Signalling transduction mechanisms that link the β-adrenoceptor agonists BRL37344 and clenbuterol to glucose uptake in mouse isolated soleus muscle

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Introduction

10 pM BRL37344 (a selective β_3-adrenoceptor [AR] agonist) and 10 pM clenbuterol (β_2-AR agonist) stimulate glucose uptake in mouse isolated soleus muscle. 100 pM of either has no effect. 10 nM BRL37344 also stimulates glucose uptake but 100 nM clenbuterol inhibits uptake. Studies using β-AR antagonists and β-AR knockout mice, show that the effects of 10 pM BRL37344 and clenbuterol do not involve β-ARs, whereas the opposite effects of 10 nM BRL37344 and 100 nM clenbuterol are both mediated by the β_2-AR (Ngala *et al.*2008 *Br J Pharmacol* 155: 395; Ngala *et al.*2009 *Br J Pharmacol,* in press). This suggests that these agonists might affect different signalling mechanisms via the β_2-AR. We have already reported that forskolin increases both cyclic AMP content and glucose uptake, 10 pM clenbuterol increased, whereas 100 nM clenbuterol decreased cAMP content, but that there were no cAMP changes associated with the effect of 10 pM and 10 nM BRL-37344 (Ngala *et al.*, 2008 *Br J Pharmacol.* 155: 395-406).

Aim

To investigate the roles of protein kinase A (PKA), phosphatidylinositol-3 kinase (PI3K), mitogen-activated protein kinases (MAPK) and AMP-activated protein kinase (AMPK) in the effects of BRL-37344 and clenbuterol on glucose uptake in soleus muscle.

Methods

Soleus muscles from male C57Bl/6 mice were preincubated for 60 min in KHB buffer. 2-deoxy [1-^{14}C] glucose (0.1 μCi/ml) uptake (termed 'glucose uptake') was measured over 45 min in KHB buffer that contained 5.5 mM glucose and 0.1 nM bovine insulin. The incubation medium contained the βAR agonists in the presence or absence of either 1μM wortmannin, 1μM LY294002 (PI3K inhibitors), 1μM compound C (AMPK inhibitor), 20μM PD98059 (prevents activation of MAPKKinase), 10μM SB203580 (p38MAPK inhibitor) or 10μM H89 (protein kinase A inhibitor).

Results are means ± S.E. of 6-8 values
*P< 0.05; ** P<0.01; ***P<0.001 for effects of β-AR agonists.
†P<0.05; ††P<0.01; †††P<0.001 for effects of inhibitors.

PKA

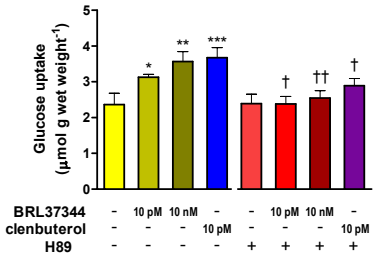

Fig 1.
The stimulatory effects of 10 pM and 10 nM BRL37344, and of 10 pM clenbuterol were blocked by H89.

PI3K

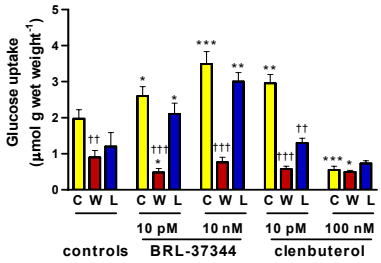

Fig 2.
Wortmannin inhibited 10 pM and 100 nM BRL37344-stimulated, and 10 pM clenbuterol-stimulated glucose uptake. LY294002 inhibited only the effect of 10 pM clenbuterol.

MAPK – clenbuterol

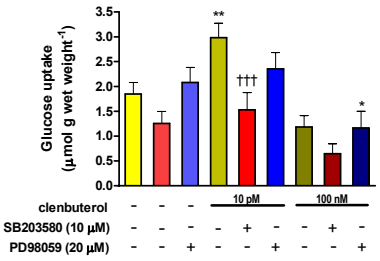

Fig 3.
The stimulatory effect of 10 pM clenbuterol was blocked by the p38 MAPK inhibitor SB203580.

MAPK – BRL37344

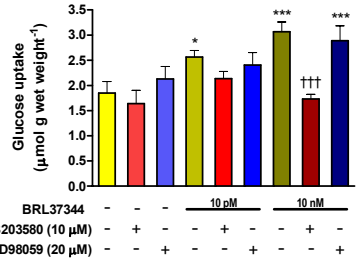

Fig 4.
The stimulatory effect of 10 nM BRL37344 was blocked by the p38 MAPK inhibitor SB203580.

AMPK

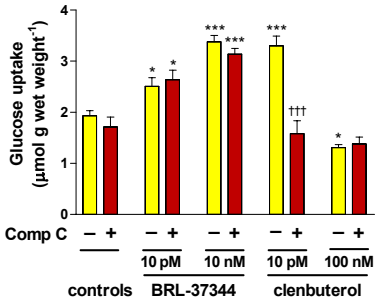

Fig 5.
Compound C blocked the stimulatory effect of 10 pM clenbuterol.

Summary

- The protein kinase A inhibitor (H-89) prevented the stimulatory effect of 10 pM clenbuterol on glucose uptake. It also inhibited the stimulatory effects of 10 pM and 10 nM BRL37344 on glucose uptake, even though BRL37344 does not affect total cyclic AMP content.
- The PI3 kinase inhibitor wortmannin blocked the stimulatory effects of 10 pM clenbuterol and of 10 pM and 10 nM BRL37344, whereas LY294002 blocked clenbuterol only.
- The p38 MAPK inhibitor SB203580 blocked the effects of 10 pM clenbuterol and 100 nM BRL37344. Compound C blocked the effect of 10 pM clenbuterol only .

Conclusions

- **10 nM BRL37344-stimulated glucose uptake (β_2-AR-mediated) requires PKA, PI3K and p38 MAPK activity.**
- **10 pM BRL37344-stimulated glucose uptake (β-AR-independent) requires PKA and PI3K.**
- **10 pM clenbuterol-stimulated glucose uptake (β-AR-independent) requires PKA, PI3K, p38 MAPK and AMPK activity.**

GPR41 and GPR43 in leptin secretory responses of murine adipocytes to SCFA

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Introduction

GPR41 (free fatty acid receptor 3; FFA3) and GPR43 (FFA2) are activated by short chain carboxylic acids. Both receptors couple to Ga_i/Ga_o but GPR43 also couples to Ga_q. Xiong *et al.* (PNAS 2004, 101: 1045) reported that GPR41 mRNA is expressed in mouse epididymal adipose tissue, and that propionate and butyrate stimulated leptin secretion. The effect of propionate was enhanced in the presence of adenosine deaminase, which was interpreted to be due to removal of the desensitising effect of adenosine, acting through the A1 receptor, on the G_i-mediated responses. Acetate was less potent or less effective than propionate or butyrate, consistent with the response being mediated by GPR41 rather than GPR43. Moreover, siRNA that targeted GPR41 almost totally prevented leptin secretion in response to propioniate in Ob-Luc cells.

It is premature to conclude that GPR41 mediates the effect of SCFA on leptin secretion, however. First, the Ob-Luc cells had been transfected with GPR41 mRNA. Secondly, Hong *et al.* (Endocrinology 2005, 146: 5092) failed to detect GPR41 mRNA in four murine adipose tissue sites. GPR43 mRNA, by contrast, was detected at all sites. They found that propionate inhibited isoproterenol-stimulated lipolysis in confluent 3T3-L1 adipocytes and that this effect was prevented by GPR43 siRNA.

Aims

We have compared responses to SCFA in epididymal and mesenteric adipocytes from wild-type and GPR41 knockout mice. To help interpret these findings, we have studied the expression of GPR41 and GPR43.

Methods

GPR41 knockout mice were produced by Deltagen and further backcrossed at AstraZeneca, Alderley Park and the University of Buckingham to give a total of nine backcrosses onto the C57BL/6 background.

The wild type mice shown in Figures 3 and 4 were littermates of the GPR41 knockout mice. Transcript levels for GPR41 and GPR43 were quantified in triplicate by real-time RT-PCR.

Epididymal and mesenteric adipocytes were isolated by collagenase digestion and incubated for one hour in the presence of adenosine deaminase and acetate (Ac), propionate (Pr) or butyrate (Bu) before measurement of leptin or glycerol in the supernatant.

N values are for the number of adipocyte preparations. *P < 0.05; **P < 0.01 vs corresponding control (C). †† P < 0.01; ††† P < 0.001 vs corresponding wild-type value.

Results

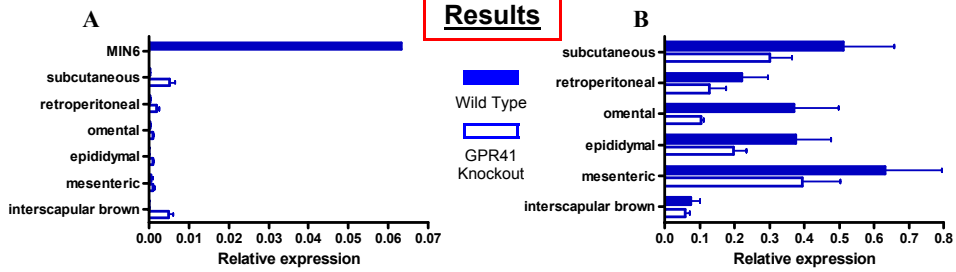

Figure 1: GPR41 and GPR43 expression in Wild-type and GPR41 Knockout Mice
GPR41 expression was easily detected in MIN6 cells (insulin secreting) but values for wild-type mice were no higher than the background seen in GPR41 knockout mice (**A**). GPR43 expression in GPR41 knockout mice was significantly reduced (P = 0.016; U-test) in omental adipose tissue and in white adipose tissue overall (P < 0.01; 2-way ANOVA); (**B**).

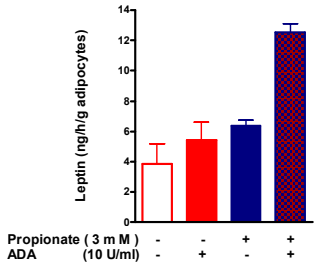

Figure 2: Leptin secretion by Rat Epididymal Adipocytes
A preliminary experiment using showed that propionate stimulated leptin secretion in the presence but not the absence of adenosine deaminase. Adenosine deaminase was used in future experiments on leptin secretion.

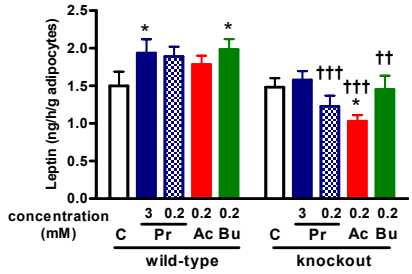

Figure 3: Leptin secretion by Mouse Epididymal Adipocytes
Propionate and butyrate stimulated leptin secretion by adipocytes from wild-type mice. The responses to all three SCFA were significantly reduced in adipocytes from GPR41 knockout mice, as indicated by the daggers. Acetate significantly reduced leptin secretion by adipocytes from the knockout mice, as indicated by the asterisk. The mechanism that mediated this reduction is not known.

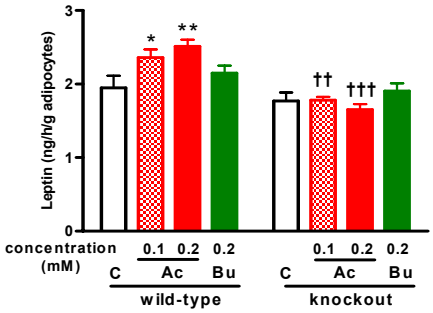

Figure 4: Leptin Secretion by Mouse Mesenteric Adipocytes
Acetate increased leptin secretion by mesenteric adipocytes from wild-type mice but not by adipocytes from knockout mice. Butyrate had no effect in adipocytes from either wild-type or knockout mice.

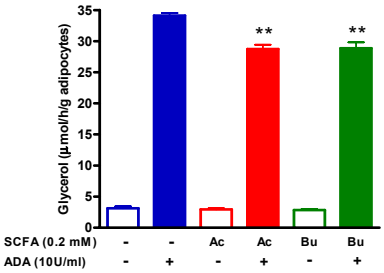

Figure 5: Glycerol Release by Mouse Epididymal Adipocytes
Adenosine released into the medium inhibits lipolysis via the A1 receptor and Gi. In the absence of adenosine deaminase, SCFA had no effect on lipolysis, but both acetate and butyrate (0.2 mM) inhibited ADA-stimulated lipolysis.

Summary

- We could not detect GPR41 mRNA in adipose tissue from five sites. Nevertheless, SCFA stimulated leptin secretion by epididymal and mesenteric adipocytes from wild-type but not GPR41 knockout mice.
- GPR43 mRNA was easily detected and its expression was reduced in GPR41 knockout mice.
- Acetate but not butyrate stimulated leptin secretion in wild-type mesenteric adipocytes, consistent with mediation of this response by GPR43.
- -SCFA inhibited adenosine deaminase-stimulated lipolysis, consistent with GPR43 signalling via Ga_i.

Conclusion:

GPR43 rather than GPR41 may mediate short chain fatty acid-stimulated leptin secretion by mouse adipocytes.