Acute 2DG-Induced Glucoprivation or Dexamethasone Abolishes 2DG-Induced Glucoregulatory Responses to Subsequent Glucoprivation

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Behavioral, neuroendocrine, and autonomic responses to glucoprivation are impaired after a glucoprivic episode. A life-threatening manifestation of this effect, known as hypoglycemia-associated autonomic failure (HAAF), occurs in diabetic patients as a result of prior inadvertent hypoglycemia resulting from insulin therapy. Glucocorticoids, which are elevated by glucoprivation, have been implicated in the pathogenesis of HAAF. The goal of the present study was to examine the effect of glucocorticoids on glucoregulatory responses in a rat model of HAAF. 2-deoxy-D-glucose (2DG; 200 mg/kg) was used to induce glucoprivation. Rats were injected with saline, 2DG, or the synthetic glucocorticoid, dexamethasone (DEX; 250 μg/rat) in the morning. Then 6 h later, rats were injected with 2DG, and their feeding and hyperglycemic responses were measured. Both 2DG and DEX in the morning eliminated glucoprivic feeding and hyperglycemic responses in the afternoon test. Epinephrine (0.3 mg/kg) administration in the afternoon elicited marked hyperglycemia in animals given 2DG that morning, demonstrating that glycogen depletion from morning glucoprivation was not responsible for the absence of the hyperglycemic response in the afternoon test. The effects of prior saline or 2DG treatment on subsequent glucoprivic feeding were also examined in adrenalectomized rats in which the source of endogenous glucocorticoids was removed. In these animals, prior glucoprivation did not attenuate 2DG-induced feeding in the afternoon test. These findings demonstrate that a single glucoprivic episode is sufficient to cause impairment in glucoregulatory responses to a second glucoprivic episode in the same day. In addition, these results strongly implicate glucocorticoids in the pathogenesis of HAAF. *Diabetes* **50:2831–2836, 2001**

Glucoregulatory responses, including adrenal medullary secretion of epinephrine, secretion of glucocorticoids, and stimulation of appetite, are elicited by glucose deficit. These responses reduce and reverse glucoprivic conditions by mobilizing glucose from storage sites, promoting calorie ingestion, and replenishing glycogen reserves. These glucoregulatory responses are essential for maintaining blood glucose concentrations that are adequate to meet the brain's continuous glucose requirement.

Hypoglycemia-associated autonomic failure (HAAF) is a reversible (1–3) impairment of glucose regulation that follows a glucoprivic episode. This condition occurs in diabetic patients as a consequence of prior inadvertent insulin-induced hypoglycemia (4–6), but can also be induced by glucoprivation in nondiabetic humans and animals (3,7). In HAAF, glucoprivic stimulation of appetite and secretion of glucagon and adrenal catecholamines is severely impaired. In addition, autonomic responses, such as cardiac palpitations and sweating, that are normally perceived by humans during a hypoglycemic event do not occur (4,5,8). The absence of these perceptible autonomic responses reduces the awareness of the hypoglycemic crisis so that palliative behavioral measures are not initiated. Thus, in HAAF, the failure of glucoregulatory responses together with hypoglycemia unawareness allows blood glucose levels to fall without detection to dangerously low or lethal levels.

The mechanisms underlying the development of HAAF are not entirely understood, but endogenous glucocorticoids, which are increased in response to glucoprivation, have been implicated. The infusion of cortisol in euglycemic humans significantly attenuates neuroendocrine and autonomic responsiveness to a subsequent hypoglycemic challenge (9), and glucocorticoids reduce feeding in response to intraventricular administration of 2-deoxy-D-glucose (2DG) (10). Furthermore, in patients with primary adrenocortical failure, hypoglycemia does not reduce responses to a subsequent hypoglycemic bout as it does in normal individuals (11).

In the present studies, we examined the effect of 2DG-induced glucoprivation on feeding and adrenal medullary−induced hyperglycemia during a second glucoprivic challenge 6 h later. We also examined feeding and hyperglycemic responses to 2DG 6 h after an injection of the synthetic glucocorticoid dexamethasone (DEX). Fi-

nally, we examined the effect of glucoprivation on subsequent 2DG-induced feeding in rats in which the source of endogenous glucocorticoids was removed by adrenalectomy. The results demonstrated that a single glucoprivic episode significantly reduces feeding and the hyperglycemic response to a subsequent glucoprivic challenge induced 6 h later. These results also support the hypothesis that endogenous glucocorticoids are causally involved in the impairment of these responses.

RESEARCH DESIGN AND METHODS

Animals and procedures. Adult male SD rats weighing 350–380 g at the start of the study were obtained from Harlan Laboratories (Indianapolis, IN). They were individually housed in suspended wire mesh cages in a temperature-controlled room ($21 \pm 1°C$) illuminated between 0630 and 1830 h. Food and tap water were available ad libitum throughout the study. Before and during the study, rats were handled and habituated to the testing environment and procedures. Feeding and blood glucose tests were conducted in separate groups of rats. Testing was conducted in the rats' home cages.

In all of the testing procedures described below, the dosage of 2DG (Sigma Chemical) was 200 mg/kg and the dosage of the synthetic glucocorticoid, DEX (dexamethasone 21-phosphate, disodium salt; Sigma), was 250 μg/rat. Both substances were injected subcutaneously in 0.9% saline at a concentration of 1 mg/ml. The control solution was saline (Sal; 0.9%; 1 ml/kg). In one study, the hyperglycemic response was assessed in the afternoon test after injection of epinephrine hydrochloride (EPI; 0.3 mg/kg s.c.; Sigma). The morning treatments were given at 0800 h, and the afternoon treatments were given at 1400 h. In experiment 1, food was removed from the cages for 2 h after the morning treatments to allow the glucoprivic stimulus to develop and not be attenuated by the consumption of food. Feeding or blood glucose was measured in separate groups of rats in the afternoon test. In experiment 2, food was withheld for the entire 6-h interval between morning and afternoon treatments. Feeding or blood glucose was measured in separate groups of rats in the afternoon test. In experiment 3, rats were adrenalectomized and feeding responses were subsequently tested according to the protocol described for experiment 1. Experimental groups are designated throughout according to which of the above treatments they received in the morning and afternoon (morning treatment/afternoon treatment).

Feeding and blood glucose tests

Experiment 1. In this experiment, rats were assigned to one of the following treatment groups for measurement of food intake: Sal/Sal ($n = 6$), Sal/2DG ($n = 6$), 2DG/2DG ($n = 6$), and DEX/2DG ($n = 6$). At 0800 h on the morning of the test day, animals were injected with saline, 2DG, or DEX. After injection, they were placed back into their cages without food for 2 h. Food was then returned and rats were allowed to eat ad libitum for the next 4 h. At 1400 h, rats were injected with saline or 2DG and returned to their home cages with a preweighed quantity of food. Food intake and spillage were measured to the nearest 0.1 g in a 3-h test between 1400 and 1700 h.

For measurement of blood glucose, the following treatment groups were used: Sal/Sal ($n = 6$), 2DG/2DG ($n = 7$), DEX/2DG ($n = 8$), and 2DG/EPI ($n = 5$). At 0800 h on the test day, rats were injected with saline, 2DG, or DEX and placed back into their home cages without food for 2 h. Food was then returned and rats were allowed to eat ad libitum for the next 4 h. At 1400 h, food was removed and the rats were injected with 2DG or EPI. Blood (50 μl) was collected from the tail 15 min before and 30, 60, 90, and 120 min after the injections. Glucose was analyzed using the glucose oxidase method (12).

Experiment 2. The design of experiment 2 was identical to that of experiment 1, except that food was withheld for the entire 6-h interval between the morning and afternoon injections. The following groups were used for the feeding tests: Sal/Sal and Sal/2DG ($n = 6$), 2DG/Sal and 2DG/2DG ($n = 6$), and DEX/Sal and DEX/2DG ($n = 6$). Food consumption was measured in a 3-h test, as described above. The following treatment groups were used for assessment of the hyperglycemic response: Sal/Sal ($n = 8$), Sal/2DG ($n = 6$), 2DG/2DG ($n = 7$), DEX/2DG ($n = 8$), and 2DG/EPI ($n = 5$). As described above, food was withheld for the entire 6-h treatment/test interval. Then 6 h later, rats were injected with saline, 2DG, or EPI, and blood was collected for glucose analysis as previously described.

Experiment 3. In this experiment, the effect of glucoprivation on subsequent 2DG-induced feeding was examined in adrenalectomized (ADX) rats. In preparation for the experiment, rats ($n = 7$) were anesthetized with 10 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa) and 2 mg/kg Rompun (xylazine; Bayer) and adrenalectomized bilaterally using a ventral approach. They were allowed at least 2 weeks recovery before testing began. ADX rats were maintained on 0.9% saline drinking water supplemented with 25 μg/ml
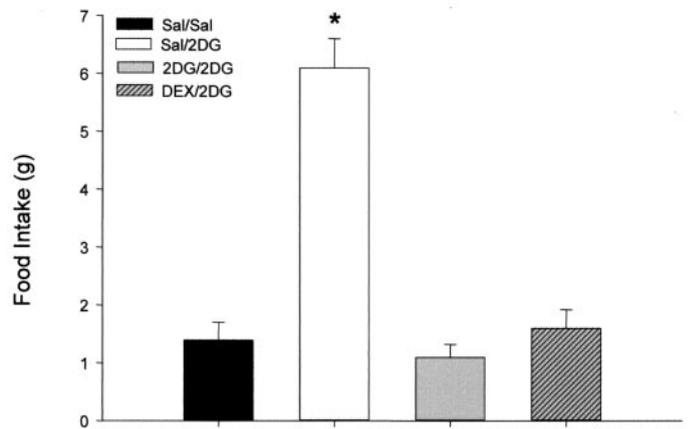

FIG. 1. Cumulative intake of pelleted rat food during the 3 h immediately after subcutaneous injection of saline (1 ml/kg) or 2DG (200 mg/kg) in animals given saline (1 ml/kg; Sal/Sal and Sal/2DG), 2DG (200 mg/kg; 2DG/2DG), or DEX (250 μg/rat; DEX/2DG) 6 h earlier (0800 h). Data are expressed as mean 3-h food intake ± SE. *$P < 0.001$ as compared with Sal/Sal, 2DG/2DG, and DEX/2DG groups.

corticosterone (4-pregnene-11 β, 21-diol-3,20-dione; Sigma). The completeness of ADX was assessed visually at autopsy and by measurement of 2DG-induced hyperglycemia, a response that is completely dependent on adrenal medullary secretion under the conditions of our test (13). Normal rats ($n = 6$) were used as controls.

ADX and control rats were tested under three experimental conditions: Sal/Sal, Sal/2DG, and 2DG/2DG. At 0800 h on the test day, rats were injected with saline or 2DG and placed back into their cages without food for 2 h. They were then allowed to eat ad libitum for 4 h before the afternoon test. At 1400 h, animals were injected with saline or 2DG and placed back into their home cages with a preweighed quantity of food. Food intake was measured for 3 h, as previously described. Because 2DG did not elevate blood glucose in ADX rats, the effects of prior 2DG and DEX on the hyperglycemic response were not assessed.

Statistical analyses. Data are expressed as means ± SE. Data were analyzed using analysis of variance with repeated measures. Significant individual differences were identified using Bonferroni's t test. The critical level for significance was set at $P < 0.05$ for all comparisons.

RESULTS

Experiment 1: feeding and blood glucose results. Figure 1 shows the results of the feeding tests conducted 6 h after 2DG or DEX treatment. We found that 6 h after saline injection, 2DG produced a feeding response that was significantly greater than that produced by a saline injection. The Sal/2DG group ate 6.1 ± 0.58 g of food and the Sal/Sal group ate 1.4 ± 0.27 g of food ($P < 0.001$). Both 2DG and DEX injections reduced the feeding response in the subsequent 2DG test to levels that did not differ from the intake of the Sal/Sal rats. The 2DG/2DG group consumed 1.1 ± 0.22 g and the DEX/2DG group consumed 1.6 ± 0.32 g of food in this test.

Results of the blood glucose tests conducted 6 h after saline, 2DG, or DEX injection are presented in Fig. 2. In this study, 6 h after saline injection, 2DG produced blood glucose elevations that were significantly greater at every sampling time ($P < 0.001$) than those produced by a saline injection. In the Sal/2DG group, blood glucose concentrations were increased from 66 ± 1 mg/dl at $t = -15$ to a maximum of 158 ± 7 mg/dl. In contrast, 2DG-induced hyperglycemia was significantly impaired in the rats treated 6 h earlier with 2DG or DEX. In the 2DG/2DG group, blood glucose concentrations rose in response to a second 2DG injection from a basal value of 82.7 ± 2 mg/dl
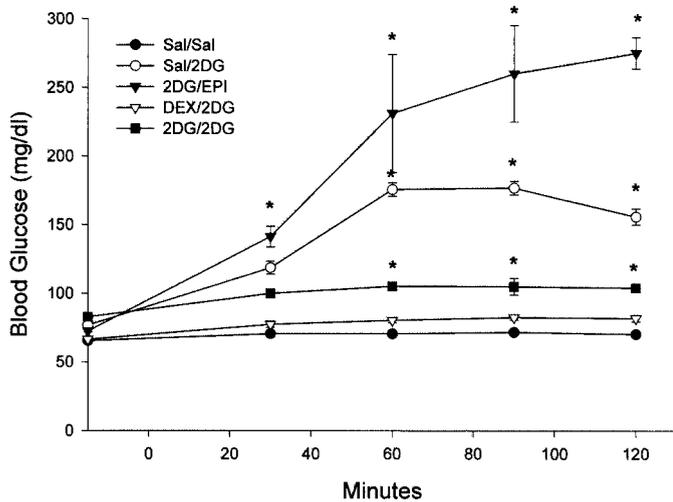
FIG. 2. Blood glucose concentrations after subcutaneous injection of saline (1 ml/kg; Sal/Sal group), 2DG (200 mg/kg; Sal/2DG, 2DG/2DG, and DEX/2DG), or EPI (0.3 mg/kg; 2DG/EPI) in animals treated with saline (1 ml/kg), 2DG (200 mg/kg), or DEX (250 μg/rat) 6 h earlier (0800 h). Immediately after morning treatment, food was removed from the animals' cages for 2 h. Blood samples were collected from the tail 15 min before the treatment injection. Tests were conducted in the absence of food. Data are expressed as the mean blood glucose concentration ± SE. *$P < 0.001$ as compared with the Sal/Sal group.

at $t = -15$ to a maximum of only 105 ± 3 mg/dl. In the DEX/2DG group, blood glucose concentrations increased from 66 ± 1 mg/dl to a maximum of 83 ± 2 mg/dl. Blood glucose levels did not differ significantly from Sal/Sal rats at any sampling time for the DEX/2DG group. Impairment of the hyperglycemic response to the afternoon 2DG injection was not attributable to the depletion of hepatic glycogen stores. Systemic administration of EPI 6 h after 2DG treatment elicited a dramatic response, raising blood glucose to a maximum concentration of 259 ± 13 mg/dl.
Experiment 2: feeding and blood glucose results. Figure 3 shows that withholding food during the entire 6-h
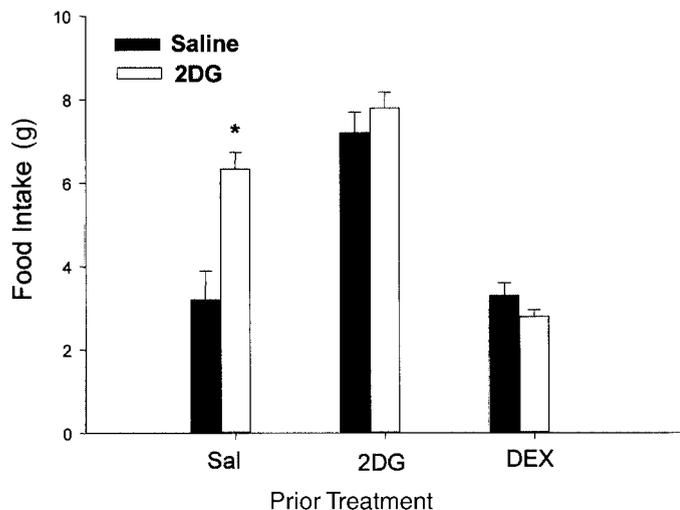

FIG. 3. Cumulative intake of pelleted rat food during the 3 h immediately after subcutaneous injection of saline (1 ml/kg) or 2DG (200 mg/kg) in animals given saline (1 ml/kg; Sal/Sal and Sal/2DG), 2DG (200 mg/kg; 2DG/Sal and 2DG/2DG), or DEX (250 μg/rat; DEX/Sal and DEX/2DG) 6 h earlier (0800 h). Food was withheld during the entire 6-h interval between the morning treatment and the afternoon test. Data are expressed as the mean 3-h food intake ± SE. *$P < 0.001$ as compared with the Sal/Sal treatment.
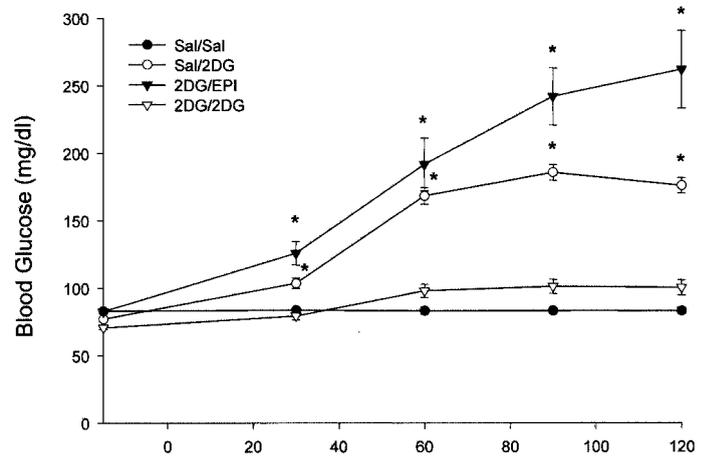
FIG. 4. Blood glucose concentrations after subcutaneous injection of saline (1 ml/kg), 2DG (200 mg/kg), or EPI (0.3 mg/kg) in animals given a prior injection of saline (1 ml/kg; Sal/Sal and Sal/2DG) or 2DG (200 mg/kg; 2DG/2DG and 2DG/EPI) 6 h earlier. Food was withheld for the entire 6-h interval between the morning treatments and the afternoon test. Blood samples were collected from the tail 15 min before subcutaneous injection of saline, 2DG, or EPI. Tests were conducted in the absence of food. Data are expressed as the mean blood glucose concentration ± SE. *$P < 0.001$ as compared with the Sal/Sal group.

interval between the treatment and the test produced feeding responses that differed in some tests from those observed in the protocol in which food was withheld for only the first 2 h of this interval. In the Sal/2DG group, food intake was significantly elevated compared with the Sal/Sal group. These two groups consumed 6.3 ± 0.7 and 2.9 ± 0.40 g of food in the afternoon tests, respectively ($P < 0.05$). In addition, injection of DEX in the morning blocked the feeding response to 2DG in the afternoon test, just as was observed above. The DEX/Sal group ate 3.3 ± 0.3 g and the DEX/2DG group ate 2.8 ± 0.2 g of food in the afternoon test ($P = 0.32$). The group that was differentially affected by this paradigm, as compared with the 2-h deprivation paradigm, was the 2DG/2DG group. The 2DG/2DG group ate 7.2 ± 0.5 g of food in the afternoon test. In other words, if access to food was denied after the first 2DG injection, 2DG-induced feeding was not attenuated in the subsequent test. The response of the 2DG/2DG group was not significantly different from the response of the 2DG/Sal group, which was included as a control for the delayed glucoprivic feeding response. The latter group consumed 7.7 ± 0.4 g of food in the response to 2DG injected 6 h earlier at the beginning of the food-deprivation period.

Figure 4 shows that withholding food during the entire 6-h interval between treatment and the test produced blood glucose results in all treatment groups that were similar to those observed in the protocol in which food was withheld for only the first 2 h. Blood glucose concentrations in the 2DG/2DG group did not differ at any time point from those observed in the Sal/Sal group. Afternoon 2DG injection produced a maximum blood glucose concentration of 98 ± 5 mg/dl in the 2DG/2DG group, compared to 171 ± 6 mg/dl in the Sal/2DG group. Injection of EPI produced a significant elevation of blood glucose in rats injected with 2DG 6 h earlier, indicating that the absence of the hyperglycemic response in the 2DG/2DG group was not caused by glycogen depletion.
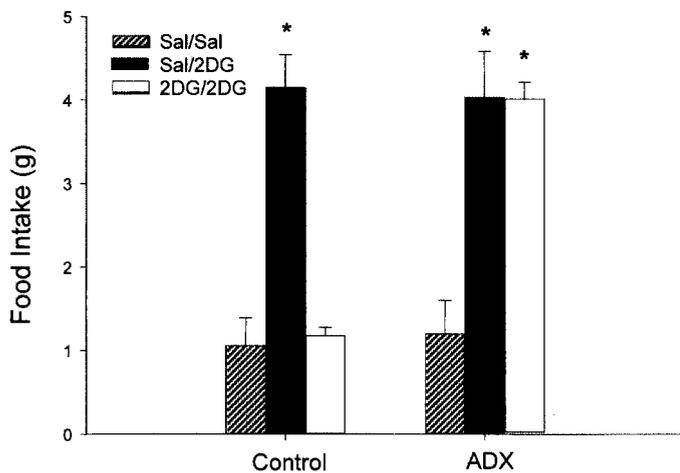
FIG. 5. Cumulative intake of pelleted rat food during the 3 h immediately after subcutaneous injection of saline (1 ml/kg) or 2DG (200 mg/kg) in control and ADX animals given saline (1 ml/kg; Sal/Sal and Sal/2DG) or 2DG (200 mg/kg; 2DG/2DG) 6 h earlier (0800 h). Food was withheld for 2 h after the morning treatment. Data are expressed as the mean 3-h food intake ± SE. *$P < 0.001$ as compared with the Sal/Sal treatment.

Experiment 3: glucoprivic feeding in ADX animals.
Figure 5 shows the effects of adrenalectomy on food intake 6 h after saline or 2DG injections. We observed that 6 h after saline injection, 2DG elicited a significant increase in food intake in both control and ADX rats ($P < 0.001$). In the Sal/Sal condition, the control group ate 1.1 ± 0.33 g and the ADX group ate 1.2 ± 0.40 g of food in the afternoon test. In the Sal/2DG condition, these groups ate 4.2 ± 0.4 and 4.0 ± 0.6 g of food, respectively, in the afternoon test. Injection of 2DG in the morning reduced the feeding response of the controls in the subsequent 2DG test to 1.2 ± 0.1 g, an amount not different from the Sal/Sal response. However, in the ADX rats, prior 2DG-induced glucoprivation did not block the feeding response. In the 2DG/2DG condition, the ADX rats ate 4.0 ± 0.2 g of food in the afternoon test. This amount of food intake did not differ from the intake in the Sal/2DG condition.

DISCUSSION
The findings presented here showed that a single glucoprivic episode in the morning abolished glucoprivic feeding and adrenal medullary−induced hyperglycemia during a second glucoprivic episode in the afternoon. The finding that a single glucoprivic episode can impair or totally block subsequent glucoregulatory responses in rats is consistent with results in nondiabetic humans, showing that one prior hypoglycemic episode reduces neuroendocrine, autonomic, and symptomatic responses during a subsequent hypoglycemic bout (14). Although we used a single dosage of 2DG in all of our experiments, studies in humans indicate that the depth of antecedent hypoglycemia is directly correlated with the magnitude of the subsequent impairment (4). Mild antecedent hypoglycemia results in significant blunting of EPI, muscle sympathetic nerve activity, and glucagon secretion during day 2 of hypoglycemia. Additional decreases in antecedent hypoglycemia lead to further neuroendocrine and autonomic impairments. Collectively, these findings have profound implications for the management of diabetes with inten-

sive insulin therapy, which can consist of multiple insulin injections per day (15). Insulin overdoses or missed meals and snacks can elicit variations in blood glucose levels that may alter the sensitivity to subsequent glucoprivation later in the day.

Endogenous glucocorticoids are elevated by glucoprivation and have been suggested to be the key pathogenic mechanism in the development of glucoprivic unresponsiveness in HAAF (9,11). The present results strongly support this hypothesis. We found that administration of DEX, the synthetic glucocorticoid, in the morning was as effective as 2DG-induced glucoprivation in suppressing responses to 2DG in the afternoon test. This finding could not be attributed to a hyperglycemic effect of DEX, which might have competitively antagonized the glucoprivic effect of 2DG, because blood glucose concentrations at the time of the second 2DG injection were not elevated. Furthermore, we found that adrenalectomy, which removes the source of endogenous glucocorticoids, prevented the reduction in glucoprivic responsiveness induced by the prior glucoprivic episode. Because adrenalectomy removes not only endogenous glucocorticoids and catecholamines, but also a host of adrenal neuropeptides and neurotransmitters, it is possible that other adrenal secretagogues may be involved in the pathogenesis of glucoprivic unresponsiveness. For example, galanin expression is rapidly induced in response to insulin-induced hypoglycemia, and galanin mRNA levels in chromaffin cells have been shown to remain elevated for at least 48 h (16). In addition, galanin administration in humans significantly reduces the norepinephrine (NE) response to insulin-induced hypoglycemia and attenuates the EPI response (17). Although our findings cannot exclude the possibility that other adrenal secretagogues may be involved in the pathogenesis of glucoprivic unresponsiveness, they are congruent with clinical observations showing that humans with primary adrenocortical failure are resistant to the development of HAAF (11). In addition, we found in the present studies that the administration of exogenous glucocorticoids was independently capable of inducing HAAF. Taken together, these findings strongly implicate endogenous glucocorticoids in the pathogenesis of HAAF.

The adrenal medullary response to glucoprivation results in a prompt elevation of plasma EPI that increases glucose concentrations by stimulation of glycogenolysis. In rats, adrenal medullary EPI secretion is the primary mediator of the hyperglycemic response to glucoprivation (13). For this reason, it was necessary in our experiments, in which two glucoprivic episodes were separated by only 6 h, to evaluate the possibility that the diminished blood glucose response in the second 2DG test was attibutable to the exhaustion of glycogen stores. However, we found that this was not the case, as exogenous EPI administered 6 h after a morning glucoprivic episode elicited a robust hyperglycemic response. Therefore, the absence of hyperglycemia in the afternoon test was caused by impairment of the neural activation of adrenal medullary secretion and not to depletion of glycogen stores. In fact, the blood glucose levels achieved during the afternoon 2DG test were similar to levels attained in rats with denervated adrenal glands (13). Insulin-induced hypoglycemia has been previously shown to reduce the adrenal medullary

EPI response to a subsequent hypoglycemic event (3–8), as well as the EPI response to central 2DG administration (18). Furthermore, EPI secretion in response to central 2DG is attenuated by prior central glucoprivic episodes (19). And finally, prior 2DG-induced glucoprivation abolishes 2DG-induced Fos expression in the adrenal medulla (20).

Two protocols were used in the present study to examine the effects of morning glucoprivation on feeding and hyperglycemic responses to a second glucoprivic episode in the afternoon. In one protocol, food was withheld for the 2 h immediately after the first 2DG injection to allow the glucoprivic stimulus to develop. After the 2-h food deprivation period, the rats were allowed ad libitum access to food. In the second protocol, food was withheld during the entire 6-h interval between 2DG tests. In both protocols, 2DG-induced glucoprivation in the morning resulted in an impairment of the hyperglycemic responses in the afternoon 2DG test. However, these protocols produced different effects on the feeding response during the afternoon 2DG test. Rats with access to food between the tests did not eat in response to the afternoon injection of 2DG, but rats denied access to food between tests ate large amounts of food in the second test. One possible interpretation of these results is that the loss of the feeding response in the second test in the fed rats was caused by the presence of satiety signals that suppressed food intake during the second test rather than by glucoprivation unresponsiveness. However, this explanation seems unlikely, because ADX rats given food between tests exhibited feeding responses in the afternoon 2DG test. A more likely explanation is that when food was withheld between the tests, the reduced responsiveness to 2DG normally seen in the second test was masked by the occurrence of a delayed feeding response to the first 2DG injection 6 h earlier. This explanation is substantiated by the fact that rats given 2DG in the morning, denied access to food for 6 h, and then given saline in the afternoon, also ate large amounts of food. Delayed glucoprivic feeding is a phenomenon described previously in studies showing that glucoprivic challenges significantly stimulate food intake, even when food is withheld for 6–8 h after an injection of 2DG or insulin (21–23). In our protocol, the delayed glucoprivic feeding response may have reflected sustained activity in components of the glucoprivic feeding circuitry that were not affected by the elevated glucocorticoids, perhaps in neurons located downstream of the glucocorticoid-sensitive sites.

The fact that 2DG-induced glucoprivation produced a deficit in the hyperglycemic response to the second glucoprivic episode, regardless of the availability of food, reflects the fact that the glucoprivic feeding and hyperglycemic responses are mediated by different neural circuits (24,25). Although the glucoreceptors controlling both responses appear to overlap in the ventrolateral and dorsomedial hindbrain, the output neurons from these sites that are responsible for feeding and adrenal medullary secretion are different. Selective destruction of the hindbrain catecholamine neurons projecting rostrally to the paraventricular nucleus of the hypothalamus completely abolishes glucoprivic feeding, while sparing the hyperglycemic response. Destruction of the spinally projecting catechol-

amine neurons abolishes the hyperglycemic response to glucoprivation, but leaves the feeding response intact (25). Because the neural pathways mediating the different responses are clearly separate, it is reasonable to expect that they may be subject to differential neuronal regulation, as indicated by our results.

Hindbrain catecholaminergic neurons in the ventrolateral and dorsomedial medulla are required neural substrates for communicating glucoprivic signals detected in the hindbrain to rostral and caudal effector nuclei (25). These same populations of neurons in the hindbrain also exhibit Fos-ir in response to both 2DG- (13,26) and insulin-induced hypoglycemia (27), but not after repeated prior 2DG (20) or hypoglycemia (28). The temporary deficits in glucoprivic responses resulting from either repeated or acute prior glucoprivation are similar to the permanent deficits observed in animals with selective lesions of hindbrain catecholamine neurons (25). Thus, hindbrain catecholamine neurons may be directly involved in the reduced glucoprivic responsiveness in HAAF.

Circulating glucocorticoids may inhibit the hypothalamic-pituitary adrenal axis and the sympathoadrenal system by their effects on hindbrain catecholaminergic neurons. Hindbrain catecholaminergic neurons possess type II glucocorticoid receptors (29), and activity of hindbrain EPI and NE neurons is inversely related to plasma glucocorticoid levels under some conditions. For example, chronic adrenalectomy increases basal activity of medullary catecholaminergic neurons (30) and enhances stress-induced activation of catecholamine metabolism in cell bodies (31). In addition, adrenolectomy enhances and prolongs hypothalamic NE release during immobilization stress (32), whereas cortisol administration reverses the augmented catecholaminergic responses in ADX animals during immobilization stress (33). Similarly, chronic elevation of corticosterone decreases basal levels and stress-induced increases in release, metabolism, turnover, and synthesis of catecholamines in the PVH (34). Corticosterone treatment also significantly attenuates stress-induced EPI and NE responses, and DEX blunts adrenolectomy-induced elevations in basal and stress-induced NE responses (35). The combined results showing that hindbrain EPI and NE neurons are crucial substrates mediating glucoprivic responses, that they possess type II glucocorticoid receptors, and that the expression of Fos-ir in these neurons is selectively inhibited by prior repeated glucoprivation, may indicate that these neurons are the anatomical substrate on which glucocorticoids act to modulate glucoprivic responsiveness.

Glucocorticoid secretion is viewed as a key glucoregulatory response because it stimulates gluconeogenesis. It is puzzling that this response appears to be the major pathogenic mechanism in HAAF. This raises further questions regarding the normal physiological role of glucocorticoid secretion during conditions of glucose deficit. To achieve overall glucose homeostasis, it may be necessary to turn off certain responses to prevent further perturbations in glucose homeostasis (36). Glucocorticoid release during glucoprivation may serve such a function during severe glucoprivation, suppressing ongoing glucoregulatory responses and decreasing their sensitivity to further activation for a period of time. By suppressing the stimuli

that drive glycogenolysis, for example, this mechanism may both conserve glycogen stores and facilitate gluconeogenesis. In this regard, glucocorticoid release during glucoprivation may serve as a "metabolic memory" for glucoreceptive sites, enabling glucoreceptors to modulate signaling output to effector neurons based on recent alterations in glucose homeostasis.

Intensive insulin therapy has proven to be the most beneficial therapy for maintaining tight glycemic control and reducing the progression and development of diabetes-related complications (15). However, HAAF is a life-threatening complication of this therapy. Although the mechanisms underlying reduced glucoprivic sensitivity in HAAF are not fully understood, mounting evidence supports a causative role for endogenous glucocorticoids in the pathogenesis of HAAF. Because hindbrain EPI and NE neurons are crucial for feeding and adrenal medullary responses to glucoprivation, these neurons should be considered as a potential neural substrate through which glucocorticoids act to produce HAAF.

ACKNOWLEDGMENTS

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