Comparative Biochemistry and Physiology, Part B 199 (2016) 67–73

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

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

Contribution of *in vitro* myocytes studies to understanding fish muscle physiology☆

Emilio J. Vélez, Esmail Lutfi, Sheida Azizi, Núria Montserrat, Miquel Riera-Codina, Encarnación Capilla, Isabel Navarro, Joaquim Gutiérrez *

Departament de Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona, 08028, Barcelona, Spain

ARTICLE INFO

Article history:
Received 30 July 2015
Received in revised form 4 December 2015
Accepted 6 December 2015
Available online 10 December 2015

Keywords:
In vitro cultures
Insulin
IGFs
Insulin and IGF-I receptors
Myogenesis
Myogenic factors
TOR
Amino acids

ABSTRACT

Research on the regulation of fish muscle physiology and growth was addressed originally by classical *in vivo* approaches; however, systemic interactions resulted in many questions that could be better considered through *in vitro* myocyte studies. The first paper published by our group in this field was with Tom Moon on brown trout cardiomyocytes, where the insulin and IGF-I receptors were characterized and the down-regulatory effects of an excess of peptides demonstrated. We followed the research on cultured skeletal muscle cells through the collaboration with INRA focused on the characterization of IGF-I receptors and its signaling pathways through *in vitro* development. Later on, we showed the important metabolic role of IGFs, although these studies were only the first stage of a prolific area of work that has offered a useful tool to advance in our knowledge of the endocrine and nutritional regulation of fish growth and metabolism. Obviously, the findings obtained *in vitro* serve the purpose to propose the scenario that will need confirmation *in vivo*, but this technique has made possible many different, easy, fast and better controlled studies. In this review, we have summarized the main advances that the use of cultured muscle cells has permitted, focusing mainly in the role of IGFs regulating fish metabolism and growth. Although many articles have already appeared using this model system in salmonids, gilthead sea bream or zebrafish, it is reasonable to expect new studies with cultured cells using innovative approaches that will help to understand fish physiology and its regulation.

1. Introduction

During the last decade, the use of muscle cells for *in vitro* studies in fish has increased importantly, with the contribution of different laboratories around the world. After the first publication in common carp by Koumans et al. (1990), many other groups have developed the technique in different species: rainbow trout (Rescan et al., 1995; Seiliez et al., 2008; Gabillard et al., 2010; Garikipati and Rodgers, 2012), Atlantic salmon (Bower and Johnston, 2009), giant danio (Froehlich et al., 2013) and zebrafish (Froehlich et al., 2014). The primary culture of fish cardiomyocytes has been also utilized as a useful technique to characterize heart physiology, and Nurmi and Vornanen (2002) explored the electrophysiological characteristics of rainbow trout cardiac myocytes, and later, Goldstein et al. (2004) studied their metabolic viability. Brette et al. (2008) proposed the ventricular myocytes in zebrafish as an ideal model for the investigation of mutated ion channels, and more recently, Sander et al. (2013) also used zebrafish cardiomyocytes to study heart regeneration.

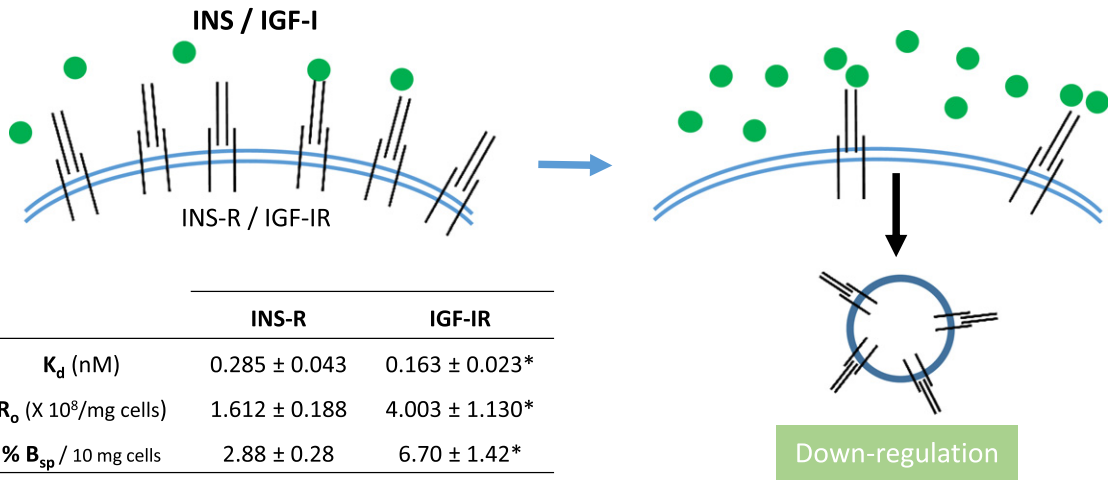
Our group has participated in the development of the field working first with fish myocytes, later with adipocytes, and more recently with osteoblasts. (Castillo et al., 2002; Montserrat et al., 2007; Bouraoui et al., 2008; Salmerón et al., 2013a; Capilla et al., 2011). However, the first paper published by our group in fish myocytes was an article with Tom Moon (Moon et al., 1996) on cardiomyocytes that was started in Seattle in collaboration with E.M. Plisetskaya in the summer of 1995 and later continued in Barcelona during the sabbatical of Tom at the University of Barcelona. In this study, both insulin and insulin-like growth factor I (IGF-I) receptors in brown trout cardiomyocytes were characterized and the capacity of down-regulation by both ligands was demonstrated (Fig. 1). This finding was later confirmed *in vivo* in the red muscle of the same species (Baños et al., 1997), and these studies were the first stage to open the line of research in cultured myocytes from skeletal muscle in our laboratory.

We continued the research on skeletal myocytes through the collaboration with INRA (B. Fauconneau, P.Y. Le Bail and P.Y. Rescan, in Scribe, Rennes). They already had developed the technique to isolate satellite muscle cells from rainbow trout (Rescan et al., 1995; Faucanneau and Paboeuf, 2000), and we adapted it to investigate insulin and IGF-I binding (Castillo et al., 2002). This first paper was focused on the characterization of IGF-I receptors (total number, binding and affinity) through myocytes development in culture,

☆ Contribution to a special issue celebrating the work of Dr. Thomas W. Moon on the occasion of his retirement after 45 years in comparative biochemistry and physiology.

* Corresponding author at: Departament de Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, 08028, Barcelona, Spain. Tel.: +34 934021532; fax: +34 934110358.
E-mail address: jgutierrez@ub.edu (J. Gutiérrez).

http://dx.doi.org/10.1016/j.cbpb.2015.12.003
1096-4959/



	INS-R	IGF-IR
K_d (nM)	0.285 ± 0.043	0.163 ± 0.023*
R_o (X 10^8/mg cells)	1.612 ± 0.188	4.003 ± 1.130*
% B_{sp} / 10 mg cells	2.88 ± 0.28	6.70 ± 1.42*

Fig. 1. Insulin and IGF-I receptors characterization in brown trout isolated cardiomyocytes. Schematic representation of receptor binding down-regulation by excess of peptide. INS: insulin; INS-R: insulin receptor; IGF-IR: IGF-I receptor; K_d: dissociation constant of high affinity binding sites; R_o: number of binding sites; % B_{sp}: percentage of specific binding. The asterisks indicate significant differences between insulin and IGF-I receptor characteristics ($P < 0.05$).

and it was the basis for the majority of the papers published later by our group and other laboratories.

The aim of this review was to summarize the main advances that the use of muscle cells cultured *in vitro* has permitted, focusing mainly in the role of IGFs regulating metabolism and development and pointing out some applications of the technique used in understanding fish physiology and growth.

2. Metabolic functions of IGFs

Once the IGF-I receptors were characterized in rainbow trout myocytes through their development in culture (Castillo et al., 2002), the next study on myocytes (Castillo et al., 2004) had the objective to investigate the significance of the abundant number of IGF-I receptors and the low number of insulin receptors in fish muscles, especially when this ratio was compared with other homoeothermic vertebrates (Párrizas et al., 1995a). In previous studies, we had established in a variety of fish species that the number of insulin receptors was significantly lower than that of the IGF-I receptors (Párrizas et al., 1995b). However, the physiological meaning of such abundant number was unknown, and the purpose then was to investigate whether IGF-I could assume some of the functions that in mammals are performed by insulin receptors. Such an objective required to set up the main techniques on metabolites uptake (e.g., glucose, amino acids (AA), thymidine and fatty acids) in cultured myocytes.

Results were very interesting and helped us to understand the role that IGF-I was playing in fish in comparison to mammals (Fig. 2). In fact, IGF-I was stronger than insulin stimulating glucose, AA and thymidine uptake in rainbow trout myocytes (Castillo et al., 2004) and also in gilthead sea bream myocytes (Montserrat et al., 2012). Both studies confirmed the specificity of the effects by means of inhibitors of glucose uptake (e.g., worthmanin, PD-98059 and cytochalasin B) that significantly reduced the entrance of glucose in myocytes. Furthermore, studies by Díaz et al. (2007, 2009) demonstrated the involvement of the facilitative glucose transporter (GLUT4) in the uptake of glucose stimulated by insulin and IGF-I in brown trout cultured myocytes, and Montserrat et al. (2012) also observed the increase of GLUT4 in response to IGFs treatment in gilthead sea bream myotubes. Our findings coincide with results in muscle cells in mammals (Ciaraldi et al., 2001) and chicken (Duclos et al., 1993). In fish, Drakenberg et al. (1997) and Degger et al. (2000) found that *in vivo* IGF-I treatment increased glucose incorporation into fish muscle glycogen. More recently using a different cellular model, Bou et al. (2014) demonstrated that in rainbow trout adipocytes IGF-I increases glucose uptake similarly than insulin.

Regarding AA, there is literature demonstrating that IGF-I stimulates their uptake in fish muscle *in vitro* and *in vivo* (Inui and Ishioka, 1983; Negatu and Meier, 1995; Degger et al., 2000). Gallardo et al. (2001) showed also the stimulatory effects of IGF-I in alanine uptake and protein synthesis in brown trout cardiomyocytes. Through culture development, higher responses of IGF-I in AA uptake were observed at early stages than once the myotubes are developed (Castillo et al., 2004). This coincides with results in mammals (Davis et al., 2002) and agrees with the fact that IGF-I can play a more important role when cells are in a proliferating stage.

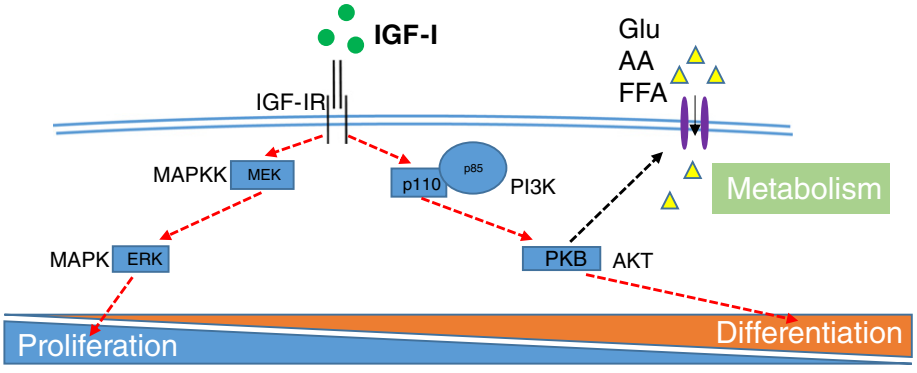


Fig. 2. IGF-I signaling through MAPK and AKT pathways during myocytes development regulating mainly proliferation and differentiation, respectively. IGF-I through AKT pathway also enhances nutrients uptake to control metabolism. Glu: glucose; AA: amino acids; FFA: free fatty acids.