Effects of the CRF₁ receptor antagonist SSR125543 on energy balance and food deprivation-induced neuronal activation in obese Zucker rats

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Abstract

The corticotropin-releasing factor (CRF) system is involved in numerous physiological and behavioral actions, including the regulation of energy balance. We examined the effects of the CRF₁ receptor antagonist, SSR125543, on energy balance and food deprivation-induced neuronal activation in obese rats. Lean (Fa/?) and obese (*fa/fa*) Zucker rats were treated orally with SSR125543 at a daily dose of 30 mg/kg for 21 days. Rats were killed either fed *ad libitum* or food deprived for 6 h in order to induce a mild stress response in obese rats. SSR125543 reduced plasma corticosterone levels in lean rats, prevented corticosterone response to fasting in obese rats, and increased CRF mRNA levels in the paraventricular hypothalamic nucleus (PVN) of both lean and obese rats, further confirming that the antagonist partially blocked CRF₁ receptors. SSR125543 increased protein gain in obese rats. Whole carcass analyses

showed reduced energy and fat gains in lean rats. Consistent with reduced fat gain, circulating triglyceride and leptin levels were reduced in SSR125543-treated lean rats. In obese rats, circulating glucose levels and the homeostasis model assessment of insulin resistance index of insulin resistance were reduced by SSR125543 treatment. CRF₁ receptor blockade increased uncoupling protein-1 mRNA levels in interscapular brown adipose tissue of obese rats. The antagonist partly blocked the fasting-induced changes in *c-fos* mRNA levels in the PVN and arcuate nucleus of obese rats. Overall, these results suggest that although SSR125543 had relatively mild effects on energy balance, CRF₁ receptor blockade attenuated several metabolic effects of short-term fasting and improved plasma variables related to the metabolic syndrome and diabetes.

Journal of Endocrinology (2007) **193**, 11–19

Introduction

The corticotropin-releasing factor (CRF) family of peptides produce a global negative energy profile by reducing food intake and increasing energy expenditure (Richard *et al.* 2002, Doyon *et al.* 2004). The effects of CRF-related peptides on energy expenditure are thought to depend on a CRF₁ receptor-mediated activation of thermogenesis. Central administration of CRF or urocortin 1 stimulated thermogenesis in brown adipose tissue (BAT), possibly through a sympathetically mediated process (LeFeuvre *et al.* 1987, Arase *et al.* 1988, Currie *et al.* 2001, De Fanti & Martínez 2002). However, prolonged CRF₁ receptor activation could also produce non-specific effects on energy expenditure that would oppose those of CRF-related peptides. For instance, the stimulation of CRF₁ receptors strongly activates the pituitary–adrenal axis and the secretion of corticosteroids, which, by contrast to CRF, promote energy deposition (Castonguay *et al.* 1986, Strack *et al.* 1995) and inhibit BAT thermogenesis (Galpin *et al.* 1983, Strack *et al.* 1995, Arvaniti *et al.* 1998).

The role of the CRF₁ receptor in feeding behavior remains unclear (Heinrichs & Richard 1999). Administration of the

selective CRF₁ receptor antagonist NBI-27914 did not attenuate the CRF-induced reduction in food intake (Smagin *et al.* 1998, Pelleymounter *et al.* 2000), and sub-chronic administration of CRA1000 did not affect food intake and body weight (Ohata *et al.* 2002). However, CRA1000 blocked anorexia induced by emotional stress (Hotta *et al.* 1999). This result supports the idea that CRF₁ receptor could mediate anorectic effects that would be non-specific and secondary to the anxiety- and fear-like behaviors triggered by non-selective CRF receptor agonists, such as CRF and urocortin 1 (Heinrichs & Richard 1999). Numerous studies showed that activation of CRF₁ receptors by stress or CRF-related peptides promotes anxiety-like behaviors (Takahashi 2001). Hence, several selective CRF₁ receptor antagonists were specifically developed for their potential use as anxiolytic agents. All selective CRF₁ receptor antagonists developed to date, including SSR125543 (Griebel *et al.* 2002), CP-154526 (Schulz *et al.* 1996, Arborelius *et al.* 2000), CRA1000 (Okuyama *et al.* 1999), and antalarmin (Deak *et al.* 1999, Habib *et al.* 2000, Griebel *et al.* 2002, Zorrilla *et al.* 2002) produced anxiolytic-like effects. Considering the role of the CRF₁ receptor in the regulation of energy balance,

these antagonists may also prove useful in the pharmacological treatment of obesity, especially in cases where obesity is associated with hyperactivity of the stress axis.

Animal investigations tend to demonstrate that the CRF₁ receptor activity is increased in obesity. Following stressful stimuli, obese rodents showed an enhanced activity of the hypothalamic–pituitary–adrenal (HPA) axis (Guillaume-Gentil *et al.* 1990), which is under the control of the CRF₁ receptor. In addition, food-deprived obese Zucker rats exhibit a neurogenic-stress-like response (Timofeeva & Richard 1997, 2001), which appears to be mediated by the CRF₁ receptor and associated with the development of obesity. The development of obesity may be linked to a dysregulation of the HPA axis. In support of this statement, adrenalectomy reverses or attenuates the obese phenotype in genetically obese (*ob/ob*) mice (Yukimura & Bray 1978, Feldkircher *et al.* 1996) and (*fa/fa*) Zucker rats (Yukimura *et al.* 1978, Marchington *et al.* 1983).

This study examines the effects of the CRF₁ receptor antagonist, SSR125543, on energy balance. In order to examine how a sub-chronic treatment with SSR125543 could block the stress response, Zucker rats were subjected to a 6-h daytime food deprivation, which represents a mild stress in obese Zucker rats. In addition to their hyper-responsive stress axis, obese Zucker rats present several characteristics of the metabolic syndrome including hypertriglyceridemia, hyperinsulinemia, and insulin resistance (Johnson *et al.* 1991).

Materials and Methods

Animals and treatments

Lean (*Fa/?*) and obese (*fa/fa*) male Zucker rats, aged 8–9 weeks, were purchased from Charles River Laboratories (St-Constant, QC, Canada). All rats were cared for and handled according to the Canadian Guide for the Care and Use of Laboratory Animals, and the protocol was approved by Université Laval Animal Care Committee. The animals were housed individually in wire-bottom cages and, unless specified, allowed unrestricted access to food and water. Throughout the study, rats were given a purified, high-carbohydrate diet, which was composed of the following (in g/100 g): 31.2 cornstarch, 31.2 DL-dextrose, 6.4 soybean oil, 20.0 casein, 0.3 DL-methionine, 1.0 vitamin mix (Teklad no. 40060; Teklad, Madison, WI, USA), 4.9 AIN-93 mineral mix (ICN Biochemicals, Montréal, QC, Canada), and 5.0 fiber (Alphacel; ICN Biochemicals). The energy content of the diet consisted of 64.9% carbohydrate, 14.5% fat, and 20.6% protein, and its density was 4.01 kcal/g. Rats were subjected to a 12 h light:12 h darkness cycle (lights on between 0700 and 1900 h) and kept under an ambient temperature of 23 ± 1 °C. Treated rats received two daily oral administrations of SSR125543 (Sanofi-Aventis, Paris, France) for 21 days; 10 mg/kg at 0830 h and 20 mg/kg at 1600 h. On the last day of treatment, 30 mg/kg SSR125543

was administered 4 h prior to killing. SSR125543 was suspended in 0.6% methyl cellulose containing 0.5% Tween 80 for the first 14 days and in 5% DMSO, 5% Cremophor EL, and 90% saline for the last 7 days of treatment. Rats were killed between 1400 and 1700 h in either an *ad libitum* fed state or after a 6-h food deprivation. Lean and obese rats were anesthetized with 2 and 4 ml of a mixture containing 20 mg/ml ketamine and 2.5 mg/ml xylazine respectively. Blood was collected by intracardial puncture into syringes coated with 0.5 M EDTA (Sigma-Aldrich), and rats were perfused intracardially for 2 min with ice-cold isotonic saline. Brain and interscapular BAT were sampled immediately after the perfusion. BAT was frozen in liquid nitrogen and stored at -86 °C.

Gains in energy, fat and protein

Carcasses were autoclaved at 125 kPa for 15 min, homogenized in two volumes of water (w/v) and freeze-dried. Carcass energy content was determined by adiabatic bomb calorimetry, whereas carcass protein was determined using a FP-2000 Nitrogen Analyzer (Leco Corporation, St Joseph, MI, USA) with 250–300 mg dehydrated carcasses. Non-protein matter energy was obtained by subtracting protein energy from total carcass energy. Values of 23.5 and 39.2 kJ/g were used for the calculation of the energy content of protein and fat respectively (Webster 1983). Initial energy, fat, and protein contents of the carcasses were estimated from the live body weight of lean and obese rats with reference to a baseline group of rats (six per phenotype) killed at the beginning of the experimental period. Such estimates allow gains in energy, fat, and protein to be determined for the treatment period. Rats in the initial group were identical in every respect (e.g. age and gender) to those of the experimental groups. Food efficiency was expressed as the ratio of energy gain to digestible energy intake multiplied by 100.

Plasma determinations

Blood was harvested by cardiac puncture, centrifuged (1500 g, 15 min at 4 °C), and plasma was stored at -20 °C until later biochemical measurements. Plasma glucose concentrations were determined using an automated glucose analyzer YSI 2300 Stat Plus (YSI Incorporated, Yellow Springs, OH, USA). Commercially available RIA kits were used to determine plasma levels of corticosterone (MP Biomedicals, Toronto, ON, Canada) and insulin (Linco Research, St Charles, MO, USA), whereas enzymatic kits were used for triglycerides (Roche) and non-esterified fatty acids (NEFAs; Wako Diagnostics, Richmond, VA, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using plasma glucose and insulin levels of food-deprived rats as previously described (Matthews *et al.* 1985).

Brain preparation

Brains were prepared essentially as previously described (Richard *et al.* 1996). After their removal, brains were placed into a 4% paraformaldehyde–3.8% borax solution for at least 7 days with frequent replacement of the solution. They were then transferred to a paraformaldehyde–borax solution containing 10% sucrose at least 12 h before cutting them into 30 µm thick coronal sections using a sliding microtome (HM 440E; Microm, Walldorf, Germany). Brain sections taken from the olfactory bulb to the brainstem were allocated to six sequential sets in 24-well tissue culture plates containing a cold sterile cryoprotecting solution (50 mM sodium phosphate buffer, 30% ethylene glycol, and 20% glycerol) and stored at -30°C .

In situ hybridization histochemistry

In situ hybridization histochemistry was used to determine *c-fos*, CRF, and CRF₁ receptor mRNA levels on tissue sections taken from the hypothalamus. The protocol, largely adapted from Simmons *et al.* (1989), is described in detail in Doyon *et al.* (2006). Briefly, brain sections (one out of every six sections) were fixed in paraformaldehyde (4%), digested with proteinase K and acetylated with acetic anhydride prior to hybridization with an antisense ³⁵S-labeled cRNA probe (10⁶ c.p.m./slide). After exposure on an X-ray film (Eastman Kodak), slides were exposed in NTB2 nuclear emulsion (Eastman Kodak) for 4 (CRF), 5 (*c-fos*), or 22 (CRF₁ receptor) days. Processed slides were examined by dark-field microscopy using an Olympus BX51 microscope (Olympus America Inc., Melville, NY, USA). Images were acquired with an Evolution QEi camera and analyzed with ImagePro plus v. 5.0.1.11 (MediaCybernetics, Silver Spring, USA). The system was calibrated for each set of analyses to prevent saturation of the integrated signal. Mean pixel densities were obtained by taking measurements from both hemispheres of one to four brain sections and subtracting background readings taken from areas immediately surrounding the region analyzed.

Antisense ³⁵S-labeled riboprobes

Complementary RNA probes were generated from rat cDNA fragments for *c-fos* (Dr I Verma, The Salk Institute, La Jolla, CA, USA; GenBank accession no. V00727), CRF (Dr K Mayo, North-western University, Evanston, IL, USA), and CRF₁ receptor (Dr M H Perrin and Dr W W Vale, The Clayton Foundation, La Jolla, CA, USA; GenBank accession no. L24096). Radiolabeled antisense riboprobes were synthesized by incubating 250 ng linearized plasmid at 37 °C for 60 min in the presence of 10 mM NaCl, 10 mM dithiothreitol, 6 mM MgCl₂, 40 mM Tris (pH 7.9), 0.2 mM ATP/GTP/CTP, α -³⁵S-UTP, 40 U RNase inhibitor (Roche), and 20 U T7 (*c-fos* and CRF₁ receptor) or SP6 (CRF) RNA polymerase (Promega). The DNA templates

were treated with 100 µl DNase solution (1 µl DNase, 5 µl of 5 mg/ml tRNA, 94 µl of 10 mM Tris/10 mM MgCl₂). Riboprobes were purified on RNeasy Mini Spin Columns (Qiagen).

Real-time quantitative RT-PCR

Total RNA was isolated from 60–90 mg BAT using the RNeasy Lipid Tissue Mini Kit (Qiagen) and on-column DNA digestion was performed using the RNase-free DNase Set (Qiagen). First-strand cDNA was synthesized from 1 µg total RNA with Expand Reverse Transcriptase and oligo(dT) (Roche). Rat uncoupling protein-1 (UCP1) and L27 amplicons were generated using the primers and the method previously described (Doyon *et al.* 2006). Amplification was carried out using Platinum Taq polymerase (Invitrogen), CYBR Green I (Cedarlane Laboratories, Hornby, ON, USA), and a Rotor Gene 3000 (Corbett Research, Sydney, Australia). Results were analyzed using Rotor-gene v. 6.0 software (Corbett Research).

Statistical analysis

Results are presented as mean values ± 1 S.E.M. Statistical differences in cumulative weight gain between control and SSR125543-treated rats were determined within each genotype using a crossed-nested design with repeated measurements. Cumulative weight gain data were log transformed and multivariate normality was verified with Mardia's test. Statistical differences within each genotype were determined by Student's *t*-test or two-way ANOVA. Data for corticosterone, insulin, and *c-fos* mRNA were log transformed, whereas a square root transformation was used for NEFA. Tukey's multiple comparison tests followed two-way ANOVAs with significant interaction effect. Results were considered significant with $P < 0.05$. Statistical analyses were performed using SAS v. 9.1.3 software package (SAS Institute, Cary, NC, USA) or SigmaStat v. 2.0 software (SPSS, Chicago, IL, USA).

Results

Body weight and energy balance

Treatment with the CRF₁ receptor antagonist, SSR125543, significantly increased cumulative body weight gain in obese rats (Fig. 1). The difference in cumulative body weight gain between treated and untreated obese rats increased throughout the study to reach 25 g at the end of the study. Despite the fact that they were heavier than their untreated counterparts, treated obese rats did not exhibit any marked changes in energy balance variables. Protein gain was nonetheless significantly increased (Table 1). One might argue that this increase contributed to the accelerated body weight gain of the treated obese rats. It is noteworthy that changes in protein gain may substantially impact on the body weight gain as they

Figure 1 Cumulative weight gain in lean (Fa/?) and genetically obese (fa/fa) Zucker rats during 20 days of daily oral administration of 30 mg/kg SSR125543. Significant effect of SSR125543 treatment was assessed by repeated-measures ANOVA. *n*=14/group.

are associated with the changes in body water. SSR125543 significantly increased the BAT UCP1 mRNA levels without significantly affecting energy expenditure or food efficiency.

Although SSR125543 did not affect weight gain (Fig. 1), total energy and fat gains were reduced in lean rats (Table 1). These results further underline the notion that the sole measurement of body weight cannot infallibly predict changes in energy balance, in particular if fat mass is affected, and they strengthen the usefulness of energy balance measurements based on carcass analyses. There was no difference in the protein gain between treated and untreated lean rats. In lean rats, SSR125543 also reduced food efficiency (19%), while it did not significantly affect energy expenditure or BAT UCP1 mRNA levels (Table 1).

Metabolic plasma variables

Treatment with SSR125543 reduced circulating triglyceride levels in lean rats (Table 2). The fasting-induced reduction in

plasma triglycerides was abolished by SSR125543 in obese rats. Circulating NEFAs were increased following food deprivation, but this increase was blunted by SSR125543 in obese rats (Table 2). Circulating glucose levels in SSR125543-treated obese rats were reduced to values similar to those of lean and untreated food-deprived obese rats (Table 2). Although SSR125543 did not significantly modify plasma insulin levels (Table 2), the HOMA-IR index was reduced in obese rats (Table 2). Both antagonist and food deprivation reduced circulating leptin levels in lean but not in obese rats (Table 2). This reduction in leptin levels was consistent with the reduction in fat mass observed in treated lean rats.

The HPA axis and neuronal activation

Blockade of CRF₁ receptors reduced plasma corticosterone levels in lean rats (Fig. 2A). To assess the ability of SSR125543 to attenuate HPA axis and neuronal activation, we measured plasma corticosterone and hypothalamic *c-fos* expression in food-deprived obese Zucker rats, which are known to exhibit a stress response to short-term food deprivation. Expression of *c-fos* has been shown to be particularly reliable to assess neuronal activations induced by stressful events (Imaki *et al.* 1992, Timofeeva & Richard 2001). The present results demonstrate the ability of SSR125543 to prevent the fasting-induced increase in corticosterone levels in obese rats (Fig. 2A). Treatment with SSR125543 also prevented the fasting-induced increase in *c-fos* mRNA levels in the parvocellular division of the paraventricular hypothalamic nucleus (PVN) of obese Zucker rats (Fig. 2B). *C-fos* expression in the parvocellular division of the PVN certainly represents one of the most reliable and valid markers of an increase in HPA axis activity. Blockade of the CRF₁ receptor increased CRF mRNA levels in the PVN of both lean and obese rats (Fig. 2C and E) but had no effect on CRF₁ receptor mRNA levels (Fig. 2D). SSR125543 reduced *c-fos* mRNA levels in the arcuate nucleus of obese Zucker rats (Fig. 2F).

Table 1 Energy balance and body composition in lean (Fa/?) and genetically obese (fa/fa) Zucker rats after 21 days of daily oral administration of 30 mg/kg SSR125543

	Lean		Obese	
	Control	SSR125543	Control	SSR125543
DEI (kJ)	6822 ± 171	6587 ± 127	10 780 ± 432	9993 ± 184
Energy gain (kJ)	1738 ± 108	1361 ± 105 *	3758 ± 202	3785 ± 256
Energy expenditure (kJ)	5084 ± 113	5225 ± 98	7021 ± 476	6208 ± 202
Food efficiency (%)	25.3 ± 1.3	20.5 ± 1.4 *	35.5 ± 2.1	37.6 ± 2.4
Fat gain (g)	34.9 ± 2.4	25.2 ± 2.4 *	91.6 ± 5.0	89.9 ± 6.5
Protein gain (g)	15.8 ± 0.9	15.9 ± 0.7	7.1 ± 0.8	11.1 ± 0.9 *
BAT weight (g)	0.79 ± 0.03	0.77 ± 0.04	1.65 ± 0.08	1.61 ± 0.07
BAT UCP1 mRNA	98.5 ± 11.0	95.3 ± 7.2	50.8 ± 4.7	71.2 ± 7.1 *

Digestible energy intake (DEI) represents 95.5% of total energy intake. Brown adipose tissue (BAT) uncoupling protein-1 (UCP1) mRNA levels are expressed as a ratio of L27 mRNA levels. *Student's *t*-test, *P*<0.05, *n*=12–14/group.

Table 2 Plasma glucose, lipids, and hormones in *ad libitum* fed (AL) and 6-h food-deprived (FD) lean (Fa/?) and genetically obese (fa/fa) Zucker rats after 21 days of daily oral administration of 30 mg/kg SSR125543

	Control		SSR125543	
	AL	FD	AL	FD
Lean				
Glucose (mmol/l)	11.6±0.6	11.0±0.6	10.8±0.7	10.5±0.7
Insulin (nmol/l)	0.29±0.06	0.16±0.02 [†]	0.21±0.05	0.11±0.02 [†]
HOMA-IR	n.a.	12.9±1.9	n.a.	8.8±1.2
Triglycerides (mmol/l)	3.58±0.56	2.48±0.22 [†]	2.51±0.26*	1.57±0.13** [†]
NEFA (mmol/l)	0.14±0.03	0.25±0.02 [†]	0.11±0.01	0.19±0.02 [†]
Leptin (ng/ml)	10.0±0.8	7.7±0.4 [†]	6.6±0.4*	5.2±0.4* [†]
Obese				
Glucose (mmol/l)	24.6±1.1	14.5±2.6 [§]	12.6±1.4 [†]	13.9±1.8
Insulin (nmol/l)	2.66±0.30	1.64±0.32 [†]	2.07±0.43	1.25±0.19 [†]
HOMA-IR	n.a.	193.9±30.1	n.a.	119.6±13.6*
Triglycerides (mmol/l)	4.77±1.03	2.26±0.57 [§]	4.34±0.73	3.98±0.48 [†]
NEFA (mmol/l)	0.22±0.02	0.54±0.06 [†]	0.22±0.02*	0.33±0.04* [†]
Leptin (ng/ml)	40.7±2.0	41.3±2.0	40.8±0.9	38.9±2.2

*Significant main effect of SSR125543 treatment and [†]significant main effect of food deprivation as assessed by two-way ANOVA or Student's *t*-test (HOMA-IR). When significant, only interaction results are shown: [‡]significant effect of SSR125543 within a specific feeding status and [§]significant effect of food deprivation within a specific treatment. *P*<0.05, *n*=6–7/group.

Discussion

The present study was carried out to assess the effects of the CRF₁ receptor antagonist, SSR125543, on energy balance and food deprived HPA axis, and neuronal activation in obese rats. Overall, SSR125543 treatment induced mild but rather beneficial effects on energy metabolism; it reduced energy and fat gains in lean rats, while it reduced circulating glucose levels and the HOMA-IR in obese rats. Consistent with reduced fat gain, circulating triglyceride and leptin levels were reduced in SSR125543-treated lean rats. CRF₁ receptor blockade increased uncoupling protein-1 mRNA levels in interscapular BAT of obese rats. In obese rats, an increase in protein was observed, which might have contributed to improve overall glucose metabolism. SSR125543 reduced plasma corticosterone levels in lean rats, prevented corticosterone response to fasting in obese rats, and increased CRF mRNA levels in the PVN of both lean and obese rats, confirming that the antagonist partially blocked CRF₁ receptors.

In lean rats, CRF₁ receptor blockade reduced total energy and fat gains without significantly affecting the body weight gain. In similar studies, administration of antalarmin (Bornstein *et al.* 1998) or CRA1000 (Ohata *et al.* 2002) did not significantly modify body weight in Sprague–Dawley or Wistar rats respectively, although no attempt was made to estimate fat gain. It is noteworthy that reductions in fat gains are not always associated with significant changes in the body weight gains, especially when they are slight and not accompanied with concomitant reductions in protein gain. In this study, protein gains in lean control and SSR125543-treated rats were comparable. Not surprisingly, fat gain reduction in SSR125543-treated rats was paralleled with a significant decrease in the levels of the adipocyte-derived hormone leptin. Changes in leptin levels have repeatedly been

reported to follow fat mass alterations (Frederich *et al.* 1995, Maffei *et al.* 1995, Considine *et al.* 1996, Kolaczynski *et al.* 1996). As opposed to our observation, 11 days of treatment with antalarmin did not modify plasma leptin levels in Sprague–Dawley rats (Bornstein *et al.* 1998). This discrepancy may be attributable to the shorter treatment period in the antalarmin study. The reduction in fat mass was due to a reduction in food efficiency. As plasma corticosterone promotes fat deposition while increasing energy intake and reducing energy expenditure (Castonguay *et al.* 1986, Freedman *et al.* 1986, Strack *et al.* 1995), lower corticosterone levels in SSR125543-treated lean rats may certainly explain part of the reduction in fat gain.

In obese rats, CRF₁ receptor blockade alters neither the body fat gain nor the circulating leptin levels. However, the body weight gain was increased following treatment with SSR125543. This increase was associated with and was likely accounted for by an increase in the body protein gain. In contrast with changes in fat gain, changes in protein gain are likely to impact more substantially on the body weight gain as they are associated with changes in body water. Changes in protein gain have, however, a lesser influence on energy gain than fat gain changes, since the energy density of protein is lower than that of fat (Webster 1983). The positive effect of the CRF₁ receptor antagonist on protein gain may relate to the suppressive effect of this class of compounds on stress-induced activation of corticosterone release. Corticosteroids exert strong catabolic effects on protein mass (Hasselgren 1999). Although SSR125543 did not normalize plasma corticosterone in obese rats, it nonetheless blunted the elevation of corticosterone levels following food deprivation, which represents a stressing condition in obese Zucker rats (Timofeeva & Richard 1997, 2001). The high basal levels of corticosterone in obese rats possibly prevent a complete

Figure 2 Plasma corticosterone (A), and mean pixel density of the hybridization signal for *c-fos* (B), corticotropin-releasing factor (CRF) (C) and CRF₁ receptor (CRF-R1) (D) mRNA in the parvocellular division of the paraventricular hypothalamic nucleus (PVN), and *c-fos* mRNA in the arcuate nucleus (F) of *ad libitum* fed (AL) and 6-h food-deprived (FD) lean (*Fa/?*) and genetically obese (*fa/fa*) Zucker rats after 21 days of daily oral administration of 30 mg/kg SSR125543. Representative hybridization signals for CRF mRNA in the PVN of obese rats (E).

*Significant main effect of SSR125543 treatment and †significant main effect of food deprivation as assessed by two-way ANOVA. When significant, only interaction results are shown (bars), as assessed by Tukey's multiple comparison tests. $P < 0.05$, $n = 6-7$ /group.

restoration of the body protein gain to the lean levels. Also consistent with a reduction in the HPA axis activity tone in obese rats treated with SSR125543 is the increased levels of BAT UCP1 mRNA. We previously showed that

corticosterone reduces BAT UCP1 mRNA levels (Arvaniti *et al.* 1998). In addition, BAT thermogenic activity is decreased by corticosterone and increased by adrenalectomy or treatment with the glucocorticoid receptor antagonist

RU-486 (Galpin *et al.* 1983, Hardwick *et al.* 1989, Strack *et al.* 1995). The increase in UCP1 expression in obese SSR125543-treated rats has probably contributed to sustain the energy efficiency despite the substantial (though not statistically significant) energy intake that occurred in these rats. UCP1 is unique to BAT and confers thermogenic properties to this tissue (Sell *et al.* 2004).

The obese Zucker rat is hyperinsulinemic (Zucker & Antoniadis 1972) and presents a mild hyperglycemia (Triscari *et al.* 1979). Here, we show that SSR125543 restored normal glycemia in obese rats. This result is in agreement with the hyperglycemic effect of central CRF administration (Brown *et al.* 1982a,b). Although the reduction in insulin levels did not reach statistical significance, the HOMA-IR index was reduced in obese rats, indicating improved insulin sensitivity. Reduced fat gain in SSR125543-treated lean rats was associated with a reduction in circulating triglyceride that was not seen in treated obese rats, which did not exhibit a reduction in fat gain. However, SSR125543 prevented the fasting-induced reduction in circulating triglycerides and blunted the fasting-induced increase in NEFA in obese rats. Together, these results indicate that CRF₁ receptor blockade improves plasma variables related to diabetes.

The action of CRF-related peptides on HPA axis activity is thought to be almost exclusively mediated through the CRF₁ receptor (Doyon *et al.* 2004). As expected, CRF₁ receptor blockade attenuated the activity of the HPA axis. Three weeks of treatment with SSR125543 reduced the basal plasma corticosterone levels in lean rats and prevented the fasting-induced corticosterone response in obese Zucker rats, which are hypercorticosteronemic (Guillaume-Gentil *et al.* 1990, Richard *et al.* 1996) and hypersensitive to stress (Guillaume-Gentil *et al.* 1990, Timofeeva & Richard 1997). Similarly, a previous study showed that SSR125543 reduced the basal adrenocorticotrophic hormone (ACTH) concentrations and attenuated the CRF- and stress-induced increase in circulating ACTH in Sprague-Dawley rats (Gully *et al.* 2002). However, sub-chronic administration of CRA1000 (Ohata *et al.* 2002) or antalarmin (Wong *et al.* 1999) did not blunt the HPA response to immobilization stress. The relatively mild nature of the stressor used in our study (6-h daytime food deprivation) may explain the discrepancy with other sub-chronic studies.

Centrally, SSR125543 increased CRF mRNA levels in the parvocellular division of the PVN of both lean and obese Zucker rats, which likely resulted from an attenuation of the negative feedback regulation of CRF synthesis by corticosterone (Jingami *et al.* 1985, Young *et al.* 1986, Herman *et al.* 1992, Arvaniti *et al.* 2001). Consistent with the corticosterone results, CRF₁ receptor blockade prevented the fasting-induced increase in *c-fos* mRNA levels in the PVN of obese rats. SSR125543 also reduced *c-fos* mRNA levels in the arcuate nucleus of obese rats. These results imply that the CRF₁ receptor is involved in stress-induced neuronal activation. Despite changes in *c-fos* mRNA levels in the arcuate nucleus, we observed no changes in neuropeptide Y,

agouti-related peptide, or proopiomelanocortin mRNA levels in this brain region (data not shown).

In conclusion, our results show that although SSR125543 had relatively mild effects on energy balance, blockade of CRF₁ receptors attenuated several metabolic effects of short-term fasting and improved plasma variables related to the metabolic syndrome and diabetes.

Acknowledgements

We wish to thank Sanofi-Aventis for providing SSR125543. This work was supported by a grant from Institut de recherche en santé du Canada (IRSC) (to D Richard) and a postdoctoral fellowship from Fonds québécois de la recherche sur la nature et les technologies (FQRNT) (to C Doyon). The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received in final form 15 January 2007

Accepted 25 January 2007

Made available online as an Accepted Preprint

26 January 2007