INTERPLAY BETWEEN ANDROGENIC/ANABOLIC STEROID AND IGF-1 SIGNALING IN AMYOTROPHIC LATERAL SCLEROSIS

Maria Pennuto, Italy
Grant: € 125 000

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by the loss of upper and lower motor neurons and skeletal muscle atrophy. We have previously shown that muscle plays an important role in ALS pathogenesis and in other neuromuscular diseases, and that intervention in muscle to enhance insulin-like growth factor 1 (IGF-1) signaling attenuates muscle atrophy in such diseases. The observation that there is a higher risk to develop ALS in males and in varsity athletes together with our finding that myocites derived from sporadic ALS (sALS) patients express abnormal levels of androgen receptor lead us to hypothesize that dysregulation of androgen signaling contributes to muscle atrophy in ALS by a mechanism that involves alteration of mIGF-1 signaling. To test our hypothesis, we will pursue the following three Specific Aims:

Specific Aim 1: To determine the effect of androgens on myocites derived from sALS patients. Our working hypothesis, based on our preliminary data, is that androgens enhance toxicity in sALS. We will test this in myocites derived from sALS patients, and we will measure cell death and cell toxicity. We anticipate that we will determine the effect of androgen stimulation on sALS myocite viability.

Specific Aim 2: To characterize the effect of androgens on ALS pathogenesis. Our working hypothesis is that androgens exacerbate muscle atrophy in ALS via a mechanism that involves androgen receptor. We will test this in a mouse model of ALS expressing mutant SOD1.

Specific Aim 3: To assess the impact of androgen dysregulation on mIGF-1 signaling in ALS muscle. We hypothesize that androgens alter the IGF-1 signaling thus promoting muscle atrophy via a mechanism that involved FOXO3a transcription factor. We will test this in SOD1 mutant mice and cells derived from sALS patients.

With the research described in this application we will shed light onto the role of androgens in sALS. The main outcome here is expected to be the identification of androgens and androgen receptor as novel risk factors for sALS. Moreover, we will determine the role of FOXO3a in ALS pathogenesis in the context of muscle atrophy and androgen signaling. The research proposed in this application will open new therapeutic avenues for treatment based on the use of anti-androgens (FDA-approved) in ALS.

TEAM

This project will be undertaken by the joined effort of four independent research groups:

Coordinator: Maria Pennuto, PhD, Italian Institute of Technology, Italy
The group of Dr Pennuto is composed of the PhD student Chiara Scaramuzzino and the undergraduate fellow Tanya Aggarwal.

Gillian Butler-Browne, PhD, Since January 1st 2009, a mixed research unit UPMC-INSERM-CNRS-AIM has been created with the primary objective of developing innovative approaches to cell, gene or pharmacological therapies, based on understanding the molecular pathophysiology of a disease and therefore adapted to it. Dr. Gillian Butler-Browne is heading the “Remodeling, Regeneration and Cell Therapy of Striated Muscle team” within this research Unit. The laboratory is located at the Myology Institute, a reference centre for international expertise on diseases and ageing of skeletal muscle. The unit has access to all of the equipment necessary to carry out this project including cell-culture facilities designed for human cell culture, molecular biology and histology laboratories dedicated to research in neuromuscular diseases, viral vector production facilities, animal facilities, image analysis platforms, as well as state-of-the-art whole body imaging and physiological evaluation platforms. We have close collaborations with Dr Pierre-François Pradat and Prof Menninger who treat the ALS patients and Dr Vincent Mouly(research director), Dr Anne Bigot and DR Kamel Mamchaoui (research assistant) are directly involved in the project.

Antonio Musaro, PhD, University La Sapienza, Rome, Italy. The group of Dr. Musarò is composed by the post-docs Gabriella Dobrowolny and Michela Aucello and by the technician Carmine Nicoletti. The undergraduate and PhD students will be also partly involved in the project.

Angelo Poletti, PhD, University of Milan, Italy. The group of Dr. Poletti is composed by the Assistant Professor Rita Galbiati, by the post-Docs Paola Rusmini, Daniela Sau, Valeria Crippa and by the PhD student Elena Bolzoni. The undergraduate students will be also partly involved in the project.

ALS/androgen/IGF-1: Interplay between Androgenic/Anabolic Steroid and IGF-1 Signaling in Amyotrophic Lateral Sclerosis

Call for projects 2010
Grant: 125 000 €
Project Duration: 2 years

Investigators: Maria Pennuto, Italian Institute of Technology, Italy, Angelo Poletti, University of Milan, Italy, Antonio Musaro`, University La Sapienza, Rome, Italy, Gillian Butler-Browne, Inserm/UMR7215 CNRS, France

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Dysregulation of androgen signaling may contributes to the mecanism of ALS. To test our hypothesis, we treated mice expressing mutant SOD1 with the synthetic androgen nandrolone and reduced serum levels of testosterone by surgical castration. We obtained evidence that androgens modify SOD-related ALS phenotype in vivo. In detail, we found that castration leads to a significant decrease of body weight in the SOD1-G93A mice, indicating that endogenous testosterone contributes to body weight maintenance in this ALS mouse model. Nandrolone treatment increased body weight only in the castrated mice, but it had no effect on the mice producing endogenous testosterone. We also analyzed motor dysfunction using hanging wire and rotarod analyses. We found that castration ameliorates performance. Surprisingly, we found that also nandrolone improves motor function. These result support a protective role of endogenous testosterone in the pathogenesis of ALS. Moreover, these data highlight a protective role of nandrolone in ALS-related pathogenesis. To elucidate the mechanism through which androgens exert their effects on the mutant SOD1 mice, we analyzed the expression of heat shock proteins. We found that motor neurons expressing mutant SOD1 express high levels of the heat shock protein B8 (HspB8). We showed that HspB8 increases the clearance of mutant SOD1. Importantly, we have evidence that the expression of HspB8 is positively regulated by estrogens. In conclusion, our results support a role for androgens in ALS pathogenesis.

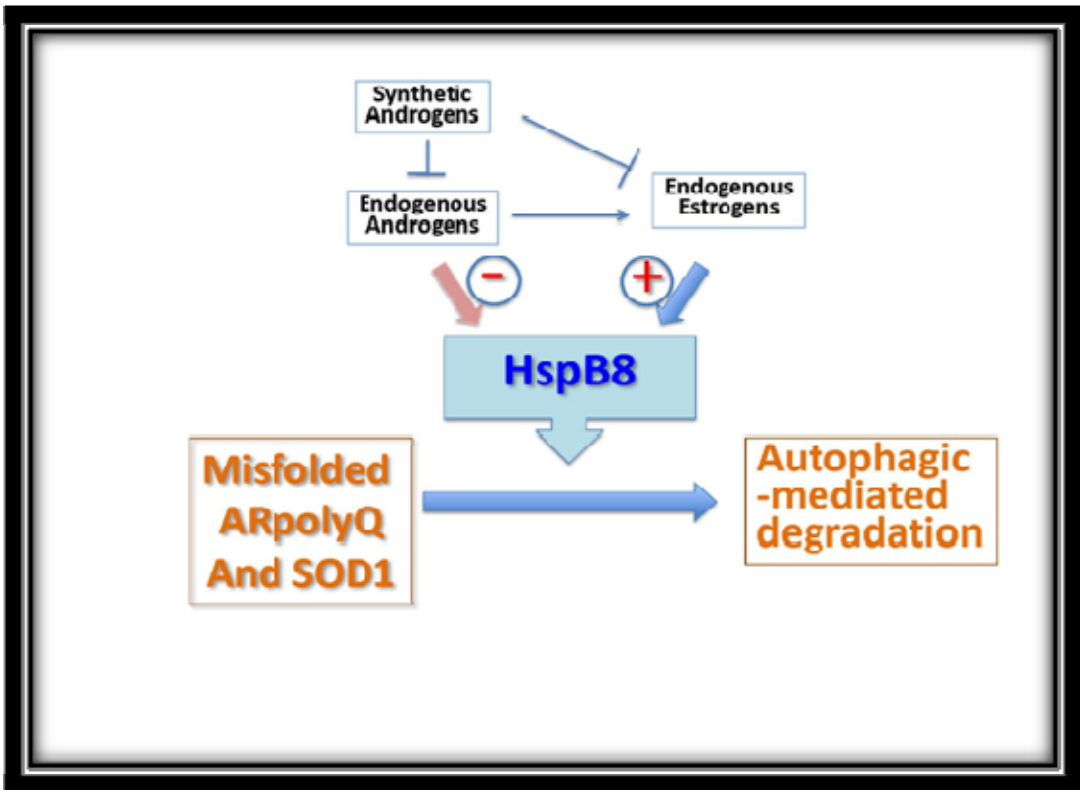

Figure 1. Effect of androgens on ALS pathogenesis.
We hypothesize that dysregulation of androgen signaling contributes to ALS pathogenesis. We hypothesize that androgens affect ALS pathogenesis via a mechanism that involves proteins prone to misfold, such as androgen receptor (AR), and proteins that regulate protein folding, such as the molecular chaperone HspB8. The synthetic steroid nandrolone inhibits secretion of endogenous androgens, which indirectly results in a reduction of endogenous estrogens, because androgens, but not nandrolone, can be converted to estrogens. Expression of HspB8, which is important for the clearance of misfolded protein, is regulated by androgens.