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Involvement of Corticosterone in the Fasting-Induced Rise in Protein Utilization and Locomotor Activity

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CHALLET, E., Y. LE MAHO, J.-P. ROBIN, A. MALAN AND Y. CHEREL. *Involvement of corticosterone in the fasting-induced rise in protein utilization and locomotor activity.* PHARMACOL BIOCHEM BEHAV **50**(3) 405–412, 1995.—During fasting, most of the energy is derived from lipids whereas proteins are efficiently spared. However, there is a late rise in net protein utilization. Fasting is also associated with an increase in locomotor activity. Because the plasma corticosterone level increases concomitantly with these metabolic and behavioral changes, the involvement of corticosterone has been hypothesized. To test this, the net protein utilization and locomotor activity were investigated in fasted adrenalectomized (Adx) rats, with or without replacement with corticosterone, and in fasted intact rats treated with RU486, an antagonist of type II glucocorticoid receptors. During the phase of fasting characterized by protein sparing, urine nitrogen loss was further reduced in Adx rats and in RU486-treated controls compared with intact rats and with Adx rats with corticosterone replacement: this indicates a catabolic effect of corticosterone through type II receptors. In the last phase of fasting, the rise in net protein breakdown was suppressed in Adx rats and restored by corticosterone replacement. The increase in locomotor activity induced by fasting in controls was suppressed in Adx and restored by corticosterone replacement. This rise in running activity was still present in RU486-treated rats. In conclusion, this study shows that corticosterone plays a critical role in the changes of both protein catabolism and locomotor activity during prolonged fasting.

Corticosterone Fasting Adrenalectomy RU486 Nitrogen excretion Wheel running

GLUCOCORTICOIDS are known to play a role in a wide variety of physiological and behavioral processes, including protein metabolism and food intake. The functional and biochemical characterization of two glucocorticoid receptor types, with different affinities for corticosterone, has allowed specifying the glucocorticoid effects (13,22). The main metabolic effect of corticosterone is an increase in net protein degradation, mainly through a suppression of synthesis but also via an increase in breakdown (32,36). Type II, or classical glucocorticoid receptors, are mainly localized in skeletal muscles (14), a major source of protein during starvation. The mediation of the catabolic effect of corticosterone through type II receptors has been hypothesized (13). In the control of food intake, corticosterone is involved, mediating dietary carbohydrate and fat intake both ad lib and after a short-term fast (5,44) through distinct effects of type I and II receptors. Corticosterone is critical in passerine birds for the stimulation of foraging when food is scarce (2). It stimulates running activity in intact fed rats (24) and enhances exercise endurance in adrenalectomized rats (12).

During prolonged fasting in birds and mammals, changes in plasma corticosterone level approximately coincide with changes in lipid and protein utilization. The possible role of corticosterone in these metabolic changes has therefore been hypothesized (3,9,10). Adaptation to long-term fasting is characterized by lipid mobilization and efficient protein sparing (10,18). There is, however, a late rise in net proteolysis (18) that is triggered before fat stores are entirely depleted (3,41). At that time, there is a large increase in the level of plasma corticosterone (3,10). In birds (e.g., penguins), sponta-

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neous behavioral changes, reflecting the search for food, have been linked with the late increase in net proteolysis (19,28). In fasted albino rats, there is also a delayed rise in locomotor activity [e.g., (42)]. This has been interpreted as reflecting an increasing drive for refeeding and grossly coincides with the rise in net protein breakdown (26). The possible involvement of plasma corticosterone in these metabolic and behavioral changes may be hypothesized from the observations on fed animals (see above). It is further supported by the observations in obese Zucker rats: although they undergo an 80-day fast, they do not exhibit the late rise in nitrogen excretion (9) and locomotor activity (Robin, personal communication), and yet show no increase in corticosteronemia (9).

Most of this evidence is correlative, however. To assess the causal relationship between corticosteronemia, protein catabolism, and locomotor activity, the protein net catabolism and locomotor activity were examined in fasted adrenalectomized rats, with or without replacement with corticosterone, and compared with those of fasted controls. To investigate the possible involvement of type II receptors, the effects of a specific type II receptor antagonist RU38486 were also analyzed in fasted adrenal-intact rats.

The results indicate the involvement of corticosterone in the rise in protein catabolism and locomotor activity characteristic of late fasting.

METHOD

Animals and General Setup

Male Sprague–Dawley rats (Iffa-Credo, Mérieux, France) were kept in a temperature-controlled room (25 ± 1°C) with an artificial 12L : 12D photoperiod. The animals were supplied ad lib with water and standard chow pellets (mass percentage: 50% carbohydrate, 5% fat, and 24% protein).

The rats were weighed daily and kept in single metabolic cages in which they had free access to a running wheel (diameter: 30 cm). The animals were housed in these cages and were accustomed to daily handling for weighing for at least 2 weeks before being fasted. Water remained available ad lib during fasting.

Spontaneous wheel running was continuously recorded during the experiment. Wheel rotations were detected by an optical device, connected to a computer via an electronic counter. Wheel rotations were recorded every 15 min.

Throughout the experiment, urine was collected daily over H_2SO_4 1 N and stored at −20°C until analysis. At the end of the experiment (see below for timing), rats were killed by decapitation in the morning. Blood was collected in heparinized tubes and centrifuged. Whole plasma was stored at −20°C until analysis. Epididymal fat pad mass was also determined, as an index of the utilization of fat stores during prolonged fasting (3).

Surgery and Treatments

Bilaterally adrenalectomized (Adx) rats were obtained from Iffa-Credo (France). After surgery, the animals were supplied ad lib with a 0.9% saline solution. Adrenalectomy was performed at least 3 weeks before experiments. Corticosterone pellets were implanted at the onset of fasting in anesthetized Adx rats according to the method of Meyer et al. (30). Each pellet of approximately 100 mg contained 50% corticosterone and 50% cholesterol. In the fed state (30), such a dose is known to produce a plasma corticosterone level similar to that observed in fasting rats during the protein-sparing stage (3). RU38486 (noted RU486 thereafter) was kindly provided by Roussel-Uclaf (Romainville, France). The crystalline powder was dissolved in aqueous solution containing 1% polysorbate 80 and 0.25% carboxymethylcellulose; 25 mg RU486/kg was administered daily by oral route in intact rats throughout the duration of fast. This dose was chosen in accordance with the tests characterizing the potent glucocorticoid antagonist effect of RU486 (34), which can cross the blood–brain barrier (Philibert, Roussel-Uclaf Co., personal communication). In vivo, the effect of RU486 (PO or SC) has been studied on many responses induced by dexamethasone in rats. For example, an injection of dexamethasone (0.01 mg/kg, IP) induces a sharp increase of hepatic tyrosine amino transferase in Adx rats; this is inhibited in a dose-dependent manner by RU486 [ED_{50} at 5 mg/kg and full antagonist effect between 10 and 25 mg/kg, PO; (34)]. RU486 (10 mg/kg, PO) also exhibits a potent antiglucocorticoid activity (dexamethasone or corticosterone) on thymus weight in intact rats (34).

Experimental Design

When their body mass reached approximately 400 g, rats were either killed as control fed animals or subjected to an experimental fast. Animals were then divided into four groups: fed vs. fasted and intact vs. adrenalectomized. The fasted intact group was further subdivided; in one of the subgroups, the rats received RU486, the other served as fasted controls. The fasted Adx group was also subdivided, with the animals receiving or not a corticosterone implant.

All experiments were performed in compliance with E.E.C. regulations on care of experimental animals, and were submitted to control by French authorities.

Timing of Sacrifice

As mentioned above, prolonged fasting is marked by a mobilization of fat stores and a reduction in body protein loss. This state (the so-called phase II or phase of protein sparing) ends with a progressive increase in nitrogen excretion; this late situation (phase III or late fasting) is still reversible after 3 days (i.e., rats can be successfully refed at this stage) (3,8,26). Sacrifice was performed after 2 days in phase III, as identified by a net increase in diurnal locomotor activity. In a pilot experiment, we had confirmed the previous finding (26) that the rise of locomotor activity during phase III in the fasted rat is associated with a modification of the circadian pattern of locomotor activity: on the second day of phase III, intact fasted rats ran more than 70% of the time during daylight. Another possible criterion is the increase in daily body mass loss per unit of body mass, which reflects the rise in protein utilization in food-deprived rats (3,6). Whenever possible, such as in control or RU486-treated rats, the behavioral criterion was confirmed by the increase of daily body mass loss for predicting the optimal timing of sacrifice.

Methods of Analysis

Urine nitrogen content was measured by the Kjeldahl's method. Nitrogen was converted to protein by multiplying by 6.25. Plasma corticosterone (Cort) was determined by radioimmunoassay (kit from ICN Biochemicals, Inc.). Glucose and β-hydroxybutyrate (β-OHB) were assayed in plasma after deproteinization with 7% perchloric acid. Glucose was determined according to Bergmeyer et al. (4) and β-OHB according to Williamson and Mellandy (45).

TABLE 1

DURATION OF FASTING AND CHANGES IN BODY MASS IN RATS

Group	Duration of Fasting (days)	Initial Body Mass (g)	Final Body Mass (g)
Controls fed	0	400.0 ± 3.7*	
Controls starved	10.1 ± 0.6*	399.8 ± 2.3*	243.9 ± 3.3*
RU486 starved	9.7 ± 0.7*	399.7 ± 2.0*	264.0 ± 3.8†
Adx fed	0	399.6 ± 1.9*	
Adx starved	7.1 ± 0.4†	398.0 ± 1.5*	302.8 ± 4.6‡
Adx starved + Cort.	6.7 ± 0.5†	399.2 ± 2.9*	293.1 ± 3.4‡

Values are means ± SEM, $n = 7$ per group. For the different groups, see the Method section. Means lacking common symbols in a given column are significantly different ($p < 0.05$).

Statistics

Values are means ± SEM with $n = 7$ rats in each group. Peritz' F-test (20) was used for multigroup comparison of data. Trends in locomotor activity changes during fasting were determined by one-way analysis of variance (ANOVA). When data were expressed in percent, arcsine transformation was performed to stabilize the variances as: $X' = 2^*\arcsin(\sqrt{X})$.

RESULTS

Duration of Fasting and Changes in Body Mass

As determined by the criteria defined in the Method section, the duration of starvation was significantly shorter in Adx than in intact rats (whether RU486 treated or not) ($p < 0.01$, Table 1). There was no difference statistically between the two adrenalectomized groups (with or without corticosterone replacement) (7 days) or between the two intact groups (10 days).

The initial body mass was close to 400 g in the four starved groups (Table 1). Starved control rats reached the lowest final mass ($p < 0.01$, Table 1), having lost 39% of their initial body mass when fasting was ended (as defined in the Method section). The RU486-treated intact rats lost 34% of their initial body mass. Their final body mass was significantly higher than in controls and lower than in both Adx starved groups ($p < 0.01$). In contrast, the two Adx groups, with or without corticosterone replacement, weighed significantly more than intact groups ($p < 0.01$) at the termination of the experiment, having lost 24% and 27% of their prefasting mass, respectively.

Urine Nitrogen Loss

In the fed state, the urine nitrogen excretion did not differ significantly between Adx and control groups (Table 2).

In control rats, three phases of starvation were characterized from both changes in daily urine nitrogen excretion and daily body mass loss, as pointed out in other works (3,6,9). In early fasting (phase I), the urine nitrogen loss greatly decreased in the four groups ($p < 0.01$ compared to the fed state; Fig. 1). At this stage, nitrogen excretion in RU486-treated rats was significantly reduced compared to Adx groups ($p < 0.05$, Table 2). During phase II, the daily nitrogen excretion in each group plateaued (Fig. 1). During this protein-sparing stage, mean daily nitrogen excretion was higher in controls than in Adx ($p < 0.05$) among the four fasted groups (Table 2). Corticosterone replacement in Adx significantly enhanced nitrogen loss ($p < 0.05$) to a value that was not statistically different from the controls. Rats receiving a daily injection of RU486 reduced nitrogen loss to a value not significantly different from the Adx group. The final increase in daily nitrogen loss of controls characterized phase III ($p < 0.01$ compared to phase II values; Fig. 1). The nitrogen loss of RU486-treated rats followed a pattern closely similar to controls (Fig. 1), from which it did not differ significantly (Table 2). By contrast, the urine nitrogen loss in the starved Adx group showed no late increase in nitrogen excretion (Fig. 1, Table 2). This increase in urine nitrogen loss was restored in Adx rats with a SC implantation of corticosterone (Fig. 1). However, it remained significantly lower than either controls or RU486-treated rats ($p < 0.05$, Table 2).

Over the fast, the cumulative nitrogen excretion in controls

TABLE 2

URINE NITROGEN LOSS DURING PROLONGED FASTING IN RATS

Starved Group	Daily Nitrogen Excretion (mg · $24h^{-1}$)			
	Fed State	Early Fasting (First Day)	Protein Sparing (Day 4)	Late Fasting (Last Day)
Controls	477 ± 20*	329 ± 26*†	224 ± 11*	443 ± 12*
RU486	446 ± 18*	265 ± 9†	183 ± 12†	429 ± 35*
Adx	476 ± 10*	336 ± 12*	181 ± 6†	144 ± 11†
Adx + Cort.	469 ± 13*	363 ± 25*	218 ± 10*	313 ± 33‡

Values are means ± SEM, $n = 7$ per group. For the different groups, see the Method section. Means lacking common symbols in a given column are significantly different ($p < 0.05$).

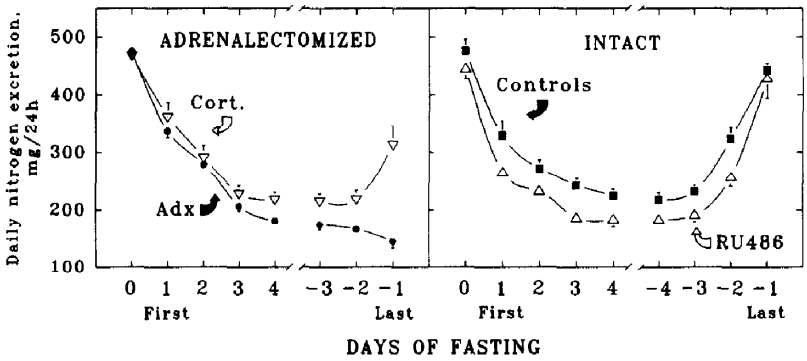

FIG. 1. Effect of fasting on daily nitrogen excretion. Abscissa: left, days counted from beginning of fasting; right, days counted to end of fasting, as determined for each individual. Day 0: fed state. Means ± SEM, $n = 7$ per group. (For the different groups, see the Method section.)

was 1.7-, 1.5- and 1.3-fold larger ($p < 0.01$) than in the Adx, corticosterone-implanted Adx and RU486-injected groups, respectively. Cumulative nitrogen excretion was significantly reduced in RU486-treated rats compared to controls (2649 ± 97 vs. 3321 ± 132 mg, respectively; $p < 0.01$). It did not statistically differ between Adx with or without corticosterone replacement (2243 ± 131 vs. 1961 ± 76 mg, respectively; $p > 0.05$).

Changes in Epididymal Fat Pad Mass

Before food deprivation, no significant difference was found in the epididymal fat mass between Adx and intact rats (Fig. 2). Fasting induced a significant loss in the mass of fat pad in each group compared to the fed state ($p < 0.01$, Fig. 2), with the loss of epididymal fat pad mass reaching approximately 90% in all starved groups.

Plasma Corticosterone and Metabolites

There was a large increase in circulating corticosterone in starved control rats compared to fed rats ($p < 0.01$, Table 3). The hypercorticosteronemia did not differ significantly between starved controls and starved RU486-treated rats. Circulating corticosterone was virtually absent in Adx rats. Corticosterone implants in starved Adx increased the level of plasma corticosterone to a higher value than that of fed controls ($p < 0.01$) but to a lower value than that of starved controls ($p < 0.05$, Table 3).

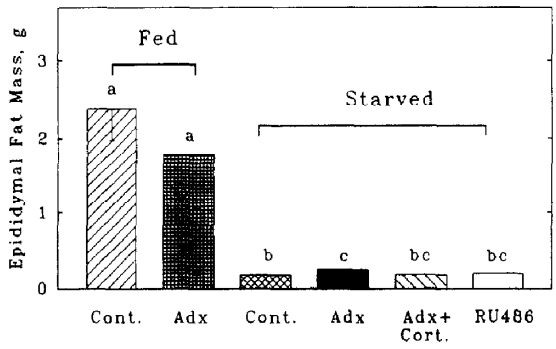

FIG. 2. Effect of fasting on epididymal fat pads mass. Histograms lacking common letters are significantly different ($p < 0.05$). Means ± SEM, $n = 7$ per group; SEM bars smaller than line thickness are not shown. (For the different groups, see the Method section.)

In the fed state, plasma glucose concentration was similar in Adx and control rats. During fasting, it decreased significantly in controls as well as in RU486-treated rats ($p < 0.01$ compared to the fed state). This decrease was larger in starved Adx rats ($p < 0.01$) and was not significantly reduced by corticosterone treatment. At the end of fasting, however, plasma glucose did not differ significantly between starved Adx with corticosterone replacement and starved controls.

In the fed state, plasma β-hydroxybutyrate was as low in controls, RU486 treated, and Adx, with or without corticosterone replacement. Similarly, plasma β-hydroxybutyrate did not differ between groups at the end of fasting (Table 3).

Total Wheel Running

In the fed state, the intact rats were more active than the Adx. Indeed, during the 5 days preceding the food deprivation, the wheel running score was sevenfold higher in intact than in Adx rats (2811 ± 369 vs. 437 ± 65 revolutions per 24 h, respectively; $p < 0.01$).

From the fed state to the end of fasting (Fig. 3), one-way ANOVA revealed a significant trend to a rise in wheel running in starved controls, $F(8, 54) = 3.75$, $p < 0.01$. In contrast, no increase in running activity was observed in fasted Adx rats, $F(7, 48) = 1.04$, $p > 0.05$. Corticosterone replacement in Adx restored the rise in locomotor activity, $F(7, 48) = 3.85$, $p < 0.01$. On the other hand, a significant increase in running scores, $F(8, 54) = 2.62$, $p < 0.05$, was observed in RU486-treated rats.

Circadian Pattern of Wheel Running

In the fed state, intact rats did 86 ± 9% of their circadian running activity during the night, against 68 ± 12% in Adx ($p > 0.05$ in Adx vs. intact rats; $n = 14$). From the fed state to the end of fasting, one-way ANOVA revealed a significant trend to decrease in the proportion of nocturnal activity in control rats [$F(8, 54) = 15.74$, $p < 0.01$; after arcsine transformation]. In the last day of fasting, this proportion was only about 20% of total activity (Fig. 4). This decrease was essentially due to an increase in diurnal wheel running, $F(8, 54) = 6.81$, $p < 0.01$, because nocturnal running scores remained constant, $F(8, 54) = 0.82$, $p > 0.05$, in fasted con-

TABLE 3

PLASMA CORTICOSTERONE AND METABOLITES IN FED AND FASTED RATS

Group	Corticosterone (nmol · L^{-1})	β-Hydroxybutyrate (nmol · L^{-1})	Glucose (mmol · L^{-1})
Controls fed	146 ± 64*	0.05 ± 0.01*	7.3 ± 0.3*
Controls starved	1608 ± 215†	0.06 ± 0.01*	3.9 ± 0.4†
RU486 starved	1310 ± 148†‡	0.07 ± 0.01*	3.9 ± 0.6†
Adx fed	4 ± 1§	0.04 ± 0.01*	6.9 ± 0.1*
Adx starved	3 ± 1§	0.08 ± 0.02*	2.3 ± 0.3‡
Adx starved + Cort.	954 ± 123‡	0.07 ± 0.01*	2.8 ± 0.4†‡

Values are means ± SEM, $n = 7$ per group. For the different groups, see the Method section. Means lacking common symbols in a given column are significantly different ($p < 0.05$).

trols. The significant change of circadian activity pattern during fasting was not inhibited by adrenalectomy because the proportion of nocturnal activity significantly decreased in fasted Adx, $F(7, 48) = 9.31, p < 0.01$. It could thus be used as a criterion for ending experimental fasts. Likewise, a significant trend to decrease in the proportion of nocturnal activity was observed in fasted Adx with corticosterone replacement, $F(7, 48) = 8.88, p < 0.01$, and in fasted RU486-treated rats, $F(8, 54) = 4.90, p < 0.01$ (Fig. 4).

DISCUSSION

This study was performed to assess the possible involvement of corticosterone in protein metabolism and locomotor activity during fasting. Results will be discussed in the following order: (i) plasma corticosterone levels and regulation of the adrenocortical system during fasting; (ii) the metabolic role of corticosterone in view of protein utilization; (iii) the involvement of corticosterone in the fasting-induced rise in locomotor activity.

Levels of Circulating Corticosterone

Our results on circulating corticosterone concentrations in fed and starved rats, Adx or intact, are in accordance with numerous previous studies [e.g. (3,9,37)]. Corticosterone replacement was performed with pellets containing 50% corticosterone. However, the implantation of these pellets in starved Adx rats, instead of producing the concentration of about 0.3 μmol·L^{-1} observed in fed conditions (30,36,37), produced a threefold higher concentration after a fasting period of 7 days. This discrepancy might be attributed to the reduction in the activity of hepatic corticosterone reductase and in the corticosterone clearance found in starved rats (17,46). The pellet implantation in starved Adx rats may thus have grossly mimicked the progressive increase in circulating corticosterone observed in starved intact rats (3,35).

RU486 is a potent blocker of type II glucocorticoid receptor. In the fed state, the feedback inhibition by corticosterone on the hypothalamo-pituitary-adrenocortical axis is mediated via type II glucocorticoid receptors of the hypothalamic paraventricular nucleus (PVN), which produces the corticotropin-releasing hormone (CRH) (11). Treatment with RU486 thus induces an increase in plasma corticosterone in fed intact rats (27) or in cortisol of monkeys (21). No such significant difference in circulating corticosterone was found between starved RU486-treated and control rats. This discrepancy in starvation may be compared to the effects of RU486 in another state with a high plasma corticosterone level, the fatty syndrome of obese Zucker rats. RU486 significantly stimulates the corticosterone secretion in lean but not in obese Zucker rats, whereas

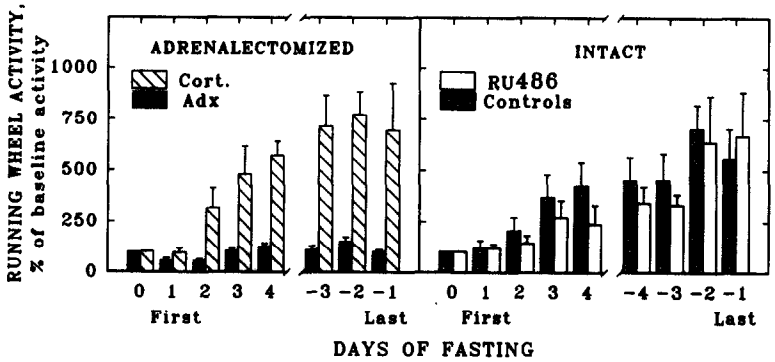

FIG. 3. Running wheel activity during fasting, expressed as percentage of the baseline locomotor activity (i.e., during the 5 days preceding the fast). Abscissa: left, days counted from beginning of fasting; right, days counted to end of fasting, as determined for each individual. Day 0: fed state. Means ± SEM, $n = 7$ per group. (For the different groups, see the Method section.)

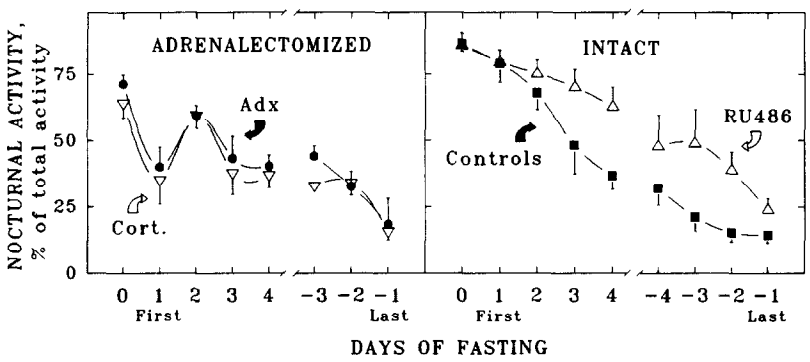

FIG. 4. Nocturnal activity during fasting, expressed as percentage of the total daily locomotor activity. Abscissa: left, days counted from beginning of fasting; right, days counted to end of fasting, as determined for each individual. Day 0: fed state. Means ± SEM, $n = 7$ per group. (For the different groups, see the Method section.)

it reduces the expression of preproCRH mRNA in the PVN in obese but not in lean rats. This means that RU486 might mediate some agonist action in obese Zucker rats (33). Thus, as for obese Zucker rats, the reason why RU486 did not induce in late fasting a larger increase of plasma corticosterone than in controls might be that changes had occurred at the glucocorticoid receptor level and that RU486 had acquired some agonist action. Further interpretations at this stage of late fasting (phase III) are difficult, because to our knowledge the effect of prolonged starvation on glucocorticoid receptors is still unknown. An increase in receptor density in the cytosol during late fasting cannot be excluded: 25 mg RU486/kg could then no longer antagonize all the glucocorticoid receptors. Still, this is not likely, because a pilot study with daily injections of 40 mg RU486/kg did not prevent the occurrence of phase III either (unpublished data).

Protein Metabolism

In the fed state, the plasma glucose, urine excretion, and epididymal fat mass of Adx rats did not differ from those of intact rats. This indicates that both Adx and intact rats, which also had the same initial body mass at the onset of the fast, were in a similar nutritional status. At the end of the experiment, it may be assumed that all four starved groups had exhausted their fat stores because approximately 90% of the initial mass of epididymal fat pads had disappeared. Indeed, a previous study in the same rat strain has shown that the decrease in fat pad mass may be used as an index of the utilization of lipid reserves during prolonged fasting (3).

The changes in urine nitrogen loss during the three phases of starvation in control rats agree with those previously described in laboratory rats (3,6,26): nitrogen loss decreases at first (phase I), plateaus at a low level (phase II), and then increases (phase III). Our data show that adrenalectomy did not suppress the initial drop in daily nitrogen excretion, with or without corticosterone replacement. By contrast, the catabolic effect of corticosterone clearly appeared in phase II, because urine nitrogen loss in Adx was reduced, whereas corticosterone implantation enhanced nitrogen excretion up to the level of controls. In RU486-treated rats, the urine nitrogen loss was reduced to the same phase II value as in Adx. These findings support the hypothesis that the catabolic action of corticosterone is mediated through type II glucocorticoid receptors (13). Furthermore, after the same duration of fast, the cumulative nitrogen excretion was lower in RU486-treated rats than in controls. This also reinforces the hypothesis of an overall catabolic role of corticosterone through type II receptors.

Corticosterone replacement in Adx restored the late increase in nitrogen excretion (phase III), therefore according with the hypothesis that corticosterone plays a key role in this process (3,9,10). It is essentially due to a drop in protein synthesis, while protein breakdown still occurs but does not increase (6); these are the two main metabolic roles of corticosterone (32,36). That the late rise in urine loss was not impaired by the daily injection of RU486 could be due, as above, to a possible agonist action of RU486 in late fasting.

The decrease in plasma glucose concentration in starved control rats agrees with previous data (7). In fasted Adx rats, hypoglycemia was even more severe. This may be related to the inability of starved Adx to release gluconeogenic amino acids and other amino acids from skeletal muscles, but also to a reduction in hepatic gluconeogenesis (15). Plasma glucose was not significantly decreased at the end of fasting in Adx with corticosterone replacement compared with controls, which suggests that gluconeogenesis was enhanced by corticosterone. However, corticosterone replacement did not statistically elevate plasma glucose in Adx after a prolonged starvation, a result that differs from previous studies during short-term fasting (43).

In late fasting, the low plasma β-hydroxybutyrate level in starved controls and RU486 as well as in Adx indicates that all the rats were close to depletion of lipid stores.

Total Locomotor Activity

The large increase in the spontaneous wheel-running activity of control rats during a fast is consistent with many previous studies (26,31,42). Importantly, this rise in locomotor activity was suppressed in fasted Adx rats, except when they were provided with corticosterone replacement. Adrenalectomy induces a decrease in the spontaneous activity in fed rats [(29,40), this study]. However, the relative predeprivation hypoactivity cannot be related to the lack of increase of wheel

running in fasted Adx because running scores during fasting were expressed proportionally to the baseline activity. Moreover, adrenal demedullated rats also display hyperactivity in response to starvation (16). Altogether, these results strongly suggest a critical role of corticosterone in this fasting-induced rise in running. It has been suggested that the fasting-induced increase in wheel running may reflect an increase in food-searching behavior (26). Interestingly, a study in wild birds suggests that corticosterone may be critical for the initiation of food foraging during periods of food deprivation (2).

The overall blockade of type II glucocorticoid receptors by RU486 treatment had only minor effects on the increase in locomotor activity, and this as soon as the fasting state was initiated. In addition to the different affinities of type I and II receptors for corticosterone [e.g., (13,22)], another striking difference between the two types is their localization in the brain and peripheral tissues. Type I receptors have been specifically localized in limbic brain, kidney, parotid gland, and heart; type II receptors are widely distributed in the brain as well as in many peripheral tissues such as skeletal muscle, heart, liver, and thymus (14,23,39). No effect of RU486 was observed on the fasting-induced increase in locomotor activity, whereas this process, absent in Adx, was restored by corticosterone replacement: this may suggest that stimulation of corticosterone took place through the type I receptor. Type I receptors have also been involved in the corticosterone negative feedback following a novel environment stress (38), another situation in which circulating corticosterone reaches a high level. However, the type I and/or type II involvement in the behavioral action of corticosterone should be further specified. On one hand, further studies should investigate the effects of a specific type I antagonist as well as effects of a combination of both receptors antagonists in intact fasted rats. On the other hand, specific agonists should also be used in fasted Adx rats.

Circadian Changes in Locomotor Activity

Prolonged fasting yielded a significant increase of diurnal activity, as rats in late fasting performed more than three-fourths of their total running activity during daylight (this study). Several authors have previously mentioned a relative increase in diurnal activity during prolonged food deprivation (1,25,26). For many years, the increase in total locomotor activity in response to fasting has been interpreted as reflecting a behavioral arousal [e.g., (16)]. That nocturnal activity in fasted rats does not increase in contrast to the rise in diurnal activity [(1), this study] does not support this arousal paradigm. Of interest, the circadian pattern of locomotor activity was modified by fasting in Adx rats although they did not concomitantly increase their total activity. This suggests that adrenal glands are not essential in the fasting-induced modification of the circadian pattern of activity.

In conclusion, the present study demonstrates that both the late increase in nitrogen excretion and the rise in locomotor activity during prolonged fasting are suppressed by bilateral adrenalectomy and restored by a corticosterone replacement. Thus, corticosterone is the critical hormone suppressed by adrenalectomy and has a key role in these metabolic and behavioral processes. Moreover, a daily injection of RU486, an antagonist of type II glucocorticoid receptor, limits the urine nitrogen loss to the Adx level in intact rats during protein sparing, but does not prevent the late increase in nitrogen excretion. From a behavioral point of view, the pattern of increased locomotor activity during fasting was unchanged in RU486-treated rats vs. controls. Further experiments are needed to settle the nature of glucocorticoid receptors involved in this behavioral process.

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