A₁ adenosine receptor antagonism improves glucose tolerance in Zucker rats

BAIYANG XU, DEBORAH A. BERKICH, GEORGE H. CRIST, AND KATHRYN F. LANOUE
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Xu, Baiyang, Deborah A. Berkich, George H. Crist, and Kathryn F. Lanoue. A₁ adenosine receptor antagonism improves glucose tolerance in Zucker rats. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E271–E279, 1998.—The A₁ adenosine receptor antagonist 1,3-dipropyl-8-(p-acrylic)-phenylxanthine (BW-1433) was administered to lean and obese Zucker rats to probe the influence of endogenously activated A1α receptors on whole body energy metabolism. The drug improved glucose tolerance as indicated by a rise in serum glycerol in obese rats. The disappearance of the response by day 7 of chronic studies was accompanied by an increase in A1α numbers. Glucose tolerance tests were administered to rats treated with BW-1433. Peak serum insulin levels and areas under glucose curves (AUGs) were 34 and 41% lower in treated obese animals than in controls, respectively, and 19 and 39% lower in lean animals. With chronic administration (6 wk), AUGs decreased 47 and 33% in obese and lean animals, respectively. There was no effect of BW-1433 in either lean or obese rats on weight gain or percent body fat. Thus the major sustained influence of whole body A1α antagonism in both lean and obese animals was an increase in whole body glucose tolerance at lower levels of insulin.

obesity; lipolysis; insulin sensitivity

A₁ ADENOSINE RECEPTORS are widely distributed in mammalian tissues and are part of a highly homologous family of G protein-linked cell membrane receptors that bind adenosine (11, 18, 31). The function of these receptors is to transmit regulatory signals from extracellular adenosine to the inside of cells. The adenosine receptors can be divided into major classes designated A₁, A₂a, A₂b, and A₃, all of which have been isolated, cloned, and expressed (41). The different receptor subtypes, which are the products of separate genes, can be distinguished on the basis of their agonist and antagonist specificities. The subtypes differ in their influences on cell metabolism and their tissue distribution (31). Whereas the A₁ and A₃ adenosine receptors transduce signals via Gₛ and Gₛ, the A₂ receptors interact with Gₛ. When A₁ or A₃ adenosine receptors predominate in a particular cell type, extracellular adenosine may inhibit adenylylate cyclase or modulate the activity of cationic channels, whereas A₂ receptors may stimulate adenylylate cyclase. Extracellular adenosine acting via the A₁ or A₃ receptors and Gₛ may also inhibit norepinephrine release from sympathetic nerve endings and influence phospholipase C. The A₃ receptors can be distinguished from A₁ by their lack of sensitivity to antagonism by alkyl xanthine derivatives (32, 41, 51). In vivo agonist concentrations are difficult to evaluate because adenosine is produced locally as an autacoid and is rapidly degraded. Previously it was also difficult to identify specific adenosine receptor subtypes responsible for functional tissue responses. However, recent advances in the synthesis of receptor-selective antagonists (11) have provided useful tools to dissect the physiological role of specific adenosine receptor subtypes (for review see Refs. 11, 31, 32, 41, and 51).

One widely used A₁ adenosine receptor antagonist, cyclopentyl-1,3-dipropylxanthine (DPCPX) (5), binds to A₁ receptors 700 times more tightly than to A₂ receptors. Thus DPCPX can be used to quantitate the numbers of A₁ adenosine receptors in isolated membranes and/or to identify physiological phenomena due to endogenous adenosine acting specifically via A₁ adenosine receptors (11). However, DPCPX, like many other xanthine derivatives, is poorly soluble in aqueous media, making its use somewhat inconvenient. In the present study a more polar compound, 1,3-dipropyl-8-(p-acrylic)phenylxanthine (BW-1433), similar in structure to DPCPX, has been employed as an A₁ selective antagonist. Although it is only 53-fold as selective for the rat A₁ adenosine receptor as for A₂ (31), BW-1433 has the advantage that it is orally available. Because of its polarity and negative charge at neutral pH, it is not likely to penetrate the blood-brain barrier (9, 31). Therefore, BW-1433 has the advantage over DPCPX in that the compound probably influences only peripheral receptors.

In the present investigation, to evaluate the influence of A₁ adenosine receptors and endogenous adenosine on triglyceride and glucose metabolism of intact animals, (Fa/?) lean and (fa/fa) obese Zucker rats were treated orally with the A₁ adenosine receptor antagonist BW-1433. Whole body lipolysis was evaluated by measuring serum glycerol levels and glucose metabolism by performing glucose tolerance tests. Zucker rats were used because previous in vitro data obtained from adipocytes (2, 26, 47) and muscle fibers (7, 8) have shown that A₁ adenosine receptors are more active in obese than in lean Zucker rats. Furthermore, excess A₁ adenosine receptor activity may contribute to the adiposity and glucose intolerance of obese (fa/ fa) Zucker rats (2, 26, 47).

Oral administration of BW-1433 rendered rats insensitive to exogenous administration of an adenosine receptor agonist, N-methylscytosine (PIA, 10 µg/kg), demonstrating BW-1433’s effectiveness as a whole body, peripheral, A₁ adenosine receptor antagonist. In agreement with several previous studies (29, 40), PIA lowered fasting serum glycerol levels by >50%. In the present study we found that BW-1433 blocked the PIA-induced decrease in glycerol in a dose-dependent way. When the antagonist was administered chronically, for 1 wk there was a significant transient increase in...
in serum glycerol in obese Zucker rats that could be observed 30 h after initiation of treatment, but not after 7 days.

Previous in vitro studies have shown that adenosine, acting via A<sub>1</sub> adenosine receptors, increases insulin sensitivity in isolated adipocytes (33, 40, 48) but decreases insulin sensitivity in isolated muscle fibers (7, 8). To determine which in vitro phenomenon might predominate in intact animals, acute and chronic tests of the effect of A<sub>1</sub> adenosine receptor antagonism were carried out by administering intraperitoneal glucose tolerance tests to lean and obese Zucker rats. The results of these tests showed that glucose utilization increased in the presence of lower levels of serum insulin when Zucker rats were treated either acutely (6 h) or chronically (6 wk). This effect did not diminish with prolonged treatment. Growth (weight gain) of animals treated with BW-1433 for 6 wk was not altered by A<sub>1</sub> adenosine receptor antagonism, nor was it possible to observe any change in percentage of body fat due to whole body A<sub>1</sub> adenosine receptor antagonism. Thus the major sustained influence of whole body A<sub>1</sub> adenosine receptor antagonism in both lean and obese animals over a 6-wk period was an increase in whole body glucose utilization, mediated possibly by changes in skeletal muscle insulin sensitivity.

### METHODS

**Animal model.** Female Zucker rats were used for the acute studies. The Zucker rat colony used is maintained in the animal facility of The Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine. Breeding pairs used to initiate the colony were obtained from Marcelle Lavau, Inserm U177, Unité de Recherches sur la Physiopathologie de la Nutrition, Paris, France. In acute experiments, 6- to 9-wk-old female animals were fasted overnight but had free access to water. They were given vehicle or vehicle plus BW-1433 by gavage at 8 AM, and experimental studies were performed 6 h later. In chronic experiments 6- to 8-wk-old male and female animals were employed. Lean and obese 6-wk-old animals were divided into groups of 8–10 each, of which 3–5 were either male or female. Each group received either vehicle or vehicle plus BW-1433 (3, 6, or 12 mg/kg every 12 h) for either 1 or 6 wk. Results of male and female animals were analyzed separately. No gender-related differences were observed except in the case of body weight and percentage of body fat. All procedures used in connection with these animals were approved by The Milton S. Hershey Medical Center Institutional Animal Use and Care Committee.

**Measurement of percent body fat.** The percent body fat of animals used in this study was measured with an EM-SCAN model SA-2 small animal body composition analyzer (4), as described previously (2). To standardize the conductivity measurements, we measured total body water, after intraperitoneal injection of 1H<sub>2</sub>O, in a series of animals of different percent body fat. The percent body fat was then plotted as a function of conductivity measured by the SA-2, and the instrument was programmed with the standard curve.

**Administration of PIA and BW-1433.** PIA (10 µg/kg) was dissolved in an aqueous solution containing 10% dimethylsulfoxide and administered to fasted animals intraperitoneally. BW-1433 was obtained as the free acid. In this form the compound is poorly absorbed after oral administration. It was neutralized with an equivalent amount of NaOH (pH 8.5) and suspended in an aqueous solution containing 10 mg/ml methyl cellulose. Various amounts were administered to the rats by gavage as specified in the table and figure legends.

**Serum assays.** Blood samples (150 µl) were taken by nicking the rats’ tails with a surgical scalpel. Blood was collected in heparinized tubes and centrifuged to obtain serum. Serum was deproteinized with an equal volume of 6% perchloric acid and, after centrifugation, neutralized with a solution containing 3 N KOH, 0.5 M 3-(N-morpholino)propanesulfonic acid, and 0.1 M EDTA. Neutralized perchloric acid extracts were assayed for glucose and glyceral by standard spectrophotometric enzymatic assays (16, 46). Insulin was assayed by use of the Diagnostic Products radioimmunoassay kit procedure with serum that had not been deproteinized. The serum concentrations of BW-1433 were measured by high-performance liquid chromatography with a Beckman System Gold Instrument. To measure BW-1433, serum samples are deproteinized with 2.5 volumes of methanol rather than with perchloric acid. After filtration, samples were injected onto a Beckman C<sub>18</sub> analytical column (4.6 × 250 mm). The drug was eluted isocratically with 60% methanol and 10 mM each of tetramethylammonium hydroxide and sodium phosphate (pH 7.0) at a flow rate of 1 ml/min. The antagonist was detected spectrophotometrically at 332 nm. The retention time of BW-1433 is 12 min and the millimolar extinction coefficient is 32.8. The areas under the chromatographic peaks were obtained from a programmable integrator and compared with those obtained from standard solutions of BW-1433.

**Glucose tolerance tests.** Glucose tolerance tests were performed after intraperitoneal injections of glucose (1.25 g/kg). Blood samples were obtained at 15, 30, 60, and 120 min after the glucose injection, and serum was assayed for glucose. Plasma glucose concentrations are plotted as a function of time (see Fig. 5) and the areas under the curve calculated (see Fig. 7). The 4 M × 120 min area was subtracted from the total area of each curve to provide an estimate of the increase due to injected glucose.

**Plasma membranes.** Both lean and obese animals were anesthetized with 50 mg/kg pentobarbital sodium. A low midline incision was made, and endometrial or epididymal fat pads were excised from anesthetized animals. Adipocytes were isolated as described previously (2, 47) after collagenase treatment in the presence of 0.2 µM adenosine. Adipocyte plasma membranes were isolated as described previously (2). Brain plasma membranes were isolated by differential centrifugation of homogenized cerebral cortices from lean and obese Zucker rats as described by Nakata (38).

**Receptor binding.** Receptor binding was studied by measuring the binding of [3H]DPCPX to plasma membranes. Membranes (25 µg) were incubated in buffer containing 50 mM tris(hydroxymethyl)aminomethane-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, and 1 M EDTA at 37°C for 60 min with radiolabeled ligand and with 10 µM adenosine deaminase. The membranes were then rapidly filtered over Whatman GF/C filters. Filters were washed three times with ice-cold buffer before being counted in 10 ml of a biodegradable scintillation cocktail. Nonspecific binding was defined by duplicate determinations with 10<sup>-4</sup> M PIA. Saturation curves were analyzed with a nonlinear curve-fitting computer program (Enzfitter) for the construction of Scatchard transformations and calculations of the binding parameters, maximal receptor binding capacity (B<sub>max</sub>) and dissociation constant (K<sub>d</sub>).

**Competition binding experiments.** Competition binding experiments were carried out using [3H]DPCPX as the radioligand and varying concentrations of nonradioactive BW-1433. Competition curves were analyzed...
Figure 1. Effect of orally administered BW-1433 on serum levels of
BW-1433. Overnight-fasted lean (○) and obese (●) Zucker rats were
given various amounts of BW-1433 by mouth. Six hours later, blood samples were taken and serum was analyzed by high-performance liquid chromatography for BW-1433. Values are means ± SE for 4–7 samples.

Fig. 2. Effect of orally administered BW-1433 on serum levels of
BW-1433. Overnight-fasted lean (○) and obese (●) Zucker rats were
given various amounts of BW-1433 by mouth. Six hours later, blood samples were taken and serum was analyzed by high-performance liquid chromatography for BW-1433. Values are means ± SE for 4–7 samples.

Table 1. Effect of nutritional status on serum glycerol levels

<table>
<thead>
<tr>
<th>Status</th>
<th>Fed 6-h Fast 18-h Fast</th>
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<tbody>
<tr>
<td>Lean</td>
<td>0.117 ± 0.013 (4)</td>
</tr>
<tr>
<td>Obese</td>
<td>0.190 ± 0.011 (4)</td>
</tr>
</tbody>
</table>

Values are means ± SE for nos. of animals in parentheses and are expressed in mM. Blood samples were taken from tails of lean and obese rats at various time intervals after removal of food. Neutralized perchloric acid extracts of serum were assayed enzymatically for glycerol.
The major site of glycerol utilization is the liver, where it is used as a substrate for gluconeogenesis. It is produced mainly in adipose tissue, but it cannot be consumed there because of the absence of glycerokinase (28).

To test the effectiveness of orally administered BW-1433 and to determine drug levels to be employed in later studies, three different amounts of the compound (5, 10, and 15 mg/kg) were administered to animals, and 6 h later 10 μg/kg PIA were injected intraperitoneally into the same animals. The agonist (PIA) when administered alone in the absence of BW-1433 was equally effective in lean and obese animals in lowering plasma glycerol, although it acted more slowly (in time course studies) in the obese animals (data not shown). Figure 3 shows that the antagonist BW-1433 blunted or completely prevented the effect of PIA on serum glycerol in both lean and obese rats and that BW-1433 was more effective at lower levels in lean than in obese animals.

Effect of A1 antagonism in lipolysis. To determine whether endogenous adenosine, as opposed to exogenously administered PIA, exerts a significant influence on whole body fasting lipolysis, lean and obese Zucker rats were given 12 mg/kg of BW-1433 by mouth after an overnight fast and every 12 h thereafter for 7 days. Glycerol levels were measured in blood samples taken before the antagonist was administered, 6 h after the initial administration, 6 h after the third dose (30 h), and 6 h after the last dose on day 7 (Fig. 4). In each case, animals were denied access to food for 14 h before the experimental procedure, and food was restored immediately after the blood sample was taken except on day 7 when animals were killed. There was a significant increase in serum glycerol observed only at 30 h in obese animals because of the administration of antagonist. The increase at 30 h observed in lean animals was not significant. At day 7 the antagonist-induced increase was insignificant for both lean and obese animals. The declining values observed in control, vehicle-treated animals may be due to a training-induced decline in the initial stress of having blood taken. The experiment was repeated with two groups of animals from different litters, and results were similar each time. The results suggest that A1 adenosine receptor activity does influence rates of whole body lipolysis but also indicate that the adipose tissue quickly adapts to the presence of the antagonist, abolishing its influence.

Changes in numbers of A1 adenosine receptors in adipose tissue and brain after administration of BW-1433. Chronic in vivo administration of A1 adenosine receptor agonists or antagonists stimulates adaptive changes in adipose tissue (20, 22, 34, 50), cardiac tissue (30), and brain (17). Therefore, we evaluated the possibility that the disappearance of the effect of BW-1433 on glycerol levels after 7 days of BW-1433 administration may be associated with increased receptor numbers. Maximal [3H]DPCPX binding to adipose tissue membranes was measured. After the 7-day treatment regimen (12 mg/kg every 12 h) of the experiment described above, in which glycerol levels first increased and then declined back to control levels, the animals were killed and samples of endometrial fat and cerebral cortex tissue were taken from vehicle-treated and antagonist-treated rats. Plasma membranes were prepared from isolated adipocytes. Cerebral cortex plasma membranes were isolated from frozen (−70°C) brain tissue by differential centrifugation. A1 adenosine receptor numbers were measured by determining the Bmax of [3H]-DPCPX binding. The results are shown in Table 2. When this experiment was repeated with male animals with epididymal fat pads (n = 2 each group), receptor numbers for epididymal fat pads were in the same range as those for endometrial fat pads, suggesting few if any male vs. female differences. Only the female data

![Fig. 3. Effect of oral BW-1433 on glycerol levels of obese (A) and lean (B) rats given A1 adenosine receptor agonist N6-isopropyl adenosine (PIA). Animals were fasted overnight and BW-1433 was administered orally. Amounts of BW-1433 given were 0 ( ), 5 ( ), 10 ( ), and 15 mg/kg ( ). After 6 h, PIA (10 μg/kg) was injected subcutaneously. Blood samples were taken at times shown. Samples were extracted with perchloric acid and neutralized with KOH. The neutralized extracts were assayed enzymatically for glycerol. Values are mean ± SE; n = 4.](http://ajpendo.physiology.org/DownloadedFrom/10.2302/32246/2017)
Activation of A1 adenosine receptors has an effect on glucose homeostasis in the intact animal are skeletal muscle, heart, adipose tissue, and liver. Studies carried with isolated adipocytes (13, 33, 48) suggest that A1 adenosine agonists decrease insulin sensitivity. The major organs responsible for glucose transport, whereas antagonists increase gluconeogenesis. Thus it was of interest to determine whether BW-1433 administration has an influence on intact animals, its reported effect on skeletal muscle fiber (7, 8) may predominate.

Table 2. Effect of chronic A1 adenosine receptor antagonism by BW-1433 on A1 adenosine receptor numbers in adipose tissue and cerebral cortex tissue.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Adipose Tissue</th>
<th>Cerebral Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lean</td>
<td>Obese</td>
</tr>
<tr>
<td>Vehicle</td>
<td>852 ± 78</td>
<td>856 ± 49</td>
</tr>
<tr>
<td>BW-1433</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12 mg/kg)</td>
<td>1,058 ± 108*</td>
<td>1,195 ± 42*</td>
</tr>
</tbody>
</table>

Values (means ± SE; n = 4) are expressed in fmol/mg. Lean and obese Zucker rats were treated every 12 h with 12 mg/kg 1,3-dipropyl-8-(p-acrylyl)phenylxanthine (BW-1433). At the end of 1 wk they were killed, and endometrial fat pads and cerebral cortices were taken. Plasma membranes were prepared as described in METHODS and frozen until later use. *P < 0.05, BW-1433 treated vs. vehicle.

For receptor numbers are shown in Table 2. As previously reported (2), the A1 adenosine receptor numbers are similar in lean and obese animals. Treatment with the A1 adenosine receptor antagonist for 1 wk increased receptor numbers in adipose tissue of obese animals by 40%. A smaller (24%) but still significant increase in lean animals was also observed. A1 adenosine receptor numbers in brain membranes were not increased by whole body A1 adenosine receptor antagonism. This provided support for the hypothesis (36) that this polar antagonist does not cross the blood-brain barrier.

Fig. 5. Effect of different oral doses of BW-1433 on serum insulin levels during glucose tolerance tests. A glucose tolerance test was administered to overnight-fasted obese (A) and lean (B) Zucker rats 6 h after oral administration of 0 (○) or 15 (●) mg/kg BW-1433. Rats were injected with 1.25 g/kg glucose, and blood samples were taken just before and at 15, 30, 60, and 120 min after injection. KOH-neutralized perchloric acid extracts of serum were assayed for glucose. Values are means ± SE; n = 4.

Influence of oral BW-1433 on insulin sensitivity. Studies of isolated muscle fibers (7, 8) have indicated that A1 adenosine agonists decrease insulin sensitivity of glucose transport, whereas antagonists increase insulin sensitivity. The major organs responsible for glucose homeostasis in the intact animal are skeletal muscle, heart, adipose tissue, and liver. Studies carried out with isolated adipocytes (13, 33, 48) suggest that activation of A1 adenosine receptors has an effect on glucose uptake opposite to that seen in muscle. Studies of in situ dog hearts show that adenosine acting through A2 receptors also stimulates glucose uptake by cardiac tissue but only in the presence of insulin (27). Adenosine increases gluconeogenesis in the liver, but this is secondary to its influence on A2 adenosine receptors (6, 37). Therefore, BW-1433, an A2-specific antagonist, is unlikely to have an effect on hepatic gluconeogenesis. Thus it was of interest to determine whether BW-1433 administration has an influence in the intact animals on glucose or insulin levels, and if so, in what direction.

In initial acute studies, lean and obese animals were fasted overnight and then given 12 mg/kg of BW-1433 or vehicle. Six hours later serum samples were taken and analyzed for glucose and insulin. Serum insulin levels were higher in control obese (20.0 ± 1.9 µU/ml) than in control lean animals (10.0 ± 0.5 µU/ml), as previously reported (21), and were not altered by the presence of BW-1433 in the serum. Fasting glucose levels were also unchanged by the A1 antagonist. However, when glucose tolerance tests were subsequently administered, A1 adenosine receptor antagonism lowered glucose levels measured during the glucose tolerance tests (Fig. 5). Serum insulin concentra-
tolerance did not diminish with time. Instead, in the case of the untreated obese animals, the areas under the glucose tolerance curves increased significantly (0.01 > P > 0.001) over the 5-wk treatment period, whereas the untreated lean animals’ glucose tolerance did not change during the 6-wk treatment regimen. In the case of the two 12 mg/kg treatment groups, the areas also remained unchanged. However, after 5 wk, the lower doses of the orally administered antagonist appeared to be less effective in the obese than in the lean animals.

At the completion of the chronic 6-wk study just described, animals were fasted overnight, and insulin, glucose, and glycerol levels were measured in serum. The results are shown in Table 3. J ust as we observed at the completion of the 1-wk study, glycerol levels at the end of the 6-wk study were the same in treated and untreated animals. Chronic A1 adenosine receptor antagonist also produced no change in fasting blood glucose levels. Likewise, the differences between lean and obese fasting serum insulin levels were not significant.

Weight gain and percentage of body fat. To determine whether the Zucker rats lost body fat as a result of the 6-wk administration of BW-1433, animals were weighed every other day during the 6-wk regimen. Male animals gained weight faster than female animals. However, there was no significant effect of oral BW-1433 at any of the three dosages on weight gain. When the data are pooled or separated according to gender, the conclusions are the same (data not shown).

Because it was possible that loss of fat in animals given BW-1433 might have been masked by an increase in muscle mass, percentage of body fat was also measured using a small body composition analyzer (EM SCAN model SA-2). The data, again analyzed separately by gender, indicate that percentage of body fat was not altered by the A1 adenosine antagonist, although there were gender-related differences in percentage of body fat (data not shown).

DISCUSSION

In vitro studies of isolated adipocytes by us (2, 47) and others (10) have shown that A1 adenosine receptor antagonists stimulate lipolysis by raising cAMP levels, even in the absence of added adenosine and in the presence of adenosine deaminase (to remove endogenous adenosine). We reported (47) that the ability of antagonists to stimulate lipolysis and increase cAMP is greater in adipocytes from obese Zucker rats than in those from lean rats. Because receptor numbers and Kd values are the same in lean and obese animals, we took this to mean that the receptors in adipose tissue of the obese animals had higher tonic activity in the absence of agonist than the lean receptors. We have speculated that this may be responsible for the low levels of cAMP observed in the obese fat cells.

In the present study, the increase in serum glycerol observed when the antagonist BW-1433 was administered to obese animals in vivo, but not when it was administered to lean animals (Fig. 4), may be due to higher tonic activity of adipocyte A1 adenosine receptors, or to higher levels of endogenous extracellular adenosine, in the obese animals.

Studies by other workers document the influence of PIA on rodent and human adipocytes. These indicate that the agonist PIA increases insulin sensitivity of

Table 3. Effect of chronic A1 antagonism on serum levels of glucose, insulin, and glycerol in lean and obese Zucker rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin, µU/ml</th>
<th>Glucose, mM</th>
<th>Glycerol, mM</th>
<th>Insulin, µU/ml</th>
<th>Glucose, mM</th>
<th>Glycerol, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>18.2 ± 2.1</td>
<td>6.5 ± 0.3</td>
<td>0.47 ± 0.05</td>
<td>6.9 ± 1.8</td>
<td>5.7 ± 0.1</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td>BW-1433, mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16.8 ± 2.7</td>
<td>6.8 ± 0.2</td>
<td>0.56 ± 0.04</td>
<td>6.9 ± 0.41</td>
<td>6.0 ± 0.2</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>14.4 ± 1.7</td>
<td>6.1 ± 0.1</td>
<td>0.36 ± 0.03</td>
<td>7.4 ± 0.43</td>
<td>6.0 ± 0.1</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>12</td>
<td>15.9 ± 2.5</td>
<td>6.4 ± 0.3</td>
<td>0.52 ± 0.02</td>
<td>7.8 ± 0.35</td>
<td>5.9 ± 0.1</td>
<td>0.54 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8. Experimental protocols were as described in Fig. 7. Blood samples were taken at end of 6-wk treatment period after an overnight fast. Listed doses of BW-1433 were given every 12 h during 6-wk regimen.
Our studies show that the blunted A1 responses are especially pronounced in white females with upper nist responses (15, 25, 39). The blunted responses are may also explain why chronic exposure to the antagonist other studies of adipocytes isolated from obese humans cate that it inhibits insulin release by lowering in the pancreas. In vitro studies (3) of the effect of low cAMP. The influence of an adenosine receptor antago

PnG proteins. This conclusion is supported by preliminary data, which demonstrated changes in G protein levels during osmotic minipump infusion of BW-1433 for 7 days (35). Upregulation of A1 adenosine receptors in fat may also explain why chronic exposure to the antagonist for 6 wk does not change growth rate or percent body fat.

Studies of the influence of A1 adenosine receptor agonists and antagonists given chronically to intact animals have been performed previously (17, 20, 22, 30, 34, 50). Downregulation of peripheral receptor numbers with chronic agonist treatment (20, 22, 34) and upregulation of receptor numbers with antagonist (17, 30, 50) have been reported.

In in vivo studies, Gerrits et al. (19) demonstrated that the decline in the expression of adipose tissue GLUT-4 that occurs when rats are made streptozotocin diabetic can be reversed by subcutaneous injections of 0.3 µmol PIA/kg every 2 h for several days. This effect was mediated by PIA-induced decreases in adipose tissue cAMP.

Other chronic studies of agonist influence on adipose tissue (with use of osmotic minipumps) indicated that the initial stimulation of lipolysis that occurred when PIA was infused decreases with time and was virtually absent after 6 days (22). This adaptation to agonist was associated with a decrease in receptor numbers and a decrease in G, (20, 22). When the adaptive mechanism was probed further by measuring mRNA levels in adipocyte cell cultures treated chronically with PIA, no decrease in mRNA levels was found corresponding to the observed decrease in receptor numbers and membrane G, (34).

The influence of the A1 adenosine receptors in the central nervous system has also been investigated during whole body agonist and antagonist administration. Because there are more A1 adenosine receptors in brain than in any other organ of the body, it is likely that the most profound influence of agonists and antagonists would be felt in that organ (17). Decreases in locomotor activity that accompany the intraperitoneal administration of A1 adenosine receptor agonists to mice have been documented (24, 36). The locomotor depression induced by intraperitoneal administration of N6-cyclohexyladenosine (CHA) is effectively reversed by coadministration of the antagonist 8-cyclopentyl-1,3-dipropylxanthine, known as CPX. This relatively nonpolar antagonist penetrated the blood-brain barrier and thereby blocked the central effects of the agonist. On the other hand, 4 mg/kg BW-1433 injected intraperitoneally (blocking the peripheral effects of CHA) was ineffective in blocking CHA's effect on locomotor activity (24). The selectivity of BW-1433 for the peripheral receptors as opposed to receptors in the central nervous system is explained by its relative polarity, which may prevent it from crossing the blood-brain barrier. In support of this supposition, we observed no increase in A1 adenosine receptors in cerebral cortex membranes of obese Zucker rats treated chronically with BW-1433, although an increase in receptor numbers was observed in adipose tissue (Table 2).

Unexpectedly, the most obvious change in energy metabolism induced by the administration of BW-1433 was not a change in adipose tissue metabolism but an increase in whole body insulin sensitivity as judged by glucose tolerance. This change was sustained throughout the 6-wk regimen of antagonist administration (Fig. 7). The whole body increase in insulin sensitivity was not the result of blocking A1 adenosine receptors in adipose tissue, because this would have produced the
opposite effect on insulin sensitivity (13, 33, 40), and it was not caused by a decrease in gluconeogenesis. Although adenosine itself stimulates hepatic gluconeogenesis, studies with specific agonists and antagonists show that the hepatic effects of adenosine are on the $A_2$ adenosine receptor (6, 37). Moreover, there are only trace levels of $A_3$ adenosine receptors in liver (49). The lack of $A_1$ receptors in liver and the apparent $A_2$ specificity of the increases in gluconeogenesis suggest that BW-1433 does not exert its effect on glucose disappearance by inhibiting hepatic gluconeogenesis.

An attractive possibility is a direct effect of BW-1433 on glucose uptake in muscle. Previous in vitro studies of isolated muscle strips indicated that $A_1$ adenosine receptor antagonists increased insulin sensitivity (7, 8). An argument against this possibility is that $A_2$ receptors are low in that tissue (49). The effect of BW-1433 might be indirectly acting via $A_2$ adenosine receptors in peripheral nerve endings, or the BW-1433 might act via muscle $A_{2b}$ adenosine receptors, which tend to have less stringency antagonist specificity than $A_{2a}$ adenosine receptors. The lack of influence of BW-1433 on the fasting serum glucose and insulin levels of Zucker rats observed in the present study (Fig. 5, Table 3) is surprising in light of the changes in intraperitoneal glucose tolerance. However, because fasting blood glucose is likely to be most strongly influenced by the balance between the rate of hepatic gluconeogenesis and the rate of insulin-independent glucose utilization by the brain and other tissues that lack GLUT-4, the finding is perhaps not unreasonable.

The molecular basis for the influence of $A_1$ adenosine receptors on insulin signaling is not known, but several instances of cross talk between the $G_i$ protein signaling and the insulin signal have been reported. A decrease in cAMP due to $G_i$ activation might be expected to increase, rather than decrease insulin sensitivity. However, $A_1$ adenosine receptor agonists have no effect on skeletal muscle cAMP (8), so that dichotomy is avoided.

In some tissues, $A_1$ adenosine receptors activate protein kinase C (31), an event associated with a decrease in insulin sensitivity (12, 23). The decrease in insulin sensitivity might be mediated by a protein kinase C-catalyzed serine or threonine phosphorylation of the insulin receptor or of an insulin receptor substrate (IRS) such as IRS-1, because these events are known to block insulin action (42). Alternatively, some recent reports suggest that $G_i$, when activated in NIH 3T3 cells, can stimulate protein tyrosine phosphorylation (14, 44). The possibility that this occurs in obese Zucker rat skeletal muscle is suggested by the fact that protein tyrosine phosphatase activity is unusually high in obese Zucker rat skeletal muscle (1).

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