Br J Clin Pharmacol 1996; **42**: 291–300

Clinical pharmacology of β_3-adrenoceptors

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1 An atypical non β_1/β_2-adrenoceptor (AR) subtype (β_3-AR) has been identified which is selectively stimulated by a group of ligands which mediate lipolytic and thermic responses in brown and white adipose tissue.
2 Molecular studies have shown that β_3-AR in man are mainly expressed in visceral adipocytes, and to a lesser extent in gall-bladder and colon. *In vitro* studies with β_3-AR agonists have shown activity at other sites including skeletal muscle and myocardium.
3 Regulation of β_3-AR may differ from β_1/β_2-AR subtypes in that continuous agonist exposure does not result in receptor down-regulation.
4 A polymorphism of the human β_3-AR gene (Trp64Arg) has been identified which is associated with obesity, insulin resistance and an earlier onset of non-insulin-dependent diabetes mellitus (NIDDM). Studies are required to establish whether expression of the mutant gene results in altered metabolic responses to β_3-AR stimulation in man.
5 There is accumulating evidence to support a therapeutic role of β_3-AR agonists in NIDDM because of anti-obesity and anti-diabetic activity, as a consequence of thermogenic effects as well as increased insulin sensitivity and glucose tolerance.
6 Selectivity studies with BRL35135 and isoprenaline in humans have demonstrated a β_3-AR mediated component to thermogenesis which is dissociated from β_1/β_2-mediated effects on carbohydrate and fat metabolism. Similar studies have suggested a functional β_3-AR mediating cardiac but not airway responses in humans. An evaluation of β_3-AR agonists in irritable bowel syndrome may be warranted in view of colonic antimotility properties *in vitro*.

Keywords β_3-adrenoceptors lipolysis thermogenesis

Introduction

Since the original classification by Lands *et al.* in 1967 [1], it has become evident that the heterogeneity of β-adrenoceptors (β-AR) extends beyond the β_1 and β_2-AR subtypes. In 1983 Tan *et al.* [2] was the first to postulate the existence of an atypical β-AR in rat adipocytes and suggested this as the putative β_3-AR. Subsequently detailed *in vitro* pharmacological studies with both animal and human tissue have identified a number of ligands which stimulate lipolysis on putative β_3-AR to a much greater degree than their effects on β-AR in atria (β_1) or trachea (β_2). The gene encoding for rat and human β_3-AR has now been sequenced and cloned which in turn has provided more detailed information on the pharmacology of the β_3-AR [3–5]. The β_3-AR is ubiquitous being present in adipocytes, skeletal muscle, as well as smooth muscle in gut, heart and airway tissue. Despite this rapid expansion of knowledge regarding the *in vitro* pharmacology of β_3-AR, *in vivo* studies in humans are more limited as a consequence of a structured development programme for these compounds within the pharmaceutical industry. Nonetheless, with increasing interest in possible therapeutic applications of β_3-AR agonists, and particularly with development of more selective agents, it now seems appropriate to review briefly the clinical pharmacology of β_3-AR. The purpose of this article is not to provide a detailed review of the pharmacology of β_3-AR, but more to highlight key *in vitro* studies and how these relate to effects of β_3-agonists in man.

Key pharmacological studies of β_3-AR

β_3-AR in adipocytes

Much of the earlier work on β_3-AR pharmacology was done with novel arylethanolamines developed by

Beecham Pharmaceuticals. Arch *et al.* [6] investigated the effects of three Beecham compounds on rat brown adipocyte tissue (BAT). The compound BRL 37344 (free acid of BRL 35135A) exhibited 400-fold selectivity for lipolysis over atrial rate (β_1) and 20.5-fold selectivity over tracheal relaxation (β_2). A contrast was clearly seen comparing lipolysis selectivity ratios for salbutamol of 0.35 for atria and 0.01 for trachea. In comparison with isoprenaline, BRL 37344 exhibited five-fold greater potency for stimulation of lipolysis, but was 5.3-fold less potent for stimulation of atrial rate. In addition, it was shown that β-AR antagonism by atenolol (β_1) ICI 118,551 (β_2) and propranolol ($\beta_1 + \beta_2$) showed much lower affinities for lipolytic than for tracheal responses to isoprenaline and BRL 28410 (free acid of BRL 26830A). Taken together these findings were a clear demonstration that an atypical β-AR in rat BAT did not conform to the usual β_1- or β_2-subtypes. Similar results were found by Wilson *et al.* [7] for effects on lipolysis in rat white adipose tissue (WAT) comparing BRL compounds with conventional β-AR agonists, as well as showing lower affinity of antagonists for adipocytes than atria or trachea.

Hollenga & Zaagsma [8] evaluated effects of β_1-AR antagonism with CGP 20712A and β_3-AR antagonism with ICI 118,551 on the lipolysis response to isoprenaline and BRL 37344. Since CGP 20712A only inhibited responses to BRL 37344 at higher concentrations (100 μM), this suggested that BRL 37344 must be acting not via β_1-AR but via an atypical β-AR. This conclusion was further supported by the effects of ICI 118,551 in that pA_2 values were well below those typically found with β_1 or β_2-AR mediated responses. Similar results were found with isoprenaline suggesting that its effects on lipolysis were also mediated by atypical β_3-AR. In a further study from Hollenga *et al.* [9] it was shown that whilst BRL 37344 was a potent β-AR agonist on lipolysis in rat WAT, it was only a weak agonist on human WAT.

Molecular pharmacological studies

In 1989 Emorine *et al.* [3] described the isolation of the human gene which encoded for the β_3-AR, and Chinese hamster ovary (CHO) cells were then transfected with this gene in order to characterize its cellular expression. Binding studies with [^{125}I]-iodocyanopindolol (ICYP), a β-AR ligand, showed an association constant for the β_3-AR which was almost 10 times greater than that for β_1 or β_2-AR. The accumulation of cyclic AMP in response to β-AR agonist stimulation showed that BRL 37344 exhibited much greater potency in CHO-β_3 cells than CHO-β_2 or CHO-β_1 cells. The effects of classical β-AR antagonists were also evaluated in terms of effects on isoprenaline induced cyclic AMP accumulation in CHO-β_1,β_2 and β_3 cell lines. This showed that out of 11 classic β-AR antagonists, only CGP 20712A and ICI 118,551 produced efficient inhibition of cyclic AMP accumulation in CHO-β_3 cells, whilst pindolol and oxprenolol exhibited partial agonist effects by augmenting cyclic AMP accumulation compared with isoprenaline. Subsequently, Granneman *et al.* [4] cloned the rat β_3-AR gene which was then expressed in CHO cells. Species differences were observed in that BRL 37344 had a much higher affinity than noradrenaline in rat β_3-AR, whilst in human β_3-AR both ligands exhibit equal affinity [3]. Granneman *et al.* [5] went on to perform further sequence analysis in cloned human and rat β_3-AR genes and showed evidence of both structural and functional homology between the two species.

Regulation of β_3-AR

It is well recognized that reduced expression of β_1/β_2-AR due to down-regulation occurs with continuous agonist stimulation and results in an attenuated cyclic AMP response and tachyphylaxis [10, 11]. The situation with respect to β_3-AR expression is less clear cut with different studies showing either down-regulation or paradoxical up-regulation. Granneman *et al.* showed that continuous exposure to noradrenaline or BRL 26830A produced a reduction in levels in mRNAof β_3-AR in BAT [12]. In a further study from the same laboratory it was found that exposure of isolated adipocytes to isoprenaline or BRL 37344 produced selective desensitization of adenylate-cyclase activity in β_1 but not β_3-AR [13]. Carpene *et al.* [14] also reported similar findings of desensitization of β_1 and β_2 but not β_3-AR for lipolytic responses in adipocytes after long-term noradrenaline exposure. Thomas *et al.* [15] found a paradoxical up-regulation in expression of β_3-AR mRNA following chronic exposure of adipocytes to isoprenaline. This phenomenon was thought to be due to specific cyclic AMP response elements within the β_3-AR gene which result in enhanced transcription of β_3-AR mRNA. Liggett *et al.* [16] went on to perform chimeric β-AR experiments to characterize the amino acid sequences of the C-terminal regions of the β_2 and β_3-AR which in turn determine agonist induced sequestration and down-regulation of receptors.

Tissue distribution of β_3-AR

There is now increasing evidence that β_3-AR are present in other sites apart from adipose tissue. In 1987 Bond & Clarke [17] suggested that relaxant effects of isoprenaline in guinea-pig ileum were not mediated by classical α or β-AR subtypes. It was subsequently shown by the same group [18] that BRL 37344 exhibited greater potency than noradrenaline and adrenaline, whilst effects of BRL 37344 and isoprenaline were resistant to a high concentration of propranolol. In 1990 Norman & Leathard [19] suggested that inhibition of spontaneous rhythmical contractions of isolated rabbit duodenum by ritodrine and salbutamol were mediated by an atypical β_3-AR. Other studies with BRL 37344 have characterised the presence of β_3-AR in rat oesophagus and guinea-pig gastric fundus [20–22]. Antagonist studies with compounds developed by Sanofi Pharmaceuticals (SR 58306A and SR 58339A) have

shown highly selective inhibition of rat colon contractility, at concentrations which have no effect on guinea-pig atrial rate or tracheal relaxation [23]. Similar results have been reported with the compound SR 58611A showing a high degree of selectivity for β_3-AR in rat colon and rat BAT [24, 25]. This correlation between effects on lipolysis and colon motility suggests that it is the same β_3-AR which mediates responses in both of these tissues [25]. The colonic antimotility properties of β_3-AR agonists *in vitro* might suggest a possible therapeutic role in irritable bowel syndrome.

There is also some evidence to suggest that β_3-AR may also be present in skeletal muscle and that this may mediate metabolic responses. Challiss *et al.* [26] showed that BRL 28410 (free acid of BRL 26830A) exhibited greater potency at β-AR receptors of rat soleus muscle in terms of lactate formation and inhibition of glycogen synthesis than at β_1 or β_2-AR in atria and uterus respectively. This was supported by a pA_2 value of propranolol for antagonism of BRL 28410 on glycogen synthesis being lower than the pA_2 value for antagonism of isoprenaline, the latter being in the expected range for β_1/β_2-AR. Roberts *et al.* [27] went on to characterise propranolol resistant [^{125}I]-iodocyanopindolol binding sites in rat soleus muscle showing that the β_3-AR agonists BRL 37344, SR 58611A and ICI D7114 all exhibited competitive binding suggesting the presence of an atypical β_3-AR at this site.

Studies have also suggested the presence of β_3-AR mediating secretary function in ferret and canine tracheal epithelium [28, 29]. It has also been shown in dose response studies with BRL 37344 in canine bronchial smooth muscle that β_3-AR mediate bronchorelaxant effects [30], whilst other data have suggested a role of β_3-AR in mediation of prejunction inhibition of non-adrenergic non-cholinergic contraction in guinea-pig bronchus [31]. However, Martin *et al.* have subsequently shown with BRL 37344 and SR 58611A that β_3-AR mediated bronchorelaxation does not occur in guinea-pig, sheep or human airway smooth muscle [32]. There are also studies with rabbit tracheal and canine bronchial epithelium to indicate that stimulation of ciliary motility is mediated by β_3-AR [33, 34]. An effect of BRL 35135 on guinea-pig eosinophil chemotaxis which is resistant to propranolol blockade has also been described again inferring a role of β_3-AR in mediating this action [35].

Kaumann was the first to speculate the existence of β_3-AR in the heart, on the basis of studies with β-AR partial agonists such as pindolol which stimulate atrial rate through non-β_1/β_2-AR [36]. This would be in keeping with the agonist activity of pindolol on human β_3-AR CHO cells [3]. It has been shown in sinoaortic denervated dogs that isoprenaline unlike BRL 37344 has a chronotopic effect, suggesting that baroreceptor mechanisms rather than cardiac β_3-AR stimulation is responsible for this effect [37]. In another study with isolated dog atria the inotropic and chronotropic effects of BRL 37344 and isoprenaline were blocked by β_1-AR but not β_2-AR antagonism [38].

Molecular pharmacology techniques have also been applied to investigate the tissue distribution of human β_3-AR [39]. In man, β_3-AR mRNA is present in BAT and WAT and is also expressed in gall bladder and colon. Furthermore mRNA for mitochondrial uncoupling protein, which is specific for BAT is present in infant and adult whole adipose tissue, suggesting that significant amounts of brown adipocytes persist throughout life. However, there was no expression of β_3-AR mRNA in quadriceps or abdominal skeletal muscle, heart, liver, lung, thyroid or lymphocytes.

β_3-AR genetic polymorphism

Recent data has emerged to suggest that polymorphism of the human β_3-AR gene at position 64 (Trp64Arg) is associated with obesity, insulin resistance and early onset of non-insulin-dependent diabetes mellitus (NIDDM). Clement *et al.* in a French study showed that obese people who were heterozygotes for the Trp64Arg gene mutation had an increased capacity to gain weight compared with those without the mutation [40]. The frequency of the Trp64Arg allele was similar in obese compared with normal subjects.

The same mutation was detected with an allelic frequency of 0.31 in Pima Indians, 0.13 in Mexican Indians, 0.12 in black Americans and 0.08 in white Americans [41]. It was also found that Pima subjects homozygous for the mutation had an earlier onset of NIDDM and had a lower resting metabolic rate. Studies in Finnish subjects showed that the mutation frequency was similar in non-diabetic subjects (12%) and subjects with NIDDM (11%), although the mean age for onset of diabetes was lower in those with the mutation [42]. In the non-diabetic subjects, those with the mutation exhibited a greater degree of obesity and impaired insulin sensitivity. It would be particularly interesting to know whether the mutant β_3-AR allele is associated with altered *in vivo* expression in terms of metabolic response to β_3-AR stimulation in obesity and NIDDM.

Effects of β_3-AR agonists in vivo

Metabolic effects

Arch *et al.* [43] evaluated the anti-obesity and thermogenic effects of BRL 26830A in genetically obese mice and rats. Repeated dosing resulted in a reduction in body weight in obese but not lean animals. These effects were mirrored by the thermic activity of BRL 26830A in terms of energy expenditure which was inhibited selectively by (±)-propranolol. Interestingly BRL 26830A was found to have no significant anorectic activity, producing no reduction in intake of food over a 7 week period.

Zed *et al.* [44] evaluated the anti-obesity activity of BRL 26830A in a double-blind placebo controlled study of 16 obese human subjects. Each group of subjects was started on an individualized slimming diet continued over 8 weeks, with either BRL 26830A 50 mg four times

daily or placebo being given for 6 weeks followed by 2 weeks of placebo in both groups. Weight loss was greater with BRL 26830A compared with placebo (mean loss of 9.34 kg *vs* 6.56 kg), whilst energy expenditure was maintained in the BRL group but fell in the placebo group. Furthermore, after switching to placebo in both groups during the last 2 weeks, energy expenditure in the BRL group was also found to fall. It was therefore concluded that the mechanism for weight loss with BRL 26830A was by preventing the fall in energy expenditure associated with reduced energy intake as occurs in obesity.

Connacher *et al.* [45] went on to assess effects of BRL 26830A (50 mg four times daily for 2 weeks followed by 100 mg four times daily for 16 weeks) or placebo in 40 obese subjects given an 800 kcal high-fibre low fat diet in a parallel group double-blind study. Weight loss was found to be greater in the BRL treated group (15.4 kg at 18 weeks) compared with placebo (10.0 kg at 18 weeks), resulting in a mean weekly additional weight loss of 0.3 kg with a combination of diet and BRL 26830A. Furthermore, after 6 weeks there was a greater fall in resting metabolic rate in the placebo group compared with the BRL group. It was also shown after a single 100 mg dose of BRL 26830A there was an 11.6% increase in energy expenditure after 2 h. This infers that the weight loss with BRL 26830A was due to enhanced thermic activity and preventing the fall in energy expenditure associated with reduced energy intake during dieting. In terms of other β-AR mediated adverse effects, BRL 26830A had no significant effect on resting pulse rate or blood pressure, although there was subjective reporting of tremor which tended to diminish after the first 10 weeks. There was no significant difference between the active and placebo groups in terms of serum cholesterol, high density lipoprotein cholesterol, triglyceride, fasting plasma glucose or insulin concentration. In a further study [46], it was shown that a single 50 mg dose of BRL 26830A produced increases in objective finger tremor measured by accelerometer, although no concomitant β_2-AR antagonist was given to enable any conclusions to be drawn regarding β-AR subtype selectivity of the drug.

Wheeldon *et al.* [47, 48] have performed studies to dissect out the β-AR subtypes mediating the metabolic effects of isoprenaline and BRL 35135. In the first of these studies normal volunteers received infusions of isoprenaline (β_1, β_2 and β_3-AR agonist) at 0.5–3.0 mg min^{-1} for 30 min, with the rate of the infusion predetermined for each subject to increase heart rate by approximately 60 beats min^{-1} [47]. Subjects were randomized in a crossover design to receive prior to the infusion single doses of either placebo, atenolol 25 mg, or nadolol in doses of 5 mg, 20 mg and 80 mg. Measurements at baseline and during isoprenaline included basal metabolic rate, heart rate, finger tremor and biochemical parameters of carbohydrate and lipid metabolism. Isoprenaline markedly increased basal metabolic rate by 30.6% and this was significantly reduced to 17.4% by β_1-blockade with atenolol but not 5 mg nadolol. Significant β_2-blockade (from tremor data) occurred with 5 mg nadolol but not with 25 mg atenolol, suggesting that β_1 but not β_2-AR were involved in mediating the thermogenic effects of isoprenaline. However the rise in basal metabolic rate was not totally blocked even by 80 mg nadolol (9.5% increase), which produced complete β_1/β_2-antagonism as evidence by the elimination of the heart rate response to isoprenaline (Figure 1a). There were also significant increases in plasma free fatty acid, glycerol, glucose, insulin and lactate which were completely abolished by β_1/β_2-blockade (Figure 1b,c). Thus, isoprenaline produced an increase in basal metabolic rate which had a non-β_1/β_2-mediated component, and which was not associated β_1/β_2-AR mediated effects on carbohydrate or fat metabolism. It is therefore implied that the thermogenic response to isoprenaline was partially mediated by β_3-AR in man.

In the second study, normal subjects were given single oral doses of BRL 35135 (8 mg) or the selective β_2-

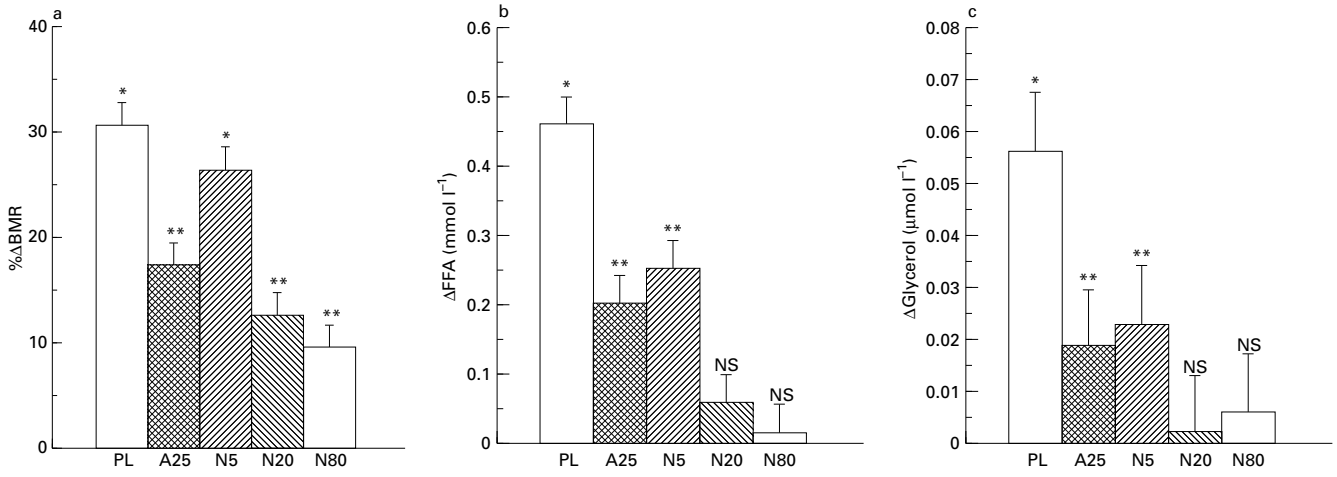

Figure 1 (a) Effects of isoprenaline on basal metabolic rate after pre-treatment with placebo (PL), atenolol 25 mg (A25), nadolol 5 mg, 20 mg, 80 mg (N5, N20, N80) $*P<0.05$ *vs* pre-treatment baseline, $**P<0.05$ *vs* placebo. (b) Effects of isoprenaline on serum free fatty acids and (c) glycerol (symbols as in Figure 1a). NS: not significantly different from baseline. Reproduced from ref. 47 (Wheeldon *et al. Quart J Med* 1993; **86**: 595–600), with permission of Oxford University Press and Association of Physicians.

agonist salbutamol (8 mg), after pre-treatment with placebo, bisoprolol (5 mg) as a selective β_1-antagonist or nadolol (20 mg) to block β_1 and β_2, but not β_3-AR [48]. Both salbutamol and BRL 35135 produced a significant fall in serum potassium in keeping with β_2-AR stimulation. Both drugs also caused an increase in serum glucose, insulin and lactate, which mirrored the hypokalaemic response in being unaffected by selective β_1-blockade but completely blocked by nadolol. BRL 35135 but not salbutamol also produced a rise in serum free fatty acid and glycerol which were also β_2-AR mediated (Figure 2a,b,c). A significant increase in basal metabolic rate was observed with both BRL 35135 (23.8% increase *vs* placebo) and salbutamol (16.7% increase *vs* placebo) (Figure 2d,e). For salbutamol this effect was mediated solely by β_2-AR, being blocked by nadolol but not bisoprolol. In contrast the thermogenic response to BRL 35135 was only partially blocked by bisoprolol (15.5% increase *vs* placebo) or nadalol (8.9% increase *vs* placebo). These results with BRL 35135 and with isoprenaline therefore support the presence of thermogenic β_3-AR in man, although these do not appear to be involved in the control of either carbohydrate or fat metabolism *in vivo*. However, more detailed assessment of fatty acid oxidation, as for example using [^{13}C]-palmitate infusion, may be required to fully characterize the role of β_3-AR stimulation in mediating lipolysis.

Hoffmann—La Roche Pharmaceuticals also developed a series of bis-phenylethanolamine compounds including Ro16–8714 and Ro40–2148. Studies in normal and obese human subjects with Ro16–8714 showed a marked dose-dependent thermic response with an increase in energy expenditure up to 27%, and associated dose-related increases in heart rate and systolic blood pressure [49, 50]. This compound was superceded by Ro40–2148 which was shown to be more β_3 selective in animal studies. A single oral 800 mg dose of Ro40–2148 given to non-obese normal subjects produced an increase in energy expenditure ranging from 2.7 to 17.3% without inducing heart rate or blood pressure effects [51].

ICI Pharmaceuticals developed β_3-AR agonists including ICI D7114 which in animals studies was not shown to selectively stimulate BAT metabolism without β_1 or β_2-AR mediated effects [52]. However in clinical trials with either lean or obese human volunteers, ICI D7714 turned out to be only a low efficacy partial agonist which had no effect on energy expenditure or lipid metabolism [53, 54]. The American Cyanamid pharmaceutical company has developed a phenylethano-

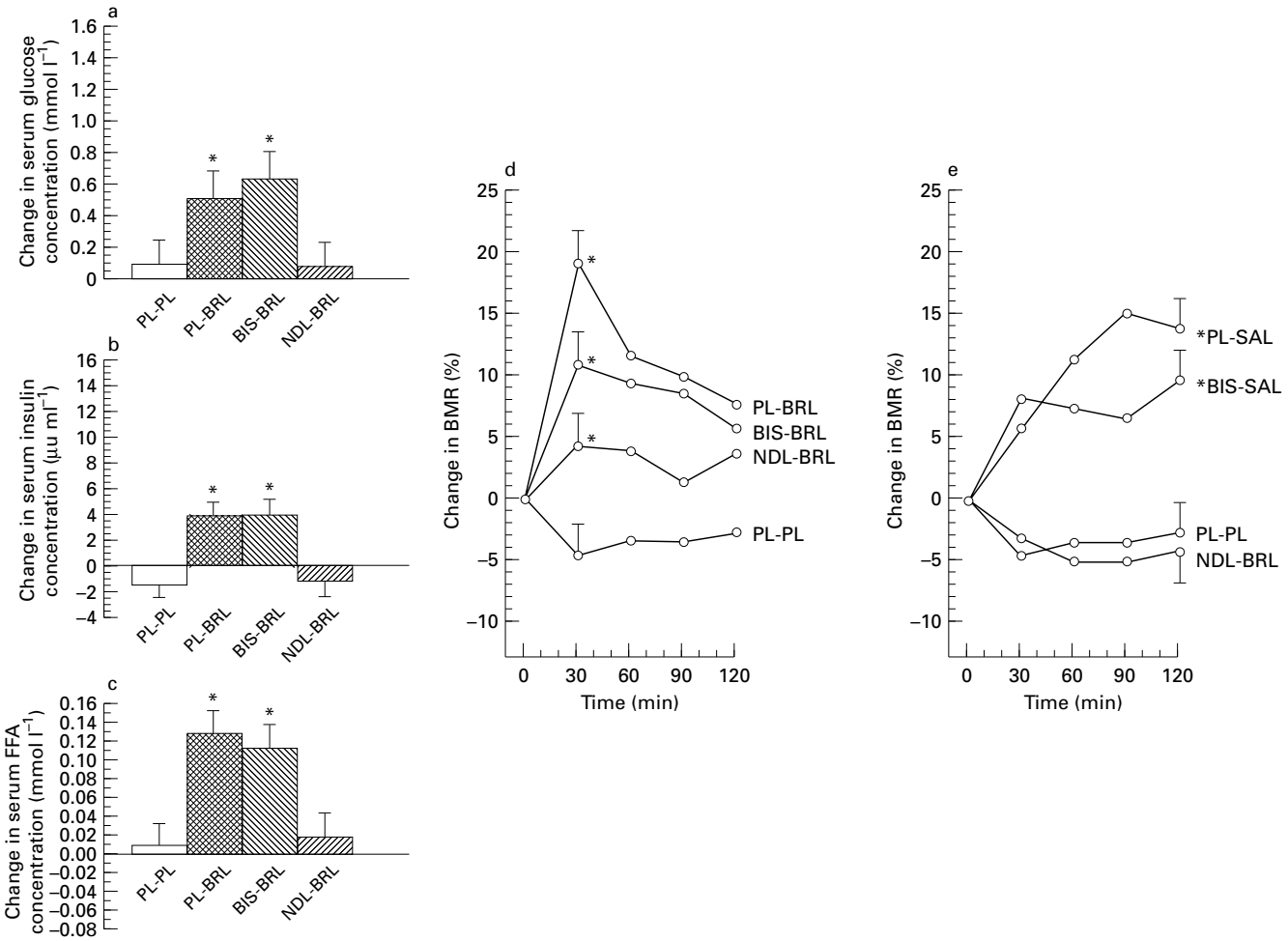

Figure 2 Effects of BRL 35135 on (a) serum glucose, (b) insulin and (c) free fatty acid concentration after pre-treatment with placebo (PL), bisoprolol 5 mg (BIS) or nadolol 20 mg (NDL). *$P<0.05$ compared with PL. Response of basal metabolic rate (BMR) to single oral doses of (d) BRL 35135 8 mg (BRL) and (e) salbutamol 8 mg (SAL)—after pre-treatment with placebo (PL), bisoprolol 5 mg (BIS) or nadolol 20 mg (NDL). *$P<0.05$ compared with PL. Reproduced from ref. 48 (Wheeldon *et al.*, *Clin Sci* 1994; **86**: 331–337), with permission from the Biochemical Society and Portland Press.

lamine related compound CL 316,243 which in initial *in vitro* and *in vivo* studies has been shown to be a potent selective β_3-AR which stimulates BAT lipolysis and thermogenesis [55].

Effects on glucose metabolism

It is now considered that defective thermogenesis which results in obesity is probably related to insulin resistance. As a consequence to this, drugs which enhance a sensitivity to endogenous insulin may restore thermogenic activity in obese individuals. In this respect β_3-AR agonists were investigated as a possible therapeutic option in patients with NIDDM because of anti-obesity activity as well as possible anti-diabetic activity, particularly in view of the insulin resistance and weight gain associated with the sulphonylurea type drugs.

The Beecham group of compounds were extensively investigated for their putative anti-diabetic properties. Studies with their BRL 26830A in normoglycaemic rats showed dose dependent improvements in glucose tolerance, and showed that the compound was a potent insulin secretagogue [56]. Studies in obese rats with BRL26830A also showed evidence of improved glucose tolerance and increased insulin sensitivity with enhanced glucose utilization [57]. Further data in rodents showed BRL 26830A to have comparable activity with glibenclamide and glipizide in terms of improving glucose tolerance [58].

Smith *et al.* [59] then went on to evaluate the effects of 10 days treatment with either BRL 26830A or placebo in normal volunteers using the hyperinsulinaemic-euglycaemic clamp technique, and showed a 20% reduction in fasting plasma insulin levels and increased insulin sensitivity during clamping. Connacher *et al.* [60] evaluated the effects of BRL 26830A in a dose of 100 mg three times daily given for 3 weeks to obese non-diabetic women who were not calorie restricted. The results showed reduced levels of insulin with unchanged glucose after treatment with BRL 26830A, along with a fall in plasma concentration of glycerol and palmitate, suggesting an improvement in insulin sensitivity.

The Beecham compound BRL 35135 also showed similar anti-diabetic properties to BRL 26830A in animal studies [61, 62]. Mitchell *et al.* evaluated the effects of BRL 35135 given for 10 days (2 mg four times daily for 5 days and 6 mg four times daily for 5 days) in 10 obese subjects with an oral 100 g glucose tolerance test performed pre- and post-treatment along with glucose oxidation and storage measured by indirect calorimetry [63]. Both plasma glucose and serum insulin concentrations showed reductions for area under curve (180 min) for comparison of pre- *vs* post-treatment with BRL 35135, indicating improved glucose tolerance and insulin sensitivity (Figure 3). These effects were associated with increased glucose storage with unchanged glucose oxidation. A further evaluation was performed in 18 obese patients with NIDDM who were given 10 days treatment with either BRL 35135 or placebo, with the active drug showing a 91% increase in insulin-mediated glucose disposal during clamping, suggesting increased insulin sensitivity [64]. BRL 35135 had no significant effects on either fasting blood glucose or insulin concentrations during the study period, whilst tremor was experienced in three out of 12 patients. It is interesting to note, that whilst the thermogenic effects

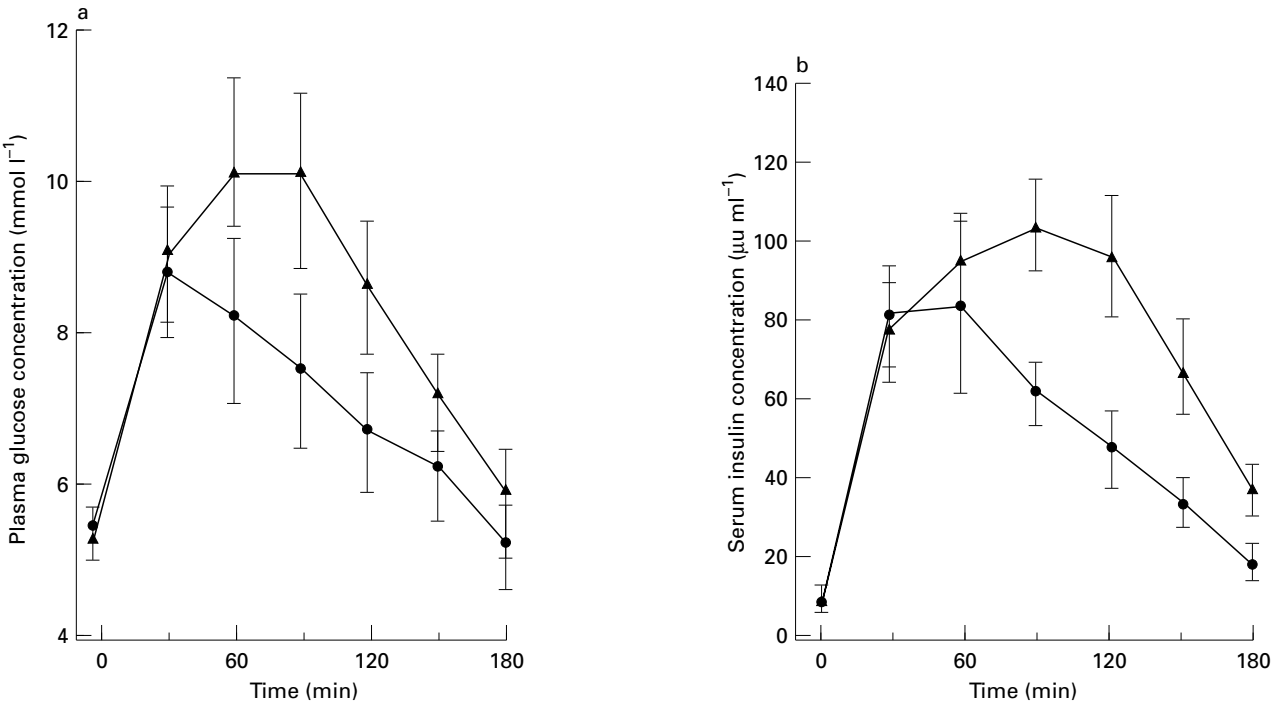

Figure 3 Effects (mean ± s.e. mean, $n = 10$) of 10 days treatment with BRL 35135 in overnight fasting obese subjects on (a) glucose tolerance and (b) insulin secretion following a 100 g oral glucose load before (triangles) and after (circles) treatment. Reproduced from ref. 63 (Mitchell *et al.*, *Int J Obesity* 1989; **13**: 757–766) with permission from the MacMillan Press.

of BRL 35135 are partially mediated by β_3-AR, the release of glucose, insulin and lactacte are mediated solely by β_2-AR [48].

Cardiac and airway effects

The possibility of putative β_3-AR was raised by Kaumann on the basis of unusual dose-related chronotropic activity with partial β_3-AR agonists [36]. Studies have subsequently been performed in normal humans with BRL 35135 and isoprenaline [65, 66]. In the first study, normal subjects were randomized to receive single oral doses of either BRL 35135 8 mg or salbutamol 8 mg, after pre-treatment with either placebo, bisoprolol 5 mg or nadolol 20 mg. Both BRL 35135 and salbutamol produced an increase in finger tremor which was blocked by nadolol but not bisoprolol and was mediated by β_2-AR. Likewise increases in systolic blood pressure and Doppler stroke distance (linear analogue of stroke volume) occurred with BRL 35135 and salbutamol which were also β_2-AR mediated. There were also chronotropic and minute distance (linear analogue of cardiac output) responses to BRL 35135 and salbutamol which were unaffected by β_1-AR antagonism. However, whereas nadolol blocked these responses to salbutamol, there was still a small significant chronotropic and minute distance response remaining with BRL 35135 plus nadolol in comparison with placebo, despite complete antagonism of co-existing β_2-AR effects. These findings suggest that BRL 35135 produced most of its cardiac effects via stimulation of β_2-AR, although there remains a possibility of β_3 mediated component to its response which was not seen with salbutamol. It is of course possible that blockade of β_2 mediated tremor responses by nadolol does not necessarily imply the same degree of blockade of cardiac β_2-AR. This hypothesis is unlikely in that other cardiac responses to BRL 35135 including systolic blood pressure and stroke distance were also abolished by nadolol, as were all cardiac responses to salbutamol.

In a further study the cardiac effects of isoprenaline infusion were investigated after prior treatment with placebo, atenolol 25 mg or nadolol in doses of 5 mg, 20 mg and 80 mg [66]. The idea behind dose ranging with nadolol was to evaluate the lowest dose required to produce significant β_2-blockade, as a high dose might conceivably cause β_3-blockade. The results showed that nadolol 5 mg produced almost complete blunting of finger tremor whilst atenolol had no significant effect. Chronotropic and minute distance responses to isoprenaline were consistent with stimulation of β_1 and β_2-AR with partial blockade by both atenolol 25 mg and nadolol 5 mg, and further blockade by nadolol 20 mg. However, isoprenaline produced an increase in systolic blood pressure and stroke distance that was not attenuated by a dose of nadolol (20 mg) which produced complete blunting or β_1 and β_2-AR responses, inferring the possibility of functional inotropic or lucitropic β_3-AR in the human heart. The problem with the above two studies is evident in terms of the lack of an available highly selective β_3-AR agonist or antagonist, and hence the reason for using conventional β_1 and β_2-AR antagonists for dissecting out β-AR subtypes in the heart. The question as to the presence of putative β_3-AR in the human heart therefore remains unresolved. The effects of isoprenaline and BRL 37344 in humans are in complete contrast with those found in isolated blood perfused dog atria, where inotropic and chronotropic responses to BRL 37344 and isoprenaline were mediated by β_1-AR in terms of blockade by bisoprolol and propranolol but not ICI 118,551 [38]. This suggests that there are likely to be species differences in β_3-AR distribution, although molecular pharmacology studies have suggested that human cardiac tissue as such is devoid of β_3-AR [39].

The effects of the Zeneca compound ZD 2079 on cardiac responses to isoprenaline and exercise in healthy volunteers have been reported in preliminary abstract form [67, 68]. A single 60 mg dose of ZD 2079 augmented the chronotropic but not inotropic response to both isoprenaline and exercise. However, since responses were also not evaluated in the presence of $\beta_1$or β_2-AR blockade, it is not possible to deduce from these data which cardiac β-AR subtypes were responsible for mediating these effects, although *in vitro* studies have suggested that the compound is selective for the β_3-AR [69].

The airway effects of BRL 35135 in man have also been investigated in a study of normal subjects who demonstrated a fall in airways resistance in response to salbutamol [70]. Airway and metabolic responses were evaluated after a single 8 mg dose of BRL 35135 on two separate days having been pre-treated with either placebo or nadolol 20 mg, or on another day after a single dose of placebo having been also pretreated with placebo. There was a 32% mean fall in airways resistance which was antagonized by nadolol, like other β_2-mediated responses including potassium and glucose, whereas the lipolysis response was not antagonized by nadolol. These results do not suggest the presence of functional β_3-AR in human airways. These *in vivo* findings in humans have now been substantiated by *in vitro* studies in human airways with BRL 37344 and SR 58611A [32].

Future prospects for β_3-AR agonists

Further clinical studies and closer co-operation with the pharmaceutical industry are required with some of the newer more potent highly selective β_3-AR agonists, some of which are currently undergoing Phase I and Phase II evaluation. In particular, careful chronic dosing evaluation will be required to investigate whether tachyphylaxis occurs, in view of conflicting *in vitro* studies regarding effects on β_3-AR regulation. There are already promising *in vivo* data to suggest a therapeutic role for β_3-AR agonists as possible anti-diabetic and anti-obesity agents and larger multicentre type studies are now indicated. It would also be interesting to know whether polymorphism of the β_3-AR gene is associated with an altered phenotypic expression in terms of *in*

vivo metabolic response to β_3-AR stimulation. Other potential areas for treatment with β_3-AR agonists include the disorders of gastrointestinal motility such as irritable bowel syndrome, on the basis of *in vitro* studies showing potent colonic antimotility activity. The next decade of research with increasing knowledge of *in vitro* and *in vivo* pharmacology, along with the development of newer more potent and selective compounds, will hopefully deliver the promise of therapeutic agents acting on β_3-AR.

The author wishes to acknowledge the assistance of Mrs J. Thomson in preparing the manuscript.

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(*Received 13 February 1996, accepted 10 April 1996*)