

**P014** Amino-acid transporter SNAT2 can regulate PI3K/Akt and mTOR independent of amino-acids

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In muscle mTOR senses low pH through inhibition of pH-sensitive SNAT2 amino-acid transporters, which impairs amino-acid dependent mTOR activation and protein synthesis. Low pH also activates proteolysis by inhibiting PI3K/Akt which is not regarded as amino-acid sensitive. However, it has been suggested that SNAT2 also signals independent of amino-acids. Here we examined SNAT2's effects on PI3K/Akt by siRNA silencing SNAT2 in L6 myocytes. PI3K was assessed by lipid kinase assay, Ser<sup>473</sup>-phosphorylated Akt by immunoblotting, and proteolysis from <sup>3</sup>H-Phe output, expressed as 10<sup>3</sup> x log% of cell <sup>3</sup>H/h.

Acidic pH (7.1), which decreases SNAT2 transporter activity by 40%, increased proteolysis to 8.4±0.5 versus 7.5±0.3 at pH 7.4 (P<0.05). PI3K inhibition (12.5µM LY294002) increased proteolysis to 9.5±0.3 (P<0.05). SNAT2 siRNA silencing (35% activity decrease) also increased proteolysis to 14.1±0.4, versus 12.7±0.4 with control siRNA (P<0.05). Acid reduced PI3K to 61±16% (P<0.05) and Akt phosphorylation to 70±6% (P<0.05) of the pH 7.4 value. Acid's effect was mimicked by SNAT2 silencing which decreased PI3K to 32±4% and Akt phosphorylation to 40±19% of control value (P<0.05).

It is concluded that, in addition to direct mTOR activation through amino-acids, SNAT2 also regulates PI3K/Akt, strongly suggesting that it can signal independent of its amino-acid transport function.