

POSTER SESSION 1

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P99**Nrg1 β enhances glucose uptake in cardiomyocytes via mTOR, Src and Akt**

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Background: Neuregulin (Nrg)1 β 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 heart failure, it remains unclear which cellular mechanisms are responsible for its cardioprotective actions. Here we tested if Nrg1 β regulates glucose uptake in cardiomyocytes and analyzed the underlying signaling mechanisms.

Methods: Neonatal rat ventricular myocytes were treated with Nrg1 β (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 Nrg1 β 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 Nrg1 β treatment.

Results: Similar to IGF-I and Insulin, Nrg1 β caused a 1.9 fold increase in 3H-D-glucose incorporation ($P < 0.01$). Nrg1 β 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 Nrg1 β -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 Nrg1 β -induced glucose uptake; PP242 partially blocked it and rapamycin did not block it at all. These results suggest that Akt is required for Nrg1 β -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 Nrg1 β -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 Nrg1 β increases glucose uptake in cardiomyocytes via Akt. We also show that Nrg1 β 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 Nrg1 β -induced phosphorylation of Akt and glucose uptake.