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

DANIEL S. FLETCHER, MARI RIOS CANDELORE, DANICA GRUJIC1, BRADFORD B. LOWELL1, SILVI LUELL, VEDRANA S. SUSULIC2 and D. EUAN MACINTYRE

Department of Pharmacology, Merck & Co., Rahway, New Jersey

Accepted for publication June 12, 1998

ABSTRACT

The effects of beta-3 adrenergic receptor (β3-AR) agonists on gastrointestinal (GI) motility, as reported 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, have 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 cause relaxation in the GI tract of rodents 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[KO] mice genetically engineered with insertion of functional β3-AR exclusively in white and brown adipose tissues only (β3-AR[WAT+BAT])...
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.

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 adenyl cyclase activity in vitro in Chinese hamster ovary cells expressing the cloned rat β3-AR are shown in table 2. The relative potencies were BRL...
TABLE 2
In vitro potency of β3-AR agonists

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

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

a Activity is expressed relative to maximal isoproterenol activation.

b Active form of pre-drug BRL 35135.

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

**Effects of β3-AR agonists on plasma glycerol levels.**

The ability of the β3-AR agonist 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 physiological 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 tissue (Emorine et al., 1989; Graneman et al., 1991; Bensaid et al., 1993). A role for adrenergic receptors in mediating muscle contractility in a variety of organs has been well documented (Arch and Kaumann, 1993; De Ponti et al., 1996). Selective β3-AR agonists have been shown to inhibit motility of isolated organs, such as guinea pig ileum, rat colon, intestine and esophageal smooth muscle (Bond and Clarke, 1988; Croci et al., 1988; van der Vliet et al., 1990; deBoer et al., 1995), as well as inhibit GI motility in vivo (Giudice et al., 1989; Croci et al., 1991; Landi et al., 1993; Manara et al., 1996). In addition, a link between β3-AR and nervous system regulation of gut motility has been proposed (Tholland et al., 1996; Yoshida et al., 1996). However, the mechanism by which β3-AR might affect muscle contractility has not been fully elucidated.

Our results in mice have confirmed the earlier reports of a role for β3-AR in the regulation of GI motility. The effect of β3-AR agonists to stimulate lipolysis in WT mice, as measured by evoked glycerolemia, was consistent with their ability to stimulate adenylyl cyclase activity in cells expressing a cloned rodent β3-AR receptor. In the in vitro assay, the relative order of activity was BRL 35135 = CL 316,243 > ICI 198,157 >> SR 58611A. A similar rank order of activity for several of these β3-AR agonists has been reported by Manara et al. (1995) for relaxation of rat colon in vitro. In our experiments, the extent of radiotracer transit through the GI tract of WT mice was decreased after administration of these selective β3-AR agonists. In addition, we have demonstrated that these agonists failed to evoke lipolysis or have any affect on GI motility in transgenic mice lacking β3-AR. Thus, all of these β3-AR agonists effects on both lipolysis and GI motility appear to be mediated exclusively through the β3-AR in the WT mice. Our results also confirm those of Susulic et al. (1995), who have reported that the ability of the selective β3-AR agonist, CL 316,243, to cause an increase in adipose lipolysis and whole body metabolism, as well as reduce food intake, is mediated exclusively by β3-AR, since these effects are absent in transgenic β3-AR knockout mice.

In our experiments, treatment of WT mice with SR 58611A (3 mg/kg; s.c.) caused no effect on GI motility. SR 58611A is effective in modulating canine and rat colonic motility both in vitro (Croci et al., 1991) and in vivo when compound was given intravenously (De Ponti et al., 1995; Manara et al., 1996). The lack of activity of SR 58611A in our experiments may be attributed to the lower in vitro potency of this compound compared to the other β3-AR agonists we tested, and perhaps to suboptimal pharmacokinetics using our dosing regimen. The α2-AR agonist, clonidine, was used as a positive control in our studies, based on its previously reported ability to increase GI transit time (Maugeri et al., 1994; Puig et al. 1996). Clonidine effectively decreased the extent of movement of radiotracer through the GI tract in both the WT and β3-AR[KO] mice, demonstrating that its mechanism of action was independent of the β3-AR receptor, and that other pathways for regulation of GI motility remain operational in the β3-AR[KO] mouse.

The GI effects of agonists selective for the atypical (non-β1, β2) beta adrenergic receptor have been linked with the detection of β3-AR mRNA in these tissues (Berkowitz et al., 1995; Cohen et al., 1995). These reports, together with our results demonstrating that these agonists affect GI motility exclusively through β3-AR, could readily be interpreted as evidence for a direct effect of β3-AR receptor activity in GI transit.
tissues. However, it is possible that the molecular identification of β3-AR in these GI tissues may be due to the wide spread distribution of adipocyte tissue throughout the digestive tract (Evans et al., 1996). Such low-level signals for β3-AR mRNA, detected in skeletal muscle from β3-AR[WAT+BAT] mice, have been attributed to adipocytes resident within or surrounding this tissue (Grujic et al., 1997). Also, the absolute pharmacological selectivity of synthetic 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 attest 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[WAT+BAT] and WT mice. Characterization of the β3-AR[WAT+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 (Grujic 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 glycolgenolysis, β3-AR agonists acutely elicit increased serum insulin levels (Arch and Kaumann, 1993), a response that is absent in the transgenic β3-AR knockout mouse (Susolic 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 and in vivo responses of GI tissues from WT, β3-AR[KO] and β3-AR[WAT+BAT] mice upon exposure to 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[WAT+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[WAT+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).

Acknowledgments

The authors thank R. Meurer and Z. Baflian for the biochemical analyses and F. Shen for statistical analyses.

References


Manara L, Badone D, Baroni M, Boccardi G, Cecchi R, Croci T, Giudice A, Guzzi U,


Send reprint requests to: Dr. Daniel S. Fletcher, R80Y-150, Merck & Co., P.O. Box 2000, Rahway, NJ 07065.