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Biphasic effects of the β-adrenoceptor agonist, BRL 37344, on glucose utilization in rat isolated skeletal muscle

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1 The effects of the selective β_3-adrenoceptor agonist, BRL 37344 (BRL) on glucose uptake and phosphorylation (i.e. glucose utilization; GU) and glycogen synthesis in rat isolated soleus and extensor digitorium longus (EDL) muscle preparations *in vitro* were investigated by use of 2-deoxy-[^{3}H]- glucose (GU) and [U-^{14}C]-glucose (glycogen synthesis).

2 Low concentrations of BRL ($10^{-11}-10^{-9}$M) significantly increased GU, with maximal increases of 30% in soleus and 24% in EDL at 10^{-11}M. Neither the selective β_1-adrenoceptor antagonist, atenolol ($10^{-8}-10^{-6}$M), nor the selective β_2-adrenoceptor antagonist, ICI 118551 ($10^{-8}-10^{-6}$M) had any effect on the stimulation of GU induced by 10^{-11}M BRL.

3 High concentrations of BRL ($10^{-6}-10^{-5}$M) caused significant inhibition (up to 30%) of GU in both soleus and EDL muscles. The inhibition at 10^{-6}M BRL was blocked completely by 10^{-6} and 10^{-7}M ICI 118551 in soleus, and by $10^{-6}-10^{-8}$M ICI 118551 in EDL; atenolol ($10^{-8}-10^{6}$M) had no effect.

4 Another selective β_3-adrenoceptor agonist, CL 316,243, also caused a significant stimulation of muscle GU, with maximal increases of 43% at 10^{-9}M in soleus and 45% at 10^{-10}M in EDL. The stimulation of GU declined with further increases in the concentration of CL 316,243, but no inhibition of GU was seen, even at the highest concentration (10^{-5}M) tested.

5 BRL at 10^{-5}M inhibited completely insulin-stimulated glycogen synthesis in both soleus and EDL, but this inhibitory effect of BRL was abolished by 10^{-6}M ICI 118551. BRL at 10^{-11}M (with or without 10^{-6}M ICI 118551) had no effect on insulin-stimulated glycogen synthesis.

6 It is concluded that: (i) low (<nM) concentrations of BRL stimulate GU via an atypical β-adrenoceptor that is resistant to conventional β_1-adrenoceptor and β_2-adrenoceptor antagonists; (ii) the stimulation of GU is negated by the activation of β_2-adrenoceptors that occurs at higher (>nM) concentrations of BRL; (iii) inhibition of GU via β_2-adrenoceptor activation is associated with inhibition of glycogen synthesis, possibly due to activation of glycogenolysis; (iv) the opposing effects of β_2-adrenoceptor and atypical β-adrenoceptor activation on GU suggest that in skeletal muscle these adrenoceptors are linked to different post-receptor pathways.

Keywords: Skeletal muscle; β-adrenoceptors; glucose; glycogen; BRL 37344; CL 316,243; atenolol; ICI 118551

Introduction

Skeletal muscle is considered to be the principal site of insulin-stimulated glucose disposal, but there is also evidence for a non-insulin-mediated pathway of glucose utilization in muscle. Both exercise and cold exposure markedly increase skeletal muscle glucose uptake despite unchanged, or even reduced plasma insulin levels and this alternative mechanism is thought to be activated by increased sympathetic activity (Wallberg-Henriksson & Holloszy, 1985; Vallerand *et al.*, 1987; Cartee *et al.*, 1989). Sympathomimetic stimulation of this pathway could explain how the novel β-adrenoceptor (βAR) agonists that were developed for the treatment of diabetes and obesity result in improved glucose homeostasis. For example, Smith *et al.* (1990) used one of these agonists (BRL 35135) to show that chronic treatment improves insulin sensitivity and glucose tolerance in rodent models of diabetes.

Apart from providing potential new agents for the treatment of diabetes and obesity, the existence of novel βAR agonists such as BRL 35135 also helped in the detection and identification of the β_3AR subtype (Arch *et al.*, 1984; Emorine *et al.*, 1989; Granneman *et al.*, 1991; Krief *et al.*, 1993). Following the molecular identification of the β_3AR there have been numerous studies investigating the nature and physiological role of this β_3AR (for reviews, see Arch & Kaumann, 1993; Yen, 1994), but little attention has been directed to the role and mechanisms of the β_3AR and selective agonist stimulation of non-insulin-mediated glucose utilization in skeletal muscle. However, this aspect was the subject of an earlier study (Liu & Stock, 1995) in which we described the acute effects of BRL 35135 on tissue glucose utilization in rats *in vivo*. The results from that series of experiments suggested that an atypical βAR (possibly the β_3AR) was involved in the stimulation of skeletal muscle glucose uptake induced by the agonist. The stimulation was resistant to the selective β_1AR antagonist, atenolol, and was inhibited only by very high doses of the non-selective βAR antagonist, propranolol. Paradoxically, the selective β_2AR antagonist, ICI 118551, was found to potentiate the effects of the agonist and suggested that as well as stimulating muscle glucose utilization via an atypical βAR, there was also a β_2AR-mediated inhibitory component to the effects of BRL 35135 on skeletal muscle.

These conflicting βAR-mediated responses to the same agonist *in vivo* could result from a complex interplay between a variety of factors, of which blood flow could be the most important. The β_3AR agonists have been shown to increase blood flow (Berlan *et al.*, 1994; Shen *et al.*, 1994), which in itself can modulate insulin-mediated glucose uptake (Baron *et al.*, 1994). This makes it difficult to decide whether BRL 35135 is acting on the atypical βAR and/or the β_2AR on skeletal muscle and/or on vascular smooth muscle. Moreover, apart from one recent report (Summers *et al.*, 1995), there is little molecular

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evidence for β_3AR in skeletal muscle, although both functional (Thurlby & Ellis, 1986; Challiss *et al.*, 1988) and ligand binding studies (Molenaar *et al.*, 1991; Sillence *et al.*, 1993; Summers *et al.*, 1995) indicate the presence of an atypical βAR.

In order to investigate further the mechanisms of βAR-subtypes involved in mediating the effects of BRL 35135 on muscle glucose metabolism, it was decided that it was necessary to avoid the problems caused by changes in muscle blood flow and other humoral responses in the *in vivo* preparation used previously. Hence, the effects of two β_3AR agonists, BRL 37344 (the active metabolite of BRL35135) and CL 316,243 (Largis *et al.*, 1994) on glucose utilization were determined in rat isolated skeletal muscle preparations *in vitro*. Selective β_1AR and selective β_2AR antagonists were used in an attempt to identify the receptor subtype(s) involved in the tissue responses and a final experiment involved determining the effects of BRL 37344 on insulin-stimulated [^{14}C]-glucose incorporation into glycogen.

Methods

Animals

Male Wistar rats (body weight 60–90g; age 21–31d) were obtained from the Biological Research Facility (St George's Hospital Medical School) and used on the same day without further treatment.

Measurement of glucose utilization (GU)

Rats were killed by cervical dislocation and the skin removed from the hind limbs. Soleus and extensor digitorium longus (EDL) muscles weighing 20–30 mg were dissected from both legs being careful to avoid any damage to the muscle. On each experimental day, eight soleus and EDL muscles (i.e. from 4 rats) were obtained and subsequently assigned in a balanced fashion to the various experimental treatments. Both proximal and distal tendons were tied onto a stainless steel clip under slight tension to maintain each muscle at resting length. The muscles were immediately placed in individual flasks containing 3 ml of Krebs-Henseleit bicarbonate buffer (KHB; pH 7.4) with 5.5 mM glucose, 0.14% bovine serum albumin and 10 mM HEPES equilibrated with 95% O_2:5% CO_2. All flasks were immediately sealed with SubaSeal and transferred to 37°C shaking water bath and gassed continuously with 95% O_2:5% CO_2 via a 21G needle, with a second (23G) needle acting as a vent in the SubaSeal. After a 30 min pre-incubation period, the muscles were transferred to fresh incubation flasks with 3 ml of the same buffer, but containing 2-deoxy-[^{3}H]-glucose (1μCi ml^{-1}), bovine insulin (0.1 nM; 20 nu ml^{-1}) with appropriate concentrations of the drugs to be tested. The muscles were then incubated for 60 min in the shaking water bath with continuous gassing. At the end of the incubation, muscles were rinsed briefly in cold saline, blotted on tissue paper and freeze-clamped in liquid nitrogen.

For analysis, muscles were weighed, digested in 0.5 ml 1 M NaOH for 45 min at 60°C and neutralized with 0.5 ml 1 M HCl. One aliquot (200 μl) of the digest was added to 1 ml of $Ba(OH)_2/ZnSO_4$ and another (200 μl) was added to 1 ml of 6% perchloric acid to determine the 2-deoxy-[^{3}H]-glucose-6-phosphate content in the tissues, as described by Issad *et al.* (1987). After centrifugation, 800 μl of each supernatant was added to 10 ml scintillation cocktail (Beckman Ready Value) and the radioactivity determined (Beckman LS6000). Tissue GU (uptake and phosphorylation) was calculated as the amount of 2-deoxy[^{3}H]-glucose-6-phosphate accumulated in the tissue (d.p.m. mg^{-1} wet weight).

Measurement of insulin-stimulated glycogen synthesis

The maximal rate of insulin-stimulated [^{14}C]-glucose incorporation into glycogen was measured as described by Challis *et al.* (1988) using muscles prepared as above. After the 30 min pre-incubation period in KHB at 37°C, the muscles were transferred to fresh incubation flasks containing [U-^{14}C]-glucose (0.25 μCi ml^{-1}) and bovine insulin (0.6 μM; 100 mu ml^{-1}) with appropriate concentrations of the test drugs, and incubated for a further 60 min in a shaking water bath with continuous gassing. The muscles were then removed, rapidly blotted and freeze-clamped. Glycogen synthesis was measured following alkaline digestion of the freeze-clamped muscles and ethanolic precipitation of glycogen prior to determination of radioactivity (as above). The results are expressed as μmol glucosyl units incorporated h^{-1} g^{-1} wet weight (Challiss *et al.*, 1988).

Materials

Radiolabelled 2-deoxy-[^{3}H]-glucose and [U-^{14}C]-glucose were purchased from Amersham International (Amersham, U.K.), and atenolol was purchased from Sigma (Poole, U.K.). BRL 37344 ([**R***,**R***]4-[2-[(2-hydroxy-2(3-chlorophenyl)ethyl)-amino]-propyl]-phenoxyacetic acid) and ICI 118551 (erythro-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol) were supplied by SmithKline Beecham (Epsom, U.K.) and Zeneca Pharmaceuticals (Macclesfield, U.K.), respectively. CL 316,243 (disodium (**R**,**R**)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethylamino]propyl]-1,3-benzodioxole-2,2-dicarboxylate) was supplied by American Cyanamid Co. (New York, U.S.A.).

Statistics

Each incubation with a particular drug concentration and combination was replicated 3–6 times, and the results have been presented as mean values ± s.e.mean. The statistical significance of differences between control and experimental groups was assessed by Student's unpaired *t* test; all probabilities quoted are two-tailed. Multiple comparisons were made by using one-way analysis of variance followed by Scheffe's test to identify significant differences between groups.

Results

When developing the method for measuring glucose uptake and phosphorylation (glucose utilization; GU) in isolated skeletal muscle preparations *in vitro*, an insulin concentration-response (0, 0.1, 1.0, 3.0 and 10 nM) experiment was carried out. The results showed that insulin stimulated GU in both soleus and EDL muscles in a dose-dependent manner. In soleus, for example, insulin increased GU by 4, 11, 24 and 46% compared to control muscles at 0.1, 1.0, 3.0 and 10 nM concentrations. As well as demonstrating the viability of the preparation, this preliminary trial allowed a low, permissive (4% stimulation of GU) concentration (0.1 nM) of insulin to be selected that could be used in the incubation media in subsequent experiments in order to maintain the metabolic viability of the muscles, as recommended by Bonen *et al.* (1994).

The first experiment examined the effects of BRL on GU in soleus and EDL by incubating the muscles in KHB plus insulin (0.1 nM; control), or with the same medium containing 10^{-12}–10^{-5} M BRL. The results for both soleus and EDL have been presented in Figure 1 in absolute units of GU (a, b), but also as percentage changes from control values (c,d); the latter make it easier to see the extent of the changes from the basal GU. BRL caused a significant increase in soleus GU at low concentrations (10^{-12}–10^{-9} M), with the peak stimulation (30% vs control) being seen at 10^{-11} M. However, the stimulation was attenuated, and eventually disappeared with further increases in agonist concentration. At the highest concentrations tested, BRL caused a significant inhibition, such that GU was lower than control values (decrease = 17% at 10^{-6} M; 27% at 10^{-5} M). Very similar responses were seen in EDL (Figure 1), where BRL also caused a significant increase in GU at 10^{-11}–

10^{-9} M, with a maximal stimulation of 24% at 10^{-11} M. As in soleus, BRL at 10^{-6} and 10^{-5} M depressed GU below control values by 12% and 31%, respectively.

Given the biphasic effects of BRL on muscle GU, it was decided to use selective β_1AR and β_2AR antagonists in an attempt to identify the βAR-subtypes responsible for the sti-

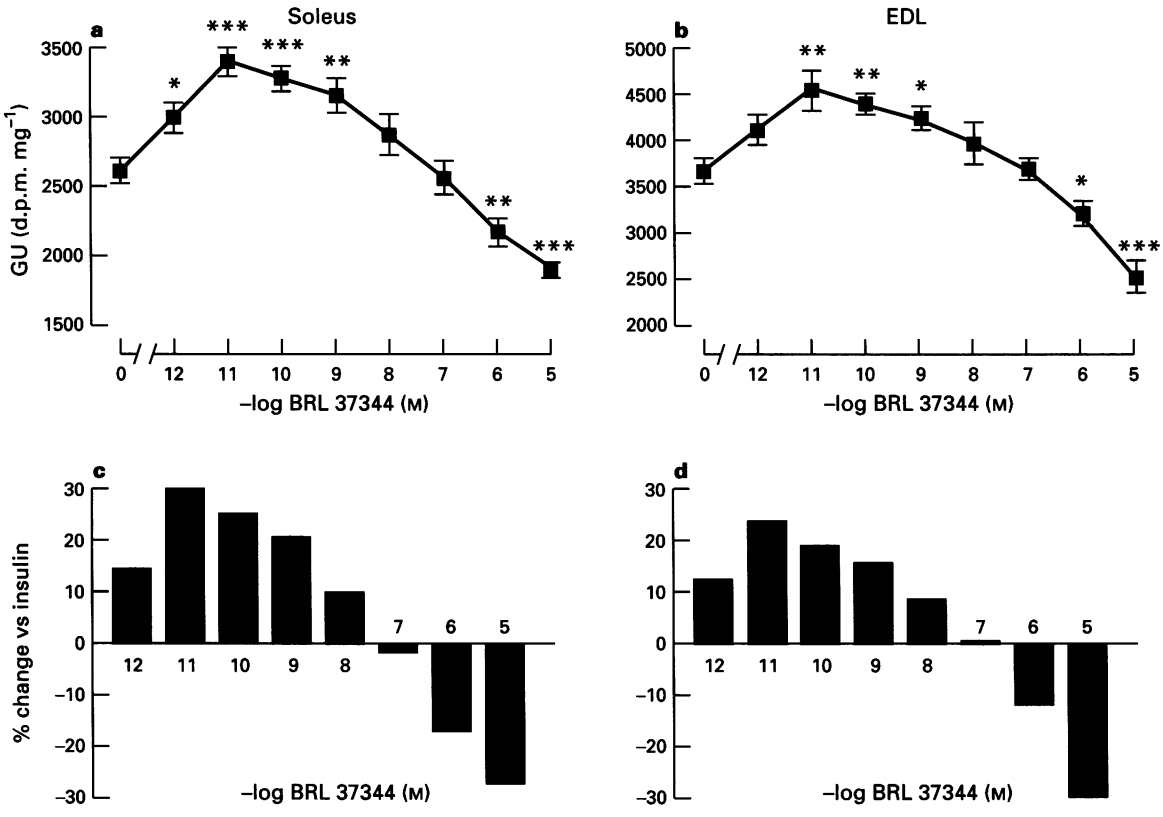

Figure 1 Effect of BRL 37344 on glucose utilization (GU) in soleus and EDL muscles in the presence of 0.1 nM insulin (insulin alone = 0). Results (a, b) are mean values ± s.e.mean ($n=6$); $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs insulin alone (Student's unpaired t test). Results are also expressed as percentage changes vs insulin alone (c, d).

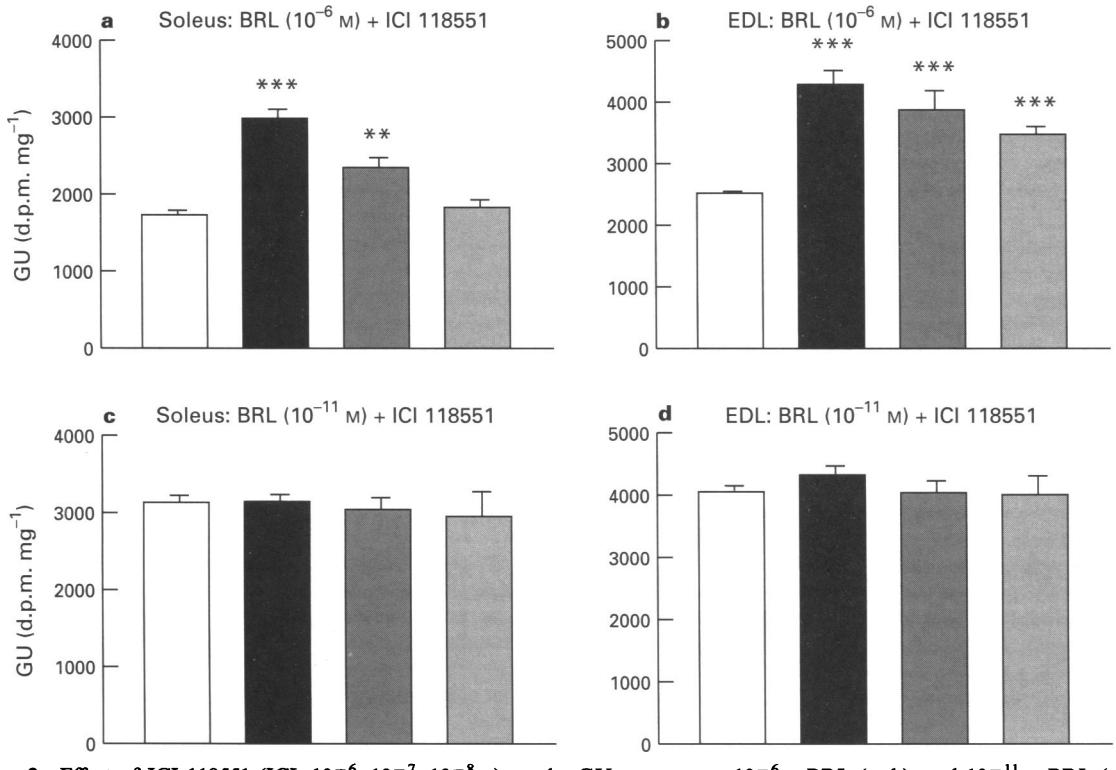

Figure 2 Effect of ICI 118551 (ICI; 10^{-6}, 10^{-7}, 10^{-8} M) on the GU response to 10^{-6} M BRL (a, b), and 10^{-11} M BRL (c, d) in soleus and EDL. Results are mean values ± s.e.mean; ($n=3-6$); $^{**}P<0.01$, $^{***}P<0.001$ compared to BRL alone (Student's unpaired t test). (a, b) Open columns BRL 10^{-6} M; solid columns BRL + ICI 10^{-6} M; cross hatched columns BRL + ICI 10^{-7} M; stippled columns, BRL + ICI 10^{-8} M. (c, d) As (a) and (b) except that BRL 10^{-11} M was used instead of 10^{-6} M.

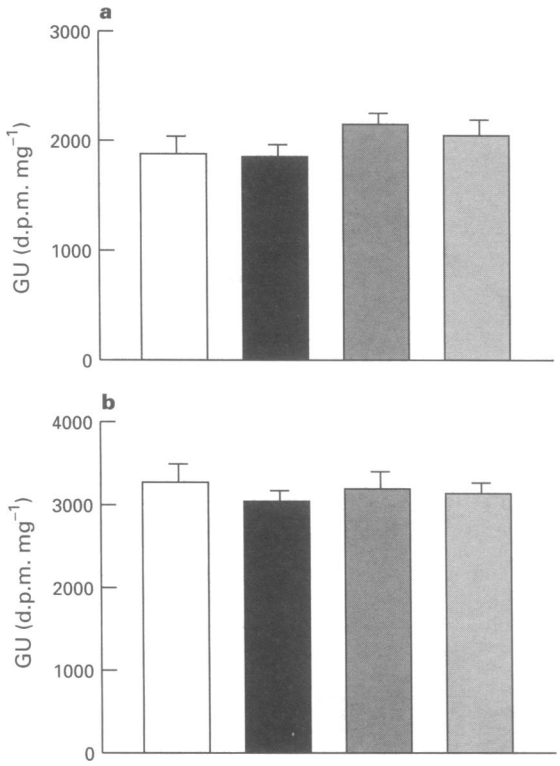

Figure 3 Effect of atenolol (Aten; 10^{-6}, 10^{-7}, 10^{-8} M) on the GU response to 10^{-6} M (a), and 10^{-11} M BRL (b) in soleus. Results are mean values ± s.e.mean ($n=6$). In (a), open column, BRL 10^{-6} M; solid column, BRL + Aten 10^{-6} M; cross-hatched column, BRL + Aten 10^{-7} M; stippled column BRL + Aten 10^{-8} M. (b) Same as (a) except that BRL 10^{-11} M used instead of 10^{-6} M.

mulatory and inhibitory effects. In the next experiment, the muscles were exposed to two concentrations of BRL (10^{-11} M or 10^{-6} M) selected for their ability to stimulate or inhibit GU (see Figure 1). With these agonist concentrations, the effects of three concentrations of the selective β_2AR-antagonist ICI 118551 (ICI; 10^{-6}, 10^{-7}, 10^{-8} M) were determined. When 10^{-6} M BRL was used (Figure 2a, b), ICI at 10^{-6} and 10^{-7} M caused a significant disinhibition of the GU response to BRL in soleus (% increase vs BRL alone: 72% at 10^{-6} M ICI; 36% at 10^{-7} M ICI), whereas ICI at 10^{-8} M had no significant effect. In EDL (Figure 2 a, b) all three concentrations of ICI disinhibited the response - i.e. blocked the reduction in GU caused by BRL (% increase vs BRL alone: 71, 54 and 40% at 10^{-6}, 10^{-7} and 10^{-8} M ICI, respectively). However, when 10^{-11} M BRL was used (Figure 2c, d), ICI had no effect on the GU response at any concentration in either soleus or EDL.

Given the similarity in soleus and EDL responses so far, the effect of the selective β_1AR-antagonist atenolol on the BRL response was tested only in soleus muscles (Figure 3). As with the ICI experiments, soleus muscles were exposed to two concentrations (10^{-6}, 10^{-11} M) of the agonist with or without three concentrations (10^{-6}, 10^{-7} and 10^{-8} M) of atenolol. Unlike the β_2AR antagonist, atenolol did not have any effect on the GU response to either 10^{-6} M or 10^{-11} M BRL - i.e. there was no inhibition or potentiation of the BRL response at any of the three concentrations of the β_1AR antagonist.

The selective β_3AR agonist CL 316,243, has been reported to have much less β_2AR activity than BRL (Largis *et al.*, 1994; Yoshida *et al.*, 1994), and in the next experiment the effects of this compound on GU in soleus and EDL was tested with the same protocol as that used in the first experiment with BRL. It should be noted (Figure 4) that the baseline GU values in this experiment were somehow lower than those observed in the experiments with BRL (see Figure 1). This may have been due to the experiment being carried out several months later with a different batch of rats at a slightly different age (28 - 31d) and

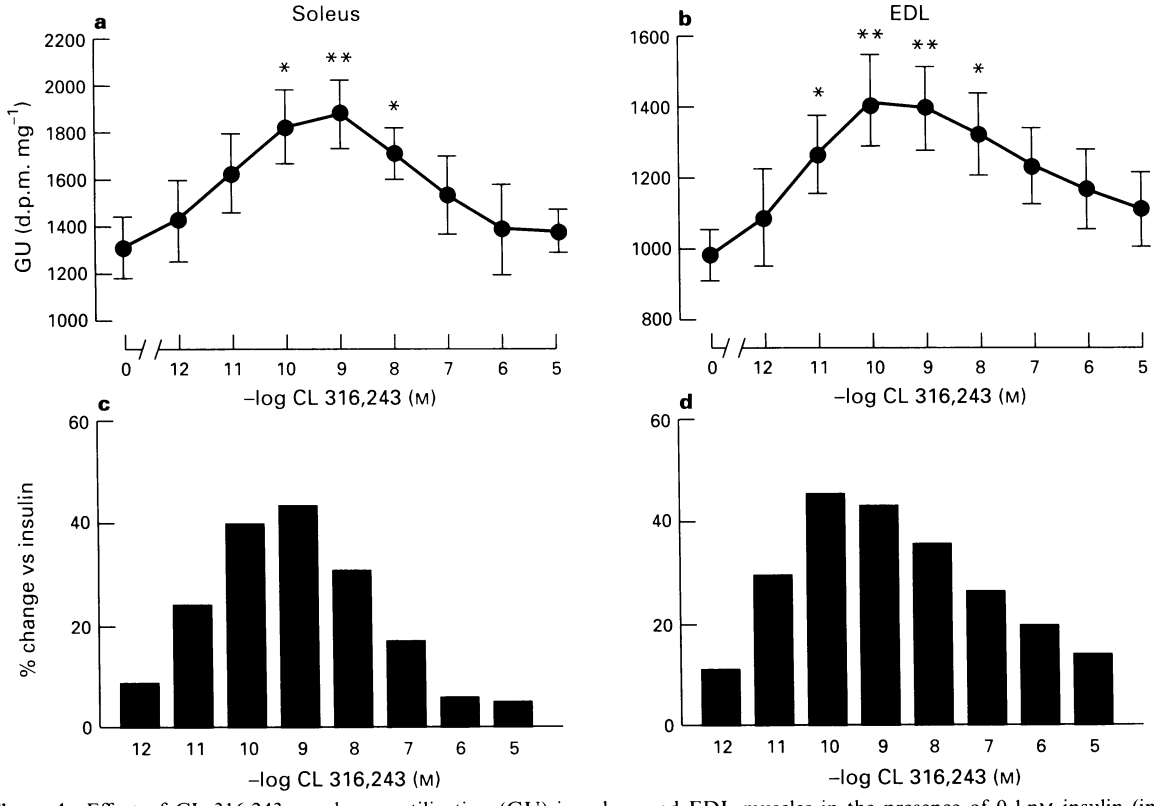

Figure 4 Effect of CL 316,243 on glucose utilization (GU) in soleus and EDL muscles in the presence of 0.1 nM insulin (insulin alone = 0). Results (a, b) are mean values ± s.e.mean ($n=6$); $^*P<0.05$, $^{**}P<0.01$ vs insulin alone (Student's unpaired t test). Results are also expressed as percentage changes vs insulin alone (c, d).

weight (80–90g) than those used earlier (21–26d; 60–80g). In spite of this difference in basal GU, the response pattern with CL 316,243 was very similar to that obtained with low concentrations of BRL, i.e. a 'bell-shaped' concentration-response curve was produced. The peak stimulation (43% in soleus; 45% in EDL) was somewhat greater than that seen with BRL (30 and 24% for soleus and EDL, respectively), but required higher concentrations to achieve this (10^{-9} M in soleus and 10^{-10} M in EDL compared to 10^{-11} M for BRL in both muscles). As with BRL, the stimulation of GU declined with increasing concentrations but GU was never inhibited below basal levels, even at the highest concentration (10^{-5} M) tested (cf. 10^{-6} and 10^{-5} M BRL in Figure 1).

Given the opposing effects on GU induced by low and high concentrations of BRL, the final experiment attempted to determine whether the changes in GU were a reflection of changes in the glucose utilized for glycogen synthesis. Soleus and EDL muscles were incubated with KHB (control), KHB with insulin (6.5 nM) or KHB plus insulin together with BRL (10^{-5} or 10^{-11} M) with and without the selective β_2AR antagonist, ICI (10^{-6} M). In soleus, insulin caused over 4.3 fold stimulation of [^{14}C]-glucose incorporation into glycogen compared to control (Figure 5). BRL at 10^{-5} M completely inhibited this effect of insulin, with glycogen synthesis decreasing to the level seen in the absence of insulin (control). However, this inhibitory effect of BRL was abolished by 10^{-6} M ICI and glycogen synthesis was restored to that seen with insulin alone. BRL at 10^{-11} M had no effect on insulin-stimulated glycogen synthesis, and no potentiation was seen when 10^{-6} M ICI was included in the incubation medium. Almost exactly the same results were obtained with EDL (Figure 5). As an adjunct to this experiment, the effect of 10^{-11} M BRL on soleus glycogen synthesis in the presence and absence (control) of a sub-maximal concentration (1 mu ml^{-1}; 6 nM) of insulin was tested in a few ($n=3$) muscles. When compared to the control level (no insulin), BRL at 10^{-11} M had no effect on the basal rate of glycogen synthesis, and neither did it potentiate the effect of the sub-maximal concentration of insulin (results not shown).

Discussion

Earlier studies on the effects of BRL on rat tissue GU *in vivo* indicated a possible direct βAR stimulation in skeletal muscle independent of changes in plasma insulin levels (Abe *et al.*, 1993; Liu & Stock, 1995). The results from the present study, together with the *in vitro* results of Abe *et al.* (1993), provide further evidence that BRL can stimulate non-insulin-mediated glucose utilization in muscle directly and independently of changes in blood flow and other humoral responses to the βAR agonist *in vivo*. The *in vitro* preparation removes the muscles from these other influences and only insulin is included at levels sufficient to maintain metabolic function. The two muscles chosen for study are examples of slow-twitch oxidative, or Type I (soleus) and fast-twitch glycolytic, or Type II (EDL) muscles, but unlike the *in vivo* responses of these two muscles to BRL 35135 (Liu & Stock, 1995), the *in vitro* responses to BRL 37344 (the metabolite of BRL 35135) in both were practically identical in almost all respects.

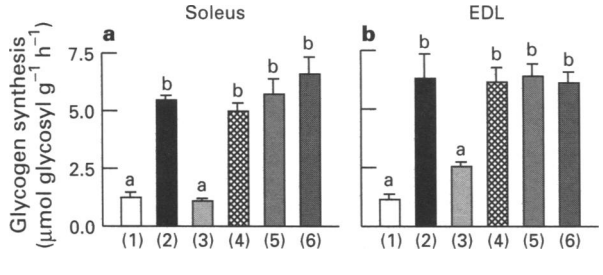

Figure 5 Effect of BRL (10^{-5}, 10^{-11} M) and BRL plus ICI 118551 (ICI; 10^{-6} M) on insulin-stimulated glycogen synthesis in soleus and EDL. Apart from the control, all muscles were incubated with insulin (0.6 μM). Results are mean values ± s.e.mean ($n=6$). Column sharing common superscript letters are not significantly different ($P>0.05$; Scheffe's test). In both panels: (1) control; (2) insulin; (3) BRL 10^{-5} M; (4) BRL 10^{-5} M + ICI 10^{-6} M; (5) BRL 10^{-11} M; (6) BRL 10^{-11} M + ICI 10^{-6} M.

BRL was found to be an exceptionally potent agonist of *in vitro* GU, with peak effects occurring as low as 10^{-11} M in both soleus and EDL. The lack of sufficient data points at the lowest concentrations used prevents estimation of EC_{50} values, but inspection of the results shown in Figure 1 suggests that the EC_{50} was approximately 10^{-12} M. This compares with nanomolar EC_{50} values for the activation of adipocyte lipolysis (Cawthorne *et al.*, 1992; Largis *et al.*, 1994), and micromolar IC_{50} values for inhibition of KCl-induced tone in rat colon (McLaughlin & MacDonald, 1990). Since adipocyte lipolysis by this agonist is thought to be mediated predominantly by the β_3AR, the potency of BRL in muscle suggests either that the muscle β_3AR is more tightly coupled to post-receptor mechanisms, or that there is a difference in the number of 'spare' receptors (as indicated by the binding studies of Roberts *et al.*, 1993 and Sillence *et al.*, 1993), or that the muscle βAR is atypical, i.e. not one of the three known subtypes (see below). The greater potency of BRL on muscle GU compared to adipocyte lipolysis may explain why its parent compound (BRL 35135) is effective in improving glucose tolerance in genetically obese rodents at doses that have no significant anti-obesity, lipid mobilizing effects (Cawthorne *et al.*, 1992). According to Largis *et al.* (1994), the other β_3AR agonist tested (CL 316,243) is slightly more potent in activating adipocyte lipolysis than BRL ($EC_{50} = 3.0\times10^{-9}$ M vs 8.4×10^{-9} M for BRL), but it proved to be 1–2 orders of magnitude less potent (10^{-9} M soleus; 10^{-10} M EDL) than BRL (10^{-11} M) in producing maximal increases in muscle GU. This also raises doubts as to whether the muscle receptor mediating the increase in GU is the β_3AR, even though the results with antagonists indicate that the receptor is clearly atypical.

Based on the pA_2 values for the two selective βAR antagonists (atenolol = 7.6 for β_1AR; ICI = 9.3 for β_2AR; Arch & Kaumann, 1993), even the lowest concentration used (10^{-8} M) should have resulted in a marked reduction (50%, or more) in the response to the agonist if the adrenoceptor involved was either the β_1AR or the β_2AR. Likewise, the highest concentration used (10^{-6} M) of either antagonist should have been sufficient to cause some inhibition of β_3AR-mediated responses (pA_2 for β_3AR = 5.1 and 5.8 for atenolol and ICI, respectively; Arch & Kaumann, 1993). In the event, neither antagonist at any of the three concentrations used had any effect on the stimulation of GU induced by 10^{-11} M BRL. This indicates that the stimulation of GU does not involve the β_1AR or β_2AR, and casts further doubt on a β_3AR involvement.

The inhibition of GU at the high (10^{-6} M) concentration of BRL was unaffected by atenolol, but was reversed by all three concentrations of ICI in EDL, and by the two higher concentrations in soleus, thereby implicating a β_2AR involvement in this inhibitory effect of the agonist. A β_2AR involvement would also explain why high concentrations of CL 316,243 failed to inhibit GU, since it has much greater selectivity for β_3AR than BRL. Based on potency ratios, CL 316,243 is 10,000 times more selective for β_3AR than β_2AR, whereas BRL is only 23 times more selective (Largis *et al.*, 1994). However, when concentrations of CL 316,243 rose above 10^{-9} M, there was a clear loss of selectivity as GU declined from peak levels towards control values.

The co-existence of two βAR subtypes with differing affinities for BRL and CL 316,243 helps explain the biphasic responses to the two agonists, but the metabolic responses underlying the opposing effects on GU have also to be considered. The cellular and molecular mechanisms by which activation of the atypical βAR stimulates glucose uptake is not known. Glucose transport across the cell membrane is mediated by a family of glucose transporters and the GLUT4 iso-

form is predominant in skeletal muscle (Fukumoto *et al.*, 1989; James *et al.*, 1989; Pessin & Bell, 1992). Both insulin and muscle contraction induce translocation GLUT4 from microsomal membranes to the plasma membrane (Klip & Paquet, 1990; Goodyear *et al.*, 1991), and it is possible that activation of the atypical βAR enhances glucose uptake via GLUT4 translocation and/or increasing the intrinsic activity of the GLUT4 present in the plasma membrane. Takahashi *et al.* (1992) and Shimazu (1994), for example, have reported that increased sympathetic activity following electrical stimulation of the ventromedial hypothalamus stimulates glucose transport in muscle and other peripheral tissues by increasing the intrinsic activity of the GLUT4 present in the plasma membrane.

Whatever the mechanism responsible for the atypical βAR-mediated increase in glucose uptake at low concentrations of BRL, the increased intracellular phosphorylation and utilization does not appear to result in increased glycogen synthesis. The failure of 10^{-11} M BRL to enhance insulin-stimulated glycogen synthesis (Figure 5) could have been because the concentration of insulin used had produced maximal rates of glycogen synthesis, i.e. the rate could not be increased further. However, a subsequent experiment (see Results) showed that 10^{-11} M BRL had no effect on basal glycogen synthesis, or when the insulin concentration was reduced to a lower, submaximal concentration. This suggests that the increased GU following activation of the atypical βAR is due to increased oxidation, and would be consistent with the suggestion (Thurlby & Ellis, 1986) that skeletal muscle is partly responsible for the thermogenic effects of selective β_3AR agonists.

The glycogenolytic effects of adrenaline are well known, and so it was not surprising to find that the loss of βAR selectivity at a high (10^{-5} M) concentration of BRL resulted in decreased GU and decreased glycogen synthesis. β_2AR activation of glycogenolysis will not only inhibit net glycogen synthesis, but also increase the intracellular level of glucose-6-phosphate, and this will inhibit hexokinase activity and glucose transport across the cell membrane. This β_2AR inhibitory effect on GU and the lower β_2AR activity of CL 316,243 compared to BRL (see above), probably explains why CL 316,243 was more effective than BRL in stimulating GU at low concentrations, even though it was less potent (10^{-10} M CL 316,243 caused a maximal 45% stimulation of GU in EDL, compared to a maximum of 24% for 10^{-11} M BRL).

The present study has shown that the co-existence of atypical βAR and β_2AR in skeletal muscle could explain the biphasic effects of BRL on GU, but even if, as seems unlikely, the atypical receptor is the β_3AR, the ability of two βAR subtypes to exert opposite effects raises questions about the immediate post-receptor mechanisms. Typically, βAR are thought to activate G_s proteins, although there is evidence that these receptors can also be coupled to G_i proteins in the same tissue (for review see Granneman, 1995). An example is the findings of Chaudhry *et al.* (1994), who have shown that β_3AR in rat adipocytes interact with both G_s and G_i, with the G_i proteins acting to limit the β_3AR stimulation of adenylyl cyclase activity. Thus, it is possible that the atypical and β_2AR are linked to different G_s and/or G_i proteins in skeletal muscle. However, even if these G-proteins were linked to different isoforms of adenylyl cyclase (for review see Milligan, 1993), this could not explain how intracellular changes in cyclic AMP induced by the two receptor subtypes results in opposing effects on GU, unless there was functional compartmentalization of cyclic AMP-dependent protein kinase(s), as has been suggested for adipocytes (Hollenga *et al.*, 1991; Langin *et al.*, 1992; Murphy *et al.*, 1993). The final possibility is that the atypical βAR may modulate transmembrane signalling pathways that are independent of adenylyl cyclase, since it has been shown that in bovine skeletal muscle membranes there was no atypical βAR-mediated increase in cyclic AMP production with isoprenaline or BRL, although there were atypical binding sites for βAR ligands (Sillence & Matthews, 1994). Clearly, further investigations are required before any conclusions can be made about the nature, physiological role and post-receptor mechanisms of this atypical βAR in skeletal muscle. More importantly, further molecular confirmation of the existence of the atypical βAR in muscle (Summers *et al.*, 1995) is required, as well as determining if its structure corresponds to that of the β_3AR.

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