Differential suppression of hyperglycemic, feeding, and neuroendocrine responses in anorexia

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Salter, Dawna, and Alan G. Watts. Differential suppression of hyperglycemic, feeding, and neuroendocrine responses in anorexia. Am J Physiol Regul Integr Comp Physiol 284: R174–R182, 2003. First published September 12, 2002; 10.1152/ajpregu.00275.2002.—We have used the anorexia shown by rats given hypertonic saline to drink to investigate central mechanisms that can inhibit feeding. Rats dehydrated in this manner for 3 or 5 days showed a severe attenuation of the compensatory feeding observed after an overnight fast compared with control euhydrated rats or rats whose food was restricted to match the intake of anorectic rats. Food intake after injections of 2-deoxy-D-glucose (2-DG) was also significantly decreased in dehydrated animals compared with that after a 2-DG injection given before dehydration. However, all the dehydrated animals demonstrated a robust eating response after water was returned whether they had received injection of 2-DG or vehicle. Despite a profound reduction in 2-DG-induced feeding, other glucoregulatory responses to 2-DG remained intact in dehydrated animals. After 2-DG injection, corticosterone secretion and blood glucose were significantly elevated from preinjection values whether or not animals were dehydrated. Thus the mechanisms responsible for anorexia in dehydrated animals specifically target stimulatory feeding pathways but leave intact other counterregulatory glucometabolic motor events.

corticosterone; plasma glucose; 2-deoxy-d-glucose; overnight fast; dehydration

ANOREXIA, THE LOSS OF APPETITE for food, is evoked by many different mechanisms. Although it is a significant complication to a variety of clinical pathologies, the underlying neural mechanisms responsible for anorexia are largely unknown. To investigate these neural substrates, we have taken advantage of a simple way to stimulate anorexia in animals. When their drinking water is replaced with hypertonic saline, rats not only become progressively dehydrated, they also exhibit a profound anorexia, which is maintained until access to water is restored (50). In an effort to conserve water and limit the addition of osmoles to an already compromised fluid compartment, dehydration implements a series of adaptive responses that target gastrointestinal function. Thus salivation, gut motility, and spontaneous circadian-driven feeding are reduced in an attempt to resolve or minimize fluid perturbations at the expense of energy balance (11, 17, 54). In this way, dehydration-anorexia offers a useful paradigm for investigating how the normal compensatory mechanisms used to trigger feeding during negative energy balance can be inhibited.

We previously demonstrated that dehydrated animals and animals that are food restricted to match the intake of anorectic rats show the same attributes of negative energy balance (54). These include body weight loss, diminished circulating leptin and insulin, and increased blood glucocorticoid concentrations and neuropeptide Y (NPY) gene expression in the arcuate nucleus of the hypothalamus (ARH). Normally, these neural and endocrine processes stimulate compensatory feeding mechanisms aimed at increasing caloric intake to match expenditure. In this way, weight loss triggers hunger to restore body energy stores through food intake. Elevated glucocorticoids and decreased insulin levels can independently stimulate food intake (4, 44), whereas falling plasma leptin levels after starvation (9, 19) or the deficiency of leptin in ob/ob mice (8) stimulates eating.

Feeding behavior is mediated in certain circumstances by the increased activity of those NPY-producing neurons in the ARH (1, 37) that express leptin receptors (6) and project to the paraventricular nucleus of the hypothalamus (PVH) and the lateral hypothalamic area (LHA) (6, 15). NPY injections into the PVH or LHA elicit robust feeding (26, 45, 46), whereas chronic infusions of NPY lead to hyperphagia and obesity (57). On the basis of their neuropeptide and endocrine profile, we have proposed that, in dehydrated-anorexic animals, some component of these NPY-mediated compensatory mechanisms is inhibited until released by subsequent water intake (52).

The present study was designed to determine whether dehydrated-anorexic rats show reduced feeding responses to two challenges that invoke feeding responses that are believed to involve NPY-mediated mechanisms: 2-deoxy-d-glucose (2-DG) and overnight starvation. NPY appears to be a key mediator of fasting-induced hyperphagia, in that food deprivation in-
increases NPY mRNA in the ARH (9) and elevates NPY levels and release in the PVH (24, 37, 56). 2-DG is a glucose analog that leads to cytoglucopenia by competitively inhibiting glucose utilization. Evidence implicates NPY in the feeding response that follows central or peripheral administration of 2-DG (2, 21, 22, 27). 2-DG feeding is mediated by central catecholamine neurons (31, 33, 34), which colocalize NPY and project to the PVH (40). Immunoneutralization of NPY in the PVH impairs 2-DG feeding (22), whereas 2-DG-induced glucoprivation increases Fos expression in ARH NPY neurons (27).

2-DG offers a further advantage as an experimental tool for delineating the neural circuits underlying anorexia, because, in addition to stimulating feeding, it rapidly elicits two other motor responses: sympathetic activation of epinephrine secretion from the adrenal medulla, which leads to hyperglycemia (41), and corticosterone secretion from the adrenal cortex (55), which is driven by increased activation of neuroendocrine corticotropin-releasing hormone (CRH) neurons in the PVH. Together, these behavioral, autonomic, and neuroendocrine motor events serve to replenish and redistribute metabolic fuels. Examining how other nonbehavioral motor control systems function during dehydration should provide a broader view of how adaptive neural mechanisms function in anorexia.

MATERIALS AND METHODS

Animals and Procedures

Adult male Sprague-Dawley rats weighing 235–260 g were obtained from Harlan Laboratories and housed in suspended Plexiglas cages with sanitized wood chips. They were maintained in a temperature-controlled room on a 12:12-h light-dark schedule with lights on at 0600. Rats were provided continuous access to food (Teklad rodent diet 8604) and water throughout the experiment, except where stated. In some animals, drinking water was replaced with 2.5% saline solution for up to 5 days; in others, the amount of food available was restricted to match that eaten by dehydrated animals (54). We previously showed that 5 days of drinking hypertonic saline increases plasma osmolality by ∼6% (53, 54). Body weights and nocturnal food intake were measured daily throughout the experiment. All procedures have been approved by the local institutional animal care and use committee.

Surgical Procedures

Rats were handled daily for ∼4 days before any surgical intervention and daily thereafter. On the 4th day after arrival, rats designated for 2-DG injections were anesthetized with an injection (100 μl/kg im) of a 50% solution of ketamine (100 mg/ml) plus xylazine (20 mg/ml), and sterile intra-atrial catheters were inserted by way of the external jugular vein. Catheters were threaded subcutaneously to the dorsal surface, exteriorized between the scapulae, and then sutured in place. Catheters were flushed daily with sterile heparinized 0.9% saline. Animals were allowed to recover to presurgical weight before further manipulation. Only rats with stable weight gains and consistent nocturnal intakes were included in the study.

Overnight Starvation

Five groups of animals were used in this experiment. Food intakes were measured in all groups for 3 days before testing. Drinking water was replaced in two groups with 2.5% saline for 3 (n = 5) or 5 days (n = 5). Body weights and food intake were then measured twice daily between 0800 and 0900 and between 1600 and 1700. Food was removed from all cages at 1700 on the evening of the final night of saline. On the following morning at 0800, a measured amount of food was returned, and the food remaining in the cage was measured to the nearest 0.1 g each hour for a total of 4 h. At the conclusion of the feeding test, saline was replaced with drinking water, and food intake was measured after a further hour.

A food restriction schedule was provided for two other groups of animals maintained on drinking water. Animals were weight matched to animals dehydrated for 3 (n = 5) or 5 days (n = 5). They were then given an amount of food at the beginning of each light and dark period equal to that eaten by the dehydrated animals (54). The amount of food was calculated for each rat as a percentage of the food eaten per 100 g of mean body weight (for 2 days before beginning the food restriction). On the evening of the 2nd or 4th day of food restriction, food was removed completely. Food was returned to animals on the following morning, and intake was measured each hour for the next 4 h. A fifth group of euhydrated animals was allowed continuous access to food (n = 5) and water. Food intake was measured in this group for 4 h on the morning after an overnight fast.

Responses to 2-DG

Four experiments were performed to determine the effect of dehydration on the responses to 2-DG. In experiment 1, a dose-feeding response curve was established for 2-DG. In experiment 2, the effect of two doses of 2-DG given 7 days apart was determined to test the validity of using each animal as its own control before and during dehydration. In experiment 3, the effects of dehydration on feeding responses to 2-DG were determined. In experiment 4, the effects of dehydration on the plasma glucose and corticosterone responses to 2-DG were measured.

All feeding tests were conducted between 0800 and 1300 as follows. At the beginning of the experiment, all food and sawdust were removed from the cages, the animals were weighed, and a measured amount of food was placed in the test cage for ∼1 h. Equal volumetric doses of vehicle or 0.1 ml/kg 2-DG were then injected into the jugular catheter. Some rats in experiments 3 and 4 were given a subcutaneous 2-DG injection because of blocked jugular catheters. There was no significant difference in response between catheter and subcutaneous injections, so these data were pooled. After the injection, food consumption was measured by weighing the food remaining in each cage to the nearest 0.1 g each hour for the next 4 h.

Experiment 1. To establish appropriate doses of 2-DG for investigating the effects of dehydration on the feeding response to 2-DG, animals maintained with continuous access to water were injected with vehicle (0.9% saline) or one of four doses of 2-DG: 50 mg/kg (n = 5), 100 mg/kg (n = 4), 200 mg/kg (n = 5), and 250 mg/kg (n = 6). Food intake was then measured as described above.

Experiment 2. One group of animals (n = 5) was tested three times over the next 9 days for food intake after injections of vehicle or 2-DG. On day 1, vehicle injections were given to measure baseline food consumption in the test cage. On the following day, each animal was injected with 200
mg/kg 2-DG. Food intake was again measured on day 9 after an injection of 200 mg/kg 2-DG.

Experiment 3. Rats (n = 28) were divided into three groups and tested three times over the next 9 days for food intake after injection of vehicle or 2-DG. On day 1, all animals were injected with vehicle to establish baseline food consumption in the test cage. On the following day (day 2), each animal was injected with vehicle or 200 or 250 mg/kg 2-DG. On day 4, drinking water was replaced with 2.5% saline. Food intake was again measured on day 9, the 5th day of dehydration after injection of vehicle or 200 or 250 mg/kg 2-DG. Each animal received the same treatment on days 2 and 9. At the conclusion of the 4-h feeding test, drinking water was returned to all dehydrated animals, and food intake was measured during the following hour.

Experiment 4. The design of experiment 4 was virtually identical to that of experiment 3, except blood samples were taken from the jugular catheter for plasma glucose and corticosterone determinations. Animals were tested three times after equal volumetric injections of 0.9% saline vehicle or 0.1 ml/kg 2-DG. Venous blood samples (150 μl) were collected from the jugular catheters of all animals immediately before and 15, 30, 60, and 120 min after injection. Food was removed for 2 h before baseline blood collection and was not returned until after the final blood sample.

On day 1, all animals were injected with vehicle to determine baseline responses; on day 3, animals were injected intra-atrially with vehicle (n = 4) or 200 mg/kg 2-DG (n = 8). Drinking water was replaced with 2.5% saline at 1200 h on the following day. On the morning of the 5th day of dehydration, rats were injected with vehicle or 200 mg/kg 2-DG. Drinking water and food were replaced at the conclusion of the blood sampling. Only animals that exhibited a feeding response of >3 g to drinking water were included in the study.

Blood samples were immediately placed on ice and centrifuged, plasma was removed, and the samples were stored at 20°C until assayed. Plasma glucose concentration was assayed using an autoanalyzer (model 2700, Yellow Springs Instruments, Yellow Springs, OH). Plasma corticosterone concentrations were determined by double-antibody radioimmunoassay (48) using a commercially available kit (ICN Pharmaceuticals). All samples were assayed in duplicate in single assays. Internal controls were within appropriate ranges.

Statistics

Values are means ± SE. Changes in body weight during dehydration, food intake suppression, food intake after overnight starvation, and amount eaten after return of water were compared between groups using one-way ANOVA. For analysis of plasma glucose and corticosterone responses, incremental increases were calculated for each animal by subtracting the maximum concentration attained from the pre-injection value. Baseline euhydration tests were compared with euhydration 2-DG tests in experimental animals using ANOVA with repeated measures at each time point. Data from euhydrated and dehydrated animals were compared using a 2 × 2 repeated-measure ANOVA. Bonferroni’s multiple comparison test was used to measure individual differences. The critical level for significance was set at P < 0.05 for all comparisons.

RESULTS

Feeding Responses to Overnight Starvation

Overnight starvation elicited significantly different amounts of compensatory feeding in dehydrated, food-restricted, and ad libitum-fed groups [at hour 4; F(df4,25)] = 45.92, P < 0.001]. Figure 1A shows that the amount of food eaten after 4 h by 5-day food-restricted animals was significantly greater than that consumed by ad libitum-fed animals (P < 0.05). However, both groups of dehydrated animals ate significantly less after overnight starvation than either food-restricted or ad libitum-fed animals, with the animals that had been dehydrated for 5 days eating significantly less than those dehydrated for 3 days (P < 0.01). This represented a 60% and 80% reduction compared with the ad libitum-fed euhydrated group (Fig. 1B). However, all dehydrated animals displayed a robust eating response during the hour after access to drinking water was restored (Fig. 1A); the size of the response was not significantly different between the two dehydrated groups.

Feeding Responses to 2-DG

Changes in body weight over the course of the feeding tests are shown in Table 1. There was no significant

Fig. 1. Hypertonic saline ingestion (dehydration) inhibits feeding in response to overnight starvation. A: cumulative weight of food eaten after overnight starvation after 5 days of dehydration (○), 3 days of dehydration (□), ad libitum feeding (●), 3 days of food restriction (▲), and 5 days of food restriction (■). Dashed lines, robust eating response 1 h after water was returned to dehydrated animals. Values are means ± SE. B: total weight of food eaten 4 h after food replacement by food-restricted (solid line) or dehydrated (dashed line) animals after overnight starvation and various times of food restriction or dehydration. Values (means ± SE) are expressed as percentage of response of ad libitum-fed animals (100%). See RESULTS for levels of statistical significance.
difference in rate of weight gain among the animals in the three treatment groups before or during the testing period. At the end of dehydration, rats in each treatment group lost a similar amount of body weight and exhibited equivalent anorexia (Table 1).

Experiment 1

Figure 2 shows the amount of food eaten after injection of increasing doses of 2-DG. 2-DG at 50 or 100 mg/kg elicited a feeding response that was not different from the response to vehicle at all time points except hour 4, when the response to 100 mg/kg 2-DG was significantly greater ($P < 0.05$) than the response to vehicle or 50 mg/kg 2-DG (Fig. 2B). Animals treated with 200 or 250 mg/kg 2-DG ate significantly more than animals treated with vehicle at all times ($P < 0.01$). Feeding responses to vehicle and 50 mg/kg 2-DG were indistinguishable. On the basis of the results of this experiment, 200 or 250 mg/kg 2-DG was used to test the effects of dehydration on feeding responses in subsequent experiments.

Experiment 2

In euhydrated animals given two injections of 200 mg/kg 2-DG separated by 7 days, the response to the second injection was indistinguishable from the response to the first injection (Fig. 3). One-way repeated-measure ANOVA indicated a significant effect of 2-DG on food intake ($F(df[2,12]) = 16.33$, $P < 0.001$). The first and second doses of 2-DG elicited significantly more food intake than vehicle at all time points ($P < 0.01$). However, the amount of food intake after the first injection was not significantly different from that after the second injection at any time.

Experiment 3

Injection of vehicle did not elicit a significant eating response in any of the three groups of animals (Fig. 4A), but after administration of 200 or 250 mg/kg 2-DG, these animals ate significantly more ($F(df[2,37]) = 48.33$, $P < 0.001$; Fig. 4B). Figure 4C shows that dehydration significantly reduced the ability of 2-DG to elicit food intake. Two-way repeated-measured

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Table 1. Effects of drinking 2.5% saline on body weight and mean nocturnal food intake

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n = 8)</th>
<th>200 mg/kg (n = 10)</th>
<th>250 mg/kg (n = 10)</th>
</tr>
</thead>
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<tr>
<td><strong>Body weight</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Beginning, g</td>
<td>248.5 ± 4.6</td>
<td>237.1 ± 2.2</td>
<td>260.5 ± 6.9</td>
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<tr>
<td>Gain, g/day</td>
<td>3.2 ± 0.4</td>
<td>2.6 ± 0.4</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>Decrease with DE, %</td>
<td>13.7 ± 2.0</td>
<td>14.8 ± 1.3</td>
<td>16.6 ± 1.6</td>
</tr>
<tr>
<td><strong>Nocturnal food intake</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline (2 day avg), g</td>
<td>17.8 ± 0.6</td>
<td>18.6 ± 0.6</td>
<td>18.2 ± 0.4</td>
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<tr>
<td>Decrease with DE, %</td>
<td>59.7 ± 4.6</td>
<td>65.8 ± 4.8</td>
<td>64.9 ± 4.2</td>
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</table>

Values are means ± SE; n, number of animals. 2-DG, 2-deoxy-D-glucose. There were no significant differences between treatment groups in body weights at the start of the experiment, the rate of increase before dehydration (DE), or the decline in body weight after DE. Similarly, DE was associated with a similar decrease in nocturnal food intake in all 3 groups.
measure ANOVA revealed a main effect of dehydration \(F_{(df(2,25)} = 44.25, P < 0.001\) and an interaction effect between 2-DG and dehydration \(F_{(df(2,25)} = 12.3, P < 0.001\). Finally, all dehydrated animals ate similar amounts of food when drinking water was returned at the end of the 2-DG-feeding test, whether they were injected with vehicle or one of the two doses of 2-DG 4 h previously (Fig. 4D).

**Experiment 4**

**Effects of dehydration on responses of plasma glucose concentrations to 2-DG.** Plasma glucose results are presented in Table 2 and Fig. 5. Vehicle injections did not significantly increase plasma glucose concentrations in any treatment group on day 1 (Fig. 5A). On day 3, animals were injected with vehicle or 200 mg/kg 2-DG (Fig. 5B). There was no significant response to vehicle, but 2-DG elicited a significant increase in plasma glucose from preinjection values \(F_{(df(4,35)} = 13.25, P < 0.001\). On day 9, preinjection plasma glucose concentrations were not different from preinjection values on days 1 and 3 (Table 2). After injection of vehicle or 2-DG, mean plasma glucose concentrations in dehydrated animals were again unaffected by vehicle injection but were significantly increased from preinjection values by 2-DG \(F_{(df(4,33)} = 9.90, P < 0.001\); Fig. 5C).

Two-way ANOVA with repeated measures indicated that 2-DG administration was a significant main effect at all time points after injection (at 60 min; \(F_{(df(1,10)} = 85.78, P < 0.001\). However, dehydration was not significant as a main or an interactive effect at any time point measured. Dehydration did not significantly alter the incremental increase in glucose elevation elicited by 2-DG from that seen in euvhydrated animals at any time point. Additionally, there was no significant difference in changes of plasma glucose after vehicle injection between euvhdydrated and dehydrated animals.

Although dehydration did not significantly alter the magnitude of the plasma glucose response to 2-DG, there was an apparent difference in the kinetics of the response. Peak mean glucose concentrations were seen at 15 min on day 3 (euvhydration), but at 120 min on

<table>
<thead>
<tr>
<th>Glucose, mg/dl</th>
<th>Corticosterone, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1 (euvhydration)</strong></td>
<td></td>
</tr>
<tr>
<td>Vehicle Pre</td>
<td>104.6 ± 3.0</td>
</tr>
<tr>
<td>Max</td>
<td>112.0 ± 3.0</td>
</tr>
<tr>
<td><strong>Day 3 (euvhydration)</strong></td>
<td></td>
</tr>
<tr>
<td>Vehicle Pre</td>
<td>112.0 ± 4.3</td>
</tr>
<tr>
<td>Max</td>
<td>114.3 ± 5.6</td>
</tr>
<tr>
<td><strong>Day 9 (dehydration)</strong></td>
<td></td>
</tr>
<tr>
<td>Vehicle Pre</td>
<td>105.5 ± 1.2</td>
</tr>
<tr>
<td>Max</td>
<td>270.0 ± 34.3</td>
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<tr>
<td><strong>Vehicle or 2-DG</strong></td>
<td></td>
</tr>
<tr>
<td>Vehicle Pre</td>
<td>98.5 ± 11.0</td>
</tr>
<tr>
<td>Max</td>
<td>113.5 ± 25.9</td>
</tr>
<tr>
<td>2-DG Pre</td>
<td>106.4 ± 4.6</td>
</tr>
<tr>
<td>Max</td>
<td>246.3 ± 29.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Injection of vehicle (0.9% saline) did not increase plasma glucose and corticosterone before [days 1 and 3 (euvhydration)] or after 2.5% saline [day 9 (dehydration)]. Similarly, 2-DG (200 mg/kg) injections significantly increased plasma glucose and corticosterone concentrations in all treatment groups. Pre, preinjection value; Max, maximum absolute value attained after injection. See RESULTS for levels of statistical significance.
day 9 (dehydration). This was partly due to very high values in the 15-min samples from two animals on day 3. Removing these values reduced the variance of the mean at 15 min in such a way that peak values were now observed at 60 min in these animals.

Effects of DE on responses of plasma corticosterone concentrations to 2-DG. Corticosterone responses to vehicle or 2-DG are illustrated in Table 2 and Fig. 6. Vehicle injections did not significantly increase plasma corticosterone concentrations from preinjection values in any treatment group on days 1, 3, and 9 (Fig. 6A). However, 2-DG injections significantly increased plasma corticosterone concentrations on day 3 in euhydrated animals \( F[\text{df}(4,35)] = 9.61, P < 0.001; \) Fig. 6B). After 5 days of dehydration on day 9, preinjection levels of corticosterone were significantly elevated from those measured on day 3 \( F[\text{df}(1,10)] = 6.8, P < 0.05; \) Table 2. However, 2-DG injections still significantly increased plasma corticosterone concentrations from preinjection values in dehydrated animals \( F[\text{df}(4,33)] = 3.95, P < 0.01; \) Fig. 6C).

Two-way ANOVA with repeated measures revealed a significant main effect of 2-DG on plasma corticosterone concentrations at the time when mean maximum value was attained after 2-DG injections [at 60 min; \( F[\text{df}(1,10)] = 82.12, P < 0.001; \) Fig. 6C]. Finally, there was no significant interaction effect at any time point. There was also no significant difference between the incremental increase of corticosterone in animals receiving 2-DG, regardless of hydration state at any time point after the preinjection measurement.

**DISCUSSION**

Our present results demonstrate two points regarding the control of energy balance in dehydrated-anorexic rats. First, normal plasma glucose concentrations are maintained during dehydration-anorexia, presumably because metabolism is now biased toward increased glycogenolysis and lipolysis. This observation, taken together with the fact that dehydrated-anorexic and paired-food-restricted animals have vir-
tually identical endocrine and neuropeptidergic responses to negative energy balance (54), shows that dehydrated-anorexic animals maintain a normal metabolic response to reduced food intake. Of course, the critical difference between food-restricted and dehydrated-anorexic animals is the decreased drive to eat in the latter.

Second, we show that dehydration for as little as 3 days results in a severe attenuation of the compensatory food intake that normally occurs after an overnight fast. Dehydrated animals also eat less in response to doses of 2-DG that elicit feeding in the same rats before dehydration and have been shown by others to produce eating (35, 43). These observations are consistent with a previous study showing that 2-DG-induced food intake is attenuated in water-deprived rats (49). Dehydrated animals, therefore, do not seek to repair an actual (from overnight starvation) or a perceived (from 2-DG) caloric deficit until after access to water has been restored. At this point, dehydrated-anorexic animals reliably begin robust compensatory feeding within 10 min after return of drinking water (50). This rapid eating response clearly demonstrates that dehydrated animals will eat with appropriate stimulation and that the mechanisms responsible for inhibiting feeding to a variety of stimuli are quickly counteracted by drinking water. The mechanisms responsible for the reversal of this anorexia are unknown.

2-DG-induced glucoprivation rapidly elicits a triad of compensatory motor responses aimed at mobilizing glucose stores and replenishing energy supplies: increased secretion of epinephrine to produce hyperglycemia, glucocorticoid release, and feeding. These complementary processes are activated more or less simultaneously and promote glucose delivery to the brain. However, the fact that under certain circumstances they can be uncoupled demonstrates that their control mechanisms are not tightly linked. For example, phlorizin and alloxan, which inhibit glucose transport and glucose oxidation, respectively, elicit eating behavior, but not hyperglycemia, when injected into the fourth ventricle (18, 36). Similarly, area postrema lesions impair feeding after 2-DG administration but leave intact the hyperglycemic and the corticosterone secretory response (13). In this regard, we show that dehydrated-anorexic animals retain the ability to mount a hyperglycemic and a glucocorticoid secretory response to the same dose of 2-DG that fails to stimulate eating. Dehydration, therefore, specifically targets pathways associated with stimulating food intake, whereas those mechanisms responsible for neuroendocrine and sympathetic glucometabolism-related motor events are left intact.

Repeated daily 2-DG administration can impair the feeding response to 2-DG (39), possibly as a result of a chronic elevation of circulating glucocorticoids (10). In the present study, we have confirmed previous reports that dehydrated animals show increased plasma corticosterone levels in the morning (51). However, the mechanisms responsible for suppressing feeding in these animals are most likely different from those arising after repeated daily 2-DG, and three observations suggest that this suppression is probably not a consequence of these increased plasma corticosterone concentrations. First, nondehydrated control rats receiving two 2-DG challenges 7 days apart show identical feeding responses to each challenge. Second, the morning elevation in plasma corticosterone levels in dehydrated animals remains well below the peak values attained after 2-DG injection in euhydrated animals (present study; 51). Finally, unlike dehydration, repeated daily injections of 2-DG not only attenuate the feeding response, they also abolish the 2-DG-induced hyperglycemia (39).

The neural mechanisms that control feeding after glucoprivation or deprivation and are not fully understood, but the large body of data implicating NPY/ catecholaminergic neurons in the hindbrain and NPY/agouti-related protein-containing neurons located in the ARH provides a framework for discussing our results with regard to the neural substrates of anorexia.

Injections of an antidopamine β-hydroxylase-saporin conjugate (D-SAP) into the terminal regions of catecholaminergic neurons will specifically destroy these neurons (31). Ritter and colleagues (31, 32) recently took advantage of this specificity to show that D-SAP injected into the PVH blocks the feeding and corticosterone responses to 2-DG but leaves intact the hyperglycemic response. In contrast, D-SAP injected into the spinal cord destroys catecholaminergic neurons with descending connections and blocks the hyperglycemic response to 2-DG but leaves the feeding and corticosterone response intact (31, 32). These data demonstrate that different subsets of hindbrain catecholaminergic neurons mediate the behavioral, autonomic, and neuroendocrine components of the glucoprivic response.

We show that hyperglycemic and corticosterone responses of dehydrated-anorexic rats to 2-DG are indistinguishable from those of controls. This demonstrates that the inhibitory mechanisms in dehydrated-anorexic rats do not impact those ascending and descending catecholamine pathways that target CRH neuronendocrine neurons and mediate glucocorticoid responses or those preganglionic neurons in the spinal cord that mediate hyperglycemia. In addition, our results do not support the view that neuroendocrine CRH neurons in the PVH are involved with compensatory feeding behaviors (54); corticosterone secretion remains viable in dehydrated animals, while feeding is markedly impaired. This notion is also supported by the fact that electrolytic lesions of the PVH do not hinder glucoprivic feeding (7, 42). Collectively, these data suggest that 2-DG-induced feeding requires sets of hypothalamic neurons located outside the PVH and that these systems are potential targets for dehydration-generated inhibition.

In conclusion, we previously showed that the anorexia that develops after drinking hypertonic saline inhibits spontaneous nocturnal feeding (50). The present study shows that this anorexia also involves an inhibition of two other types of feeding: compensatory...
feeding in response to overnight starvation and the feeding that usually follows glucoprivation. The fact that hyperglycemic and glucocorticoid responses to 2-DG remain intact in dehydrated-anorexic animals shows that dehydration specifically targets those mechanisms that control the motor events of feeding behavior, but not neuroendocrine or autonomic motor responses.

Perspectives

Evidence suggests that specific alterations to feeding mechanisms in the ARH are not responsible for dehydration-anorexia (54). Instead, dehydration-anorexia appears to be generated by activity in separate inhibitory circuits. These circuits may involve CRH, neurotensin, or oxytocin neurons found in those parts of the perifornical LHA and PVH that are targeted by plasma osmolality-sensitive rostral hypothalamic afferents (25, 28, 29, 54). In this regard, several lines of evidence support the idea that a critical component for the development of dehydration-anorexia is located within the LHA, particularly its perifornical part (LHApf). NPY-containing projections from the ARH to the LHApf are important for stimulating those types of feeding initiated by changes in the levels of circulating hormones such as leptin (15, 16). Similarly, hindbrain adrenergic and noradrenergic neurons activated by 2-DG colocalize NPY (40) and project to the PVH and LHA (14, 47). Furthermore, neurons within the LHA express NPY receptors (12, 20), and injections of NPY into the LHApf produce strong feeding responses (46). We suggest that dehydration in some way inhibits the output of those NPY-containing circuits that normally elicit food intake in response to caloric deficits. This hypothesis is consistent with certain other types of anorexia where animals exhibit a suppressed feeding response to central injections of NPY, increased NPY gene expression in ARH, and increased NPY release in the PVH (3, 5, 23, 30). However, unlike these other models of anorexia, dehydration-anorexia is rapidly and completely reversed within minutes simply by restoring access to drinking water, making it a particularly useful model with which to investigate the neural substrates of anorexia.

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Some of these results have been presented in abstract form (38).

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