Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice

CHRISTINE RAVINET TRILLOU, MICHELE ARNONE, CLAIRE DELGORGE, NADINE GONALONS, PETER KEANE, JEAN-PIERRE MAFFRAND, and PHILIPPE SOUBRIÈ

Central Nervous System Research, Sanofi-Synthelabo, 31036 Toulouse Cedex, France

Submitted 6 September 2002; accepted in final form 17 October 2002

SR141716, a CB1 receptor antagonist, in diet-induced obese mice. Am J Physiol Regul Integr Comp Physiol 284: R345–R353, 2003. First published October 24, 2002; 10.1152/ajpregu.00545.2002.—Because the CB1 receptor antagonist SR141716 was previously reported to modulate food intake in rodents, we studied its efficacy in reducing obesity in a diet-induced obesity (DIO) model widely used for research on the human obesity syndrome. During a 5-wk treatment, SR141716 (10 mg·kg⁻¹·day⁻¹ orally) induced a transient reduction of food intake (~48% on week 1) and a marked but sustained reduction of body weight (~20%) and adiposity (~50%) of DIO mice. Furthermore, SR141716 corrected the insulin resistance and lowered plasma leptin, insulin, and free fatty acid levels. Most of these effects were present, but less pronounced at 3 mg·kg⁻¹·day⁻¹. In addition to its hyperphagic action, SR141716 may influence metabolic processes as the body weight loss of SR141716-treated mice was significantly higher during 24-h fasting compared with vehicle-treated animals, and when a 3-day treatment was compared with a pair feeding. SR141716 had no effect in CB1 receptor knockout mice, which confirmed the implication of CB1 receptors in the activity of the compound. These findings suggest that SR141716 has a potential as a novel anti-obesity treatment.

Obesity is a health problem of epidemic proportions in the industrialized world. It is associated with an increased risk of life-threatening pathologies such as diabetes, hypertension, and heart diseases, and weight loss has been reported to ameliorate these associated conditions. Reducing weight by caloric restriction generally fails as most obese patients regain their lost weight thereafter (53). Thus medicinal treatment of obesity has become a necessity. Among the numerous targets explored in this way are the central regulators of food intake. One of them is the cannabinoid system with its putative endogenous ligands anandamide and 2-arachidonoyl glycerol (2-AG). In addition to a wide variety of pharmacological activities, this system has been implicated in food intake regulation. Stimulation of appetite is one of the most commonly related effects of marijuana in humans (1) and Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the main active component of this drug, has been reported to produce hyperphagia (25, 27, 59). The endogenous cannabinoids anandamide and 2-AG also stimulate feeding when administered to rats (26, 56, 58).

SR141716, a potent and selective central cannabinoid (CB1) receptor antagonist (39), has been widely used to investigate the role of CB1 receptors in appetite regulation. SR141716 antagonizes the hyperphagia induced by anandamide, 2-AG and Δ⁹-THC (26, 56, 57). These results provide strong evidence for the involvement of CB1 receptors in the regulation of feeding. In addition to modulating the effects of cannabinoids in animals, SR141716 has been shown to produce changes in ingestive behaviors when administered alone. Several studies have reported that SR141716 selectively attenuates the consumption of palatable food or drink. It decreases sucrose intake in rats (8), alcohol consumption in mice (8), and sweet diet intake in marmosets (43) while having little effect on bland food consumption. These results suggest that the blockade of the central cannabinoid system may alter the rewarding value of foods and so reduce eating. As the majority of human obesity is partly due to difficulty in regulating intake in the face of an increased availability of palatable foods (5), SR141716 may provide a new interesting way for the treatment of this disorder.

Recently, a strong relationship between the endocannabinoid system and leptin was reported (15). Genetically obese rodents with defective leptin signaling exhibit elevated hypothalamic endocannabinoid levels, and these levels are reduced in the leptin-deficient ob/ob mice after an acute leptin treatment. In one of these genetically obese animals, the Zucker rat, SR141716 induced a significant decrease in food intake and body weight gain when administered orally for 4 wk (personal results). The compound has also been shown to reduce the intake of a high-fat diet in these obese rats as previously reported (7). In the present work, we chose to assess the effect of SR141716 in a diet-induced obesity (DIO) model. Rodents fed a high-
fat diet develop moderate obesity with an increase in energy intake and in insulin resistance (2, 33, 47). At present, the implication of the endocannabinoid system in the pathogenesis of DIO is unknown. Interestingly, a deficient leptin function has been widely described in DIO animals (30, 32, 33, 51, 55). Furthermore, this model has proven to be a useful one for the study of the human disorder (22, 38). Thus the effect of a chronic SR141716 treatment was investigated in C57BL/6 DIO mice, with emphasis on changes in food intake. The most effective dose tested in the Zucker rat study (10 mg/kg orally) was used in this experiment. In a subsequent experiment, which included plasma analyses, the effect of a lower dose (3 mg/kg) on adiposity was also assessed. Finally, to confirm whether the effects of SR141716 were mediated by CB1 receptor blockade, the compound was administered to CB1 receptor knockout mice fed a high-fat diet.

**MATERIALS AND METHODS**

**Experimental Procedures**

In all experiments, mice were housed on a reverse light-dark cycle (lights off 0900–2100) in a room with temperature (22 ± 2°C) and humidity control. They were fed either a high-fat diet (HFD) of 4.7 kcal/g energy density (TD 97366, Harlan; 49% fat, 18% protein, 33% carbohydrate) or a standard mouse diet (STD) containing 2.9 kcal/g (A04C, UAR; 8% fat, 19% protein, 73% carbohydrate). SR141716 [N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] was administered orally in distilled water with 0.1% Tween 80 just before the onset of the dark phase. A habituation to treatment with the vehicle was performed during the week before the start of treatment. All procedures have been approved by the Animal Care and Use Committee of Sanofi-Synthélabo Research. They were carried out in accordance with the French legislation, with the European Community Guidelines (86/609/EEC) and with the American Physiological Society's guiding principles for research (4).

**SR141716 Treatment in DIO Mice**

Six-week-old C57BL/6J male mice, purchased from Ifa Credo (France), were given HFD or STD diets for 12–17 wk before drug treatment started.

In the first experiment, mice were weight matched and assigned to one of the following three groups: high-fat diet fed/vehicle treated (HFD-Veh), high-fat diet fed/SR141716 10 mg/kg treated (HFD-SR 10 mg), and standard diet fed/vehicle treated (STD-Veh). Body weight and individual food intake were recorded daily. The energy intake was determined taking into account the caloric density of each diet and the average daily energy intake was calculated weekly for each of the 5 wk of treatment. To balance the difference in body weight between the groups, the relative energy intake was calculated by correcting for body weight and was expressed as kilocalories per gram mouse per day × 100. After a 30-day treatment, body fat and lean masses were estimated using a small-animal body composition analyzer (model SA-3000, EM-SCAN) in anesthetized mice. The measurement principle is based on the high electrical conductivity in all lean tissues relative to lipids when exposed to an oscillating radio frequency (16, 49). We previously calibrated the device by using carcass analysis of mice fed a standard or a high-fat diet. Regression models integrating total body electrical conductivity (TOBEC) values and body weights were fitted to chemically analyzed whole body protein and fat contents (adjusted $r^2 = 0.96, P < 0.0001$ for protein, adjusted $r^2 = 0.93, P < 0.0001$ for fat). Food deprivation was shown to produce a reduction of metabolic rate in rodents (60), and body weight loss during fasting was reported to be an indicator of this metabolic rate (19). Thus body weight change during a 24-h fast was measured after 5 wk of treatment. Finally, additional 18-h-fasted mice were injected intraperitoneally with human recombinant insulin (Sigma) at the dose of 0.6 U/kg. Glycemia was measured before and 30, 60, 90, 120, and 180 min after insulin injection using a glucose analyzer (Bayer Diagnostics) to assess insulin sensitivity.

A second experiment tested two doses of SR141716 (3 and 10 mg·kg$^{-1}$·day$^{-1}$) in dietary obese mice (HFD-SR 3 mg and HFD-SR 10 mg groups) compared with obese and lean vehicle-treated groups. After a 40-day treatment, mice were killed by decapitation. White adipose tissues (epididymal, lumbar, and perirenal) and skeletal muscles (soleus and gastrocnemius) were removed and weighed. Insulin and leptin plasma levels were determined using radioimmunoassays (respectively, Amersham and Linco). Enzymatic assays were used to assess cholesterol, triglycerides (Sigma Diagnostics), and nonesterified fatty acids (NEFA) (Roche).

A third experiment compared a 3-day SR141716 treatment with a caloric restriction. Obese mice were randomized into one of the three treatment groups: a group treated with vehicle, a group treated with SR141716 at the dose of 10 mg·kg$^{-1}$·day$^{-1}$ for 3 days, and a vehicle-treated pair-fed group (HFD-PFVeh) that was fed the same quantity of diet as that consumed by the animals receiving SR141716. Food intake and body weight were monitored daily; lumbar white adipose pads were weighed after death.

**SR141716 Treatment in CB1 Receptor Knockout Mice**

CB1 receptor knockout (CB1$^{-/-}$) mice were generated as described previously (40). Briefly, for constructing the targeting vector, a 9-kb KpnI-KpnI fragment including the entire coding region of the CB1 receptor gene was cloned from a 129SvJ6 mouse genomic library. An 18-base fragment containing part of the coding region was inactivated by substitution with a neomycin resistance cassette. The targeting vector was transfected into embryonic stem cells from the 129/Ola line. Then, mutated ES cells were injected into C57BL/6 blastocysts to generate chimeric founder mice. Germline transmission of the targeted allele was determined by PCR analysis of mouse tail genomic DNA. The homozygous CB1$^{-/-}$ mice used in this work were on a C57BL/6X129/Ola F2 genetic background. The lack of binding of the synthetic CB1 receptor agonist [3H]CP55,940 in these mice was checked by autoradiography in various brain sections. After 6 wk of high-fat diet, 15-wk-old male CB1$^{-/-}$ mice were either given SR141716 orally at the dose of 10 mg·kg$^{-1}$·day$^{-1}$ or treated with vehicle alone. Body weight and food intake were monitored daily throughout the 3-wk treatment period as previously described.

**Statistical analyses**

ANOVA and post hoc analyses were performed using SAS version 8.2. A repeated two-way ANOVA for treatment and time (repeated measures on food intake and body weight) or one-way ANOVA for treatment (all other variables) was used to determine statistical significance. When a single dose of SR141716 was included in the experiment, post hoc comparisons between the SR141716-treated group, obese, and lean
control groups were performed after Holm’s adjustment. When two doses of SR141716 were studied, comparisons among dietary obese mice receiving each of SR141716 doses vs. vehicle were performed by Dunnett’s test. When the hypotheses necessary for the application of parametric tests were not achieved, appropriate transformations were applied. The results are presented as means ± SEM.

RESULTS

SR141716 Treatment in DIO Mice

Experiment 1. Before treatment, mean body weights were 37.5 ± 0.4 g for the dietary obese mice and 26.9 ± 0.2 g for the lean animals (+39%, P < 0.01). SR141716 significantly decreased the body weight of HFD-fed mice (Fig. 1A). After 5 wk of treatment, HFD-SR 10 mg mice were 20% lighter than the HFD-Veh group (−8 g, P < 0.01) and only 14% heavier than lean animals (+4 g, P < 0.01). In each vehicle-treated group, the average daily energy intake per mouse (Fig. 1B) appeared constant over the study period: 15.3 ± 0.2 kcal/day in the HFD group and 10.9 ± 0.1 kcal/day in the STD group (P < 0.01 between both groups). SR141716 produced a fall in energy intake of HFD mice during the first week of treatment (−48% compared with HFD-Veh group, P < 0.01). Thereafter, energy intake of the SR141716-treated group returned close to the HFD-Veh level (−12% on week 5, P < 0.01). When food consumption was corrected for differences in body weight (Fig. 1C), the two vehicle-treated groups ate 0.40 ± 0.00 kcal·g mouse−1·day−1 over the treatment period. The relative energy intake of the HFD-SR 10 mg mice was reduced to 0.23 ± 0.01 kcal·g mouse−1·day−1 (P < 0.01 vs. the 2 vehicle-treated groups) during the first week of treatment. This effect then progressively reversed, and at the end of the treatment period, the ratio increased to 0.45 ± 0.01 kcal·g mouse−1·day−1 (during weeks 4 and 5, P < 0.05 vs. HFD-Veh and STD-Veh groups).

The body composition estimated by the TOBEC analyzer is shown in Table 1. Compared with lean animals, the total fat content was significantly higher (P < 0.01) in dietary obese mice when expressed as absolute or relative values. Total protein mass was slightly increased in obese animals but relative content was lower (P < 0.01 for absolute and relative values). SR141716 markedly reduced the total fat content in HFD mice (P < 0.01) as well as their relative adiposity (P < 0.01). Protein content was also slightly decreased in HFD-SR 10 mg mice compared with the HFD-Veh group (P < 0.01) and the relative value was increased (P < 0.01). Thus the changes in fat and lean contents in HFD-SR 10 mg mice brought the body composition profile of these mice close to that of lean animals although the differences between the two groups remained significant (P < 0.01).

During a 24-h fast, the body weight of obese mice was decreased by 6.5% while lean mice lost 12.8% of their initial body weight (Fig. 2). The difference between the two control groups was highly significant (P < 0.01). SR141716 increased the response of HFD mice to fasting and the body weight loss in HFD-SR 10 mg animals reached 8.8% (P < 0.01 vs. each vehicle-treated group).

As shown in Fig. 3, a significant increase of fasting glucose concentration was observed in obese animals compared with lean mice (P < 0.01). After the treatment with SR141716, the fasting glycemia of HFD-fed mice returned to the STD-Veh group’s value. The sensitivity to insulin assessed by calculation of the area...
under the curve of glycemia during the 3 h after an intraperitoneal insulin injection was significantly reduced in DIO mice (P < 0.01). This decrease in insulin sensitivity was reversed by SR141716 treatment.

Experiment 2. Before treatment started, obese mice were 34% heavier than lean animals (41.0 ± 4 g vs. 30.6 ± 4 g, P < 0.01). SR141716 induced a marked decrease in body weight of HFD-fed mice (Fig. 4A). This effect was dose dependent over all the study period. At the end of the 40-day treatment, HFD-Veh mice weighed 40.6 ± 1.2 g while body weight was 36.6 ± 0.8 g in the SR 3 mg group (−10% vs. HFD-Veh mice, P < 0.01) and 33.4 ± 0.6 g in the SR 10 mg group (−18% vs. HFD-Veh mice, P < 0.01). As expected, the weight of standard animals remained low (29.8 ± 0.4 g). The decrease in food intake was dose dependent only during the first few days of treatment (Fig. 4B). Thereafter, as was observed during the first experiment, the effect gradually attenuated and food intake levels of both SR141716-treated groups reached values close to that of the vehicle-treated group. The weight of white adipose tissues collected confirmed the increased adiposity of dietary-obese mice (P < 0.01 compared with lean animals for all white adipose pads) and the effect of SR141716 treatment (Table 2). The dose of 3 mg/kg lowered the weight of epididymal, perirenal, and lumbar white adipose pads by −30% (P < 0.05 compared with HFD-Veh mice) and the dose of 10 mg/kg induced a 50% decrease of these pads (P < 0.01 vs. the obese group). In marked contrast, skeletal muscle weight was not changed after SR141716 treatment.

Insulin, leptin, and total cholesterol plasma levels were higher (P < 0.01) in dietary-obese mice while circulating NEFA were lower (P < 0.01) compared with lean animals (Table 3). SR141716 decreased insulin levels by 29% in the HFD-SR 3 mg group (P < 0.05 vs. HFD-Veh mice) and by 50% in the HFD-SR 10 mg group (P < 0.01 compared with HFD-Veh mice). Leptin levels were similarly lowered (−22% in HFD-SR 3 mg group, not significant, and −53% in the HFD-SR 10 mg

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**Table 1. Body weight and body composition after a 5-wk treatment with SR141716 (10 mg·kg⁻¹·day⁻¹) in dietary obese mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Fat, g</th>
<th>Protein, g</th>
<th>Fat, %</th>
<th>Protein, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFD-Veh</td>
<td>38.7 ± 1.0*</td>
<td>9.3 ± 0.8*</td>
<td>7.0 ± 0.1*</td>
<td>23.7 ± 1.5*</td>
<td>18.1 ± 0.2*</td>
</tr>
<tr>
<td>HFD-SR (10 mg)</td>
<td>31.0 ± 0.7†</td>
<td>3.9 ± 0.4†</td>
<td>6.2 ± 0.1†</td>
<td>12.3 ± 1.2†</td>
<td>19.9 ± 0.2†</td>
</tr>
<tr>
<td>STD-Veh</td>
<td>27.2 ± 0.3‡</td>
<td>1.3 ± 0.1‡</td>
<td>5.8 ± 0.0‡</td>
<td>4.7 ± 0.3‡</td>
<td>21.4 ± 0.1‡</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n = 10). Fat and protein contents were estimated by the TOBEC system and expressed as absolute (g) or relative (% body weight) values. Transformations were applied on values [x² for % protein, log (x) for other variables]. Different symbols indicate significant differences (P < 0.01). HFD, high-fat diet; STD, standard mouse diet.

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**Fig. 2. Effect of SR141716 (10 mg·kg⁻¹·day⁻¹) on body weight change during a 24-h fast in dietary obese mice (n = 8). Weight loss was expressed as a percentage of initial body weight. Values are means ± SEM. Bars with different letters are significantly different (P < 0.01)**

**Fig. 3. Effect of SR141716 (10 mg·kg⁻¹·day⁻¹) on fasting glycemia (A) and insulin sensitivity (B) in dietary obese mice (n = 8). Insulin sensitivity was assessed by calculating the area under the curve (AUC) of glycemia during the 3 h after intraperitoneal insulin (0.06 U/kg) injection in fasted mice. Values are mean ± SEM. Bars with different letters are significantly different (P < 0.01)**
group, \( P < 0.05 \). Plasma cholesterol was unchanged by SR141716 treatment but a significant reduction (\( P < 0.05 \)) was observed in NEFA of the HFD-SR 10 mg group compared with obese control animals.

Experiment 3. Before treatment started, the mean weight of obese mice was 35.6 ± 0.2 g. As shown in Fig. 5A, by the end of day 3, the HFD-SR 10 mg group had lost 4.1 ± 0.2 g (11.6 ± 0.5\% of their initial body weight), whereas pair-fed mice lost only 3.3 ± 0.1 g (9.3 ± 0.2\% of their body weight). The difference between the two groups was significant (\( P < 0.01 \)). Hence, although pair-fed mice were given the same quantity of food as that consumed by the SR141716-treated group, they lost ~20\% less than SR141716-treated mice (Fig. 5B). The weight of lumbar white adipose pads confirmed that weight loss was accompanied by a decreased adiposity (Fig. 5C) but no significant difference was found between the HFD-SR 10 mg and HFD-PFVeh groups.

**SR141716 Treatment in CB1\(^{-/-}\) Mice**

The autoradiography confirmed the absence of \([3H]CP55,940\) binding in CB1\(^{-/-}\) mice (Fig. 6). At the start of high-fat diet, the mean body weight of CB1\(^{-/-}\) mice was 24.0 ± 0.4 g. When treatment started 6 wk later, the mean body weight was 27.6 ± 0.6 g. As shown in Fig. 7, the 3-wk treatment with SR141716 (10 mg·kg\(^{-1}\)·day\(^{-1}\)) had no effect on the body weight of CB1\(^{-/-}\) mice. Furthermore, the energy intake per mouse remained unchanged compared with the vehicle-treated animals.

**DISCUSSION**

This work describes the effect of blockade of CB1 receptors with SR141716 in a nongenetic model of obesity using highly palatable diet-fed mice. The ability of the compound to reduce food intake in this model was expected, as previous studies have already demonstrated such an effect in rodents and primates (7, 12, 43). However, in the present study, although SR141716 produced a marked acute hypophagia, it appears that such an effect tends to attenuate over a chronic administration. In contrast, SR141716 induced a sustained effect on body weight, which remained low until the end of the 5-wk experiment (with 10 mg·kg\(^{-1}\)·day\(^{-1}\) SR141716, up to a 20\% difference with DIO control mice). The weight loss was associated with a depletion of fat stores reaching ~50\% after the dose of 10 mg·kg\(^{-1}\)·day\(^{-1}\) SR141716, as indicated both by the weight of abdominal fat pads and by the total body fat content estimated with the TOBEC method. The lowest tested dose (3 mg·kg\(^{-1}\)·day\(^{-1}\)) had no effect on the body weight of obese animals, but the fractional contribution of lean tissue to body mass was increased. However, the weight of the gastrocnemius and soleus muscles was unaffected by the treatment. This small effect on the

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**Table 2. Tissue weights after a 40-day treatment with SR141716 (3 and 10 mg·kg\(^{-1}\)·day\(^{-1}\)) in dietary obese mice**

<table>
<thead>
<tr>
<th></th>
<th>White Adipose Tissues, g</th>
<th>Muscles, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epididymal</td>
<td>Perirenal</td>
</tr>
<tr>
<td>HFD-Veh</td>
<td>2.123 ± 0.153</td>
<td>0.362 ± 0.036</td>
</tr>
<tr>
<td>HFD-SR (3 mg)</td>
<td>1.566 ± 0.089*</td>
<td>0.235 ± 0.020*</td>
</tr>
<tr>
<td>HFD-SR (10 mg)</td>
<td>1.136 ± 0.071†</td>
<td>0.164 ± 0.014†</td>
</tr>
<tr>
<td>STD-Veh</td>
<td>0.653 ± 0.039</td>
<td>0.147 ± 0.011</td>
</tr>
</tbody>
</table>

Values are means ± SEM (\( n = 12 \)). Transformations [\( \log (x) \)] were applied on white adipose tissue values. Significant difference of SR141716-treated groups vs. HFD-Veh (\*\( P < 0.05 \), †\( P < 0.01 \)).
Table 3. Plasma analyses after a 40-day treatment with SR141716 (3 and 10 mg·kg⁻¹·day⁻¹) in dietary obese mice

<table>
<thead>
<tr>
<th></th>
<th>Insulin, ng/ml</th>
<th>Leptin, ng/ml</th>
<th>Cholesterol, g/l</th>
<th>Triglycerides, g/l</th>
<th>NEFA, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFD-Veh</td>
<td>3.4 ± 0.3</td>
<td>19.1 ± 3.4</td>
<td>1.62 ± 0.06</td>
<td>0.428 ± 0.033</td>
<td>0.573 ± 0.036</td>
</tr>
<tr>
<td>HFD-SR (3 mg)</td>
<td>2.4 ± 0.3*</td>
<td>14.9 ± 1.4</td>
<td>1.64 ± 0.07</td>
<td>0.355 ± 0.049</td>
<td>0.471 ± 0.044</td>
</tr>
<tr>
<td>HFD-SR (10 mg)</td>
<td>1.6 ± 0.2†</td>
<td>9.5 ± 0.9*</td>
<td>1.56 ± 0.07</td>
<td>0.467 ± 0.032</td>
<td>0.435 ± 0.031</td>
</tr>
<tr>
<td>STD-Veh</td>
<td>1.1 ± 0.1</td>
<td>3.2 ± 0.4</td>
<td>0.89 ± 0.03</td>
<td>0.525 ± 0.071</td>
<td>0.771 ± 0.054</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n = 12). Transformations [log (x)] were applied on insulin and leptin values. Significant difference of SR141716-treated groups vs. HFD-Veh (*P < 0.05, †P < 0.01). NEFA, nonesterified fatty acids.

total lean mass is expected when mice with different degrees of adiposity are compared. In accordance with our results, previous studies have reported that lean mass is increased in association with obesity and reduced by diet-induced weight loss (31, 37, 41).

Interestingly, we found a transient decrease of food intake but a sustained effect on body weight gain. This suggests that, although reducing food intake may be the main initial cause for reducing body weight, it is probably not the only mechanism for the long lasting anti-obesity effect of SR141716. One additional mechanism is likely to be an activation of metabolic processes. This may be supported by our observations that 1) SR141716 significantly increased the relative energy intake during the last 2 wk of the experiment, 2) SR141716-treated mice had an accelerated weight loss during a 24-h fast, and 3) weight loss was increased in SR141716-treated mice compared with pair-fed animals.

The mechanisms by which cannabinoid systems may modulate food intake and metabolic processes are under investigation. SR141716 is a very selective ligand for CB1 receptors (39). This work confirmed that SR141716 acts via these receptors as mice with complete deletion of the CB1 receptor are insensitive to a chronic treatment with the compound. A previous report also described this lack of effect in CB1⁻/⁻ mice in a food-restriction paradigm (15). Thus both hypophagic and metabolic effects appear to be mediated by CB1 receptors. The localization of cannabinoid receptors and endogenous ligands in the brain may shed further light on these mechanisms. One particular brain region highly linked to appetite and metabolism regulation is the hypothalamus. The endocannabinoids anandamide and 2-AG and the CB1 receptors have been localized in this area (21, 26, 36, 54). Although autoradiographic experiments have shown a relatively low density of these receptors in the hypothalamus, their coupling efficiency is as high as or even higher than that observed in more receptor-dense areas (9). Other studies have shown that the endocannabinoid anandamide, like other cannabinoid agonists, significantly increases Fos expression in various brain structures including the paraventricular nucleus of the rat hypothalamus (6, 35). This structure is richly supplied by axons from arcuate nucleus neuropeptide Y/agouti-related peptide and proopiomelanocortin/cocaine- and amphetamine-regulated transcript neurons (17, 18) and is part of the neuronal pathway that generates an
integrated response to changing energy balance (42). Finally, endocannabinoids have been implicated in the neural circuitry regulated by leptin (15). Defective leptin signaling is associated with elevated hypothalamic endocannabinoid levels in obese \textit{db/db} and \textit{ob/ob} mice and in Zucker rats. Fasting also increases endocannabinoid levels in the limbic forebrain and the hypothalamus of rats, two regions highly linked to feeding control (26). A defective leptin signaling seems to induce a chronic state of what has been described as perceived starvation (20). Genetically obese rodents exhibit a continuous motivational state for eating that induces hyperphagia, and the endocannabinoid system seems to be implicated in this mechanism. Interestingly, a deficient leptin function has also been reported in DIO animals (30, 32, 33, 51, 55). It is tempting to speculate that the hyperphagia exhibited by the obese mice used in our model may also arise from an increased motivation for eating, with a possible increase in the endocannabinoid hypothalamic or limbic activity. The high efficacy of the CB1 receptor blockade by SR141716 in this model further supports this hypothesis. However, the activity of endocannabinoids in eating-related regions of the brain remains to be determined in diet-induced obese rodents.

Previous work reported a normal body weight in CB1\textsuperscript{+/−} mice (28, 62), whereas other studies noted a significantly lower body weight of the knockout animals (52). The food intake of CB1\textsuperscript{−/−} mice with unlimited access to food was found to be similar to that of CB1\textsuperscript{+/+} mice, but was reduced in a fasting-refeeding paradigm (15). In our study, CB1\textsuperscript{−/−} mice increased their body weight by 15% during the 6-wk period of high-fat feeding. This value sharply contrasts with that observed usually in our laboratory with C57BL/6 DIO mice (+40 to +60% of the initial body weight when fed the same diet) and with other reports on DIO mice (51). Thus a full investigation of these parameters in CB1\textsuperscript{−/−} mice maintained on a standard laboratory diet and on an obesity-promoting diet should be performed to clarify these findings and to bring further indications on a possible key role of the endocannabinoid system in the regulation of both feeding and body weight.

The effects of SR141716 on central nervous system neurotransmitters have not been completely elucidated. It is noteworthy that microdialysis experiments in rats did not show any change in dopamine release in the nucleus accumbens (3) or in the outflow of 5-hydroxytryptamine in the anterior hypothalamus (50).
when animals were treated with SR141716. In contrast, SR141716 has been reported to increase extracellular concentrations of norepinephrine in this brain region (50). Considering the role of norepinephrine in thermogenesis (13, 61), it is tempting to speculate that this system might play a role in the actions of SR141716.

Hyperinsulinemia, hyperglycemia, and insulin resistance are frequently associated with human obesity (23) and these features were also manifested in the DIO murine model used in our work (10). In this model, SR141716 corrected the hyperglycemia, improved insulin resistance, and reduced plasma insulin levels. Because insulin sensitivity correlates negatively with the degree of adiposity (24, 29, 44), the SR141716-induced weight loss may contribute to the improvement of glucose homeostasis. A direct effect of the treatment, however, remains possible and is currently under investigation. In our model, the NEFA level was significantly lower in DIO mice compared with the lean animals. This may be due to the increase in β-oxidation described in high-fat diet-fed rodents (48). SR141716 treatment reduced the circulating NEFA of DIO mice, which suggests that the compound could further enhance fatty acid oxidation. Such an effect has been demonstrated with a chronic leptin treatment as leptin decreases circulating NEFA and increases fatty acid oxidation in rats (11, 46).

Overall, there are many indications showing that endocannabinoids are key components of systems that regulate both feeding and body weight and belong to the wide family of orexigenic substances. A further demonstration is the clear anti-obesity effect of the CB1 receptor antagonist SR141716 in a nongenetic model of obesity reported in our studies. SR141716 sharply decreased body weight and adiposity of obese mice without sustained hypophagia and improved their insulin resistance. These effects were already seen at 3 mg·kg⁻¹·day⁻¹ and appeared more pronounced at 10 mg·kg⁻¹·day⁻¹. In contrast, no effect of SR141716 was observed in CB1 receptor knockout mice. Dietary-obese mice develop the characteristics of the abdominal obesity syndrome found in humans, including marked visceral obesity and diabetes (22, 38). The high efficacy of SR141716 in this model suggests that CB1 receptor antagonists may constitute a new alternative for the treatment of appetite and body weight disorders in humans.

The authors thank M. Kopf for constructing the CB1 receptor knockout mice, D. Colongo and N. Boussac for help with the statistical analyses, C. Agut for help with the statistical method for TOBEC system calibration, and C. Dumontet for preparation of the manuscript.

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