BIO101, a drug candidate targeting sarcopenic obesity through Mas receptor activation

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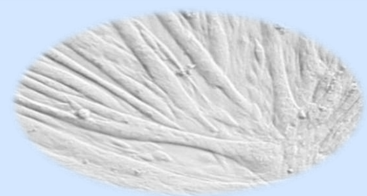
INTRODUCTION

The steroid hormone 20-Hydroxyecdysone (20E) plays a key role in insect development through nuclear ecdysone receptors (EcRs) and at least one membrane receptor (DopEcR). Although mammals lack EcR, 20E displays pharmacological effects on mammals: for example, it stimulates protein synthesis and is marketed as a physical performance enhancer. Based on 20E physiological effects on muscle mass, a 20E based-drug termed BIO101 has been designed for the treatment/prevention of muscle pathologies, e.g. sarcopenia. However, the mechanism of action on mammals has not been elucidated. Thus, the goal of this study was to identify a receptor involved in 20E effects.

MATERIAL & METHODS



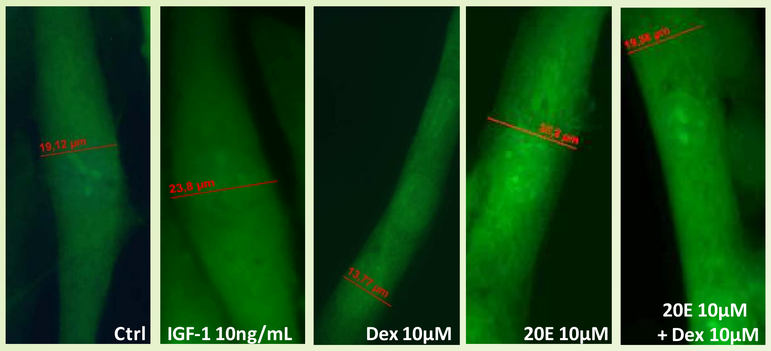
Cell line: C2C12 cells were induced into differentiation by decreasing FBS in culture media for 5 days. Appropriate treatment was applied during 6h (for gene expression analysis) or 4 days (for immunofluorescence).

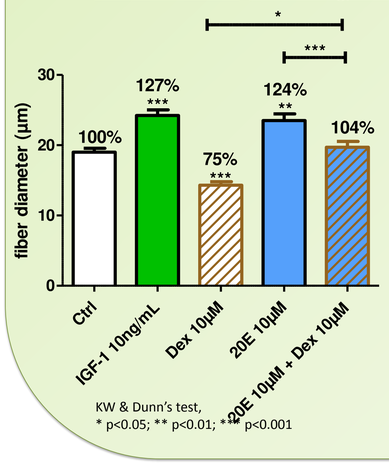
Gene expression: RNAs were extracted and purified using the phenol/chloroform method. Subsequently, RNAs were converted into cDNAs and gene expression was analyzed by q-PCRs. β-Actin was used as housekeeping gene.

Immunofluorescence: Cells were grown, differentiated and treated in 8 well chamber. At the end of incubation, cells are fixed in a solution of glutaraldehyde 2.5%/triton 0.1% for 1h at room temperature and covered by a mounting media. After 24h in a dark, cells were observed under microscope

In vivo model: c57Bl/6J under high fat diet treated with cyclodextrin (control) or 20E (5-50mg/kg) for 6 weeks .

ANABOLIC EFFECT OF 20E ON C2C12 MYOTUBE SIZE





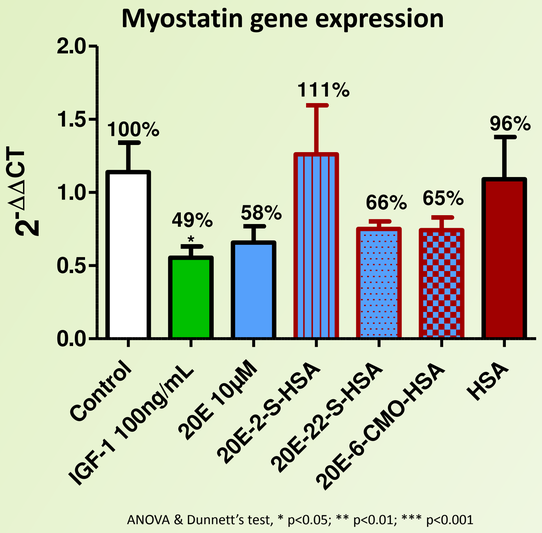
KW & Dunn's test,
* p<0.05; ** p<0.01; *** p<0.001

•10ng/mL IGF-1 treatment induced a significant enlargement of myotube diameter of C2C12 (+27%, p<0.001) and dexamethaosne induced a significant decrease of myotube diameter (-25%, p<0.001) as expected and described in the literature.

•20E 10µM, induced a significant enlargement of fiber diameter of C2C12 (+24%, p<0.001) and was able to counteract the dexamethasone effect.

EVIDENCE FOR INVOLVEMENT OF A MEMBRANE RECEPTOR

We previously documented a dose-dependent inhibition of myostatin gene expression after treatment with 20E. In order to determine if this effect was mediated by a membrane receptor, we compared the effects of three 20E-protein conjugates respectively involving positions 2, 6 and 22 of the molecule on myostatin gene expression.



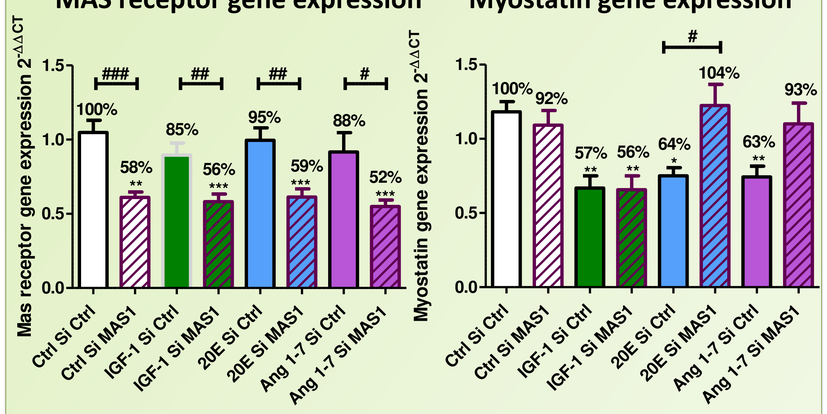
ANOVA & Dunnett's test, * p<0.05; ** p<0.01; *** p<0.001

• We observed that two of them retained an activity close to that of free 20E, whereas the third one was inactive.

• These results show that the presence of a bulky protein does not prevent 20E activity, provided that the A ring remains free.

INVOLVEMENT OF MAS RECEPTOR IN MEDIATING 20E EFFECT

There are plenty of GPC receptors, thus to reduce our field of investigation, we performed a wide literature survey focusing on control of muscle cells, insulin sensitivity and fat mass gain. A set of 9 receptors was thus selected , and the only receptor which gave positive data was *Mas*, the receptor of angiotensin-(1-7)

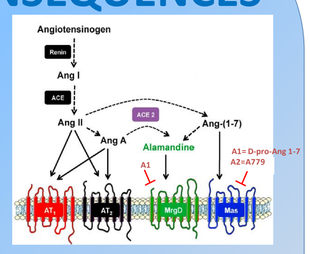


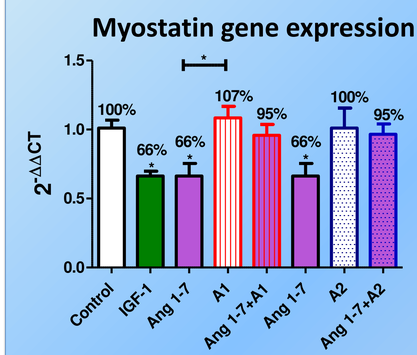
• Mas receptor down regulation by Si RNA led to significant decrease of MAS receptor gene expression in all transfected group by directed SiRNA versus scramble SiRNA.

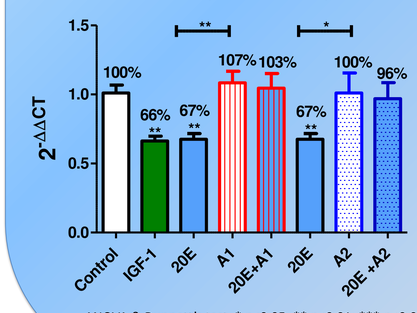
• IGF-1, 20E or Ang 1-7 inhibited significantly myostatin gene expression in cells transfected with scramble SiRNA.

• Down-regulation of Mas receptor gene did no allow inhibition of myostatin gene after treatment with 20E or Ang 1-7.

PHARMACOLOGICAL INHIBITION OF MAS RECEPTOR AND CONSEQUENCES ON 20E EFFECT







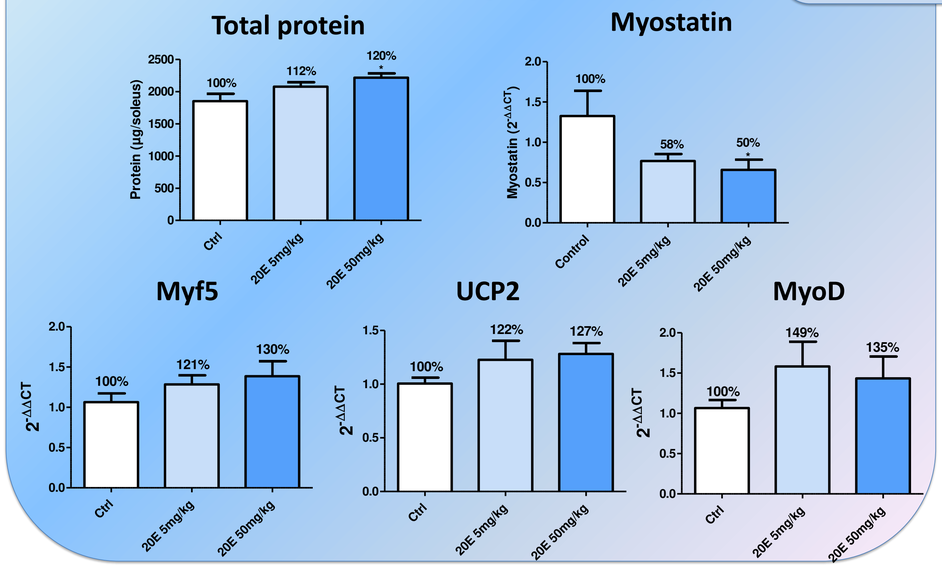
ANOVA & Dunnett's test, * p<0.05; ** p<0.01; *** p<0.001

To confirm the importance of MAS receptor signaling in 20E mediating inhibition of myostatin gene expression, we performed pharmacological inhibition of this receptor.

• Angiotensin 1-7, as previously reported with 20E inhibited myostatin gene expression.

• This inhibition was completely abolished when the molecules were incubated in the presence of Ang 1-7 antagonists (A1 or A2 10 µM).

MOLECULAR EFFECT OF 20E TREATMENT IN VIVO ON MUSCLE



***In vivo*, 20E oral treatment significantly increased protein content and decreased myostatin gene expression in *soleus* muscle, whereas several markers of myogenesis were enhanced.**

CONCLUSION

20E was shown to enlarge fiber size *in vitro* and increase protein amounts *in vivo*. These effects are associated with the inhibition of myostatin gene expression. Cellular signaling studies showed that 20E involves the activation of MAS receptor. These results led us to design a clinical trial on obese sarcopenic patients with BIO101.