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Effects of Adrenalectomy on Macronutrient Selection Patterns in the Rat

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TEMPEL, D. L., M. YAMAMOTO, T. KIM AND S. F. LEIBOWITZ. *Effects of adrenalectomy on macronutrient selection patterns in the rat.* PHARMACOL BIOCHEM BEHAV **40**(4) 861–866, 1991.—The present studies examined the effects of adrenalectomy (ADX) on nutrient selection of rats over the 24-h period, as well as during the first 2 h of the nocturnal feeding cycle. Results indicate that ADX, in rats showing generally similar preferences for carbohydrate and fat, equally suppresses intake of both of these nutrients over the 24-h period. The relative impact of ADX on carbohydrate and fat intake may shift depending upon baseline, with carbohydrate-preferring rats showing a stronger decrease in intake of this diet after ADX and fat-preferring rats exhibiting a greater decline in fat intake after ADX. Acute injections of corticosterone (CORT) and aldosterone (ALDO) are both found to restore carbohydrate as well as fat intake to ADX rats over the 24-h period. However, in the first 2 h of the dark feeding cycle, carbohydrate intake is found to be selectively suppressed after ADX, and CORT injection (0.5 and 2.0 mg/kg, SC) restores carbohydrate intake during this early dark period, while producing a small increase in fat intake only at the higher dose. This is in contrast to ALDO administration at dark onset, which has a stronger stimulatory effect on fat intake in the ADX rat but does not fully restore carbohydrate intake. These findings indicate that CORT and ALDO have differential effects on nutrient intake in ADX rats particularly at the onset of the dark cycle, and it is suggested that these effects are mediated, respectively, by the type I and type II steroid receptor systems in the brain.

Adrenalectomy Feeding behavior Fat Carbohydrate Corticosterone Aldosterone Steroid receptors

THE adrenal steroids play an important role in the control of food intake (3, 12, 20, 28, 34), the metabolism of nutrients (10, 26, 33), and the maintenance of body weight (3, 8, 9). Studies have indicated that removal of endogenous glucocorticoids by surgical adrenalectomy (ADX) results in alterations in feeding behavior, fat deposition and body weight (4, 8, 12, 21, 34). In addition, glucocorticoids are involved in the development and/or maintenance of genetic and experimentally induced obesities (2, 5, 12, 15, 31).

In fa/fa Zucker obese rats, which consume a large portion of their daily intake in the form of fat, it appears that the intake of high-fat diets are particularly affected after ADX surgery (3,4). In addition, there is evidence in normal-weight rats showing a strong suppressive effect of ADX on carbohydrate intake as well as fat intake (6, 20, 21, 28, 34).

Administration of the glucocorticoid, corticosterone (CORT), restores normal food intake and body weight gain to ADX rats (3, 12, 21, 31, 34). Moreover, recent evidence indicates that the mineralocorticoid aldosterone (ALDO) or its specific receptor system may also be involved in mediating food intake (6,34). While most of these studies have focused on total 24-h caloric intake of ADX rats, this laboratory has been studying the effects of ADX on feeding behavior during specific periods of the natural feeding cycle, when variations in circulating CORT levels as well as different patterns of nutrient intake are known to exist (1, 19, 23, 35).

The present experiments were specifically designed to study the effects of ADX on macronutrient selection over the 24-h period, as well as during the first 2 h of the nocturnal feeding cycle when circulating CORT is known to peak. In addition, the effects of CORT and ALDO injection were examined with the aim of further clarifying the specific function of these steroids and their receptors in the feeding process.

METHOD

Subjects

Forty adult male albino Sprague-Dawley rats, weighing 300–350 g at the start, were used in these studies. Animals were individually housed in hanging wire mesh stainless steel cages in a temperature-controlled room with a constant 12:12-h light/dark cycle, with lights on at 0200.

Diets

Animals were maintained ad lib on three pure macronutrient diets of protein, carbohydrate and fat and tap water. The protein diet (3.7 kcal/g) consisted of 93% casein (granular, enzymatic casein; National Casein Co.) mixed with 4% minerals (USP XIV Salt Mixture Briggs, ICN Pharmaceuticals), 2.97% vitamins (Vitamin Diet Fortification Mixture, ICN Pharmaceuticals), and 0.03% cysteine (L-cysteine hydrochloride, ICN Pharmaceuticals). The carbohydrate diet (3.7 kcal/g) was composed of 28% dextrin (ICN Pharmaceuticals), 28% corn starch (ICN Pharma-

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ceuticals) and 37% sucrose (Domino) mixed with 4% minerals and 3% vitamins. The fat diet (7.7 kcal/g) consisted of 86% lard (Armor) mixed with 8% minerals and 6% vitamins. All three diets were simultaneously available in separate glass food cups anchored at the front of the cage. The order of food cups within the cage was changed daily to prevent position preference.

Adrenalectomy

In Experiment 1, after 7 days of stable preoperative nutrient intake measures, all rats received bilateral ADX surgery. In Experiment 2, animals were randomly selected to undergo either ADX or sham surgery. Under Metofane anesthesia, adrenal glands were removed by means of two dorsal incisions caudal to the costal margin. Sham surgery consisted of bilateral dorsal incisions, plus location and slight manipulation of the adrenal glands. Following surgery, all animals were supplied with a 0.9% saline solution in addition to tap water and were given a 3–5-day recovery period before further testing.

Completeness of ADX surgery was verified at the end of each experiment by radioimmunoassay (RIA). Animals were removed from their home cages, rapidly decapitated, and trunk blood collected. Blood samples were refrigerated in glass test tubes for 12 h and were then centrifuged at 4°C. Serum was separated and frozen until RIA was performed according to the method of Krey et al. (18). Corticosterone (Sigma) was used as the standard, and 1,2,6,7,-3H cortisol (Amersham) was the tracer. The antiserum was generated against cortisol 21-succinate bovine serum albumin (Antisera P21–53 Endocrine Sciences) and has approximately a 60% cross-reactivity with corticosterone. ADX rats had a mean CORT level of 0.8 μg%, and sham rats were found to have blood CORT levels of 7.9 μg% significantly more than ADX rats ($p<0.05$).

Procedures

The first experiment was designed to study the effects of ADX on macronutrient ingestion patterns over the 24-h cycle. Since rats display individual differences in their 24-h nutrient intake patterns (32), rats (n = 20) were used as their own controls and were compared with respect to their pre- and post-ADX measures. This direct comparison permitted a more precise analysis of the effects of ADX on nutrient intake in animals exhibiting differential baseline feeding of carbohydrate and fat. Measures of 24-h food intake were recorded daily, for 7 days prior to and 21 days after ADX surgery for 20 rats.

Based on presurgery intake scores rats were separated into three groups: 1) The majority of rats (n = 12, 60% of the total group) consumed similar amounts of all three diets over the 24-h period, ingesting at least 20% of total intake from each macronutrient source. These rats were termed "no preference" rats. 2) A small percentage of rats (20%, n = 4) consumed significantly more ($p<0.05$) carbohydrate (64 kcal; 60% of total) and less fat (15 kcal; 15% of total) than the no preference rats, and they were, therefore, referred to as "high carbohydrate/low fat" rats. 3) The remaining 20% of the group (n = 4) displayed an opposite pattern, consuming significantly ($p<0.05$) more fat (86 kcal; 68% of total) and less carbohydrate (12 kcal; 10% of total) than the no preference rats and were termed "high fat/low carbohydrate" rats. Body weights were recorded 3 times/week and indicated that all rats gained an average of 5.8 g/day prior to ADX and only 1.6 g/day after ADX.

In the second experiment, 15 rats were selected before surgery from an original group of 20 rats. Only those rats consuming at least 20% of their total daily intake from each macronutrient diet, and thus exhibiting no strong dietary preferences relative to carbohydrate or fat were used. This selection process allowed us to examine differential effects of ADX, as well as steroid replacement, on intake of these two nutrients. After 7 days of stable baseline intake measures, rats were randomly selected to undergo sham or ADX surgery. Food intake over the 24-h period, as well as intake during the first 2 h of the dark feeding cycle, were recorded on days 4–12 and comparisons were made between sham and ADX scores. Following this period, ADX rats were given steroid replacement.

Steroid Replacement

Adrenalectomized rats were subcutaneously injected, once a day, 1 h prior to the onset of the dark period, with CORT (Sigma) at doses of 0.5 or 2.0 mg/kg, ALDO (d-aldosterone, Sigma), at 10 or 50 μg/kg, or propylene glycol vehicle in counterbalanced order. Sham rats received daily vehicle injections. Steroid injections were separated by at least 2 days of vehicle treatment, to allow complete clearance of steroid from the blood. Food intake was recorded 2 h after injection (after the 2nd h of the dark period) as well as 24 h after injection.

Statistical Analyses

Pre- and postoperative as well as sham and ADX intake scores were compared using a two-way analysis of variance (ANOVA) for repeated measures. The effects of various doses of steroids were also analyzed by two-way ANOVA. Specific comparisons were made using Duncan's New Multiple Range Test or, where appropriate, Student's *t*-test.

RESULTS

Effect of ADX on 24-h Nutrient Intake Patterns

This experiment studied the effects of ADX on three groups of rats, exhibiting different baseline nutrient intake patterns. As illustrated in Fig. 1, ADX surgery was found to have differential effects on 24-h nutrient intake in these three subgroups of rats. In "no preference" rats, which consumed >20% from each macronutrient source (Fig. 1, left panel), ADX decreased total 24-h kcal intake by 35% ($p<0.05$) relative to pre-ADX scores. This effect was due to an essentially equal decline in carbohydrate intake (−14.5 kcal; −40%; $p<0.05$) and fat intake (−19.7 kcal; −50%; $p<0.05$), while protein intake remained essentially unaltered.

In "high carbohydrate/low fat" rats, eating >60% carbohydrate and <15% fat (Fig. 1, middle panel), the predominant effect of ADX was to suppress intake of the carbohydrate diet. In these rats total caloric intake was suppressed by 40%, due to a predominant suppression of 24-h carbohydrate intake (−34 kcal; −53%; $p<0.05$). Fat intake, in contrast, was suppressed by only 5 kcal, which relative to a low baseline of 15 kcal was statistically insignificant (−30%, $p>0.05$). Once again, protein intake was unaffected.

The converse occurred in the "high fat/low carbohydrate" rats, that ate >60% fat and <15% carbohydrate (Fig. 1, right panel). In these rats total intake was again suppressed by 40% relative to pre-ADX measures. In this group, however, ADX most strongly suppressed intake of the fat diet (−46 kcal, −55%, $p<0.05$), while a smaller and insignificant decline was observed in the less preferred carbohydrate diet (−1.5 kcal, −30%, $p>0.05$). No effect on protein intake was observed. From these results, it becomes evident that baseline patterns of macronutrient intake are important in determining the relative

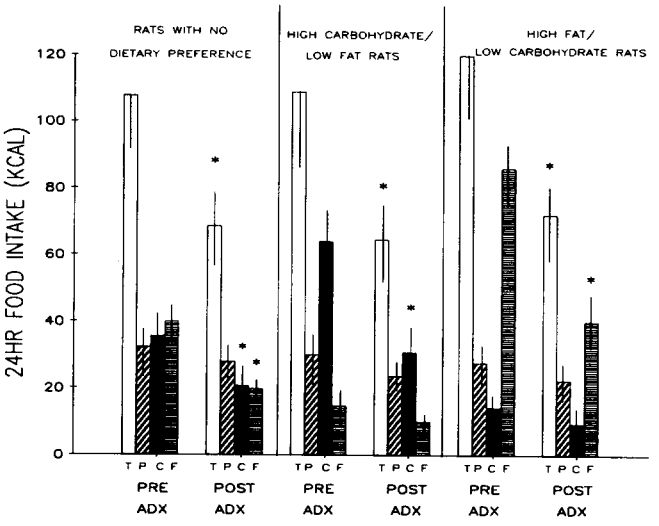

FIG. 1. In rats displaying no particular dietary preference over the 24-h period, ADX significantly suppresses daily intake of carbohydrate and fat (left panel). In high carbohydrate/low fat rats, ADX predominantly suppresses intake of the carbohydrate diet (middle panel). In high fat/low carbohydrate rats, ADX selectively suppresses fat intake (right panel). ADX is found to have no effect on 24-h protein ingestion. *$p<0.05$, pre- vs. post-ADX comparisons. T = total, P = protein, C = carbohydrate, F = fat.

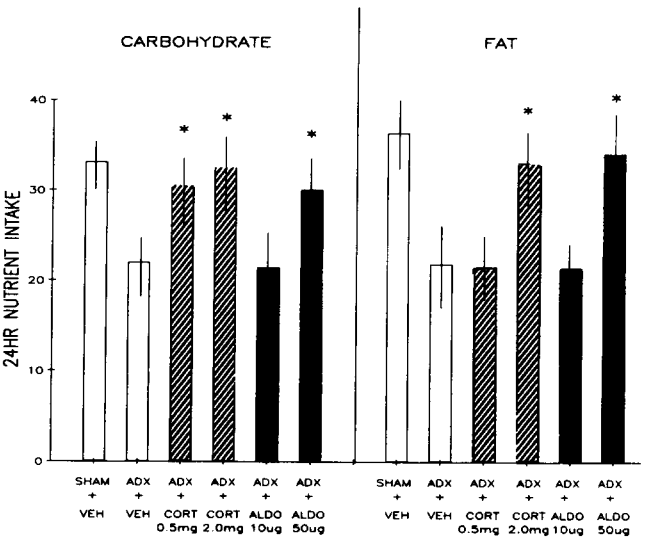

FIG. 2. Corticosterone at both 0.5 and 2.0 mg/kg restores daily carbohydrate intake to ADX rats, as does ALDO injection at a dose of 50 μg/kg (left panel). Fat intake is restored only by the higher dose of CORT and also by 50 μg/kg ALDO (right panel). No effects on protein ingestion were observed after steroid injection. *$p<0.05$, relative to ADX + VEH injection scores.

impact of ADX on daily carbohydrate and fat intake.

Effect of CORT and ALDO Replacement on 24-h Feeding Patterns

This experiment studied the effects of ADX and subsequent CORT and ALDO injection on nutrient intake patterns. Since the preoperative baseline intake patterns of these rats were generally similar, comparisons were made between the postoperative scores of sham (n = 6) and ADX (n = 9) rats, rather than between pre- and postoperative scores, as in Experiment 1.

Similar to results obtained in Experiment 1, the ADX rats in this experiment consumed 30% fewer total calories over the 24-hr period than did the sham rats (102.1 vs. 72.8 kcal/day; $p<0.05$). Daily intake of carbohydrate (−11.1 kcal, −34%, $p<0.05$) and fat (−14.5 kcal, −40%, $p<0.05$) were both suppressed, while protein intake was similar in the ADX (28.9 kcal) and sham (32.6 kcal) rats.

In these subjects, 24-h nutrient intake was measured after acute subcutaneous injection, just prior to dark onset, of CORT (0.5 or 2.0 mg/kg), ALDO (10 or 50 μg/kg) or propylene glycol vehicle in ADX rats, and propylene glycol vehicle in sham rats. Whereas these steroids had little impact on ingestion of protein (data not shown), CORT and ALDO both affected carbohydrate and fat intake in ADX rats, as shown in Fig. 2. The lower dose of CORT (0.5 mg/kg) selectively stimulated carbohydrate intake (+8.5 kcal; $p<0.05$, relative to vehicle injection), restoring 24-h intake of this diet to a level similar to that of the sham rats (Fig. 2, left panel). The higher dose of CORT (2.0 mg/kg) also stimulated carbohydrate intake in ADX rats (+10.5 kcal; $p<0.05$, relative to vehicle injection), although this effect was not significantly larger than that seen after injection of 0.5 mg/kg of CORT, and thus no dose-response relationship was observed.

In contrast to carbohydrate intake, 24-h fat consumption was not significantly altered by the 0.5 mg/kg dose of CORT in ADX rats (Fig. 2, right panel). Only the higher dose (2.0 mg/kg) affected fat ingestion, increasing intake of this diet by 11.1 kcal ($p<0.05$ relative to vehicle-treated ADX rats) and restoring fat intake to the level seen in vehicle-treated sham rats. Only at this higher dose was CORT, due to its impact on both carbohydrate and fat intake, effective in fully restoring total 24-h caloric intake of the ADX rats (96.0 kcal) to the level of the sham rats (102.1 kcal). Daily protein intake for ADX rats under vehicle conditions was 28.9 kcal/day and was not significantly affected by either 0.5 mg/kg (29.0 kcal) or 2.0 mg/kg (30.3 kcal) dose of CORT.

Acute injection of the mineralocorticoid ALDO at a dose of 10 μg/kg was without effect on 24-h nutrient intake of ADX rats. However, a higher dose of 50 μg/kg increased total intake by 18.9 kcal ($p<0.05$). As shown in Fig. 2, daily carbohydrate intake was increased by 8.1 kcal and fat intake by 12.3 kcal, ($p<0.05$ relative to vehicle baseline) and thus total 24-h caloric intake (91.7 kcal) was similar to that of sham rats (102.1 kcal). As with CORT, ALDO had no impact on the ingestion of protein (28.9, 26.6 and 27.4 kcal after vehicle, 10 μg/kg and 50 μg/kg ALDO, respectively).

Effect of ADX on Feeding Patterns in the Early Dark Period

The 15 rats used in Experiment 2 were also examined during the first 2 h of the nocturnal feeding cycle. This particular time was chosen for specific analysis since it is associated with a large burst of feeding (1, 29, 42), as well as a natural peak in circulating levels of CORT (25). Thus it may be at this time that CORT has its strongest effects on food intake. As in Experiment 2, comparisons were made between the intake scores of sham versus ADX rats.

As previously shown in intact rats (35), the first 2 h of the dark feeding period in sham rats (Fig. 3, left panel) were char-

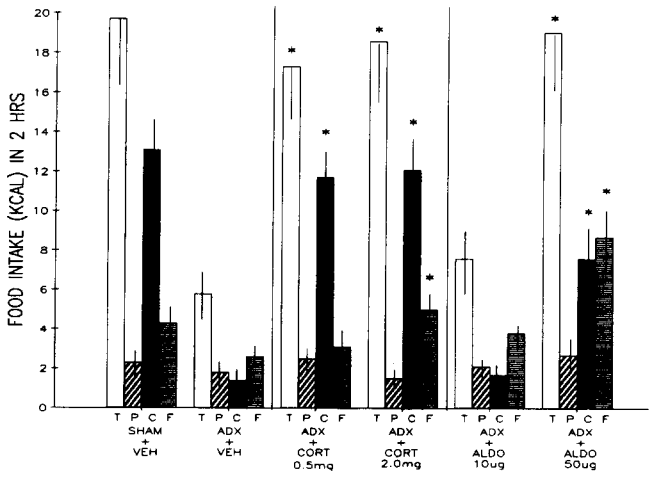

FIG. 3. During the first 2 h of the dark period, ADX predominantly suppresses intake of carbohydrate (left panel), which can be restored by CORT at 0.5 and 2.0 mg/kg. Fat intake is also stimulated by the higher dose of CORT (middle panel). ALDO at 10 μg/kg is without significant effect, but at 50 μg/kg ALDO stimulates (but does not fully restore) carbohydrate intake in ADX rats, and also strongly stimulates fat intake, increasing intake of this diet to levels greater than those of sham rats (right panel). *$p<0.05$, relative to ADX + VEH injection scores. T = total, P = protein, C = carbohydrate, F = fat.

acterized by a preference for the carbohydrate diet. These animals consumed a total of 19.7 kcal, 15% as protein, 65% as carbohydrate and 20% as fat. Calculation of these data relative to total 24-h intake showed that carbohydrate intake during this brief period accounted for 45% of these rats' total daily carbohydrate consumption. This is in contrast to only 12% for the fat diet and 7% for the protein diet, indicating that most of the fat and protein intake of these rats occurred later in the feeding cycle. During this early dark period, ADX strongly suppressed total intake by 70% (−13.9 kcal; $p<0.05$) relative to sham scores (Fig. 3, left panel). This suppressive effect of ADX was due predominantly to a strong suppression (−11.7 kcal; −90%; $p<0.05$) of carbohydrate intake, which accounted entirely for the 24-h suppression of this diet (−11.1 kcal) seen in these rats. In contrast, the effect on fat intake, which, although similarly strong over the 24-h period, was relatively weak at the onset of the dark feeding cycle (−1.7 kcal; −40%; $p>0.05$) and accounted for only 12% of the 24-h fat suppression in these rats. No change in protein intake was observed during this 2-h period.

These data suggested that ADX had a specific effect on carbohydrate intake during the early dark period. The possibility existed, however, that the smaller effect on fat may have been due to low baseline intake of this diet at this time. To further test this, 5 additional rats which displayed particularly high concentrations of fat intake during this 2-h early dark period prior to ADX were examined. These rats ingested 3.1 ± 0.7 kcal of protein, 4.2 ± 0.8 kcal of carbohydrate and 7.9 ± 1.1 kcal of fat during this time. This pattern of high fat intake was found to persist after ADX, with these rats consuming 2.5 ± 0.4 kcal of protein, 0.4 ± 0.1 kcal of carbohydrate and 5.2 ± 1.0 kcal of fat. Although a significant decline in fat intake (−40%, −3.1 kcal, $p<0.05$) was observed, a stronger ($p<0.05$) and almost complete suppression of carbohydrate intake was the predominant effect of ADX in these high-fat rats (−90%, −3.8 kcal, $p<0.05$) during this early dark period. These data, therefore, indicated that the strong and selective decrease in carbohydrate intake seen in ADX rats at dark onset was not a function of baseline nutrient intake and suggested a specific role for CORT in the regulation of carbohydrate intake at the onset of the feeding cycle.

Effect of CORT and ALDO Replacement on Early Dark Feeding Patterns

During the first 2 h of the dark period, CORT was found to have a strong stimulatory effect on carbohydrate intake, while ALDO had a more potent effect on fat intake (Fig. 3). Corticosterone injection of 0.5 and 2.0 mg/kg, significantly increased 2-h carbohydrate intake (+10.3 and +10.7 kcal, respectively, $p<0.05$) relative to vehicle injection, and restored total 2-h caloric intake to a levels similar to that of the sham rats (Fig. 3, middle panel). The higher dose of CORT (2.0 mg/kg) also had a small stimulatory effect on fat ingestion (+2.4 kcal, $p<0.05$, relative to vehicle scores). Protein intake was unaffected by CORT at either of the two doses tested.

While ALDO injection at a dose of 10 μg/kg had no effect on nutrient intake, the 50 μg/kg dose strongly increased total 2-h intake in ADX rats by 13.2 kcal ($p<0.05$) relative to vehicle injection (Fig. 3, right panel), restoring total intake to a level similar to that of sham rats (Fig. 3, right panel). This dose of ALDO increased carbohydrate intake to 7.6 kcal ($p<0.05$) relative to the 1.4 kcal baseline score of vehicle-treated ADX rats; however, this was not sufficient to completely restore carbohydrate intake in the ADX rats, which still ate significantly fewer kcal (7.6 vs. 13.1 kcal, $p<0.05$) of carbohydrate than did vehicle-injected sham rats during this 2-h period (Fig. 3, left panel). This stimulatory effect of ALDO on carbohydrate intake was also significantly smaller ($p<0.05$) than that seen after injection of 0.5 or 2.0 mg/kg of CORT. Fat intake was strongly potentiated after 50 μg/kg of ALDO (8.7 kcal, relative to 1.4 kcal for vehicle-treated ADX rats ($p<0.05$). This effect was significantly stronger that the small stimulation of this diet observed after injection of 2.0 mg/kg CORT, and it was also significantly greater than that seen in vehicle-treated sham rats (4.3 vs. 8.7 kcal, $p<0.05$) during this 2-h early dark period.

DISCUSSION

Impact of ADX on Nutrient Intake

The present data indicate that in male Sprague-Dawley rats, ADX decreases total 24-h caloric intake by 30–40%. This is similar to results obtained in other studies using various strains of rats, including Zucker obese rats, and in rats maintained on various diets (3, 4, 6, 20, 21, 34).

In rats given pure macronutrient diets, ADX is found to suppress 24-h intake of both the carbohydrate and fat diets, while minimally affecting protein intake. A similar effect on both carbohydrate and fat intake has been observed after ADX in other studies (20, 21, 34). An additional finding of this study is that baseline patterns of nutrient consumption can determine the magnitude of the effect of ADX on nutrient intake. It is found that in rats preferring carbohydrate relative to fat, ADX most strongly suppresses the intake of the carbohydrate diet, having smaller suppressive effects on the less-preferred fat diet (Fig. 1). This is consistent with the study of Devenport et al. (6), which reported a stronger suppression of carbohydrate intake (−11 kcal) than fat intake (−5 kcal) after ADX, in rats with a high carbohydrate/fat baseline. In contrast, rats in the present study, which prefer fat (>50% of total intake) over carbohydrate (<15%), exhibit a selective suppression of fat intake after ADX. This

specific suppression of fat intake, seen here in a subpopulation of male Sprague-Dawley rats, has also been described in both lean and obese Zucker rats that consume 60–70% of their total daily intake in the form of fat and only 10% in the form of carbohydrate (3,4).

Effects of CORT and ALDO Injection on 24-h Nutrient Selection Patterns

Injections of either CORT or ALDO are found to normalize, in ADX rats, intake of both carbohydrate and fat over the 24-h period. Numerous studies have reported that CORT administration, by a variety of methods including subcutaneous injection, pellet implant, or in drinking solution, dose-dependently restores total food intake in ADX rats (3, 4, 11, 12, 20, 21, 36). In the present study, consistent with Kumar and Leibowitz (20), doses of 0.5 and 2.0 mg/kg CORT are effective in restoring 24-h carbohydrate intake, while only the higher dose significantly stimulates fat intake and fully restores 24-h total caloric intake to normal levels. In Zucker obese rats, higher doses of CORT (10 mg/kg) appear to be necessary to completely restore total caloric intake to ADX rats (3), although in another study (12) obese rats were reported to be more responsive to CORT than normal weight controls.

Similar to CORT, the mineralocorticoid ALDO, at a dose of 50 μg/kg, stimulates 24-h food intake in ADX rats via increases in both carbohydrate and fat intake. Devenport et al. (6) have reported 25–100 μg/kg/day to be effective in restoring both carbohydrate and fat intake in ADX rats to control levels, while a higher dose of 125 μg/kg/day potentiated 24-h fat intake to a level significantly greater than that of sham rats.

Effects of ADX on Early Dark Feeding Patterns

The suppression of carbohydrate and fat intake seen after ADX over the 24-h period, is in contrast to the changes observed in the 2-h early dark period, when ADX strongly and selectively suppresses intake of the carbohydrate diet, while having little effect on fat intake. This strong suppressive effect of ADX on carbohydrate intake at this time, which has been previously described (21,34), accounts for the entire suppression of this diet observed over the 24-h period. This is in contrast to fat intake which, although similarly reduced over the 24-h period, is only weakly affected during this early dark period. This effect of ADX on food intake, and specifically carbohydrate intake, at dark onset can also be distinguished from the end of the dark feeding cycle, when ADX has little impact on total caloric intake or specific nutrient ingestion (34). Taken together, these data indicate that ADX does not suppress feeding behavior uniformly across the nocturnal feeding cycle. They suggest a specific time-dependent effect of ADX on carbohydrate intake at dark onset, while changes in fat intake appear to occur at different times during the natural feeding cycle.

The Effect of CORT and ALDO Replacement on Early Dark Feeding Patterns

While both CORT and ALDO are similarly effective in restoring 24-h caloric intake in ADX rats, clear differences are seen in their effects at dark onset, with CORT having a stronger stimulatory effect on carbohydrate intake and ALDO having a stronger effect on fat intake. At doses of 0.5 and 2.0 mg/kg, CORT predominantly stimulates carbohydrate ingestion in ADX rats during the first 2 h of the dark phase and fully restores 2-h caloric intake to levels equal to those of the sham rats. Only the higher dose has a small stimulatory effect on fat ingestion at this time of the cycle. These effects of CORT on carbohydrate ingestion have also been observed after continuous CORT replacement (21), as well as after direct CORT administration into the hypothalamic paraventricular nucleus (PVN), where CORT may be acting to produce its effects (34).

In contrast to CORT, ALDO injection dramatically increases fat intake in ADX rats, to levels greater than those of sham rats, but has only a small stimulatory effect on carbohydrate intake. This potentiating effect of ALDO, specifically on fat intake, is consistent with the work of Devenport et al. (6) over the 24-h period and also with Tempel and Leibowitz (34) after PVN ALDO implant at dark onset. Furthermore, a similar potentiating effect of ALDO on fat ingestion has been observed after PVN administration of ALDO in sham animals (34), indicating a role for this hormone or its receptor system in stimulating fat intake in intact as well as ADX rats.

Relation of Steroid Receptor Subtypes to Feeding Behavior Induced by CORT and ALDO

Although CORT and ALDO display similar restorative effects on nutrient intake over the 24-h period, their effects on nutrient intake at dark onset clearly differentiate these two hormones and their receptor systems (13, 16, 24, 30). The two steroid receptor systems, type I and type II, have been shown to have different affinities for CORT and ALDO (17,24) and may have differential functions in mediating the effects of circulating steroids (7, 16, 17, 25, 29, 30). The type I receptor has a far greater affinity for ALDO than does the type II, and, therefore, any effects produced by ALDO are likely to be mediated specifically by the type I receptor (17). In contrast, CORT binds and activates both receptor subtypes and may have differential functions depending on the receptor activated (7, 17, 24, 29, 30).

Aldosterone, through activation of the type I receptor (17), has been shown to produce anabolic effects, including increases in food intake, body weight and body fat content (7–9). The type II receptor also appears to have a stimulatory action on body weight and the maintenance of obesity (22,27), although the opposite pattern of effects, decreases food intake and body weight gain, are seen after administration of high doses of type II agonists (7,14).

Although interactive effects of these two receptor systems in mediating CORT's effects on food intake cannot be completely ruled out, it is suggested that the effects of CORT observed in these studies, particularly on carbohydrate intake at the onset of the dark feeding period, are mediated by the type II receptor. This is supported by studies demonstrating that: 1) the type II receptor becomes increasingly occupied and activated at the circadian peak of CORT release (25, 29, 30); 2) specific type II receptor activation, via injection of RU28362 in ADX rats, produces a pattern of effects at dark onset (preferential increase in carbohydrate intake) similar to those of CORT (Tempel and Leibowitz, unpublished data); 3) specific blockade of type II receptors, via acute PVN implant of RU28486 at the onset of the dark period, results in a suppression of carbohydrate intake at the onset of the dark feeding period, an effect similar to that seen in ADX rats (Tempel and Leibowitz, unpublished data); and 4) as shown here, ALDO or specific type I stimulation does not completely normalize feeding in ADX rats at this time of the cycle, while CORT completely restores normal feeding patterns to the ADX rat.

In conclusion, these data suggest that the type I receptor may

be involved in modulating fat intake at various points across the 24-h period, while the type II receptor seems to have a specific function in controlling carbohydrate intake at the start of the nocturnal feeding cycle.

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REFERENCES

1. Armstrong, S. A. A chronometric approach to the study of feeding behavior. Neurosci. Biobehav. Rev. 4:27–53; 1980.
2. Bray, G. A.; York, D. A. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol. Rev. 59:719–809; 1979.
3. Castonguay, T. W.; Dallman, M. F.; Stern, J. S. Corticosterone prevents body weight loss and diminished fat appetite following adrenalectomy. Nutr. Behav. 2:115–125; 1984.
4. Castonguay, T. W.; Dallman, M. F.; Stern, J. S. Some metabolic and behavioral effects of adrenalectomy on obese Zucker rats. Am. J. Physiol. 251:R923–R933; 1986.
5. Dallman, M. F. Viewing the ventromedial hypothalamus from the adrenal gland. Am. J. Physiol. 246:R1–R12; 1984.
6. Devenport, L.; Knehans, A.; Thomas, T.; Sundstrom, A. Macronutrient intake and utilization by rats: interactions with type I adrenocorticoid receptor stimulation. Am. J. Physiol. 260:R73–R81; 1991.
7. Devenport, L.; Knehans, A.; Sundstrom, A.; Thomas, T. Corticosterone's dual metabolic actions. Life Sci. 45:1389–1396; 1989.
8. Devenport, L.; Manes, G.; Thomas, T.; Mena, S.; Kem, D.; Knehans, A. Aldosterone and the mobilization of energy. Appetite 8:81–90; 1987.
9. Devenport, L. D.; Goodwin, K. G.; Hopkins, P. M. Continuous infusion of aldosterone: Correlates of body weight gain. Pharmacol. Biochem. Behav. 22:707–709; 1985.
10. Fain, J. N.; Czech, M. P. Glucocorticoid effects on lipid mobilization and adipose tissue metabolism. In: Handbook of physiology and endocrinology. sect. 7, vol. VI, Chapt. 12. Washington, DC: American Physiological Society; 1975:169–178.
11. Freedman, M. R.; Castonguay, T. W.; Stern, J. S. Effect of adrenalectomy and corticosterone replacement on meal patterns of Zucker rats. Am. J. Physiol. 249:R584–R594; 1985.
12. Freedman, M. R.; Horowitz, B. A.; Stern, J. S. Effect of adrenalectomy and glucocorticoid replacement on development of obesity. Am. J. Physiol. 250:R595–R607; 1986.
13. Funder, J. W.; Sheppard, K. Adrenocortical steroids and the brain. Annu. Rev. Physiol. 49:397–411; 1987.
14. Grigson, S. P.; Johnson, D. R.; Collier, G. H.; Flaherty, C. F. Effect of dexamethasone-21-acetate on meal size, meal frequency and macronutrient self-selection in rats. Physiol. Behav. 46:211–216; 1989.
15. King, B. M. Glucocorticoids and hypothalamic obesity. Neurosci. Biobehav. Rev. 12:29–37; 1988.
16. deKloet, E. R. Brain corticosteroid receptor balance and homeostatic control. Front. Neuroendocrinol. 12(2):95–164; 1991.
17. deKloet, E. R.; Ratka, A.; Reul, J. M. H. M.; Sutanto, W.; van Eekelen, J. A. M. Corticosteroid receptor types in brain: Regulation and putative function. Ann. NY Acad. Sci. 512:351–361; 1987.
18. Krey, L.; Lu, K. H.; Hotchkiss, W.; Piva, J.; Knobill, E. Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. II. GH and cortisol secretion. Endocrinology 96:1088–1094; 1975.
19. Krieger, D. T.; Hauser, H. Comparison of synchronization of circadian corticosteroid rhythms of photoperiod and food. Proc. Natl. Acad. Sci. USA 75:1577–1581; 1978.
20. Kumar, B. A.; Leibowitz, S. F. Impact of acute corticosterone administration on feeding and macronutrient self-selection patterns. Am. J. Physiol. 254:R222–R228; 1988.
21. Kumar, B. A.; Papamichael, M.; Leibowitz, S. F. Feeding and macronutrient selection patterns in rats: Adrenalectomy and chronic corticosterone replacement. Physiol. Behav. 42:581–9; 1988.
22. Langley, S. C.; York, D. A. Effects of antiglucocorticoid RU 486 on development of obesity in obese fa/fa Zucker rats. Am. J. Physiol. 259:R539–R544; 1990.
23. Le Magnen, J. The metabolic basis of dual periodicity of feeding in rats. Behav. Brain Sci. 4:561–607; 1981.
24. McEwen, B. S.; deKloet, E. R.; Rostene, W. Adrenal steroid receptors and actions in the nervous system. Physiol. Rev. 66:1122–1188; 1986.
25. Magarinos, A. M.; Monica, F.; De Nicola, A. F. Corticosteroid receptors and glucocorticoid content in microdissected brain regions: Correlative aspects. Neuroendocrinology 50:673–678; 1989.
26. Munck, A.; Guyre, P. M.; Holbrook, N. J. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5(1):25–44; 1984.
27. Okada, S.; Bray, G. A.; York, D. A. Blockade of glucocorticoid receptors with RU-486 prevents dietary obesity. Fed. Proc. A918; 1990.
28. Richter, C. P. Decreased carbohydrate appetite of adrenalectomized rats. Proc. Soc. Exp. Biol. Med. 48:557–579; 1941.
29. Reul, J. M. H. M.; van den Bosch, F. R.; deKloet, E. R. Relative occupation of type-I and type-II corticosteroid receptors in rat brain following stress and dexamethasone treatment: Functional implications. J. Endocrinol. 115:459–467; 1987.
30. Reul, J. M. H. M.; deKloet, E. R. Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. Endocrinology 117:2505–2511; 1985.
31. Shimomura, Y.; Bray, G. A.; Lee, M. Adrenalectomy and steroid replacement in obese (ob/ob) and diabetic (db/db) mice. Horm. Metab. Res. 19:295–299; 1987.
32. Shor-Posner, G.; Ian, C.; Brennan, G.; Cohn, T.; Moy, H.; Ning, A.; Leibowitz, S. F. Self-selecting albino rats exhibit differential preferences for pure macronutrient diets: Characterization of three subpopulations. Physiol. Behav., in press; 1991.
33. Steele, R. Influences of corticosteroids on protein and carbohydrate metabolism. In: Handbook of physiology and endocrinology. sect. 7, vol. VI, Chapt. 11. Washington, DC: American Physiological Society; 1975:153–167.
34. Tempel, D. L.; Leibowitz, S. F. PVN steroid implants: Effect on feeding patterns and macronutrient selection. Brain Res. Bull. 23: 553–560; 1989.
35. Tempel, D. L.; Shor-Posner, G.; Dwyer, D.; Leibowitz, S. F. Nocturnal patterns of macronutrient intake in freely feeding and food deprived rats. Am. J. Physiol. 256:R541–R548; 1989.
36. Yamamoto, Y.; Tempel, D. L.; Leibowitz, S. F. Effects of corticosterone in drinking water on natural feeding patterns. Soc. Neurosci. Abstr. 15(2):1130; 1989.