β-Adrenergic receptor subtypes that mediate ractopaminestimulation of lipolysis

S. E. Mills, M. E. Spurlock and D. J. Smith


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ABSTRACT: Ractopamine HCl is a β-adrenergic receptor (βAR) ligand that was recently approved for use in swine to enhance carcass leanness. The RR stereoisomer of ractopamine is the most active of the four stereoisomers exhibiting the highest affinity and signaling response. The RR isomer exhibits selective activation of the porcine β2AR, which might limit the lipolytic response to ractopamine because the β1AR is the predominant subtype in swine adipocytes and may mediate most of the lipolytic response. Therefore, we determined the βAR subtypes that mediate the lipolytic response to ractopamine in swine adipocytes. In order to confirm the predominant role of the β1AR in porcine adipocytes, isoproterenol-stimulated lipolysis was inhibited by increasing doses of subtype-selective antagonists. Inhibition curves were biphasic using β1AR antagonists (CGP 20712A and bisoprolol) and curve analysis indicated that both β1AR and β2AR contributed to lipolysis with 50 to 60% of the response coming from the β1AR. Inhibition with the β2AR antagonist clenbuterol revealed only one class of βAR that closely approximated the kinetics of the β1AR. When the RR isomer of ractopamine was the lipolytic agent, similar results to isoproterenol were observed, except that the estimated contribution of the β1AR was 38%. That β2AR antagonists did not detect a contribution of the β2AR to lipolysis may indicate that the β1AR masked the response to the β2AR. Dose titration with the RR isomer in the presence of a saturating concentration of β1AR or β2AR antagonists indicated that each subtype was present in sufficient quantities to stimulate lipolysis near maximally. Data indicate that both the β1AR and β2AR are functionally linked to lipolysis in swine adipocytes and that ractopamine activates each subtype. The RR isomer of ractopamine stimulated adenosine 3',5'-cyclic phosphate accumulation with equal efficacy to isoproterenol through the cloned porcine β2AR, but was only 35% as efficacious through the cloned porcine β1AR. These data confirm the β2AR selectivity of the RR stereoisomer, but suggest the partial agonism through the β1AR is sufficient to activate lipolysis through both subtypes in swine adipocytes.

Key Words: β-Adrenergic Receptors, Lipolysis, Pigs

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Introduction

Triglyceride hydrolysis in adipocytes is regulated by catecholamines that bind to β-adrenergic receptors (βAR) to activate hormone-sensitive lipase (Fain and Garcia-Sainz, 1983). Adipocytes express three βAR subtypes, but the role for each subtype is not clearly defined and may differ among species. For rodents, the β3AR is the predominant subtype and mediates the majority of the lipolytic response (Hollenga and Zaagsma, 1989). In the pig, the β1AR represents nearly 80% of the total receptors (McNeel and Mersmann, 1999; Liang and Mills, 2002) and seems to be the primary subtype mediating lipolysis. The β2AR may be uncoupled from the lipolytic cascade, a conclusion based on the finding that the β2AR-selective ligand BRL-37344 did not interfere with isoproterenol-stimulated lipolysis at low concentrations (β2AR-mediated; Mills, 2000). Because this conclusion was based on data using only one selective ligand, it was of interest to confirm these results with additional subtype-selective ligands.

Ractopamine is a phenethanolamine βAR agonist that promotes muscle growth in swine (Moody et al., 2000). The commercial product is a mixture of four ste-
reoisomers, of which, one (RR) seems to be the functional compound (Ricke et al., 1999; Mills et al., 2002). Ractopamine binds porcine βAR and stimulates lipolysis in vitro (Liu et al., 1989; Spurlock et al., 1993b). It is not clear which βAR mediates the response to ractopamine. For species other than swine, ractopamine is suggested to have some selectivity for the βAR (Smith et al., 1990; Moody et al., 2000). For the pig, the β2AR provides the best signal transduction (Mills et al., 2002), whereas binding affinity is similar for the β1AR and β2AR (Spurlock et al., 1993b; Liang et al., 2000; Mills et al., 2002). It was of interest, therefore, to determine which βAR mediates the activation of lipolysis in swine adipocytes.

Materials and Methods

Materials

Ractopamine—[1R*,3R*], [1R*,3S*]-4-hydroxy-α-(((3-(4-hydroxy[14C]phenyl)-1-methylpropyl)amino)methyl)benzenemethanolhydrochloride—stereoisomers were synthesized as described by Ricke et al. (1999). Other drugs were purchased from the following companies: clenbuterol and (−)isoproterenol from Sigma Chemical (St. Louis, MO), bisoprolol from TOCRIS (Ballwin, MO), and GCP20712A from RBI (Natick, MA).

Adipocytes and Lipolysis

The middle layer of subcutaneous adipose tissue (fourth to 10th ribs) was taken at the time of death from market-weight barrows (PIC line 337 sires × York-Landrace dams). Pigs were killed by exsanguination following electrical stunning at the Purdue University abattoir. Adipocytes were isolated by collagenase digestion, and cell number was determined from the average cell size as measured from osmium tetroxide-fixed cells and total lipid (Liu et al., 1989). Adipocytes (approximately 10⁵ cells/mL) were washed and suspended in incubation buffer (Krebs-Ringer bicarbonate [KRB] containing 1.25 mM CaCl₂, 0.5 mM ascorbic acid, 10 mM HEPES, 5 mM glucose, and 3% BSA, pH 7.4, as described by Liu et al. (1989). To quantify rates of lipolysis, duplicate 0.5-mL aliquots of the cell suspension were incubated in 17 × 100 mm polyethylene tubes in an atmosphere of 5% CO₂ in oxygen. Tubes contained theophylline (0.4 mM) and βAR ligands as specified in Figures 1 through 4. Vials were shaken in a gyratory water bath at 37°C for 2 h. Incubations were stopped by adding 0.025 mL of 35% (vol/vol) HClO₄, and glycerol was quantified in neutralized, protein-free extracts using a commercial kit adapted for 96-well plates (GPO-Trinder triglyceride kit; Sigma Chemical). Lipolytic rates were expressed as nmoles glycerol released min⁻¹·10⁶ cells⁻¹.

Cell Lines

Stably transfected Chinese hamster ovary cell lines expressing the porcine β₁AR or β₂AR were grown in an atmosphere of 95% air and 5% (CO₂ at 37°C in a 1:1 F12:Dulbecco’s modified Eagle’s medium DMEM medium containing 100 U/mL of ampicillin, 200 U/mL of penicillin, 200 μg/mL of streptomycin, 10⁻⁸ M Se, and 1.2 mg/mL of NaHCO₃ in 1.5 mM HEPES, pH 7.4), plus 10% FBS and G418 (0.5 mg/mL; Liang et al., 2000). Confluent cells were washed twice with warm F12:DMEM and detached with 0.4% trypsin in F12:DMEM. Cells were centrifuged at 300 × g at 4°C for 5 min and washed twice in KRB media containing 0.1% BSA. Whole cells were suspended in the same media and 0.1 mL was distributed to 1.5-mL microcentrifuge tubes for determination of adenyl cyclase activation. Tubes contained theophylline (0.4 mM) and test ligands in a final volume of 0.15 mL and were incubated for 40 min at 37°C. Incubations were stopped with 1 N NaOH and neutralized with 1 M acetic acid containing universal indicator (Sigma Chemical). Neutralized extracts were used for the assay of adenosine 3’,5’-cyclic phosphate (cAMP) by RIA using the protocol supplied with the cAMP antibody (Calbiochem, San Diego, CA). Samples and standards were acetylated to increase the sensitivity of the assay. A portion of the cell suspension was used to determine cell number by determination of DNA content. Cells were sonicated for 10 s to disrupt the cell structure. DNA was quantified using the fluorescent dye H 33258 (Sigma Chemical) according to Labarca and Paigen (1980). Data were expressed as fentamoles of cAMP per 10⁶ cells.

Data were analyzed using the GLM procedures of SAS for a completely randomized design (SAS Inst., Inc., Cary, NC). Lipolytic response curves were analyzed for best-fit using nonlinear regression analysis and kinetic parameters were determined (Prism, GraphPad Software Inc., San Diego, CA). Lipolysis studies were conducted using adipocyte preparations from two or three pigs. The effect of βAR subtype inhibitors on kinetic parameters was determined using single degree of freedom orthogonal contrasts for differences in activation of adenyl cyclase in cultured cells. Cells were maintained in culture and cells propagated for each experiment. Replicate experiments represented cells plated at different times.

Results

The β₁AR and β₂AR comprise nearly 95% of the βAR in swine adipocytes (McNeel and Mersmann, 1999), so determination of the contribution of these two subtypes to lipolysis will likely account for essentially all of the lipolytic response. To determine the contribution of the β₁AR and β₂AR to lipolysis, subtype-selective ligands were used to inhibit lipolysis stimulated by the nonselective agonist isoproterenol. As shown in Figure 1, iso-

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proterenol stimulated lipolysis in a dose-response fashion with a half-maximal dose of approximately $10^{-8} \text{M}$. Based on this dose response, subsequent experiments were conducted using $10^{-7} \text{M}$ isoproterenol to achieve a near maximal rate. The plots for the competitive inhibition of isoproterenol-stimulated lipolysis by two $\beta_1$AR-selective antagonists, CGP 20712A and bisoprolol (400- and 33-fold selective for the $\beta_1$AR; Cao, 1998; Liang and Mills, 2001) and the $\beta_2$AR-selective ligand clenbuterol (25-fold selective for the $\beta_2$AR; Cao, 1998; Liang and Mills, 2001) are shown in Figure 1. Both $\beta_2$AR antagonists exhibited biphasic displacement curves indicative of the titration of two $\beta$AR subtypes. Nonlinear analysis of competition curves yielded affinity estimates for the high- and low-affinity sites that were reasonably close to expected values for the porcine $\beta_1$AR and $\beta_2$AR (Table 1). Values for the inhibition constant, $K_i$, for the high- and low-affinity sites, respectively, were 1.6 and 2,630 nM with CGP 20712A as competitor, and 13.6 and 807 nM with bisoprolol as competitor. Expected values are based on data for each drug using the cloned $\beta_1$AR and $\beta_2$AR (Cao, 1998; Liang et al., 2000). Using $\beta_1$AR selective antagonists, these data suggest that both the $\beta_1$AR and $\beta_2$AR contribute to the lipolytic response stimulated by isoproterenol. The percentage contribution of each receptor subtype was also determined from the kinetic analysis and indicated that approximately 50% of the response was contributed by each subtype (Table 1). Clenbuterol is a partial agonist toward porcine $\beta_1$AR (Liu et al., 1989; Spurlock et al., 1993b), and under the conditions of this assay, clenbuterol is a functional antagonist. Inhibition by clenbuterol did not reveal two classes of receptors as for the $\beta_2$AR antagonist and the inhibition curves modeled best to one site (Figure 1). The calculated $K_i$ of 430 nM was closer to the value for the $\beta_1$AR (300 nM) than to the $\beta_2$AR (10 nM), suggesting that the $\beta_1$AR was contributing to the observed response (Table 1). Thus, a different picture emerges when a $\beta_1$AR or $\beta_2$AR antagonist is used.

To determine which $\beta$AR mediates the lipolytic response to the active stereoisomer of ractopamine, competitive inhibition experiments were conducted using the RR stereoisomer with $\beta_1$AR-selective and $\beta_2$AR-selective ligands CGP 20712A and clenbuterol for both $\beta_1$AR and $\beta_2$AR. The $\beta_1$AR-selective ligand CGP 20712A yielded a two-component inhibition curve (Figure 2). Kinetic analysis of these curves showed affinity estimates that were reasonably close to the expected values for $\beta_1$AR and $\beta_2$AR and a contribution by the $\beta_1$AR of 38% (Table 2). Once again, clenbuterol yielded a single-component displacement curve with an estimated $K_i$ of 262 nM, which is close to the dissociation constants $K_i$ of clenbuterol for the porcine $\beta_1$AR. Results are similar to the data when using isoproterenol as the agonist and indicate that both the $\beta_1$AR and $\beta_2$AR contribute to the stimulation of lipolysis under conditions of a high concentration of the RR stereoisomer of ractopamine. To examine the functional activity of each $\beta$AR subtype over a range of ractopamine concentrations, dose response experiments were conducted with the RR stereoisomer in the presence of concentrations of CGP 20712A (50 nM) and BRL 37344 (2,000 or 5,000 nM) calculated to block RR binding to the $\beta_1$AR or $\beta_2$AR respectively. We substituted BRL 37344 for clenbuterol because this $\beta_2$AR ligand has a greater selectivity for

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**Table 1.** Estimates of binding affinity for adenosine $\beta$-adrenergic receptor ligands obtained from competitive inhibition of isoproterenol-stimulated lipolysis

<table>
<thead>
<tr>
<th></th>
<th>CGP 20712A</th>
<th>Bisoprolol</th>
<th>Clenbuterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>High affinity, nM</td>
<td>1.6 ± 0.5</td>
<td>13.6 ± 5.2</td>
<td>432 ± 216</td>
</tr>
<tr>
<td>Expected$^a$</td>
<td>1</td>
<td>30</td>
<td>10 - $\beta_1$AR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300 - $\beta_2$AR</td>
</tr>
<tr>
<td>Low affinity, nM</td>
<td>2,630 ± 245</td>
<td>807 ± 145</td>
<td>—</td>
</tr>
<tr>
<td>Expected$^a$</td>
<td>400</td>
<td>1,500</td>
<td>—</td>
</tr>
<tr>
<td>$\beta_1$AR, %</td>
<td>57 ± 12</td>
<td>49 ± 10</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$Values for $K_i$ are means ± SEM of three independent experiments with different pigs. Data were generated from nonlinear regression of competitive inhibition curves of isoproterenol-stimulated lipolysis as illustrated in Figure 1.

$^a$Expected values are dissociation constants $K_i$ for the porcine $\beta_1$- and $\beta_2$-adrenergic receptors expressed in Chinese hamster ovary cells and were taken from Cao (1998) and Liang et al. (2000).
the $\beta_2$AR (75-fold) than has clenbuterol (Liang et al., 2000). The RR isomer stimulated lipolysis in a dose-dependent manner in the presence of antagonist, confirming that both subtypes contribute to the lipolytic response (Figure 3). Both antagonists shifted the dose response curve to the right. The shift in EC$_{50}$ (the concentration of ligand that results in half-maximal stimulation) was greater when the $\beta_1$AR was blocked (sixfold) than when the $\beta_2$AR was blocked (threefold), indicating a greater contribution of the $\beta_1$AR (Table 3). The presence of either antagonist prevented full lipolytic expression at RR stereoisomer concentrations up to $10^{-6}$ M. The fact that a near-full lipolytic response is achieved than when the $\beta_1$AR using membranes from cell lines expressing each receptor subtype (Mills et al., 2002). In fact, adenyl cyclase activation was undetectable when $\beta_1$AR were used. These results differ from the present findings in that the RR isomer stimulated lipolysis through both the $\beta_1$AR and $\beta_2$AR. To clarify whether RR activates the cloned $\beta_1$AR, we quantified cAMP accumulation in intact Chinese ovary cells that express either the $\beta_1$AR or $\beta_2$AR. Using intact cells, the RR isomer stimulated cAMP accumulation through either the $\beta_1$AR or $\beta_2$AR (Figure 4). The RR isomer was more efficacious through the $\beta_2$AR, however, being equal to isoproterenol, but was only about 35% as effective as isoproterenol through the $\beta_1$AR. These data indicate that RR may be a full agonist through the $\beta_2$AR but a partial agonist through the $\beta_1$AR.

**Discussion**

Adipocytes of all species studied to date express the three family members of $\beta$AR, although the relative expression of each subtype differs considerably across species (Strosberg, 1990; Lafontan and Berlan, 1993). The coexistence of multiple $\beta$AR suggests that a unique role exist for each subtype, but clearly distinct roles have not been identified. All three $\beta$AR subtypes share a common signaling pathway through activation of ade-

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**Table 2.** Estimates of binding affinity for $\beta$-adrenergic receptor ligands obtained from competitive inhibition of ractopamine-stimulated lipolysis

<table>
<thead>
<tr>
<th></th>
<th>CGP 20712A</th>
<th>Clenbuterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>High affinity, nM</td>
<td>1.2 ± 0.02</td>
<td>262 ± 241</td>
</tr>
<tr>
<td>$\text{Expected}^a$</td>
<td>1</td>
<td>10 - $\beta_2$AR</td>
</tr>
<tr>
<td></td>
<td>300 - $\beta_1$AR</td>
<td></td>
</tr>
<tr>
<td>Low affinity, nM</td>
<td>12,000 ± 1,300</td>
<td>400</td>
</tr>
<tr>
<td>$\text{Expected}^b$</td>
<td>38 ± 9</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Values for $K_i$ are means ± SEM of two independent experiments with different pigs. Data were generated from nonlinear regression of competitive inhibition curves of isoproterenol-stimulated lipolysis as illustrated in Figure 2.

$^b$Expected values are dissociation constants for the porcine $\beta_1$- and $\beta_2$-adrenergic receptors expressed in Chinese hamster ovary cells and were taken from Cao (1998) and Liang et al. (2001).

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**Table 3.** Kinetic variables for the stimulation of lipolysis by ractopamine through the $\beta_1$- or $\beta_2$-adrenergic receptor

<table>
<thead>
<tr>
<th></th>
<th>RR isomer</th>
<th>+BRL</th>
<th>+CGP</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC$_{50}$, nM$^b$</td>
<td>3.26 ± 0.3</td>
<td>10.6 ± 4.7</td>
<td>16.6 ± 4.9$^a$</td>
</tr>
<tr>
<td>Maximum$^b$</td>
<td>100</td>
<td>83 ± 7*</td>
<td>81 ± 4.7*</td>
</tr>
</tbody>
</table>

$^a$Significant ($P < 0.05$) deviation from the RR isomer alone.

$^b$Values are means ± SEM for three independent experiments with different pigs and were derived from dose-response curves with varying concentrations of ractopamine (RR stereoisomer) in the absence and presence of BRL 37344 (2 or 5 µM) or CGP 20712A (50 nM) as illustrated in Figure 3.
Figure 3. Effect of dose on stimulation of lipolysis in porcine adipocytes by the RR stereoisomer of ractopamine in the absence and presence of antagonist for the β<sub>1</sub>-adrenergic receptor (CGP 20712A; CGP) or the β<sub>2</sub>-adrenergic receptor (BRL 37344; BRL). Data are representative of three independent experiments with different pigs. Curves were fit by nonlinear regression, and results are presented in Table 3. The concentrations of CGP (50 nM) and BRL (2 or 5 μM) were selected to block the β<sub>1</sub> or β<sub>2</sub>AR adrenergic receptor respectively without interference of the other subtype and were determined from the affinity of each ligand and the concentration of RR according to the equation of Cheng and Prusoff (1973).

Figure 4. Activation of adenylyl cyclase by isoproterenol or the RR stereoisomer of ractopamine through the porcine β<sub>1</sub>- and β<sub>2</sub>-adrenergic receptor expressed in Chinese hamster ovary cells. Intact cells were incubated for 40 min in the absence or presence of ligand (10<sup>-4</sup> M), and adenosine 3'-5'-cyclic phosphate was quantified by radioimmunoassay. Data are the means ± SEM for three independent experiments with cloned cells grown on different dates.

nyl cyclase so that the greatest differences between subtypes seem to be in the relative level of expression, regulation of gene expression, and binding and signaling kinetics to natural and synthetic ligands (Granneeman, 1995). The predominant β<sub>AR</sub> expressed in porcine adipocytes is the β<sub>1</sub>AR (McNeel and Mersmann, 1999; Liang and Mills, 2002). We have presented evidence that the β<sub>1</sub>AR is the primary regulator of lipolysis in the pig and that the β<sub>2</sub>AR may not be linked to the lipolytic cascade (Mills, 2000). We interpreted these data to suggest that the β<sub>2</sub>AR did not participate in the stimulation of lipolysis. We further speculated that intracellular compartmentalization might uncouple the β<sub>2</sub>AR from lipolysis. Evidence for a difference in the coupling efficiency of different β<sub>AR</sub> subtypes, perhaps due to compartmentalization, has been reported (Hollenga et al., 1991). In their work, ligands that activated the β<sub>3</sub>AR had a 10-fold greater coupling efficiency (rate of lipolysis/cAMP concentration) than did the nonselective agonist isoproterenol. Alternatively, Minneman and colleagues have suggested that β<sub>AR</sub> subtypes do not function independently and that one subtype may prevail over the others depending on conditions (Zhong et al., 1996).

In contrast to our earlier conclusion, the present data do not provide evidence for compartmentalization and clearly demonstrate that both the β<sub>1</sub>AR and β<sub>2</sub>AR are functionally linked to lipolysis in the pig adipocyte. The participation of both β<sub>AR</sub> subtypes was apparent when inhibiting isoproterenol-stimulated lipolysis with β<sub>1</sub>AR antagonists, but not β<sub>2</sub>AR antagonists (clenbuterol in this study and BRL 37344 in Mills, 2000). Why the β<sub>2</sub>AR antagonist did not reveal two β<sub>AR</sub> subtypes contributing to lipolysis is not clear. It does not appear to be due to low selectivity by the β<sub>2</sub>AR antagonists because
clenbuterol and BRL 37344 have a 30- to 75-fold selectivity for the β2AR, whereas bisoprolol and CGP 20712A have a 50- to 400-fold selectivity for the β1AR (Cao, 1998; Liang et al., 2000). One explanation may be that the β2AR response is masked when the β1AR is maximally activated and that inhibition by clenbuterol does not reduce lipolysis until the β1AR is titrated. It should be pointed out that we were measuring a response to the binding of ligand and not binding itself, so not all βAR may be accounted for if βAR are in excess of the requirements for maximal lipolysis. The number of βAR are typically in excess of what is required to stimulate lipolysis, and binding of only a small percentage of the total is sufficient to stimulate lipolysis maximally (Arner et al., 1976). Therefore, it is reasonable that because the β1AR is the predominant subtype that this receptor alone could stimulate lipolysis maximally. Data in Figure 1 would suggest that the number of β2AR is insufficient for maximal lipolysis. However, data from Figure 3 indicates that the β1AR and β2AR are equally efficacious in stimulating lipolysis. An alternative explanation may be that under conditions of maximal lipolysis, the β1AR is the primary contributing subtype and that only when the number of functional β1AR is reduced with an antagonist is a β2AR component observed.

Analysis of the inhibition curves with isoproterenol as an agonist indicated that the β1AR contributed 50 to 60% of the total lipolytic response. This value is less than the predicted value of 75% based on messenger RNA abundance (McNeel and Mersmann, 1999) or receptor number (Liang and Mills, 2002). Although we are not certain of the percentage of each βAR subtype in the pigs used in the current studies, it is possible we have underestimated the contribution of the β1AR because of spare receptors. It is possible that both CGP 20712A and bisoprolol reduced the number of functional β1AR before a reduction in the rate of lipolysis was observed. If true, the contribution of β1AR would have been underestimated. Alternatively, we cannot rule out the possibility that with collagenase digestion the number and/or ratio of βAR may be altered, although we have observed greater βAR density in membrane preparations from adipocytes than from adipose tissue (Spurlock et al., 1993a).

The commercial preparation of ractopamine is a mixture of four stereoisomers that result from the presence of two chiral carbons (Colbert et al., 1991; Smith, 1998). The RR isomer seems to be the active isomer because it has the highest affinity and greatest efficacy for adenyl cyclase activation for pig βAR (Mills et al., 2002), and because it can account for the growth response in rodents (Ricke et al., 1999). Ractopamine is reported to have β1AR selectivity (Smith et al., 1990; Moody et al., 2000), but these data are based on rodent models and differ from data in the pig. The RR isomer has equal affinity for the porcine β1AR and β2AR, but more effectively couples to adenyl cyclase through the β2AR (Mills et al., 2002). Using membrane preparations from CHO cells expressing each subtype, the RR isomer was a partial agonist through the β2AR, but no response was observed through the β1AR (Mills et al., 2002). Results from the present study confirm and refine the previous observation by demonstrating that the RR isomer does signal better through the β2AR. Using the more physiological assay of cAMP accumulation in intact cells, the RR isomer is shown to be a partial agonist through the β1AR and a full agonist through the β2AR (Figure 4). The same pattern of preferential activation of the β2AR by ractopamine in rodent tissues was reported by Colbert et al. (1991).

Despite the RR isomer having only partial agonist activity through the β1AR, both the β1AR and β2AR contributed to the stimulation of lipolysis by RR. Unlike isoproterenol, however, stimulation of lipolysis by the RR stereoisomer of ractopamine was mediated predominantly through the β2AR (63%) rather than the β1AR. Again, the contribution of the β1AR may be underestimated in these experiments. One consequence of RR being only a partial agonist through the β1AR is that a full agonist response may not be realized, particularly in adipose tissue where the β1AR predominates. Although ractopamine is acutely lipolytic in the pig (Veenuizen et al., 1987), we have demonstrated that responses appear to be lost quickly and that, in some cases, chronic feeding of ractopamine has no apparent effect on the metabolism or rate of accretion of adipose tissue (Dunshea, 1993; Liu et al., 1994). It is possible that a ligand with full agonist activity through the β1AR would have an enhanced capacity to reduce lipid accretion in adipose tissue.

**Implications**

It is not clear why cells express more than one subtype of β-adrenergic receptor, but the existence of multiple subtypes provides an opportunity to target the tissues and processes that are most desirable while eliminating the less desirable responses. Previous data suggested that ractopamine might preferentially target the β2-adrenergic receptor, which may not be linked to lipolysis, and therefore may have limited effectiveness to reduce fat accretion in growing pigs. We report here that the β2-adrenergic receptor is linked to lipolysis, and despite the fact that ractopamine is selective for the β2-adrenergic receptor, the RR isomer can stimulate lipolysis through both the β1-adrenergic receptor and β2-adrenergic receptor. The β1-adrenergic receptor may be the preferred target because it is the most abundant subtype in swine adipocytes, but targeting the β2-adrenergic receptor should also result in reduced fat accretion in swine.

**Literature Cited**


