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Predominately glucocorticoid agonist actions of RU-486 in young specific-pathogen-free Zucker rats

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Departments of Nutrition and Internal Medicine; Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine; and Section of Neurobiology, Physiology, and Behavior, Division of Biological Sciences, University of California, Davis 95616; Department of Physiology, University of California, San Francisco, California 94143; and Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee 37132

Havel, Peter J., Bonnie L. Busch, Donald L. Curry, Patricia R. Johnson, Mary F. Dallman, and Judith S. Stern. Predominately glucocorticoid agonist actions of RU-486 in young specific-pathogen-free Zucker rats. *Am. J. Physiol.* 271 (*Regulatory Integrative Comp. Physiol.* 40): R710–R717, 1996.—Previous studies have demonstrated antiglucocorticoid actions for the progesterone receptor antagonist RU-486. In one study, daily administration of this drug for 2 wk decreased food intake (FI) and body weight gain (ΔBW) in obese, but not lean, conventionally housed 5-wk-old female Zucker rats. We recently found that 2-wk administration of RU-486 attenuated ΔBW in lean but not obese 12-wk-old male Zucker rats without affecting FI. To examine the actions of RU-486 and its effects on FI and ΔBW in young (5 wk old) specific-pathogen-free (SPF) male and female Zucker rats, RU-486 was administered at 30 $mg \cdot kg^{-1} \cdot day^{1}$ subcutaneously for 14 days. RU-486 did not affect FI in obese or lean male or female rats. RU-486 increased adrenal weight ($P < 0.05$) overall and in lean female rats and modestly decreased inguinal fat weight overall and in obese female rats ($P < 0.01$), suggesting some antiglucocorticoid activity in these animals. However, RU-486 also decreased thymus weight by 18–31% ($P < 0.0001$), increased plasma glucose by 10–16 mg/dl ($P < 0.002$), and increased plasma insulin by 47% in obese male rats ($P < 0.028$), demonstrating glucocorticoid agonist actions for the drug. Plasma corticosterone (B) and adrenocorticotropic hormone (ACTH) were elevated in vehicle-treated obese female and male rats by 150–360% ($P < 0.0025$) and 32–38% ($P < 0.05$), respectively, compared with lean rats. RU-486 treatment lowered the elevated plasma B and ACTH levels in obese female and male rats (both $P < 0.02$ vs. vehicle), a glucocorticoid agonist effect. We conclude that in young SPF Zucker rats *1*) RU-486 administration does not alter FI or ΔBW, *2*) RU-486 has predominately glucocorticoid agonist actions in several tissues, *3*) obese animals have increased hypothalamic-pituitary-adrenal (HPA) axis activity (plasma B and ACTH), and *4*) RU-486 administration suppresses the HPA axis in obese rats.

mifepristone; thymus; adrenal; glucose; insulin; glucagon; pancreatic polypeptide; corticosterone; adrenocorticotropic hormone

THE FATTY (*fa/fa*) Zucker rat has been used for over 35 years for investigation of the physiological and biochemical features of obesity. Recent evidence indicates that the primary genetic defect responsible for obesity in fatty Zucker rats is a mutation in the gene coding for a receptor for the adipose tissue protein leptin (10). Leptin is likely to act as a humoral signal from body fat stores to the central nervous system, where it acts to limit food intake and increase energy expenditure (38). The hypothalamic-pituitary-adrenal (HPA) axis is also involved in the regulation of body weight and adiposity (6). For example, obese Zucker rats have elevated basal and stimulated plasma corticosterone levels (16, 34), and surgical adrenalectomy attenuates food intake, body weight, and adiposity and increases energy expenditure in several animal models of obesity including obese Zucker rats (13), *ob/ob* mice (45), and obesity resulting from ventromedial hypothalamus lesions (43). Reduced feedback inhibition of central corticotropin-releasing hormone (CRH) by circulating glucocorticoids is likely to contribute to the antiobesity effects of adrenalectomy, because central administration of CRH reduces body weight (2, 39) and increases energy expenditure (40) in obese Zucker rats and increases sympathetic outflow in normal rats (7).

A number of studies have demonstrated antiglucocorticoid actions for the progesterone receptor antagonist RU-486 both in vivo and in vitro (3, 9, 14, 28). In one study, daily administration of RU-486 for 2 wk decreased food intake and body weight gain in obese but not lean 5-wk-old female conventionally housed Zucker rats (27). Plasma corticosterone was increased in both obese and lean rats treated with RU-486, suggesting that the actions of the drug in the obese animals were due to glucocorticoid antagonist effects on the HPA axis, presumably by blocking negative feedback inhibition of CRH and adrenocorticotropic hormone (ACTH) by endogenous corticosterone.

In contrast, we have found that daily administration of RU-486 did not affect food intake but prevented body weight gain in lean, but not obese, 12-wk-old male Zucker rats (18), suggesting that the actions of RU-486 in Zucker rats are more complex than a pure glucocorticoid receptor antagonist and may be dependent on variables related to the subject animals. Therefore, to examine the effects of RU-486 on food intake and body weight in younger animals, we administered RU-486 or vehicle daily for 2 wk to 5-wk-old male and female obese and lean specific-pathogen-free (SPF) Zucker rats (41). SPF animals were studied because these animals are known to be free of a large number of endemic viral and other pathogens that can induce chronic illness and stress in conventionally housed rodents (23, 32). Adrenal weight, thymus weight, plasma insulin, and glucose were measured as physiological indexes of glucocorticoid or antiglucocorticoid activity. Plasma corticoste-

Table 1. *Pathogens screened for in SPF Zucker rat breeding colony*

Pathogen	Pathogenicity
Mycoplasma pulmonis	Respiratory pathogen
Cilia-associated respiratory bacillus	Respiratory pathogen
Sendai virus	Immunosuppressive virus
Kilham rat virus	CNS and gonadal pathogen
H-1 rat parvovirus	Similar to Kilham rat virus
Rat corona virus	Salivary gland pathogen
Pneumonia virus of mice	Subclinical disease in rats
Mouse adenovirus	Subclinical disease in rats
Reovirus-3	Immunosuppressive virus
Lymphocytic choriomeningitis virus	CNS pathogen
Theiler murine encephalitis virus	CNS pathogen
Syphacia muris (rodent pinworm)	Usually subclinical
Radfordia ensifera (rat fur mite)	Pruritis, self-trauma, skin ulcers

SPF, specific-pathogen free; CNS, central nervous system.

rone and ACTH were measured to determine the effects of RU-486 on the HPA axis.

MATERIALS AND METHODS

Animals. Thirty-seven obese (*fa/fa*) and thirty-nine lean (*Fa/?*) male and female Zucker rats from standardized litters of 8–10 pups/dam were used for this study, starting at 5 wk of age. The colony supplying the animals was derived and maintained under SPF conditions (41). This colony is part of the Animal Models Core, a National Institutes of Health (NIH)-funded Clinical Nutrition Research Unit. The founder animals were cesarean derived. The colony is housed with microisolator filters covering all cages. Air entering the rooms is hepa filtered with positive pressure ventilation between the animal rooms and the corridors. All materials entering the animal rooms are autoclaved. Sentinel rats are routinely screened in a laboratory animal health surveillance program, and the colony is known to be free of the pathogens listed in Table 1 (23, 32). Rats were individually housed in hanging wire cages and were provided a stock diet (Ralston Purina) and deionized water ad libitum. The animals were maintained on a 12:12-h light-dark cycle (lights on at 6:00 A.M.) at 24–26°C. The experimental protocols were approved by the Institutional Animal Use and Care Committee of the University of California Davis and were conducted in accordance with the NIH "Guide for the Use and Care of Laboratory Animals."

Experimental protocol. Food intake and body weight were measured for 2–4 days prior to RU-486 or vehicle administration to obtain baseline values. RU-486 (mifepristone, Roussel UCLAF) (30 $mg \cdot kg^{-1} \cdot day^{-1}$) or vehicle was administered subcutaneously for 14 days as reported by Langley and York (25). To ensure rapid initial uptake of the drug, the vehicle was 70% ethanol (1 ml/kg) and the RU-486 was administered in divided doses (15 mg/kg) twice daily for the first 2 days (25). Thereafter, RU-486 (30 mg/kg) was administered in peanut oil (2 ml/kg) once daily (25). Body weight and food intake were measured daily. After 2 wk of treatment, the animals were brought to a separate room and killed by decapitation within 1 min. Trunk blood was collected in EDTA tubes immediately after decapitation. Adrenal glands, thymus glands, inguinal and gonadal fat depots, interscapular brown adipose tissue (BAT), and ovaries from female rats were removed and weighed.

Assays and data analysis. Blood samples for glucose and insulin, glucagon, pancreatic polypeptide (PP), corticosterone, and ACTH determination were placed in tubes containing EDTA and 50 μl aprotinin (24 trypsin inhibitory units/ml) per milliliter whole blood. All samples were kept on ice until centrifugation (2,500 revolutions/min for 20 min at 4°C). The plasma was frozen at −20°C until assayed.

Plasma glucose was assayed by the glucose oxidase method with a glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin, glucagon, and PP were measured in the Radioimmunoassay Core Laboratory of the Diabetes Research and Training Center at Washington University School of Medicine, St. Louis, MO. Plasma insulin was measured with a radioimmunoassay using a specific rat insulin antibody and rat insulin standards (Linco Research, St. Louis, MO). Plasma immunoreactive glucagon was measured by radioimmunoassay with an antibody that has a high degree of specificity for the COOH-terminal portion of the glucagon molecule (Linco Research). Plasma immunoreactive PP was measured in unextracted plasma with a sensitive and specific radioimmunoassay for rat PP using a guinea pig-derived antisera as previously described (1). Plasma corticosterone (46) and ACTH (35) were measured by radioimmunoassay. The data in Tables 2–5 and Figs. 1–3 are expressed as means ± SE. The data were first examined for overall effects of genotype, sex, or treatment with a three-factor analysis of variance (ANOVA). Comparisons between specific groups were made by one-way ANOVA with a Fishers protected least-significant difference posttest.

RESULTS

Food intake and body weight. Initial food intake measurements revealed that both male and female obese rats were hyperphagic at 5 wk of age ($P < 0.0001$). There were significant overall effects of genotype and sex on food intake measured from *day 7* to *day 14* of the experiment. Obese animals were hyperphagic relative to lean animals ($P < 0.0001$), and male rats ate more than female rats ($P < 0.0001$). There was no overall effect of treatment, and RU-486 did not affect food intake when RU-486-treated rats were compared with the corresponding vehicle-treated obese or lean male or female Zucker rats (Table 2). Initial and final body weights were greater both in obese female than

Table 2. *FI in Zucker rats before and during 14-day administration of RU-486 or vehicle*

Treatment	*n*	Baseline FI, g/day	Mean Daily FI (*Days 7–14*), g/day	Total FI (*Days 7–14*), g
		Female rats		
Lean vehicle	10	12.1 ± 0.3	15.0 ± 0.5	104.8 ± 3.2
Lean RU-486	11	13.5 ± 0.7	15.0 ± 0.3	105.1 ± 2.2
Obese vehicle	9	20.5 ± 1.0*	25.2 ± 0.7*	176.4 ± 4.6*
Obese RU-486	9	20.5 ± 1.2*	25.1 ± 0.7*	176.0 ± 4.6*
		Male rats		
Lean vehicle	8	15.2 ± 0.6	19.8 ± 0.7	138.6 ± 5.1
Lean RU-486	13	15.7 ± 0.3	19.7 ± 0.4	131.4 ± 3.7
Obese vehicle	7	21.6 ± 1.1*	27.1 ± 1.5*	189.4 ± 10.6*
Obese RU-486	9	21.7 ± 1.1*	26.3 ± 0.9*	184.2 ± 6.3*

Values are means ± SE; *n* = no. of animals. FI, feed intake. *$P <$ 0.01 vs. lean.

lean female and obese male than lean male rats (both $P < 0.0001$). There were overall effects of genotype and sex on body weight gain over the course of the experiment, with obese rats gaining more weight than lean rats ($P < 0.0001$) and male rats gaining more weight than female rats ($P < 0.0001$). There was no overall effect of RU-486 on body weight gain ($P = 0.5707$). RU-486 treatment had no significant overall effect on body weight gain or any effect when obese and lean male or female rats were compared with their corresponding vehicle-treated controls. (Fig. 1, *A* and *B*).

White adipose tissue and BAT weights. Inguinal and gonadal fat depot weight and total white adipose tissue (WAT; inguinal + gonadal weights) were significantly larger in obese vs. lean rats (both $P < 0.0001$). Inguinal fat depot weight was modestly reduced by RU-486 treatment in obese female ($P < 0.01$) rats but not in lean male or female or obese male rats. There was no significant effect of RU-486 on gonadal fat depot weight or on total WAT in any group. BAT weight was larger in both obese males and females than in lean rats ($P < 0.0001$). RU-486 treatment was without effect on BAT weight (Table 3).

Adrenal, thymus, and ovary weights. Obese animals had larger adrenal glands than lean animals ($P < 0.0001$), and female rats had larger adrenals than male rats ($P < 0.0001$). RU-486 significantly increased adrenal weight overall ($P < 0.02$) and specifically increased adrenal weight in lean female rats ($P < 0.05$). Males had larger thymus glands than female rats ($P < 0.0001$). Overall, RU-486 treatment decreased thymus weight ($P < 0.0001$) and specifically decreased thymus weight in obese females ($P < 0.005$), obese males ($P < 0.01$), and lean males ($P < 0.005$ vs. vehicle). RU-486 administration did not significantly affect ovarian weight in lean or obese female rats (Table 4).

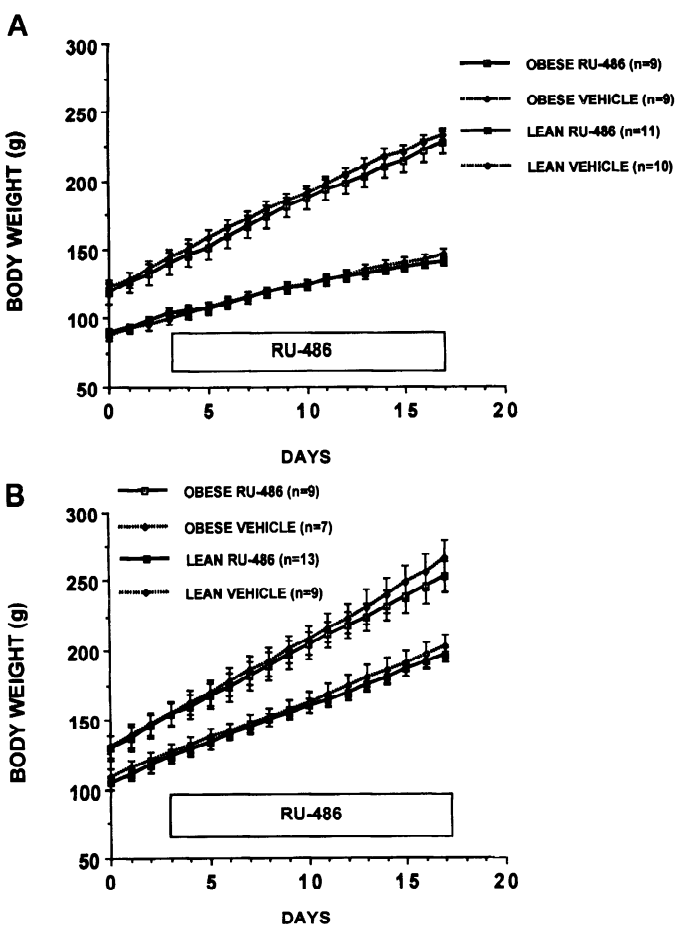

Fig. 1. Body weight before and during a 2-wk period of daily subcutaneous injections of RU-486 (30 $mg \cdot kg^{-1} \cdot day^{-1}$) or vehicle in female (*A*) and male (*B*) lean (*bottom plots*) and obese (*top plots*) specific-pathogen-free (SPF) Zucker rats; *n*, no. of rats in each group.

Plasma glucose and pancreatic hormone concentrations. Overall, obese animals had higher plasma glucose concentrations than lean animals ($P < 0.0001$), and male rats had higher plasma glucose concentrations than female rats ($P < 0.0001$). RU-486 significantly increased plasma glucose concentrations overall ($P < 0.002$) and specifically increased glucose concentrations in obese male ($P < 0.05$) and obese female rats ($P < 0.05$) but not in lean male or female rats (Table 5). Obese rats were hyperinsulinemic relative to lean animals ($P < 0.0001$), and male animals had higher plasma insulin concentrations than females ($P < 0.0001$). RU-486 significantly increased plasma insulin in obese male rats ($P < 0.028$). Obese rats had elevated plasma glucagon concentrations relative to lean animals ($P < 0.0001$). Male rats had lower plasma PP concentrations than females ($P < 0.001$). RU-486 treatment did not affect plasma glucagon or PP concentrations (Table 5).

Plasma corticosterone and ACTH concentrations. Plasma corticosterone concentrations were higher in obese Zucker rats relative to their lean littermates ($P < 0.0025$). Plasma corticosterone concentrations were higher in female than in male animals ($P < 0.025$). RU-486 administration suppressed the elevated plasma corticosterone concentrations in both obese male and obese female rats to the concentrations in lean animals (both $P < 0.02$ vs. vehicle) (Fig. 2, *A* and *B*). Overall, Plasma ACTH concentrations were elevated in obese vs. lean rats ($P < 0.05$). Plasma ACTH was significantly elevated in obese female ($P < 0.05$) and tended to be elevated in obese male ($P < 0.1$) rats relative to their lean littermates (Fig. 3, *A* and *B*). RU-486 administration suppressed the elevated plasma ACTH concentrations in obese male and female rats (both $P < 0.02$ vs. vehicle). RU-486 had no effect on plasma corticosterone or ACTH in lean animals (Figs. 2, *A* and *B*, and 3, *A* and *B*). Antiglucocorticoid and glucocorticoid agonist effects of RU-486 observed in the experiment and the significance levels of the effects are compared in Table 6.

DISCUSSION

In a previous study (25), Langley and York reported that RU-486 decreased body weight gain in obese but not lean 5-wk-old female Zucker rats. We have previously reported that administration of RU-486 prevented body weight gain in lean, but not obese, 12-wk-old male Zucker rats and did not affect food intake in either the obese or lean animals (18). The present study was conducted to examine and clarify the effects of

Table 3. *Weight of WAT depots and interscapular BAT in SPF Zucker rats after 2-wk administration of RU-486 or vehicle*

Treatment	n	Inguinal WAT	Gonadal WAT	Total WAT	BAT
			Female rats		
Lean vehicle	10	0.65 ± 0.05	0.53 ± 0.03	1.14 ± 0.10	0.33 ± 0.03
Lean RU-486	11	0.60 ± 0.02	0.39 ± 0.03	0.99 ± 0.04	0.35 ± 0.02
Obese vehicle	9	5.80 ± 0.31*	4.55 ± 0.31*	10.35 ± 0.56*	1.02 ± 0.09*
Obese RU-486	9	4.80 ± 0.41*†	4.38 ± 0.33*	9.18 ± 0.87*	0.98 ± 0.07*
			Male rats		
Lean vehicle	8	1.09 ± 0.15	1.26 ± 0.15	2.25 ± 0.28	0.43 ± 0.31
Lean RU-486	13	0.88 ± 0.05	0.96 ± 0.06	1.84 ± 0.09	0.41 ± 0.26
Obese vehicle	7	6.40 ± 0.42*	4.01 ± 0.30*	10.41 ± 0.68*	1.07 ± 0.11*
Obese RU-486	9	5.78 ± 0.42*	3.87 ± 0.30*	9.65 ± 0.55*	0.95 ± 0.55*

Values are means ± SE. WAT, white adipose tissue; BAT, brown adipose tissue. *$P < 0.0001$ vs. lean; †$P < 0.01$ vs. vehicle.

RU-486 on body weight gain and food intake in obese and lean male and female 5-wk-old SPF Zucker rats that are documented to be free of a large number of pathogenic agents. Because our previous results in the older, male animals contrasted with those of Langley and York in 5-wk-old female Zucker rats, we also examined physiological indexes of glucocorticoid activity (plasma glucose, thymus and adrenal weight) and measured plasma corticosterone and ACTH levels to assess the effects of RU-486 on the HPA axis. We found no appreciable effect of RU-486 on food intake or body weight gain overall or in any of the groups of animals. RU-486 has also been reported to be similarly ineffective in reducing body weight gain in genetically obese (*ob/ob*) mice (11). In other experiments in non-SPF animals, RU-486 reduced body weight gain without reducing food intake in 12-wk-old male Sprague-Dawley rats (44), similar to our previous findings in 12-wk-old lean male Zucker rats (18), whereas, in obesity-susceptible Osborne-Mendel rats fed a high-fat diet, RU-486 reduced both food intake and body weight gain (31). In another species, Siberian hamsters, RU-486 had a small effect on body weight but no effect on food intake (4). Thus the effects of RU-486 on body weight, if observed, can either parallel or occur independently of decreased food intake. The effects observed appear to be highly dependent on the animal model tested.

If RU-486 had significant antiglucocorticoid effects in the present study, we would have expected increased adrenal size as a consequence of reduced feedback inhibition by endogenous glucocorticoids on hypothalamic CRH and pituitary ACTH, leading to increased trophic actions of ACTH on the adrenal cortex. Adrenal weight was modestly but significantly increased overall and in lean female animals by RU-486 administration. Because glucocorticoids have potent thymolytic actions and adrenalectomy results in thymic hypertrophy, antiglucocorticoid treatment would be expected to result in increased thymic weight. However, we found that RU-486 reduced thymus weight in SPF Zucker rats, suggesting a glucocorticoid agonist action of the drug on the thymus. Because RU-486 decreased inguinal fat depot weight but decreased thymus weight in obese female rats, RU-486 may have both glucocorticoid agonist and antagonist actions within the same animals. RU-486 did not exhibit significant antiprogesterone activity in female rats as reflected by increased ovarian weight. Increased ovarian weight after RU-486 has been observed in another strain of rats (29); however, these animals were considerably older than those in the

Table 4. *Adrenal, thymus, and ovary weights in SPF Zucker rats after 2-wk administration of RU-486 or vehicle*

Treatment	n	Adrenal Wt, mg	Thymus Wt, mg	Ovary Wt, mg
		Female rats		
Lean vehicle	10	34.2 ± 1.8	296.5 ± 7.5	83.6 ± 4.2
Lean RU-486	11	40.2 + 1.9*	244.5 ± 12.3	100.2 + 5.9
Obese vehicle	9	42.2 ± 1.7†	381.7 ± 26.4	85.7 ± 6.4
Obese RU-486	9	47.8 ± 3.7	263.7 ± 10.7*	84.3 ± 8.6
		Male rats		
Lean vehicle	8	27.1 ± 1.1	494.3 ± 43.4	
Lean RU-486	13	26.6 ± 1.3	351.3 ± 31.6*	
Obese vehicle	7	36.3 ± 2.3†	426.1 ± 35.9	
Obese RU-486	9	40.3 ± 2.2†	348.2 ± 29.5*	

Values are means ± SE. *$P < 0.05$ vs. vehicle; †$P < 0.05$ vs. lean.

Table 5. *Plasma concentrations of glucose and pancreatic hormones in SPF Zucker rats after 2-wk administration of RU-486 or vehicle*

Treatment	n	[Glucose], mg/dl	[Insulin], μU/ml	[Glucagon], pg/ml	[PP], pg/ml
			Female rats		
Lean vehicle	10	124 ± 1	40 ± 4	150 ± 7	57 ± 3
Lean RU-486	11	128 ± 3	45 ± 4	150 ± 5	60 ± 4
Obese vehicle	9	127 ± 3	487 + 79*	223 + 18*	62 ± 3
Obese RU-486	9	143 ± 2*†	533 ± 109*	223 ± 11*	59 ± 5
			Male rats		
Lean vehicle	8	129 ± 5	64 ± 9	157 ± 8	49 ± 3
Lean RU-486	13	139 ± 3	65 ± 8	164 ± 5	53 ± 3
Obese vehicle	7	160 ± 8*	757 ± 110*	226 ± 21*	50 ± 5
Obese RU-486	9	176 ± 10*†	1115 ± 156*†	220 ± 10*	48 ± 5

Values are means ± SE. Brackets indicate concentration; PP, pancreatic polypeptide. *$P < 0.05$ vs. lean; †$P < 0.05$ vs. vehicle.

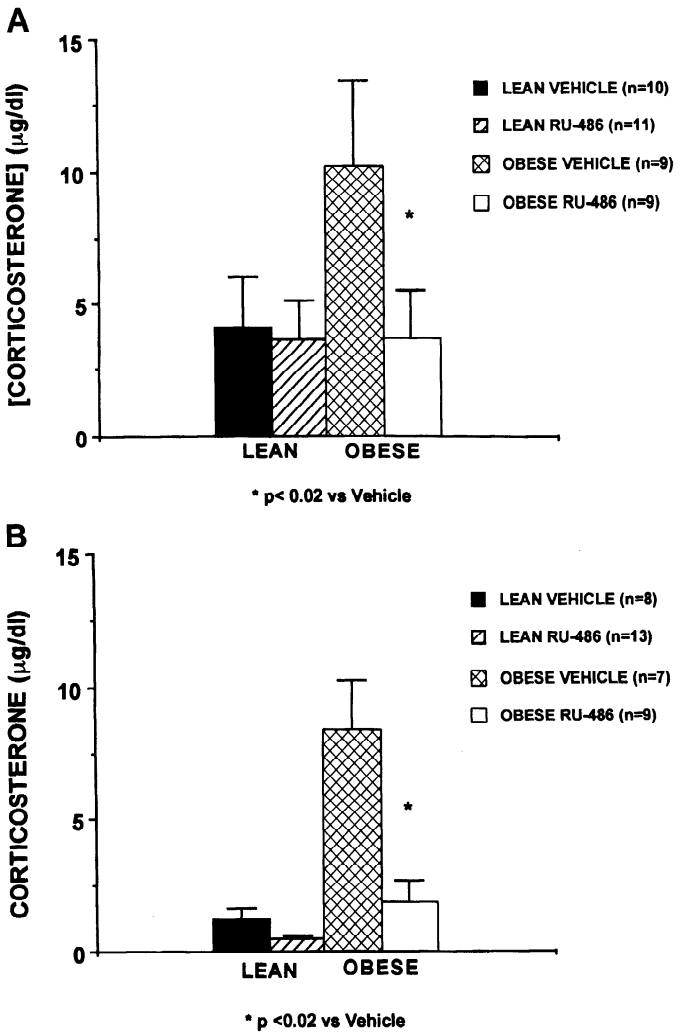

Fig. 2. Plasma corticosterone levels at the end of a 2-wk period of daily subcutaneous injections of RU-486 (30 $mg \cdot kg^{-1} \cdot day^{-1}$) or vehicle in female (*A*) and male (*B*) lean and obese SPF Zucker rats.

present study, and estrus does not occur in rats before 6–8 wk of age.

Plasma glucose was significantly increased overall and specifically in obese female and lean and obese male rats, suggesting glucocorticoid agonist actions on hepatic glucose metabolism or insulin-mediated glucose uptake in these animals. The HPA axis influences autonomic activity and insulin action and therefore plasma insulin levels. Zucker obese rats are hyperinsulinemic and insulin resistant (8). RU-486 treatment further elevated plasma insulin levels in obese male rats, suggesting that glucocorticoid agonist action of the drug exacerbated insulin resistance and/or insulin hypersecretion in these animals. Central administration of CRH is known to influence the activity of the autonomic nervous system (7, 40), which in turn can affect pancreatic insulin, glucagon, and PP secretion (20). Plasma glucagon levels were consistently higher in obese vs. lean rats, as has been previously described (8, 37), but were unaffected by RU-486 administration in any of the groups of animals. Some studies have suggested that obese Zucker rats have increased parasympathetic input to the pancreatic islets (12, 36). Plasma PP, an index of parasympathetic neural input to the pancreas (17, 19), was not different between obese and lean animals and was unaffected by RU-486; however, plasma PP levels were lower in male than in female rats. The physiological significance of this previously unreported gender difference in PP levels in rats is unknown at this time. Based on measurements of pancreatic hormone levels, RU-486 did not appear to affect autonomic nervous system activity in this study.

Plasma corticosterone was elevated in both obese female and male vehicle-treated rats relative to lean rats. RU-486 decreased the elevated plasma corticosterone to levels similar to those in lean animals in both female and male obese rats. In one previous study, oral administration of RU-486 for 4 days increased plasma corticosterone in lean but not obese 22-wk-old male Zucker rats (33), suggesting an antiglucocorticoid action on the HPA axis in lean rats, whereas in the same study prepro-corticotropin releasing factor mRNA levels in the paraventricular nucleus of the obese rats were reduced by RU-486, a glucocorticoid agonist ac-

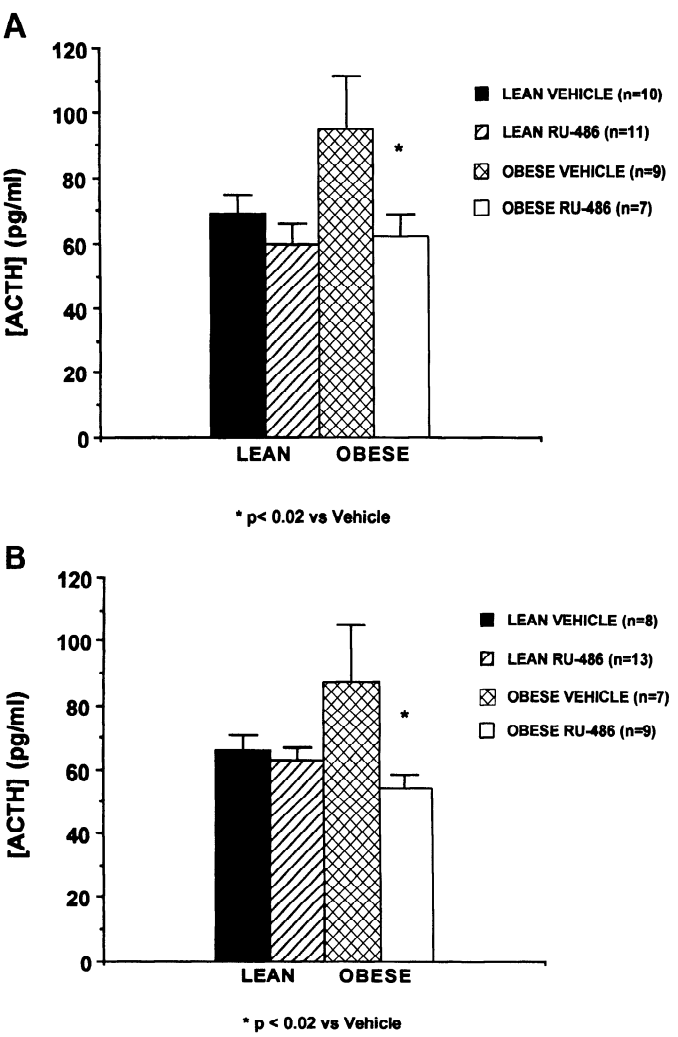

Fig. 3. Plasma ACTH levels at the end of a 2-wk period of daily subcutaneous injections of RU-486 (30 $mg \cdot kg^{-1} \cdot day^{-1}$) or vehicle in female (*A*) and male (*B*) lean and obese 5-wk-old SPF Zucker rats.

Table 6. *Antiglucocorticoid and glucocorticoid agonist effects of RU-486 administration observed in the experiment*

Group	*P* Values				
	Overall	Lean females	Obese females	Lean males	Obese males
Glucocorticoid agonist effect					
Decreased thymus wt	0.0001*	0.1501	0.0032*	0.0008*	0.0631*
Increased plasma glucose	0.0018*	0.5163	0.0307*	0.1519	0.0396*
Increased plasma insulin	0.0571	0.9820	0.6722	0.9594	0.0028*
Decreased plasma corticosterone	0.0113*	0.5912	0.0182*	0.7101	0.0108*
Decreased ACTH	0.0053*	0.4683	0.0194*	0.9871	0.0200*
Antiglucocorticoid effect					
Increased adrenal wt	0.0138*	0.0360*	0.0699	0.8599	0.2138
Decreased inguinal fat wt	0.0097*	0.8892	0.0072*	0.5345	0.1123
Decreased total fat wt	0.0377*	0.7461	0.0679	0.4670	0.2328
Antiprogesterone effect					
Increased ovary wt	0.2456	0.0617	0.8829		
Not affected by RU-486					
Food intake	0.3575	0.9517	0.9653	0.2388	0.5078
Body wt gain	0.1643	0.0741	0.6290	0.4637	0.4122
Gonadal fat wt	0.2100	0.6107	0.5725	0.3030	0.6584
Brown fat wt	0.3555	0.7496	0.5589	0.9521	0.1674
Plasma glucagon	0.7878	0.5078	0.9999	0.7624	0.7287
Plasma PP	0.8176	0.5633	0.6420	0.4102	0.7072

All *P* values by analysis of variance. Order of sensitivity to glucocorticoid agonist actions of RU-486: obese males > obese females > lean males > lean females. *Values are significant ($P < 0.05$).

tion. RU-486 has been reported to reduce plasma corticosterone levels in another animal model of obesity, the *ob/ob* mouse (11).

Similar to the corticosterone levels, plasma ACTH levels in the present study were increased in both female and male vehicle-treated obese animals. RU-486 administration significantly decreased plasma ACTH in both female and male obese rats but had no effect in lean animals in the absence of increased HPA activity. The suppressive effect of RU-486 on the HPA axis along with the thymolytic actions and effects on glucose metabolism strongly suggest that RU-486 acted primarily as a glucocorticoid agonist in this study. In contrast, Langley and York (25) found that plasma corticosterone was substantially increased in animals that received RU-486 injections. Although plasma ACTH levels were not measured in the Langley and York study, plasma corticosterone levels in the vehicle-treated animals were up to five times higher than in the present study and were not elevated in obese vs. lean animals as has been reported in other studies (16). This is a major difference between the animals used for the two studies, which suggests that the SPF Zucker rats used in the present study respond differently to RU-486 than conventionally housed animals of the same age. It is possible that in conventionally housed animals the activity of the HPA axis is increased, perhaps due to chronic stress induced by endemic pathogens (23, 32), and that RU-486 can act as a glucocorticoid antagonist under these conditions. However, in SPF animals without elevated HPA activity, RU-486 clearly has a number of glucocorticoid agonist actions. Furthermore, in contrast to the SPF animals, the conventionally housed animals in the Langley and York study had low initial body weights in both lean and obese rats, reduced final body weight in vehicle-treated obese animals, and an absence of hyperphagia at 5 wk of age (25). Other abnormalities included very low plasma glucose levels in all animals (62–69 mg/dl) and fourfold higher insulin levels in the lean rats than in our lean SPF animals. It is therefore likely that the conflicting effects of RU-486 in the two studies reflect major differences between SPF and conventionally maintained animals. Another possible explanation for the divergent effects of RU-486 in the two studies could be differences in the drug itself. Although the same batch of RU-486 was used for the two studies, differences in the storage conditions could have influenced the potency and therefore the relative antagonist/agonist actions of the drug. However, this cannot be determined in the absence of receptor binding studies and dose-response curves.

RU-486 has been shown to have both glucocorticoid antagonistic and agonist actions in experimental trials in human subjects (3, 24, 26) and in nonhuman primates (21, 27). Although potent glucocorticoid antagonist actions for RU-486 have been reported both in vivo and in vitro (3, 9, 14, 28), many of these experiments have examined the ability of RU-486 to antagonize the effects of glucocorticoid agonists such as dexamethasone on glucocorticoid-mediated events but did not evaluate the action of RU-486 in the presence of basal levels of endogenous glucocorticoid hormones. In adrenalectomized rats with or without low-dose corticosterone replacement, RU-486 had glucocorticoid receptor agonist actions to decrease thymus weight and inhibit plasma ACTH concentrations (5). In another study examining dexamethasone-induced hypertension in rats, RU-486 effectively blocked the hypertensive effects of concurrent dexamethasone administration, but RU-486 alone actually caused small but significant increases of blood pressure (22). Thus, when ambient glucocorticoid levels are low, RU-486 can display significant glucocorticoid agonist effects (42). Several studies have implicated activation of a protein kinase A (PKA)

adenosine 3′,5′-cyclic monophosphate-dependent pathway in the agonist actions of RU-486 (15, 30). These studies suggest that the phosphorylation of a coactivator by PKA potentiates the response to agonists and can also cause transcriptional activation by type II antagonists, resulting in agonist actions by antagonists (29, 30). The agonist effects of RU-486 observed in several tissues in the present study could be dependent on the activation state of the PKA signal transduction pathway in those tissues.

In summary, RU-486 does not affect food intake or body weight gain in 5-wk-old SPF Zucker rats. In addition, RU-486 has primarily glucocorticoid agonist activity in SPF Zucker rats of this age. The conflicting effects of RU-486 found in the present study with those found in a previous study may reflect marked differences in the activity of the HPA axis between conventionally maintained and SPF animals. Therefore, the net effect of this mixed agonist/antagonist on glucocorticoid-sensitive tissues is highly dependent on the physiological state of the test subjects. Caution should be exercised in interpreting the results from experiments utilizing this compound, and independent physiological measurements of glucocorticoid agonist and antiglucocorticoid actions should be monitored along with indexes of HPA activity.

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