067P β_2-ADRENOCEPTOR MEDIATED INCREASES IN GLUCOSE TRANSPORT IN L6 SKELETAL MUSCLE CELLS INVOLVES COUPLING TO BOTH Gs AND Gi.

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Stimulation of β_2-Adrenoceptors (AR) increases glucose transport (GT) in the rat skeletal muscle cell line L6 (Nevzorova *et al.,* 2002). Classically, β_2-ARs couple to Gs to increase intracellular levels of cyclic AMP. In this project we examined the signalling mechanisms of β_2-AR-mediated increases in GT in differentiated L6 cells. GT was measured by uptake of 2-deoxy-[^{3}H]-D-glucose after 180 min incubation with agonists and was increased by the selective β_2-AR agonist zinterol (% max 218±5, pEC_{50} = 9.7±0.2, n=6) and the cell permeable cyclic AMP analogues 8-bromo-cAMP (% max 212±16, pEC_{50}=4.1±0.4, n=4) and dibutyryl cyclic AMP (210±25, pEC_{50}=4.6±0.5, n=4). The cell permeable adenylate cyclase inhibitor 2',5'-dideoxyadenosine (50μM) failed to inhibit zinterol stimulated GT (two-way ANOVA, p=0.35, n=4). The PKA inhibitor 4-cyano-3-methylisoquinoline (100nM) did not affect the maximal response to zinterol, but shifted the concentration-response curve to the left (pEC_{50}=7.5±0.2 vs 8.8±0.2; two-way ANOVA, p<0.001, n=5). These results suggest that the cyclic AMP/PKA pathway is not the only mechanism involved in β_2-AR mediated GT. A number of studies show that continuous agonist stimulation of β_2-AR causes receptor desensitisation, following phosphorylation by protein kinase A (PKA) and G protein receptor kinases (GRK), which switches coupling to Gi and results in activation of other signalling pathways (Daaka *et al*., 1997; Zhu *et al*., 2001). In L6 cells, the levels of cyclic AMP were increased by zinterol (100nM) after 30 minutes, but were then decreased at times up to 180 min indicating β_2-AR desensitization (table 1). In contrast, GT was increased after incubation with zinterol for 30 minutes and continued to rise to a significantly higher maximal response after 3 hours (one-way ANOVA, p<0.01, n=4; Table 1). Pertussis toxin reduced zinterol-stimulated GT (% max increase at 180 min, 183±6 vs 159±6, p<0.0001, n=6) indicating that Gi was involved in the process.

In conclusion, the study showed that GT in L6 myotubes can be increased by cyclic AMP analogues and Gs coupled receptors, and that β_2-AR mediated GT may also involve coupling to Gi that occurs following receptor desensitisation.

Table 1. Time-course of cyclic AMP and glucose transport (GT; 2-deoxy-[^{3}H]-D-glucose uptake) in L6 myotubes following stimulation with zinterol (100nM).

Time (min)	0	30	60	90	120	150	180
Cyclic AMP (pmol/mg protein) n=4	35 ±21	2105 ±334	1538 ±238	1049 ±319	930 ±378	894 ±311	724 ±257
GT (% basal) n=4	100	133±20	134±11	148±31	175±28	162±35	219±27

Daaka, Y *et al* (1997) Nature 390, 88-91
Nevzorova, J *et al* (2002) Br J Pharmacol., 137, 9-18
Zhu, WZ *et al* (2001) PNAS 98, 1607-1612