EFFECT OF BOVINE SOMATOTROPIN AND FOOD DEPRIVATION ON β-ADRENERGIC AND A1 ADENOSINE RECEPTOR BINDING IN ADIPOSE TISSUE OF LACTATING COWS


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ABSTRACT

Lactating Holstein cows were used to assess the effect of bovine somatotropin (bST; n = 8) and fasting (FAST; n = 4) on ligand binding to β-adrenergic (BAR) and Type-1 adenosine (A1R) receptors in adipose tissue. Cows received exogenous bST (sometribove; 40 mg/d) or no hormone (control) for 4 d in a single-reversal design with a 7-d interval between treatment periods. Subcutaneous adipose tissue biopsies were taken on day 4 of each treatment. Eight d after the bST regimen, 4 cows were fasted for 3 d and adipose biopsies were taken. Ligand binding was quantified with a postnuclear, total adipose tissue membrane preparation (100,000 × g pellet). Binding to BAR and A1R was assessed with the antagonists [125I]iodocyanopindolol (ICP) and [3H]8-cyclopentyl-1,3-dipropylxanthine (DCPCX), respectively. The binding affinity (Kd) of BAR for ICP was not affected by bST but was enhanced by FAST; maximal binding (Bmax) was increased with bST treatment (P < 0.06) and reduced by FAST (61%, P < 0.01). Kd values for DCPCX binding to A1R were not changed by bST or FAST. bST did not affect Bmax for A1R; however, FAST reduced the Bmax by 38%. Data highlight the differential regulation of BAR and A1R by bST and FAST.

INTRODUCTION

Somatotropin (ST) is a homeorrhetic effector of metabolism that acts to partition nutrients toward productive functions such as milk production during lactation and lean tissue accretion during growth. One mechanism by which ST regulates nutrient use is by altering tissue response to homeostatic signals such as the catecholamines and insulin. In the case of lipolysis, ST acts to enhance adipose tissue response to a β-adrenergic challenge, resulting in enhanced lipolysis (1,2). ST treatment also relieves the tonic inhibition of lipolysis imposed by adenosine (3–5). The cellular mechanisms by which ST elicits these effects on the signal transduction system for lipolysis have not been adequately elucidated.

The adenosine receptor (A1R) and β-adrenergic receptors (BAR) represent potential loci for ST effects in the regulation of lipolysis. We are not aware of any studies that have examined these two receptor types after the in vivo ST treatment of ruminants. In fact, ligand binding to A1R on adipocytes has not been described in cattle. In the case of BAR, binding was increased with the onset of lactation in dairy cows, a period when endogenous ST is elevated (6). Watt et al. (7) reported that the in vitro culture of adipocytes from
adult ewes with (bST) for 48 hr increased BAR binding with no alteration in A₁R binding when compared with adipocytes incubated with no hormones.

The objectives of this study were to: 1) determine the effect of in vivo bST treatment on ligand binding to BAR and A₁R in adipose tissue membranes prepared from lactating cows and 2) compare receptor changes after bST treatment with those occurring after fasting, a state known to result in enhanced lipolysis and a reduced antilipolytic response to adenosine.

MATERIALS AND METHODS

Animals and experimental design. This experiment was approved by the Cornell University Institutional Animal Care and Use Committee. Multiparous Holstein cows (n = 8) more than 150 d postpartum were used. Cows were housed at the Cornell Teaching and Research Center in individual tie stalls and milked twice daily with the University herd. Unless otherwise indicated, cows were fed ad libitum a total mixed diet that was balanced to meet requirements (8) for protein and energy. A single-reversal design was used to determine the effects of bST; treatment periods were 4 d in length with a 7-d interval between periods to allow cows to return to pretreatment status. The first group of cows (n = 4) was used to evaluate treatment effects on BAR, and a second group (n = 4) was used to evaluate effects on A₁R. Sometribove (recombinant bovine methionyl-ST; Monsanto Company, St. Louis, MO; 40 mg/d) was administered subcutaneously in the hip region at 6:00 p.m. daily. Adipose tissue biopsies were taken during both the control and the bST treatment periods. For the bST period, biopsies were taken approximately 15 hr after the fourth bST injection.

Eight days after the bST treatment regimen (time allowed for incisions to heal and for cows to return to pretreatment status), the second group of cows (n = 4) was used to evaluate the effect of fasting on receptor binding for both BAR and A₁R. In this study, cows were deprived of feed for 3 d (water provided ad libitum) before adipose tissue biopsy. The fasting (FAST) treatment was not randomized with the control treatment because preliminary studies demonstrated that cows did not return to pre-FAST milk production levels after realimentation.

Biopsies. Samples of subcutaneous adipose tissue were taken by surgical biopsy from the tailhead region. Cows were administered xylazine (30 to 40 mg, intravenously) approximately 15 min before biopsy, and a 20-ml lidocaine HCl subdermal block was administered in a circular pattern surrounding the surgical site. Subcutaneous adipose tissue was removed, immediately frozen in liquid nitrogen, and stored at −80°C until membrane preparation and analysis.

Adipose tissue membrane preparation. Frozen tissue was weighed, minced, and placed in tubes containing homogenization buffer (2 ml/g tissue at pH 7.4) that consisted of 10 mM Trizma HCl, 20 mM EDTA, 200 mM sucrose, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), or PEFABLOC (PMSF substitute; Boehringer Mannheim, Indianapolis, IN). Tissue was homogenized with a Polytron (Brinkmann Instruments Co., Westbury, NY) using three 10-s bursts at 50% power, and the crude homogenates were centrifuged at 1,500 × g for 5 min (4°C). The fat cake was removed, and the infranatant was then centrifuged at 100,000 × g for 1 hr (4°C). Pellets were resuspended with five strokes of a Potter-Elvehjem tissue homogenizer (Thomas Scientific, Swedesboro, NJ) in either BAR assay buffer (75 mM Trizma base, 7.5 mM EGTA, 2.5 mM MgCl₂, 0.2 mM PMSF or PEFABLOC; pH 8.0) or standard resuspension buffer (10 mM Trizma HCl, 90 mM NaCl, 0.2 mM PMSF or PEFABLOC; pH 7.4). Suspensions were aliquoted to Eppendorf tubes and snap frozen before storage at −80°C. All membrane preparations were made
within 5 d of biopsy, and all ligand binding assays were conducted within 3 months of membrane preparations.

The protein concentration of the membrane preparations was determined by the method of Bradford (9) with the Bio-Rad kit (Bio-Rad laboratories, Hercules, CA) with bovine serum albumin as a standard. Representative samples (12 biopsies from six cows) were also analyzed by the Lowry method as modified by Peterson (10). Estimates of protein using the Bradford method averaged 89.9 ± 4.6% (mean ± standard deviation) of values obtained by the Lowry procedure.

**β-adrenergic ligand binding assay.** BAR binding was assessed by the BAR antagonist, [125I]-(−)-iodocyanopindolol (ICP; specific activity, 2,200 Ci/mmol; Dupont, NEN Research Products, Boston, MA; #NEX-189), as described by Mersmann and McNeel (11). The final assay volume was 0.15 ml, which consisted of BAR assay buffer (75 mM Trizma base, 2.5 mM MgCl₂, 7.5 mM EGTA, and 0.2 mM PMSF or PEFABLOC; pH 8.0), ICP (12.5 to 800 pM), and crude membrane preparation (30 μg of protein/assay tube). The range of ICP concentrations chosen for the binding assay was based on a preliminary study that used adipose tissue membranes from a nonlactating cow and used a wide range of ICP concentrations (data not shown).

The binding assay commenced with the addition of adipose membranes, and tubes were incubated at 37 °C for 30 min. Reactions were terminated with the addition of 4 ml of ice-cold wash buffer (75 mM Trizma base, 2.5 mM MgCl₂; pH 8.0). Bound ligand and free ligand were separated by vacuum filtration with a Millipore filtration system and glass fiber filters (2.5 cm in diameter, 1 μm thickness). The brand of filter (Boehringer Mannheim or Whatman GF/C) was kept constant within an experiment. Filters were washed four times with 4 ml of wash buffer, and radioactivity on the filters was quantified for 1 min in a gamma spectrometer. Total binding (T) was determined in triplicate, and nonspecific binding (NSB) was determined in duplicate. NSB was measured in the presence of 10⁻⁵ M (+)-propranolol. Specific binding was calculated as T minus NSB for each ligand concentration. Maximal binding (BMAX) and binding affinity (K_D) were estimated by Scatchard analysis with the EBDA program (12).

**Adenosine ligand binding assay.** Binding of the A₁R antagonist [³H]8-cyclopentyl-1,3-dipropylxanthine (DPCPX) to crude adipose tissue membranes was as described Lohse et al. (13) with modifications (14). Crude membranes (40 μg of protein/assay tube) were preincubated with adenosine deaminase (50 U/mg membrane protein) for 15 min at 37° C to remove receptor-bound adenosine. DPCPX (98.1 Ci/mmol; Dupont, NEN Research Products) was diluted in buffer (50 mM Trizma base, 0.1% 3-[3-Cholamidopropyl]-dimethylammonio]-1-propane-sulfonate (CHAPS); pH 7.4) to achieve assay doses. Concentrations of DPCPX used in binding assays were based on a preliminary study that used a much wider range of ligand concentrations and adipose membranes from a nonlactating cow (data not shown). Total ligand binding was assayed in a 250-μl final volume (pH 7.4) consisting of DPCPX (0.1 to 10 nM), 5 mM EDTA, 0.03% CHAPS, and 50 mM Trizma base. The assay was initiated with the addition of the preincubated membrane preparation to assay mix that was prewarmed to 37° C. Nonspecific binding was measured in the presence of 10⁻³ M (±)-propranolol. Specific binding was calculated as T minus NSB for each ligand concentration. Maximal binding (BMAX) and binding affinity (K_D) were estimated by Scatchard analysis with the EBDA program (12).
10 ml of scintillation fluid, and radioactivity was quantified over 20 min in a liquid scintillation spectrometer. Total binding and NSB were measured in duplicate for each concentration of ligand. Specific binding was calculated as T minus NSB. Data were analyzed by Scatchard analysis with the ENZFITTER program (BIOSOFT, Cambridge, United Kingdom).

**Statistical analysis.** Binding parameter estimates were calculated for each animal, and treatment effects were determined with the GLM procedure of SAS (15). For studies comparing control and bST treatments (single-reversal design), the model included the effects of animal, period, order of treatment, and treatment (treatment is represented as period*order). For the determination of the effect of fasting, the model included animal, order (previous order of control and bST treatments), and treatment (control or FAST).

**RESULTS**

The treatment of cows with exogenous bST for 4 d resulted in a 27% increase in milk production (27.5 and 34.9 kg/d for control and bST groups, respectively; SE = 3.3 kg/d; P < 0.05). Food deprivation for 3 d resulted in an 86% reduction in milk production.

To evaluate the completeness of the crude membrane preparation, the specific activity (disintegrations per minute (dpm) specifically bound per microgram of protein) of BAR and A1R binding to the various fractions was measured at a single, saturating dose of each ligand (Figure 1). Enrichment was calculated as the percent increase in specific activity for each cellular fraction as compared with the crude homogenate. BAR binding (dpm/microgram of protein) was essentially absent from the 100,000 x g supernatant, and A1R

![Figure 1]( lugares/1.png)

**Figure 1.** Enrichment of specific binding of the BAR antagonist ICP and the A1R antagonist DCPCX to fractions from the adipose tissue membrane preparation scheme. Enrichment is defined as the percent increase in specific activity (dpm bound/microgram of protein) compared with the crude homogenate. Concentrations of ligand used were 800 pM and 8 nM for ICP and DCPCX, respectively.
binding was minimal in this fraction as well. The final membrane preparation (100,000 x g pellet) averaged 550% specific binding capacity (per microgram of protein) for BAR and A1R compared with the crude homogenate. These data using ICP are in strong agreement with estimates of the enrichment of binding of [3H]dihydroalprenolol (BAR antagonist) to BAR in adipose tissue membrane preparations from pigs (approximately 700% enrichment in the 100,000 x g pellet; 16). Thus, the membrane preparation used in this study represented a highly enriched source of adipose BAR and A1R.

An example of BAR equilibrium binding curves and Scatchard analysis from one animal is depicted in Figure 2. These data are representative of all animals and treatments in that NSB was linear and averaged less than 50% of total binding, and Scatchard

![Equilibrium binding and Scatchard analysis](image)

Figure 2. Equilibrium binding of ICP (BAR antagonist) to adipose tissue membranes from lactating cows. The figure illustrates representative data from one animal. Equilibrium binding curves (upper panel) represent total binding, NSB (in the presence of 10^-5 M (+)-propranolol), and specific binding (total minus nonspecific). Scatchard analysis of the equilibrium binding curves is illustrated in the lower panel.
analysis revealed no indication of multiple ligand binding sites as evident from the linear relationship between bound/free and bound \( (r = 0.95) \).

The effect of treatment on ICP equilibrium binding constants is presented in Tables 1 and 2. Estimates of apparent binding affinity (\( K_d \)) and \( B_{\text{MAX}} \) were similar for the two groups of cows (compare estimates for control treatments in Tables 1 and 2). Treatment with bST did not alter the \( K_d \) for the BAR (Table 1; \( P = 0.76 \)); however, treatment with bST did result in a 60% increase in \( B_{\text{MAX}} \) (\( P < 0.06 \)). In contrast, fasting resulted in significant alterations in both the apparent affinity and the maximal binding of the BAR (Table 2). The \( K_d \) was significantly lower (\( P < 0.03 \)) for FAST vs. fed animals, indicating a higher binding affinity for BAR in adipose membranes from feed-deprived cows. Furthermore, the \( B_{\text{MAX}} \) was reduced by 61% in membranes from FAST animals compared with those from fed animals (\( P < 0.01 \)).

Representative equilibrium binding curves and Scatchard plot for the A\(_r\)R are illustrated in Figure 3. NSB was linear and averaged less than 10% of total binding. The linear Scatchard plot \( (r = 0.98) \) gave no evidence of multiple binding sites.

Equilibrium constants for A\(_r\)R binding are presented in Table 3. The apparent affinity was not altered by treatment with bST or by feed deprivation (\( P = 0.25 \)). In addition, bST treatment did not alter the \( B_{\text{MAX}} \) compared with control (\( P = 0.97 \)); however, maximal binding was reduced by 38% for membranes from FAST animals as compared with either control or bST treatments (\( P < 0.01 \)).

**DISCUSSION**

Delineating the mechanism(s) by which a hormone elicits its various effects requires examination of multiple points in its signal transduction cascade(s). The treatment of lactating cows with bST dramatically alters adipose tissue response to in vivo adrenergic challenges, with the net result of enhanced lipolytic responses to adrenergic (epinephrine) stimuli (1,2). Potential regulatory sites where ST could be acting to enhance the lipolytic response to adrenergic stimuli include the \( \beta \)-adrenergic and A\(_r\) adenosine receptors in adipose tissue. Data on adrenergic and adenosine receptor binding to the adipose tissue of ruminants in various physiologic states are limited, and A\(_r\)R-binding studies have not been reported with bovine adipose tissue. Furthermore, methodologic considerations complicate the interpretation of much of the published data. The few experiments that have been conducted using ruminant tissue used a single dose of radioligand, which prevents an estimation of receptor binding affinity (7,17). Furthermore, many studies have not considered the possible artifactual effects of using agonists vs. antagonists as ligands, the effects of guanine nucleotides and magnesium on agonist binding to the BAR or A\(_r\)R, or the effect of endogenous adenosine on A\(_r\)R binding kinetics (13,14,18,19). Understanding the regulation of BAR and A\(_r\)R is further complicated by inconsistent results from in vivo and in vitro studies. Within the extensive rodent literature, results on

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**Table 1. Effect of ST treatment on equilibrium binding constants from Scatchard analysis of ICP binding to \( \beta \)-adrenergic receptors in adipose membranes.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Control</th>
<th>bST</th>
<th>Pooled</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_d ) (pM)</td>
<td></td>
<td>183.3</td>
<td>171.0</td>
<td>38.1</td>
<td>0.76</td>
</tr>
<tr>
<td>( B_{\text{MAX}} ) (fmol/mg of protein)</td>
<td></td>
<td>34.6</td>
<td>54.3</td>
<td>6.8</td>
<td>0.06</td>
</tr>
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</table>

\( a^{[125]}(\cdot)\)-iodocyanopindolol, \( \beta \)-adrenergic receptor antagonist.

\( b \) \( n = 4 \) lactating cows.

\( c \) Treatments were control (no hormone) or daily injections of bST (40 mg/d for 4 d). Adipose tissue biopsies were taken at 9:00 a.m. (15 hr after bST injection).
TABLE 2. EFFECT OF FASTING ON EQUILIBRIUM BINDING CONSTANTS FROM SCATCHARD ANALYSIS OF ICP\(^a\) BINDING TO \(\beta\)-ADRENERGIC RECEPTORS IN ADIPOSE MEMBRANES.\(^b\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>FAST</th>
<th>Pooled SE</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_d) (pM)</td>
<td>169.3</td>
<td>64.6</td>
<td>18.5</td>
<td>0.03</td>
</tr>
<tr>
<td>(B_{\text{max}}) (fmol/mg of protein)</td>
<td>39.2</td>
<td>15.3</td>
<td>7.2</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(\text{[125]}\text{I}(-)\text{-iodocyanopindolol, } \beta\)-adrenergic receptor antagonist.  
\(\text{n} = 4\) lactating cows.  
\(\text{Treatments were control (ad libitum feed intake) or FAST (feed deprivation for 3 d, water provided ad libitum). Adipose tissue biopsies were taken at 9:00 a.m. (72 hr after initiation of feed deprivation).}

The regulation of the various receptor subtypes during fasting (duration ranging from 12 hr to 8 d), obesity, exercise, and altered thyroid status are equivocal (see review, Ref. 20), and no one has described the ontogeny of changes in BAR and/or \(A_1\)R binding with the advancement of these physiologic or disease states.

This study investigated the regulation of BAR and \(A_1\)R using the bST-treated lactating dairy cow, a model that has been extensively characterized (see review, Ref. 1). Binding to \(\beta\)-adrenergic receptors was evaluated with ICP, a nonsubtype-specific, \(\beta\)-adrenergic antagonist with high specific activity. Ruminant adipose tissue has been reported to express predominantly the \(\beta_2\) receptor subtype (17). However, no one has tested for the presence of the \(\beta_3\) receptor subtype that stimulates lipolysis in rat adipose tissue and mRNA abundance for the \(\beta_1\) subtype is predominant in adult bovine perirenal adipose tissue (21). Scatchard analysis of data from this study is consistent with a homogeneous class of receptors (Figure 2) or essentially equal affinity for each of the subtypes present.

In vivo treatment with bST for 4 d resulted in no alteration in BAR binding affinity, but there was a small increase in maximal binding. These data are consistent with the findings of Watt et al. (7), who used a single dose of ligand ([\(^3\)H]dihydroalpindolol, antagonist) and reported that binding to membranes from sheep adipocytes (mature, nonpregnant, nonlactating ewes) that had been cultured with bST for 48 hr was slightly higher than that of cells that had been cultured with no hormones. Furthermore, binding to BAR is enhanced with the onset of lactation when plasma concentrations of somatotropin are elevated in cows (6) and ewes (17).

Fasting also results in a net increase in the lipolytic response to adrenergic challenge (22–24). However, our results showed that BAR binding after FAST was very different than after bST treatment (Table 2). FAST increased the binding affinity of the receptor (reduced \(K_d\)) and reduced the number of receptors by 61% data from experiments with humans indicated that fasting resulted in no alteration in receptor affinity, but a significant reduction in receptor number (25). It has been hypothesized that the reduction in BAR number with fasting is due to the down-regulation induced by prolonged exposure to catecholamines. In vitro studies demonstrate that the exposure of cells or reconstituted lipid vesicles to \(\beta\)-adrenergic agonists leads to desensitization as well as to sequestration and down-regulation of BAR (26,27). However, it is not clear to what extent fasting results in the adipocyte being exposed to prolonged adrenergic stimulation in vivo. A reduction in sympathetic/adrenal activity occurs in fasted rats, as evidenced by reduced norepinephrine turnover in heart and spleen and reduced plasma epinephrine turnover (28,29). Klein et al. (30) reported the chronic elevation of epinephrine in plasma during fasting in humans. In the case of ruminants, Blum et al. (24) reported that the infusion of a fixed dose of epinephrine resulted in higher plasma concentrations of epinephrine in steers fasted for 4 d as compared with controls. One logical explanation would be that fasting results in reduced rates of the clearance of epinephrine, thereby exposing adipose cells to higher circulating epinephrine concentrations.
Figure 3. Equilibrium binding of DCPCX (A1R antagonist) to adipose tissue membranes from lactating cows. The figure illustrates representative data from one animal. Equilibrium binding curves (upper panel) represent total binding, NSB (in the presence of 10 μM (−)-N6-2-R-PIA), and specific binding (total minus nonspecific). Scatchard analysis of the equilibrium binding curves is illustrated in the lower panel.

Fasting the lactating dairy cow, even for only 24 hr, results in extensive metabolic changes. Given the extreme demands of milk production, these metabolic changes are not buffered by nutrient supply from rumen fill. Fasting results in enhanced plasma ST due to a reduction in clearance rather than a change in release (31,32). Fasting is also characterized by a reduction in plasma glucose and insulin with no reported change in cortisol in cows (32–34); however, fasting (72 hr) enhanced plasma levels of glucocorticoids in heifers (35). In contrast, bST treatment caused no alterations in plasma concentrations of cortisol, prolactin, glucagon, thyroxine, or triiodothyronine (see review, Ref 36). Studies have indicated that the expression of the BAR gene is regulated by glucocorticoids, cAMP, and thyroid status (see review, Ref. 27). Thus, the combined changes in the
Table 3. Effect of ST treatment and fasting on equilibrium binding constants from Scatchard analysis of DPCPX binding to A<sub>1</sub> adenosine receptors in adipose membranes.<sup>b</sup>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>bST</th>
<th>FAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_d ) (nM)</td>
<td>0.10 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>( B_{MAX} ) (fmol/mg of protein)</td>
<td>541.4 ± 73</td>
<td>544.4 ± 86</td>
<td>333.9&lt;sup&gt;d&lt;/sup&gt; ± 74</td>
</tr>
</tbody>
</table>

<sup>a</sup> A<sub>1</sub> adenosine receptor antagonist.
<sup>b</sup> n = 4 lactating cows. Values are least-square means ± SE.
<sup>c</sup> Treatments were control (no hormone, ad libitum feed intake), bST (ad libitum feed intake plus four daily injections of sometribine (40 mg/d), or FAST (feed deprivation for 3 d). Control and bST treatments were in a single-reversal design with adipose tissue biopsies taken at 9:00 a.m. (15 hr after the fourth bST injection). FAST treatment followed 8 d after the control/bST regimen. Adipose tissue biopsies were also taken at 9:00 a.m. (12 hr after initiation of feed deprivation).
<sup>d</sup> Significantly different from control, P < 0.01.

metabolite and endocrine status of fasted animals vs. bST-treated animals may explain the differential changes in BAR binding in these two situations.

Lipolysis is under tonic inhibition by adenosine, and enhanced lipolysis is possible when inhibition is relieved. Indeed, studies have demonstrated that the ability of adenosine to inhibit lipolysis is reduced in adipose tissue from bST-treated cows (4,5). The mechanism for this relief of adenosine inhibition of lipolysis could occur at the level of the adenosine receptor. Adenosine receptor subtypes have not been characterized in ruminant adipose tissue; however, A<sub>1</sub>-adenosine receptors are thought to be the predominant adenosine receptor subtype in fat (37,38). The binding of adenosine to A<sub>1</sub>R activates the inhibitory GTP-binding protein (G<sub>i</sub>) and inhibits lipolysis by inhibiting adenylyl cyclase. The A<sub>1</sub>R may exist in high- and low-affinity states (38-40), and the affinity of the receptors is influenced by G<sub>i</sub>. As in the case of BAR, antagonist but not agonist binding to A<sub>1</sub>R is relatively unaffected by G protein status. Scatchard analysis of our data is consistent with receptors of homogenous affinity (Figure 3). Furthermore, our estimates of \( K_d \) and \( B_{MAX} \) for DCPCX binding obtained with subcutaneous adipose tissue from lactating cows (control, Table 3) are consistent with reports for DCPCX binding to rat epididymal fat (\( K_d = 0.19 \) nM, \( B_{MAX} = 580 \) fmol/mg of protein; Ref. 13) and porcine subcutaneous adipose (\( K_d = 0.87 \) nM, \( B_{MAX} = 479 \) fmol/mg of protein; Ref. 14), but not human adipose tissue (\( K_d = 2.8 \) to 5.2 nM, \( B_{MAX} = 72 \) to 130 fmol/mg of protein; Refs. 19 and 41).

Treatment with bST did not alter the binding affinity or the maximal binding to the A<sub>1</sub>R in adipose tissue (Table 3). The only previous report in ruminants was a limited study by Watt et al. (7) that used one dose of ligand ([3H]phenylisopropyladenosine, agonist) and compared bST treatment against no hormone treatment in a 48-hr culture of ovine adipose tissue explants. They, too, found no effect of bST on A<sub>1</sub>R. This same group reported similar results for ST effects on A<sub>1</sub>R binding to rat adipose (42,43). Thus, it appears that the bST-induced relief of inhibition by PIA is not due to an alteration in A<sub>1</sub>R.

Fasting also results in a reduced ability of adenosine to inhibit in vitro rates of lipolysis in adipose tissue from rats (44) and cows (5). In contrast to bST treatment, feed deprivation did affect adenosine receptors. Fasting resulted in a 38% reduction in the maximal binding of A<sub>1</sub>R, but binding affinity was not altered. Like the BAR, the A<sub>1</sub>R are down-regulated with prolonged exposure to agonist (44,45). Adenosine is thought to be produced by the adipocyte and works in an autocrine/paracrine manner to inhibit lipolysis (46). Therefore, any treatment that results in an enhanced production of adenosine could result in the down-regulation of the A<sub>1</sub>R. In obese humans, for example, adenosine concentration is increased and A<sub>1</sub>R number is reduced in adipose tissue, compared with
normal-weight controls (41). Thus, it is interesting to speculate that fasting may alter adenosine production by the fat cell. To our knowledge the local production of adenosine by the adipocyte has not been directly measured during fasting. Green et al. (47) reported that the activities of 5'-nucleotidase and adenosine deaminase are increased in fat from rodents after a 24-hr fast, thus suggesting that both the production and the degradation of adenosine are enhanced with fasting.

In contrast to these results, Chohan et al. (44) did not observe an effect of fasting (24 hr) on maximal A$_1$R binding ([H]$P_{IA}$, agonist) to adipose tissue from nonpregnant, nonlactating rats. However, those authors did observe curvilinear Scatchard plots and this effect was exaggerated with fasting. This suggests that multiple binding sites exist and that fasting induces an alteration in adenosine receptors in rats. Given the differences in nutritional and endocrine status in rats vs. cows, not to mention effects of the physiologic state (lactating vs. nonlactating) and the duration of fasting, the lack of agreement with the results from this study is not surprising.

Lipolytic inhibition by adenosine and stimulation by catecholamines are altered by many physiologic and disease states. The antilipolytic effects of adenosine are enhanced by lactation, obesity, hypothyroidism, and adrenalectomy, whereas they are reduced by bST, food deprivation, exercise, streptozocin diabetes, and hyperthyroidism. The lipolytic effects of catecholamines appear enhanced in vivo by bST, lactation, and food deprivation, but may be unaltered or reduced when evaluated in vitro. Factors such as duration of treatment, adipose depot, and cell size may affect in vitro results. The exact mechanisms regulating lipolysis (both stimulation and inhibition) in various physiologic states have only been examined in a limited manner. Our data highlight the differential regulation of proteins in the signalling cascades for BAR and A$_i$R. ST treatment resulted in only a modest increase in BAR, whereas feed deprivation resulted in a substantial reduction in BAR number as well as increased BAR affinity. Furthermore, the attenuation of the antilipolytic effects of adenosine can be explained by reduced A$_i$R with fasting but not by treatment with bST. Some other adaptation in adenosine signaling must be in place to account for the bST-induced relief of lipolytic inhibition by adenosine. Recent data suggest that the mechanism involves alteration in the inhibitory GTP-binding protein G$_i$ (5,43).

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