Cardiovascular Research Supplements (2014) **103**, S9–S46

POSTER SESSION 1

Session held on 4 July 2014

doi:10.1093/cvr/cvu082

P99

Nrg1beta enhances glucose uptake in cardiomyocytes via mTOR, Src and Akt

L. Pentassuglia; C. Morandi; L. Xu; S. Lebboukh; M. Brink
University of Basel, Department of Biomedicine, Basel, Switzerland

Background: Neuregulin (Nrg)1β is a growth factor that activates PI3K/Akt and Src/FAK via the ErbB2/ErbB4 receptors. Although it is currently in clinical trial to treat haert failure, it remains unclear which cellular mechanisms are responsible for its cardioprotective actions. Here we tested if Nrg1β regulates glucose uptake in cardiomyocytes and analyzed the underlying signaling mechanisms.
Methods: Neonatal rat ventricular myocytes were treated with Nrg1β (10ng/ml) in combination with the mTOR inhibitors PP242 (2mM) and rapamycin (20ng/ml), the ErbB2 inhibitor lapatinib (1mM), the Src inhibitor PP2 (5mM), the Akt inhibitor VIII (20mM), or vehicle. Cells were pre-incubated for 30 min with the inhibitors and proteins extracted 30 min after the addition of Nrg1β for analysis by Western blot. Glucose uptake was assessed by measuring the incorporation of 3H-D-glucose for 30 min. ErbB2 or ErbB4 receptors were knocked down with siRNA for 48h before Nrg1β treatment.
Results: Similar to IGF-I and Insulin, Nrg1β caused a 1.9 fold increase in 3H-D-glucose incorporation ($P < 0.01$). Nrg1β induced phosphorylation of mTOR (S2448), Akt (S308) and FAK (Y861), as well as of the mTORC1 targets 4E-BP1, p70-S6K1 and ULK and the mTORC2 target Akt (S473). Lapatinib, PP242 and Akt inhibitor VIII blocked the Nrg1β-induced Akt-, mTOR-, p70-S6K1-, ULK-, and 4E-BP1-phosphorylation, indicating that these effects require ErbB2 and are mediated by Akt and mTOR. However, only lapatinib and Akt inhibitor VIII fully blocked the Nrg1β-induced glucose uptake; PP242 partially blocked it and rapamycin did not block it at all. These results suggest that Akt is required for Nrg1β-induced glucose uptake, and that mTORC2-dependent Akt phosphorylation mediates, at least in part, this response. PP2 blocked phosphorylation of FAK as expected, and it also partially blocked phosphorylation of Akt (S473) and p70-S6K1. PP2 also decreased general glucose uptake (0.6-fold of Ctl, $p < 0.05$) and Nrg1β-induced glucose uptake (1.06-fold of Ctl, p=ns). Knock-down of ErbB4 receptor alone was sufficient to decrease both mTORC1 and mTORC2 signaling, whereas knock-down of ErbB2 affected only the mTORC2 targets.
Conclusions: Our results show that Nrg1β increases glucose uptake in cardiomyocytes via Akt. We also show that Nrg1β activates mTORC1 via ErbB4 and mTORC2 via the ErbB2/ErbB4 heterodimer. Our data also support the hypothesis that Src/FAK is upstream of mTORC2 and mediates the Nrg1β-induced phosphorylation of Akt and glucose uptake.