**Beta-3 Adrenergic Receptor Agonists Cause an Increase in Gastrointestinal Transit Time in Wild-type Mice, But Not in Mice Lacking the Beta-3 Adrenergic Receptor**

**ABSTRACT**

The effects of beta-3 adrenergic receptor (β3-AR) agonists on gastrointestinal (GI) motility, as measured by stomach retention and intestinal transit of radiolabelled charcoal, were compared in wild-type (WT) mice and in transgenic mice lacking β3-AR (β3-AR[KO]) or having β3-AR in white and brown adipose tissue only (β3-AR[WAT+BAT]). After s.c. administration of 3 mg/kg of the selective, rodent specific β3-AR agonists BRL 35135, CL 316,243 or ICI 198,157, WT mice exhibited a significant decrease in the extent of movement of radiotracer through the stomach and intestines, indicative of decreased GI motility. These compounds also caused an increase in plasma glycerol levels in the WT mice, suggesting that increased lipolysis in adipose tissue had been evoked. None of these compounds had an effect on GI motility or evoked lipolysis in the β3-AR[KO] mice. Treatment of WT mice with SR 56811A, a β3-AR agonist that exhibited a relatively lower affinity for rodent β3-AR in vitro, did not affect GI motility or plasma glycerol levels in WT or β3-AR[KO] mice when administered s.c. at 3 mg/kg. Clonidine, an alpha-2 adrenergic receptor agonist, used as a positive control in these GI studies, caused a decrease in GI motility in both WT and β3-AR[KO] mice. These results are consistent with a postulated role for β3-AR in regulation of GI motility in the mouse. However, treatment of β3-AR[WAT+BAT] mice with 3 mg/kg BRL 35135 resulted in elevated plasma glycerol levels, as well as increased stomach retention and decreased intestinal transit of radiotracer. These results suggest that this β3-AR agonist may exert its effects on the GI tract indirectly, through an unknown signaling mechanism activated by agonism of β3-AR in adipose tissue.

Pharmacological evidence, obtained predominantly using selective agonists, has suggested that atypical beta adrenergic receptors are located in rodent GI tissue and function to regulate GI motility (Arch and Kaumann, 1993; Croci et al., 1988; Giudice et al., 1989). That these atypical beta adrenergic receptors may be the same as those that mediate lipolysis in white and brown adipose tissues throughout the body, i.e., the β3-AR, is supported by the results of mRNA localization methods that identified β3-AR in adipose and GI tissues of various species (Evans et al., 1996; Granneman et al., 1991; Cohen et al., 1995; Berkowitz et al., 1995), and by the similar relative potencies of selective β3-AR agonists to mediate adipocyte lipolysis and inhibit motility of GI tissues in vitro (Lezama et al., 1996; Landi et al., 1993; Cohen et al., 1995). It has also been demonstrated that selective β3-AR agonists cause a decrease in GI tract movement in vivo (Thollander et al., 1996; Giudice et al., 1989; Manara et al., 1995). These results suggest that the effects of selective β3-AR agonists on GI motility are due to activation of β3-AR present in the GI tissue. The availability of β3-AR[KO] and β3-AR[WAT+BAT] mice (Susulic et al., 1995; Grujic et al., 1997), offered us the unique opportunity to obtain direct proof of the involvement of β3-AR in regulating GI motility. We describe the effects of several selective β3-AR agonists on the transit of radiolabelled charcoal through the GI tract, as indicative of GI motility, in normal mice and in these two types of genetically engineered mice. Our results confirm the previous reports that selective β3-AR agonists are capable of causing a decrease in GI motility in rodents, as well as demonstrate that these agents are indeed acting through β3-AR. However, our results also suggest that the modulation of GI motility by β3-AR agonists in vivo can occur exclusively as an indirect consequence of activation of β3-AR in adipose tissue.

**Materials and Methods**

β3-AR[KO] mice, generated using homologous recombination in the FVB/N background (Susulic et al., 1995), as well as β3-AR[WAT+BAT] mice genetically engineered with insertion of functional β3-AR exclusively in white and brown adipose tissues only (β3-AR[WAT+BAT])

**ABBREVIATIONS:** β3-AR, beta-3 adrenergic receptor; GI, gastrointestinal; WT, wild-type mouse; β3-AR[KO], transgenic mice lacking β3-AR; β3-AR[WAT+BAT], transgenic mice lacking β3-AR in all tissues except white and brown adipose tissue, GC, geometric center.
GI motility studies were performed following the method of Miller et al. (1961). Mice were fasted 24 hr before bolus oral administration of 0.25 ml 1% methocel containing 0.5 μCi [51Cr]sodium (ICN Biomedicals, Inc., Irvine, CA), 5% acacia and 10% charcoal. Forty five minutes after administration of radiotracer, at which time no radiotracer had moved past the small intestine, mice were euthanized by CO₂ asphyxiation and the GI tract was removed. The small intestines were divided into 10 segments of equal length. The radiotracer had moved past the small intestine, mice were euthanized by CO₂ asphyxiation and the GI tract was removed. The small intestines were divided into 10 segments of equal length. The radioactivity within each segment, as well as within the stomach, was determined by counting in a gamma counter. The mean cpm ± S.E.M. for the radioactivity retained in the stomach, and the GC of intestinal transit were calculated for each treatment group (n = 5–15 animals).

Under these conditions, a decrease in GC corresponds to less rapid intestinal transit of radiotracer (increased intestinal transit time). An increase in stomach retention of radiotracer, together with a decrease in the extent of movement of radiotracer through the small intestine, was taken to be indicative of an overall decrease in GI motility. The β₃-AR agonists BRL 35135, CL 316,243, ICI 198,157 and SR 56811A were administered in saline, at 3 mg/kg, by s.c. injection 1 hr before oral administration of charcoal. Clonidine was administered at 0.2 mg/kg. An equal volume of saline was injected into vehicle controls. The β₃-AR agonists were provided through the Department of Medicinal Chemistry, Merck & Co., Rahway, NJ. Clonidine was purchased from Sigma Chemical Co., St. Louis, MO.

For measurement of plasma glycerol levels, heparinized blood was obtained by cardiac puncture after carbon dioxide euthanasia and centrifuged at 3000 × g for 15 min at room temperature. The plasma was stored at −70°C until assay. Plasma glycerol levels were determined spectrophotometrically using a commercially available assay kit (Triglyceride/GPO-Trinder, Sigma Diagnostics, St. Louis, MO).

The in vitro potency of the rodent-specific β₃-AR agonists were quantified in terms of the stimulation of adenylyl cyclase activity, in Chinese hamster ovary cells expressing a cloned rat β₃-AR receptor (Candelore et al. 1996).

Statistical analysis of the data was performed using a two-way analysis of variance analysis on rank-transformed data (Normal-Quantile). Statistical difference between groups was considered to be significant at *P < .05, **P < .01, and highly significant at ***P < .001.

Results

Normal GI transit and the effects of clonidine treatment. No significant difference was seen in the stomach retention of radioactive charcoal in saline-treated WT and β₃-AR[KO] mice (table 1). Similarly, normal intestinal transit of radiotracer was not significantly different in the saline-treated WT and β₃-AR[KO] mice. Clonidine administration caused a highly significant increase in retention of radiotracer in the stomach and reduced the extent of intestinal transit of radiotracer (decreased GC) in both WT and β₃-AR[KO] mice when compared to vehicle-treated controls.

Effects of β₃-AR agonists on GI transit in WT and β₃-AR[KO] mice. Subcutaneous administration of 3 mg/kg BRL 35135, CL 316,243 or ICI 198,157 caused a significant increase in stomach retention of radiotracer and reduced the extent of intestinal transit of radiotracer in WT mice, but had no effect on either motility parameter in β₃-AR[KO] mice (table 1). SR 56811A was without significant effect on either motility parameter in WT or β₃-AR[KO] mice when administered under these same conditions. The relative order of these compounds to affect overall GI motility in WT mice after a single subcutaneous dose of 3 mg/kg was BRL 35135 > CL 316,243 > ICI 198,157 >> SR 56811A (inactive).

Effects of BRL 35135 on GI transit in WAT/BAT mice. As BRL 35135 exerted the most profound effects on GI transit in WT mice, it was chosen to evaluate the effects of a β₃-AR agonist on GI transit in β₃-AR[WAT+BAT] mice. Administration of BRL 35135 to β₃-AR[WAT+BAT] mice produced a significant increase in stomach retention of radiotracer together with a significant decrease in the extent of intestinal transit of radiotracer, similar to that seen when WT mice were treated with this compound (table 1). In contrast, BRL 35135 treatment in β₃-AR[KO] mice affected neither motility parameter. Although saline-treated β₃-AR[WAT+BAT] controls appeared to exhibit a higher retention of radiotracer in the stomach than saline-treated WT controls, the increase was not statistically significant (P = .089). Similarly, the GC values for normal intestinal transit in the saline-treated controls for WT, β₃-AR[KO] and β₃-AR[WAT+BAT] mice were not significantly different.

Potencies of β₃-AR agonists in an in vitro receptor assay. The potencies of the β₃-AR agonists used in these experiments to stimulate adenylyl cyclase activity in vitro in Chinese hamster ovary cells expressing the cloned rat β₃-AR receptor are shown in table 2. The relative potencies were BRL

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**TABLE 1**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Treatment</th>
<th>n</th>
<th>Stomach Retention (%)</th>
<th>Intestinal Transit (GC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Vehicle</td>
<td>14</td>
<td>9.37 ± 1.56</td>
<td>6.39 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Clonidine</td>
<td>15</td>
<td>23.41 ± 3.27***</td>
<td>2.30 ± 0.13***</td>
</tr>
<tr>
<td></td>
<td>BRL 35135</td>
<td>10</td>
<td>39.84 ± 5.80***</td>
<td>4.91 ± 0.29***</td>
</tr>
<tr>
<td></td>
<td>CL 316,243</td>
<td>10</td>
<td>29.79 ± 5.49***</td>
<td>4.87 ± 0.32***</td>
</tr>
<tr>
<td></td>
<td>ICI 198,157</td>
<td>10</td>
<td>18.34 ± 3.63*</td>
<td>5.43 ± 0.27**</td>
</tr>
<tr>
<td></td>
<td>SR 56811A</td>
<td>5</td>
<td>8.68 ± 2.76</td>
<td>5.72 ± 0.32</td>
</tr>
<tr>
<td>β3-AR[KO]</td>
<td>Vehicle</td>
<td>19</td>
<td>7.12 ± 1.79</td>
<td>6.00 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Clonidine</td>
<td>9</td>
<td>39.76 ± 4.23***</td>
<td>1.89 ± 0.15***</td>
</tr>
<tr>
<td></td>
<td>BRL 35135</td>
<td>15</td>
<td>13.52 ± 4.32</td>
<td>6.08 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>CL 316,243</td>
<td>5</td>
<td>6.68 ± 3.14</td>
<td>6.10 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>ICI 198,157</td>
<td>5</td>
<td>3.55 ± 0.57</td>
<td>5.81 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>SR 56811A</td>
<td>5</td>
<td>4.17 ± 1.13</td>
<td>5.43 ± 0.15</td>
</tr>
<tr>
<td>β3-AR[WAT+BAT]</td>
<td>Vehicle</td>
<td>9</td>
<td>22.14 ± 6.52</td>
<td>6.10 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>BRL 35135</td>
<td>9</td>
<td>58.31 ± 7.81***</td>
<td>4.01 ± 0.27***</td>
</tr>
</tbody>
</table>

Stomach retention and intestinal transit (GC) determinations were made 45 min after an oral bolus of radioactive charcoal was administered. Mice received injections s.c. with saline vehicle, 0.2 mg/kg clonidine or 3 mg/kg β₃-AR agonist 60 min before charcoal administration. Values are the mean ± S.E.M.; n = number of mice per group; *P < .05, **P < .01, ***P < .001 in comparison to vehicle-treated control group.
TABLE 2
In vitro potency of β3-AR agonists

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC50 (nM)</th>
<th>Activation (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol</td>
<td>85.8 ± 14.2</td>
<td>100</td>
<td>14</td>
</tr>
<tr>
<td>BRL 37344</td>
<td>4.2 ± 1.2</td>
<td>84.0 ± 7.9</td>
<td>5</td>
</tr>
<tr>
<td>CL 316,243</td>
<td>4.4 ± 1.4</td>
<td>88.0 ± 6.8</td>
<td>4</td>
</tr>
<tr>
<td>ICI 198,157</td>
<td>10.8 ± 2.7</td>
<td>39.3 ± 4.7</td>
<td>3</td>
</tr>
<tr>
<td>SR 58611</td>
<td>82.3 ± 21.3</td>
<td>90.3 ± 3.8</td>
<td>5</td>
</tr>
</tbody>
</table>

Stimulation of adenyl cyclase activity in CHO cells expressing the cloned rat β3-AR receptor. Values are the mean ± S.E.M.; n = number of experiments.

35135 = CL 316,243 > ICI 198,157 >> SR 58611A.

Effects of β3-AR agonists on plasma glycerol levels.

The ability of the β3-AR agonists to induce lipolysis in adipose tissue was demonstrated by the hyperglycerolemia that was evident 60 min after administration of the agonists to nonfasted WT mice in a manner identical to that used for the GI transit studies. Elevations in plasma glycerol levels were highly significant compared to saline-treated controls after administration of BRL 35135, CL 316,243 or ICI 198,157 (table 3). Although preliminary studies in WT mice indicated that plasma glycerol elevation is maximal within 30 to 60 min after β3-AR agonist challenge and declines rapidly thereafter, plasma glycerol levels were also determined on samples taken at the time of euthanasia of mice undergoing GI transit studies involving BRL 35135 treatment. At this time after treatment of mice with BRL 35135 (i.e., 105 min post-compound administration), plasma glycerol levels were elevated 25% in WT mice and 50% in β3-AR[WAT+BAT] mice, but were not elevated in the β3-AR[KO] mice.

Discussion

The biochemical pathways which regulate lipid metabolism in response to adrenergic receptor activity are fairly well understood (reviewed by Lafontan and Berlan, 1993). Selective agonists have been used to demonstrate the involvement of the more recently discovered “atypical-” or β3-AR in regulating metabolic rate and lipolysis in adipose tissue (Arch et al., 1984; Bond and Clarke, 1988; Howe et al., 1992). Based on the relative expression of β1-, β2- and β3-AR mRNA transcripts in white and brown adipose tissue of the mouse, β3-AR appear to play a dominant role in modulating metabolism in these tissues (Collins et al., 1994). This conclusion is further strengthened by the report that a mouse strain having a selective disruption of the β3-AR gene is unresponsive to the typical physiolological and biochemical changes related to metabolism that occur in normal mice after administration of β3-AR agonists (Susulic et al., 1995). Although β3-AR are located mainly in white and brown adipose tissue (Muzzin et al., 1991; Nahmias et al., 1991), β3-AR have also been identified in GI tract tract tissue (Emorine et al., 1989; Granne...
Therefore, it is plausible that circulating hormones, such as insulin, associated with regulation of GI motility or evoking lipolysis in mice totally lacking 3-AR agonists are capable of causing reduced GI motility independent of receptors located in the GI tract via mechanisms secondary to their direct effects on adipose tissue, and presumably consequent upon mediators released during a general increase in whole body metabolic activity. Our results do not negate the possibility that β3-AR agonists may be reasonably questioned when attributing their effect on GI function to the activity of specific receptor populations. Therefore, we had originally postulated that the use of transgenic mice lacking β3-AR, and a range of rodent-specific β3-AR agonists, would validate the presence of β3-AR in the GI tract and their potential role in modulating GI motility. The differential effect of three synthetic, rodent-specific β3-AR agonists on GI motility in the WT and β3-AR[KO] mice are consistent with this supposition, as well as at least to the selectivity of these agonists for the β3-AR. However, the most effective of these β3-AR agonists, BRL 35135, caused enhanced lipolysis and decreased GI motility to an equivalent extent in both the β3-AR[WT+BAT] and WT mice. Characterization of the β3-AR[WT+BAT] transgenic mouse has shown that β3-AR are present only in brown and white adipose tissue, and that these mice respond to administration of the selective β3-AR agonist, CL 316,243, with the full range of increased lipid metabolism and thermogenesis that is seen in normal, WT mice (Grjalic et al., 1997). Our results suggest that β3-AR agonists, or at least BRL 35135, is capable of regulating GI motility indirectly and exclusively as a consequence of its action on β3-AR in adipose tissue. It is known that, along with up-regulation of lipolysis and glycogenolysis, β3-AR agonists acutely elicited increased serum insulin levels (Arch and Kaumann, 1993), a response that is absent in the transgenic β3-AR knockout mouse (Susulic et al., 1995). Both hyperinsulinemia and hyperglycemia have been shown to cause decreased GI motility (Eliasson et al., 1995; Chang et al., 1995). Therefore, it is plausible that β3-AR agonists are capable of causing reduced GI motility independent of receptors located in the GI tract via mechanisms secondary to their direct effects on adipose tissue, and presumably consequent upon mediators released during a general increase in whole body metabolic activity. Our results do not negate the possibility that β3-AR are normally present in GI tissue and may also contribute to regulation of GI motility. A comparison of the in vitro responses of GI tissues from WT, β3-AR[KO] and β3-AR[WT+BAT] mice upon exposure to the these β3-AR agonists would be highly enlightening on this point.

In summary, the effect of several selective β3-AR agonists on GI motility were compared in WT, β3-AR[KO] and β3-AR[WT+BAT] mice. The ability of these agonists to cause a decrease in the extent to which radiotracer moved through the GI tract reflected their ability to stimulate lipolysis in WT mice. None of these agonists were effective in modulating GI motility or evoking lipolysis in mice totally lacking β3-AR. However, BRL 35135 effectively increased lipolysis and decreased GI motility in β3-AR[WT+BAT] mice, suggesting that β3-AR agonists are able to effect GI motility as a consequence of their effects on adipose tissue alone. We postulate that these effects may include the products of increased lipolysis and thermogenesis in adipose tissue, and a change in circulating hormones, such as insulin, associated with regulation of overall body metabolism, and which are known to modulate GI motility. Therefore, our results are further evidence that adipocytes, not only other specialized groups of cells, can modulate the physiological responses of other tissues and organs through humoral mechanisms (Spiegelman and Flier, 1996).

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References

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