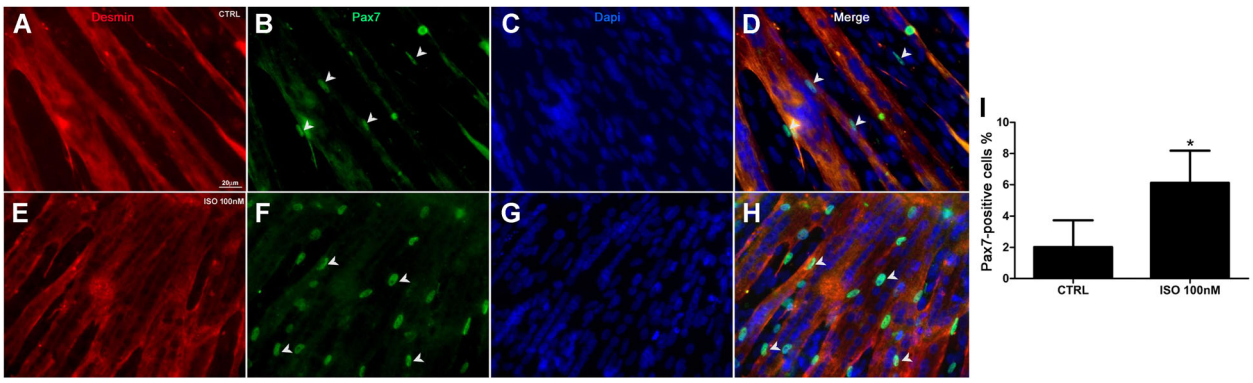
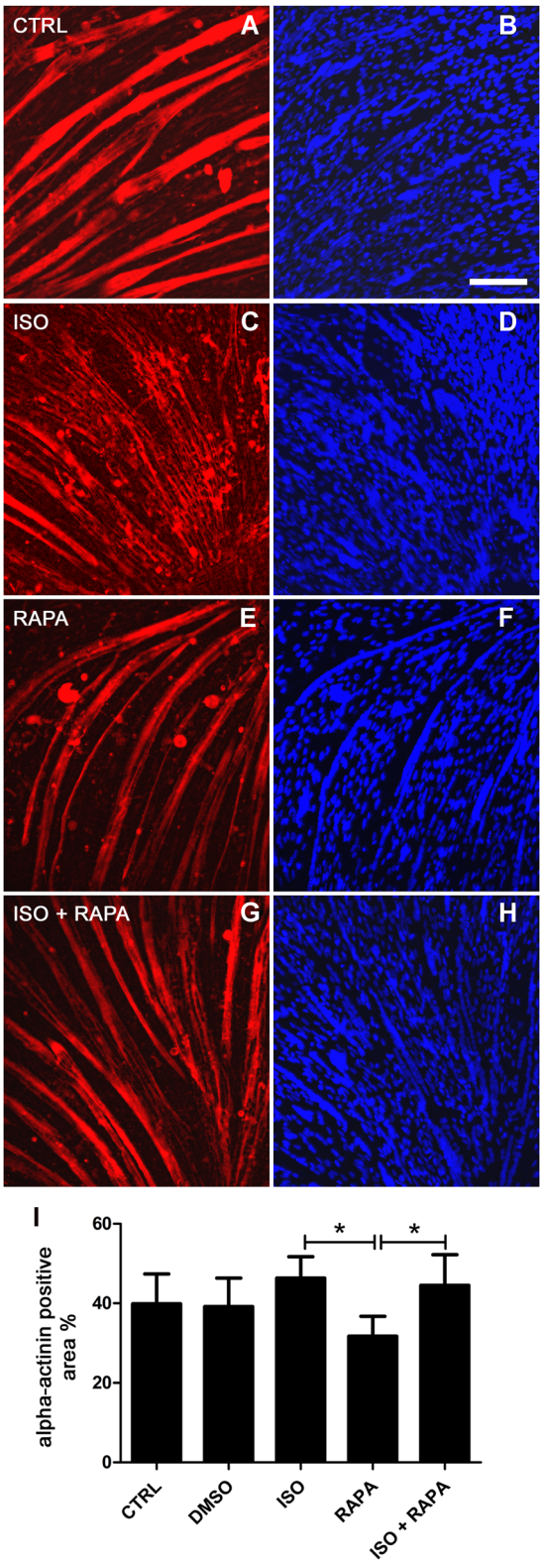


Figure 5 Isoproterenol induces the proliferation of Pax7-positive cells. Myogenic cells were grown for 24 h and treated with isoproterenol (ISO) 100 nM for the next 48 h (A–H). Control cells (CTRL) were left untreated (A–D). Seventy-two-hour cells were labeled with an anti-desmin antibody (red; A and E), an anti-Pax7 antibody (green, B and F) and the nuclear dye 4,6-diamino-2-phenylindole dyhydrochloride (DAPI) (blue; C and G). Merged images are shown in D and H. Arrowheads point to Pax7-positive cells in each condition (B, D, F, and H). Note that ISO 100 nM induces an increase in the number of Pax7-positive cells (F and H). Scale bar in A represents 20 μm. The percentage of Pax7-positive cells was quantified in CTRL and in cells treated with ISO 100 nM (I). $*P < 0.05$, paired Student *t* test; $n = 3$. At least 50 microscopic fields for each culture condition were scored in at least three independent experiments.



(Maltzahn et al., 2012). Previous data from our group showed that Wnt5a inhibits the formation of chick muscle fibers (Portilho et al., 2007), and therefore we hypothesized that Wnt5a could inhibit the effects of ISO in relation to the size of muscle fibers. Twenty-four-hour myogenic cells were treated with ISO 100 nM, or Wnt5a-conditioned medium (10% v/v), or with ISO and Wnt5a concomitantly and labeled for desmin. Our results show that Wnt5a alone induced an evident decrease in myotube size (Figure 7D–F), which is in accordance with previous data from our group (Portilho et al., 2007). Interestingly, ISO and Wnt5a added together induced an increase in the number and size of muscle fibers (Figure 7J–L), showing that Wnt5a did not inhibit the effects of ISO in myotube size. These results lead us to conclude that Wnt5a signaling pathway is not involved in the hypertrophic events induced by ISO in chick skeletal muscle cells.

In conclusion, here we show that the β-adrenergic receptor agonist ISO induces an increase in myoblast proliferation, in the percentage of Pax7-positive myoblasts and in the size of skeletal muscle fibers. Interestingly, treatment with ISO did not alter the number of fibroblast cells, suggesting that ISO effects are specific to muscle cells in the case of chick myogenic cell culture. We also showed that the ISO-induced effects on chick muscle fiber size are not mediated by the mTOR or the Wnt5a signaling pathways.

Finally, in relation to protein concentration, we found that its increase after ISO treatment was lower than the increase in the number of nuclei in the myotubes (20% and 50%, respectively), a fact supporting a hyperplasic process. Although hypertrophy and hyperplasia are two distinct processes, they frequently occur together. Thus, we suggest that ISO induces first the proliferation of Pax7-positive cells and subsequently some of these cells will fuse into multinucleated muscle fibers with increased size and increased protein synthesis. The collection of these results provides new insights into the role of β-adrenergic signaling during skeletal muscle proliferation and differentiation.

Figure 6 Rapamycin does not inhibit the effects of isoproterenol. Myogenic cells were grown for 24 h and treated with isoproterenol (ISO) 100 nM, or rapamycin 3 μM (RAPA), or with ISO and RAPA concomitantly for the next 48 h (A–H). Control cells were left untreated (A–B). Seventy-two-hour cells were labeled with an anti-sarcomeric-alpha-actinin monoclonal antibody (red; A, C, E and G) and the nuclear dye 4,6-diamino-2-phenylindole dyhydrochloride (DAPI) (blue; B, D, F and H). Note the decrease in the size of myotubes when cells were treated with RAPA (E and F). Scale bar in B represents 100 μm. $^*P < 0.05$, One-way analysis of variance (ANOVA) followed by Tukey's post-test, $n = 3$. At least 50 microscopic fields for each culture condition were scored in at least three independent experiments.

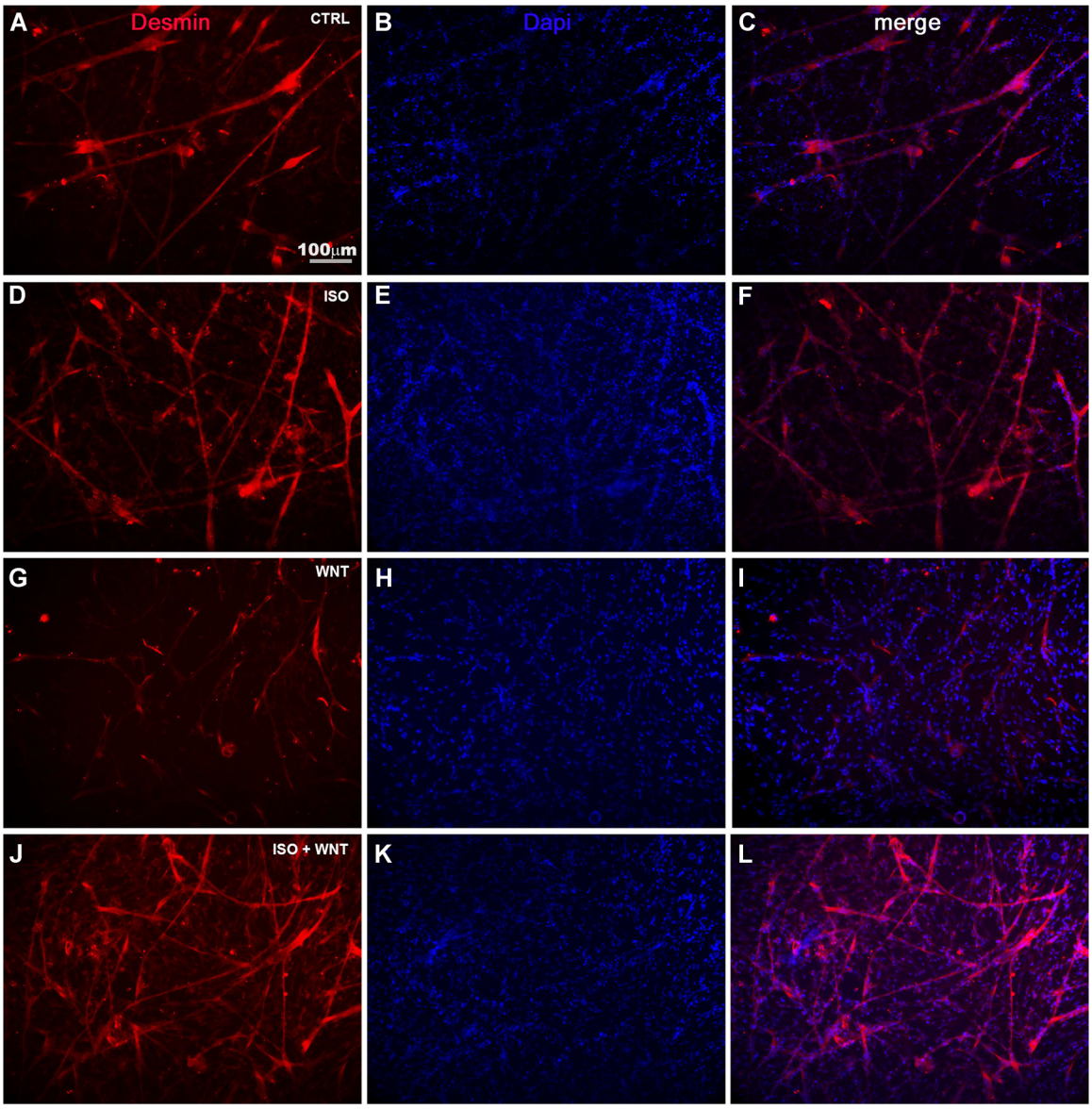


Figure 7 **Wnt5a does not inhibit the effects of isoproterenol**. Myogenic cells were grown for 24 h and treated with isoproterenol (ISO) 100 nM, or Wnt5a-conditioned medium, or with ISO and Wnt5a concomitantly for the next 24 h (A–L). Control cells were left untreated (A–C). Forty-eight-hour cells were labeled with an anti-desmin antibody (red; A, C, D, F, G, I, J, and L) and the nuclear dye DAPI (blue; B, C, E, F, H, I, K and L). Note the decrease in the size of myotubes when cells were treated with Wnt5a (G–I). Scale bar in (A) represents 100 μm.

Acknowledgments and funding

This work was supported by Brazilian grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)—grant number 302115/2017-0 for Claudia Mermelstein; and the Fundação Carlos Chagas Filho de Apoio à Pesquisa do Estado do Rio de Janeiro (FAPERJ)—grant number 203.185/2015 for Claudia Mermelstein.

Author contribution

I.R.A., G.G., J.D.T., and U.B. carried out the cell culture, immunofluorescence, and quantification of cells. M.L.C. revised the manuscript critically. C.M. and L.E.Q. conceived the study, participated in the analysis of data, and coordination of the study. All authors read and approved the final manuscript.

Data availability statement

All data generated or analysed during this study are included in this published article.

Conflict of interest

The authors declare that there are no conflicts of interests.

References

Archile-Contreras AC, Mandell IB, Purslow PP (2010) Phenotypic differences in matrix metalloproteinase 2 activity between fibroblasts from 3 bovine muscles. J Anim Sci 88: 4006–15.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–54.

Choi J, Costa ML, Mermelstein CS, Chagas C, Holtzer S, Holtzer H (1990) MyoD converts primary dermal fibroblasts, chondroblasts, smooth muscle, and retinal pigmented epithelial cells into striated mononucleated myoblasts and multinucleated myotubes. Proc Natl Acad Sci USA 87: 7988–92.

Cuenda A, Cohen P (1999) Stress-activated protein kinase-2/p38 and a rapamycin-sensitive pathway are required for C_2C_{12} myogenesis. J Biol Chem 274: 4341–6.

Cunliffe IA, McIntyre CA, Rees RC, Rennie IG (1995) Pilot study on the effect of topical adrenergic medications on human Tenon's capsule fibroblasts in tissue culture. Br J Ophthalmol 79: 70–5.

Curtis DH, Zalin RJ (1981) Regulation of muscle differentiation: stimulation of myoblast fusion in vitro by catecholamines. Science 214: 1355–7.

Curtis DH, Zalin RJ (1985) The differentiation of avian skeletal muscle in culture: changes in responsiveness of adenylyl cyclase to prostaglandin E1 and adrenergic agonists. J Cell Physiol 123: 219–27.

Guerra FS, Sampaio LS, Konig S, Bonamino M, Doria Rossi MI, Costa ML, Fernandes P, Mermelstein C (2016) Membrane cholesterol depletion reduces breast tumor cell migration by a mechanism that involves non-canonical Wnt signaling and IL-10 secretion. Transl Med Commun 1: 3.

Harper JM, Mackinson I, Buttery PJ (1990) The effects of β agonists on muscle cells in culture. Domest Anim Endocrinol 7: 477–84.

Ji SQ, Orcutt MW (1991) Effects of the β-adrenergic agonist isoproterenol on protein accretion, synthesis, and degradation in primary chicken muscle cell cultures. J Anim Sci 69: 2855–64.

Johnson BJ, Smith SB, Chung KY (2014) Historical overview of the effect of β-adrenergic agonists on beef cattle production. J Anim Sci 27: 757–66.

Jones TR, Kang IH, Wheeler DB, Lindquist RA, Papallo A, Sabatini DM, Golland P, Carpenter AE (2008) CellProfiler Analyst: data exploration and analysis software for complex image-based screens. BMC Bioinformatics 9: 482.

Kumar S, Sharma S, Katoch SS (2003) Early onset of the maximum protein anabolic effect induced by isoproterenol in chick skeletal and cardiac muscle. Acta Physiol Hung 90: 57–67.

Leicht M, Greipel N, Zimmer H (2000) Comitogenic effect of catecholamines on rat cardiac fibroblasts in culture. Cardiovasc Res 48: 274–84.

Lynch SG, Ryall JG (2008) Role of beta-adrenoceptor signaling in skeletal muscle: implications for muscle wasting and disease. Physiol Rev 88: 729–67.

Maltzahn JV, Chang NC, Bentzinger CF, Rudnicki MA (2012) Wnt signaling in myogenesis. Trends Cell Biol 22: 602–9.

Mermelstein CS, Portilho DM, Medeiros RB, Matos AR, Einicker-Lamas M, Tortelote GG, Vieyra A, Costa ML (2005) Cholesterol depletion by methyl-β-cyclodextrin enhances myoblast fusion and induces the formation of myotubes with disorganized nuclei. Cell Tissue Res 319: 289–97.

Mersmann HJ (1998) Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. J Anim Sci 76: 160–72.

Ojima K, Lin ZX, Andrade IR, Costa ML, Mermelstein C (2016) Distinctive effects of cytochalasin B in chick primary myoblasts and fibroblasts. PLOS One 11: e0154109.

Parent JB, Tallman JF, Henneberry RC, Fishman PH (1980) Appearance of beta-adrenergic receptors and catecholamine-responsive adenylate cyclase activity during fusion of avian embryonic muscle cells. J Biol Chem 255: 7782–6.

Portilho DM, Martins ER, Costa ML, Mermelstein CS (2007) A soluble and active form of Wnt-3a protein is involved in myogenic differentiation after cholesterol depletion. FEBS Lett 581: 5787–95.

Possidonio AC, Soares CP, Portilho DM, Victor Midlej V, Benchimol M, Butler-Browne G, Costa ML, Mermelstein C (2014) Differences in the expression and distribution of flotillin-2 in chick, mice and human muscle cells. PLOS One 9: e103990.

Purslow PP, Archile-Contreras AC, Cha MC (2012) Meat Science and Muscle Biology Symposium: manipulating meat tenderness by increasing the turnover of intramuscular connective tissue. J Anim Sci 90: 950–9.

Rosa de Andrade I, Corrêa S, Fontenele M, de Oliveira Teixeira JD, Abdelhay E, Costa ML, Mermelstein C (2018) γ-Secretase inhibition induces muscle hypertrophy in a notch-independent mechanism. Proteomics 18: 1700423.

Ryall JG, Church JE, Lynch GS (2010) Novel role for β-adrenergic signalling in skeletal muscle growth, development and regeneration. Clin Exp Pharmacol Physiol 37: 397–401.

Turner NA, Porter KE, Smith WH, White HL, Ball SG, Balmforth AJ (2003) Chronic beta2-adrenergic receptor stimulation increases proliferation of human cardiac fibroblasts via an autocrine mechanism. Cardiovasc Res 57: 784–92.

Yang YT, McElligott MA (1989) Multiple actions of β-adrenergic agonists on skeletal muscle and adipose tissue. Biochem J 261: 1–10.

Yoon MS (2017) mTOR as a key regulator in maintaining skeletal muscle mass. Front Physiol 8: 788–97.

Zammit PS, Relaix F, Nagata Y, Ruiz AP, Collins CA, Partridge TA, Beauchamp JR (2006) Pax7 and myogenic progression in skeletal muscle satellite cells. J Cell Sci 119: 1824–32.

Received 16 April 2019; accepted 2 June 2019.
Final version published online 20 June 2019.

Cell Biol Int **43** (2019) 1425–1434 © 2019 The Authors. *Cell Biology International* published by John Wiley & Sons Ltd on behalf of International Federation of Cell Biology