Research report

Effects of corticosteroid synthesis inhibitors on the sensitization of reward by food restriction

Glenn C. Abrahamsen *, Kenneth D. Carr

Millhauser Laboratories, Department of Psychiatry, New York University Medical Center, 550 First Avenue, New York, NY 10016, USA

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Abstract

Chronic food restriction sensitizes animals to the rewarding effects of food, drugs and lateral hypothalamic electrical stimulation. The present study employed a curve-shift analysis of lateral hypothalamic self-stimulation (LHSS) to evaluate whether the elevated plasma corticosterone levels that accompany food restriction mediate the sensitization of reward. In Experiment 1, two adrenocorticoid synthesis inhibitors, aminoglutethimide and metyrapone, were administered to food-restricted rats and the magnitude of plasma corticosterone suppression was determined at two post-administration time points. In Experiment 2, these compounds were administered to ad libitum fed and food-restricted rats whose LHSS behavior was evaluated at a time coincident with suppression of corticosterone. It was found that neither compound reversed the sensitizing effect of food-restriction on the rewarding efficacy of brain stimulation. However, aminoglutethimide (50 mg/kg) produced an increase in maximal response rates (a performance factor) across groups while metyrapone (100 mg/kg) produced a decrease. The most interesting result of this study was that 2 h after aminoglutethimide administration, when corticosterone levels had recovered from suppression, the rewarding efficacy of LHSS increased markedly in food-restricted rats. Possible explanations for this effect, including adrenocortical rebound, alterations in neurosteroid synthesis, and exacerbation of metabolic need are discussed.

Keywords: Self-stimulation; Corticosterone; Food restriction; Sensitization; Aminoglutethimide; Metyrapone

1. Introduction

Food restriction facilitates a number of reward related phenomena including drug-induced locomotion [17,52], drug self-administration [13], and intracranial electrical self-stimulation [1,6,11,12]. The facilitation of reward by chronic food restriction, as indexed by the lowering of threshold for lateral hypothalamic self-stimulation (LHSS), is mediated by the endogenous opioid system inasmuch as the decrease in threshold is reversed by nonspecific [12] as well as receptor type-selective opioid antagonists [11]. Activation of the brain opioid system by chronic food restriction is further indicated by regional changes in opioid receptor binding [57] and peptide levels [5].

Food restriction produces a number of metabolic and endocrinological changes that may trigger the CNS events involved in the sensitization of reward. Among these are decreases in circulating insulin [55], glucose [33] and thyroid hormone [25], as well as increases in free fatty acids [25] and corticosterone [26]. Elevations in circulating corticosterone are particularly interesting in light of the long known involvement of this glucocorticoid in mediating a multitude of stress effects (see [39] for a review) and recent evidence that corticosterone levels modulate the behavioral effects of psychostimulants and opiates [17–20,42,43,52]. Moreover, the sensitization of drug-induced locomotion [17] and nucleus accumbens dopamine release [52] by food restriction have been shown to depend upon elevated plasma corticosterone.

The effects of naturally occurring fluctuations in plasma corticosterone on the sensitization of LHSS by food restriction were recently investigated [1]. In agreement with previous reports [26], circulating corticosterone in food-restricted rats was markedly elevated just prior to delivery of the single daily meal and substantially reduced in the immediate post-meal period. However, the sensitized response to LHSS persisted in the post-meal period despite the decline in corticosterone. As a further test of corticosterone involvement in reward sensitization, the effect of the adrenocorticoid synthesis inhibitor aminoglutethimide
was evaluated (unpublished). In a single pilot test, aminoglutethimide was administered 2 h prior to blood sampling and LHSS testing. The drug had no effect on circulating corticosterone but potentiated the sensitization of LHSS. While this result was intriguing, the effect of acute pharmacological suppression of corticosterone synthesis on LHSS sensitization remained to be determined.

The purpose of the present study was to investigate the effects of adrenal steroidogenesis blockade on the sensitization of LHSS by food restriction. Two drugs known to block the synthesis of corticosterone – aminoglutethimide and metyrapone – were used. Aminoglutethimide blocks the initial step in the adrenocorticoid biosynthetic pathway by inhibiting mitochondrial cytochrome P-450scc activity, thus interfering with the conversion of cholesterol to pregnenolone. Metyrapone inhibits the final step in corticosterone synthesis by interfering with 11β-hydroxylase activity thereby blocking the conversion of 11-deoxycorticosterone to corticosterone. While the immediate effects of metyrapone are relatively specific to circulating corticosterone, aminoglutethimide suppresses the synthesis of a number of intermediate steroids in the biosynthetic pathway which have recently been demonstrated to affect neuronal excitability [7,27,32,35,48,50].

In Experiment 1 the effects of aminoglutethimide and metyrapone on plasma corticosterone levels in food-restricted rats were determined. For each compound a dose was selected that appeared to be the highest a food-restricted rat could tolerate without showing signs of sedation or motor impairment. These doses have previously been shown to reduce plasma corticosterone in a variety of experimental settings [2,3,10,16,24,28,40,46,49,54]. Using information obtained from Expt. 1, Expt. 2 assessed the effects of each of these drugs on LHSS in ad libitum fed control and food restricted rats. LHSS was evaluated using curve-shift methodology which can distinguish between changes in nonspecific performance factors and reward efficacy (see [1,21,34,37] for a review). Curve-shift methodology entails the monitoring of self-evaluated response rate which generate response rates ranging from maximal to asymptotic portion of the function up or down and may alter the slope of the ascending portion. Given the information of this method and its increasingly frequent application in studies of the pharmacology of reward [14,45,53], the effects of adrenocorticoid synthesis inhibitors on food-restriction-induced reward sensitization were assessed using this paradigm.

2. Materials and methods

2.1. Subjects

Adult male Sprague-Dawley rats (300–350 g) were used in both experiments. All animals were singly housed under a 12:12 h light/dark schedule and were provided ad libitum access to food and water prior to the implementation of food restriction.

2.2. Drugs

Aminoglutethimide and metyrapone (Sigma Chemical Co., St. Louis, MO) were each dissolved in 50% dimethylsulfoxide (DMSO) vehicle at concentrations of 25 mg/ml and 50 mg/ml vehicle, respectively. Each drug was administered subcutaneously in 2 ml/kg volumes. Thus, the administered doses were 50 mg/kg aminoglutethimide and 100 mg/kg metyrapone.

2.3. Experiments

2.3.1. Experiment 1: effects of aminoglutethimide and metyrapone on blood corticosterone in food restricted rats

2.3.1.1. Procedure. Fifteen rats were used in Expt. 1. Following 1 week of acclimation to the laboratory and handling, all animals were switched from ad libitum to a restricted access feeding regimen. Normal diet – pelleted Purina rat chow – was now provided in the laboratory for just a single 1-h period per day between the hours of 13.00 and 14.00 h. Each animal’s intake was limited to a maximum of 10 g per meal received in a clean Plexiglas cage. Water continued to be available ad libitum throughout each 24 h period. Animals were habituated to the handling involved in blood sampling for two days prior to drug administration.

Drug administration and blood sampling occurred on day 8 of food restriction. This is a time point at which prior research [1,26] has shown that food-restricted animals display elevated corticosterone levels, particularly in the pre-meal period. Approximately 2 h prior to the scheduled meal, three randomly assigned groups of restricted animals received either vehicle (group Veh, n = 5), 50 mg/kg aminoglutethimide (group AG, n = 5), or 100 mg/kg metyrapone (group MP, n = 5). Blood was sampled at 30 and 120 min post-injection. At these times, each animal
was placed in a clean Plexiglas cage and the tip of the tail was sliced with a clean razor blade and 50 µl of blood was drawn in a micropipet from the wound.

2.3.1.2. Plasma corticosterone determinations. Blood samples were centrifuged at 14000 CPS for 20 min and approximately 15 µl of serum was extracted and immediately frozen. Samples were later thawed and serum concentrations of corticosterone were determined using a corticosterone 125I Radioimmunoassay Kit (ICN Biomedicals, Costa Mesa, CA). Given the well-established effects of metyrapone and aminoglutethimide on circulating corticosterone, serum corticosterone data was analyzed using planned comparisons between the vehicle group and each of the two drug groups at the two time intervals.

2.3.2. Experiment 2: assessment of the effects of aminoglutethimide and metyrapone on the sensitization of LHSS by food restriction

2.3.2.1. Subjects and surgical procedures. Twelve male Sprague-Dawley rats (300–350 g) were anesthetized with ketamine (75.0 mg/kg, s.c.) and xylazine (5 mg/kg, s.c.) and stereotaxically implanted with monopolar stimulating electrodes bilaterally in the lateral hypothalamus, dorsal and lateral to the fornix. With the bregma and lambda suture landmarks in the same horizontal plane, coordinates for implantation of lateral hypothalamic electrodes were: 3.0 mm posterior to bregma, 1.6 mm lateral to the sagittal suture, and 8.4 mm below skull surface. An uninsulated jeweler’s screw placed rostrally in the skull served as ground. Electrodes and ground were permanently secured to the skull by applying dental acrylic around them and three anchoring jeweler’s screws.

2.3.2.2. Behavioral test apparatus. Behavioral testing was conducted in two 11 × 8.25 × 8.25 operant test chambers each with a retractable lever mounted on a side wall of the chamber. A Med-Associates (Georgia, VT) programmable constant current stimulator was used to deliver lever-press-contingent trains of 0.1 ms capacitance-coupled cathodal pulses. Pulses were conducted from the stimulator to implanted electrodes by way of a 14K gold electrical swivel (Stoelting) and flexible wires. Lever position, duration of test trials, delivery of ‘primes’ and response-contingent brain stimulation, and counting of reinforced lever-presses were under the control of an IBM PC using Med-Associates software and interface modules. All stimulation parameters were constantly monitored on a Tektronix 5113 oscilloscope.

2.3.2.3. Intracranial self-stimulation and drug testing. A. Training. Following recovery from surgery, LH electrodes were evaluated for the elicitation of self-stimulation. Rats displaying vigorous lever-pressing for 0.5 s trains of 100 pps stimulation, with no signs of aversive or motoric side effects, were retained and trained daily for 2 weeks to leverpress for 1.0 s trains of decreasing frequencies. During the initial days of training, each rat was assigned a current intensity that would reliably support a high response rate when frequency was 80 pps. The stimulation intensities thus assigned ranged from 150 to 240 μA. During the following 2 weeks, rats were trained daily using a rate–frequency procedure in which the frequency of brain stimulation varied systematically while intensity was held constant. Although lever-pressing was reinforced on a continuous schedule, response rate was often higher than rate of reinforcement since some animals emitted responses during a train of brain stimulation. The rates recorded and reported in this study are therefore rates of reinforcement. The series of trials comprising each rate–frequency curve was initiated by extension of the response lever accompanied by 2 s of priming stimulation of 90 pps. All animals lever-pressed at rates greater than 25/min for 1 s trains of this stimulation. Every 90 s, stimulation frequency was decreased by 8% of frequency value. Responding during the final 60 s of each 90-s trial was used to determine rate of reinforcement at the given frequency. Trials in which a criterion number of reinforced responses (5 responses/min) were not emitted were designated as negative trials and were followed by 2 s of priming stimulation of the same frequency. Following the prime, a second trial at the same frequency was conducted. Only the second of these trials was included in the analysis. Two consecutive negative trials resulted in the termination of the rate–frequency procedure and retraction of the response lever. Two rate–frequency determinations, separated by a 5 min interval, were conducted on each animal per day and results were averaged. The last 2 days of training served as baseline once animals displayed stable rate frequency curves over several consecutive training days.

B. Drug testing. Testing began when body weights for the food restricted animals had decreased 15% from baseline. Animals from both groups received two self-stimulation tests following vehicle administration, 6 days apart. Effects of aminoglutethimide and metyrapone were tested 24 h following each vehicle test. All animals were administered both drugs in a counterbalanced design. Metyrapone and aminoglutethimide were administered using the doses and injection volumes of Expt. 1. Expt. 1 revealed that aminoglutethimide suppresses corticosterone at 30 min with recovery by 120 min, while metyrapone produces a relatively stable decrease across the two time points. Thus, aminoglutethimide and metyrapone were administered 15 and 60 min prior to behavioral testing, respectively. Each animal’s corresponding vehicle test was conducted following the same post-injection interval.

Five days following the second vehicle-drug series, all animals received a third vehicle–drug test series in which only aminoglutethimide was administered. Injection dose and volume were identical to the prior aminoglutethimide
test. The only difference between this and the prior aminoglutethimide test was that testing was conducted 2 h following injection.

2.3.2.4. Histology. Lateral hypothalamic stimulation sites were localized by cutting an appropriate block of tissue from the brain and soaking it for at least 48 h in 10% buffered formalin. Frozen sections, 30 µm thick, were cut on an IEC Minotome, thaw-mounted on gelatinized slides and stained with cresyl violet.

2.3.2.5. Data analysis. The two rate–frequency curves per day were averaged to yield one curve per rat. Each of these curves were subsequently partitioned into two line segments. All sequential values within 10% of the maximal reinforcement rate obtained during the session defined the asymptotic range of responding. The average of these values was used to define the maximum response rate from which M-50 values were derived. The response rate at the lowest frequency in the asymptotic range and the response rates for all lower frequency values defined the ascending portion of the curve. A best-fit regression line was plotted through these points. Using the regression equation, the slope of the line and the frequency values corresponding to half the behavioral maximum (M-50) were computed. The food restriction effect was analyzed by deriving change scores for the M-50, slope and maximum response rate parameters comparing pre-restriction baseline to each of the two vehicle test days. These change scores were subsequently subjected to a repeated measures ANOVA, with test day (2) as the within subject factor, and group (2) as the between subject factor.

Drug test data were analyzed by repeated measures analysis of variance (ANOVA), with group (2) as the between subjects factor, and vehicle–drug (2) tests as within subject factor. Unique changes between the two groups across the vehicle–drug test days were interpreted by a significant Group × Test-day interaction. Overall drug effects were interpreted by a significant test days effect. Like the food restriction data, these data are graphically presented as group percent change in each of the parameters from vehicle to drug day.

3. Results

3.1. Experiment 1: effect of synthesis inhibitors on circulating corticosterone in food restricted rats

Body weights of rats declined from the pre-restriction mean of 380.8 to 347.4 g on the day of drug testing. Fig. 1 displays the mean serum corticosterone levels (ng/ml) obtained at 30 and 120 min post-injection. Aminoglutethimide substantially reduced the serum corticosterone level, by about 75% at 30 min, but had a negligible effect at 120 min. Metyrapone exerted a more prolonged yet less pronounced effect, reducing levels by about 30% at 30 min and 40% at 120 min. Planned comparisons confirmed these observations. Aminoglutethimide (F(1,8) = 18.70, P < 0.01) but not metyrapone (F(1,8) = 1.91, P > 0.10) produced a significant reduction in circulating corticosterone relative to vehicle at 30 min post-injection. At 120 min metyrapone significantly decreased corticosterone (F(1,8) = 6.11, P < 0.05) while the change produced by aminoglutethimide was not significant (F(1,8) = 1.45, P > 0.10).

3.2. Experiment 2: effect of aminoglutethimide and metyrapone on food restriction induced sensitization of LHSS

3.2.1. Effect of food restriction

Body weights of food-restricted rats declined from the pre-restriction mean of 400.6 to 337.4 g on vehicle day 1 and to 319.2 g on vehicle day 2. Body weights of ad libitum fed rats increased from 411 to 455.1 g and then to 465.4 g.

Fig. 2 displays representative rate–frequency curves for a control (top panel) and a food-restricted (bottom panel) animal. Here it can be seen that food restriction shifted rate–frequency curves to the left relative to baseline. Fig. 3 displays the overall group change in threshold relative to baseline. Effects of food restriction on LHSS were evident on each of the two vehicle days. A repeated measures ANOVA with vehicle day (2) as the within- and group (2) as the between-subject factors confirmed this general observation. This analysis revealed an overall effect of group on LHSS threshold (F(1,10) = 12.50, P < 0.01). A differential group change across the two test days was not evident as the interaction was not significant (F(1,10) = 1.10, P > 0.30). General changes in performance parameters were not present, since between-group changes in the
Fig. 2. Rate–frequency curves for a representative ad libitum fed control rat (top) and food-restricted rat (bottom) are displayed. In each case, the number of reinforced lever presses per min is plotted as a function of the lateral hypothalamic pulse stimulation frequency delivered. Data are displayed for pre-restriction baseline rate–frequency testing and for two vehicle tests run 6 days apart during food restriction.

The slope of the functions, maximal response rates or any interaction of these factors across test days were not observed (all \( P > 0.15 \)). Given that each of these test days served as the vehicle comparison for drug tests, between

group changes in threshold relative to baseline were analyzed for each day. This analysis revealed that LHSS threshold in food-restricted animals was substantially decreased relative to controls on both the first vehicle test \( (F(1,10) = 13.49, P < 0.01) \) and the second vehicle test \( (F(1,10) = 10.84, P < 0.01) \).

### 3.2.2. Effect of synthesis inhibitors

Fig. 4 displays the mean group change in LHSS threshold produced by aminoglutethimide and metyrapone. While neither drug appeared to differentially affect the two groups, aminoglutethimide produced an overall decrease in threshold of about 7%. These impressions were confirmed by a repeated measures ANOVA. This analysis revealed that neither group was uniquely affected by either drug as each Group X Test-day interaction was non-significant (both \( P > 0.15 \)). Additionally, aminoglutethimide produced a significant overall decrease in threshold from the vehicle to drug test \( (F(1,10) = 19.54, P = 0.001) \), while metyrapone \( (F(1,10) = 0.004, P > 0.90) \) failed to affect threshold.

Fig. 5 depicts the change in average maximal response rate produced by each drug. Neither drug produced differential group changes in performance. The absence of a significant Group X Day interaction produced by aminoglutethimide \( (F(1,10) = 1.44, P > 0.25) \) or metyrapone \( (F(1,10) = 0.03, P > 0.80) \) confirmed this impression. Both drugs produced overall changes in performance with aminoglutethimide increasing \( (F(1,10) = 8.35, P = 0.016) \) and metyrapone decreasing response rate \( (F(1,10) = 12.94, P < 0.01) \). No significant change in slope between the two groups for either drug was detected as the interaction across days was not significant (both \( F < 0.39 \)). While no overall effect of metyrapone on the slope of the function was detected \( (F(1,10) = 0.14, P > 0.70) \), aminoglutethimide did produce a significant increase in the slope of the functions \( (F(1,10) = 7.23, P < 0.05) \). This change
in slope cannot, however, explain the decrease in threshold in that, all other factors being equal, only a decrease in slope can decrease threshold.

3.2.3. Two-hour post-aminoglutethimide test

Fig. 6 displays representative rate–frequency curves for a control and food-restricted rat obtained 2 h following the administration of aminoglutethimide. As in our prior study (unpublished data), aminoglutethimide produced a pronounced decrease in LHSS threshold in food restricted, but not control rats. Fig. 7 displays the mean group changes in LHSS threshold observed in the two studies. An analysis of data from the current experiment revealed a significant Group × Day (F(1,10) = 6.26, P < 0.05) interaction suggesting a differential change from vehicle between the two groups. A simple effects analysis determined the source of this interaction to stem from aminoglutethimide producing a significant decrease in threshold in food restricted animals (F(1,4) = 49.91, P < 0.01) but not in control subjects (F(1,6) = 3.18, P > 0.12). No overall change in the average maximum response rate or the slope of the functions was produced by aminoglutethimide, nor did the group factor interact with either of these variables (all P > 0.10). It should be noted that a similar interaction was also observed in our prior study (F(1,8) = 5.51, P < 0.05; both groups n = 5).

As discussed earlier, results of Expt. 1 indicate that serum corticosterone recovers from aminoglutethimide-induced suppression by 2 h post-injection. In our previous study serum corticosterone was also measured 2 h post-injection, immediately prior to LHSS testing. Consistent with the results of Expt. 1, the suppression of corticosterone by aminoglutethimide had recovered by 2 h post-injection. Mean corticosterone levels (ng/ml) for food-re-
stricted animals were 218.5 and 216.0 following vehicle and aminoglutethimide, respectively, while levels in ad libitum fed control animals were 100.3 and 86.7.

4. Discussion

Inhibition of steroid biosynthesis by aminoglutethimide and metyrapone produced several notable effects on circulating corticosterone (Expt. 1) and behavior (Expt. 2). Using doses that have previously been shown to reduce corticosterone levels in stressed [16,20,42,49] and non-stressed [3,10,24,28] animals, Expt. 1 demonstrated the effects of these drugs in chronically food-restricted animals. Aminoglutethimide produced a robust, short-term decrease in corticosterone, while metyrapone produced a less pronounced but more persistent decrease. Other studies that have assessed corticosterone levels in rats treated with aminoglutethimide [16,28,46] and metyrapone [10,24,40,54] support this difference in magnitude and duration of effect.

The results of Expt. 2, in agreement with numerous prior reports [1,6,11,12], demonstrate that chronic food restriction sensitizes LHSS. Furthermore, while aminoglutethimide and metyrapone each produced general perfor-

mance effects, neither reversed the sensitization of LHSS by food restriction when testing was conducted at a time coincident with decreases in circulating corticosterone. This is consistent with our prior finding [1] that the sensitization of LHSS by food restriction is unaffected by a post-meal decrease in corticosterone. Collectively, these findings contrast with observations that blocking stress-induced elevations in corticosterone can reverse the sensitization of psychostimulant and opiate-induced locomotion [17]. They also contrast with findings that acute inhibition of corticosterone synthesis by metyrapone can decrease the locomotor response produced by cocaine administration [52]. Thus, these results reinforce doubts that have recently been raised regarding the homology between rewarding and locomotor effects of reinforcing stimuli [4,30,31,51,56].

While both drugs failed to uniquely change LHSS threshold in food-restricted animals, each drug had general, yet differential effects on performance parameters and LHSS threshold. Aminoglutethimide elevated maximum response rate and modestly decreased LHSS threshold, while metyrapone decreased maximum response rate and failed to affect LHSS threshold. These observations are consistent with previously reported effects of glucocorticoid synthesis inhibitors on general motor activity [2,49].

One possible mechanism that may explain the general facilitatory effects of aminoglutethimide on LHSS could be an increase in ACTH secretion. In this regard, it is known that ACTH secretion is increased 30 min following the administration of aminoglutethimide [16,28]. The effects of ACTH on self-stimulation behavior are, however, less clear. Those studies that have assessed the effects of ACTH on medial forebrain bundle self-stimulation have utilized ACTH fragments that share many of the effects of the parent molecule without stimulating steroidogenesis. Differing effects of these fragments have been reported in that ACTH4-9 increased self-stimulation rate [29] while ACTH4-10 had no effect on current threshold [41]. The likelihood that ACTH mediates the short term sensitization produced by aminoglutethimide is decreased somewhat by the fact that metyrapone also increases ACTH [28] at a time in which LHSS threshold was unaffected in the present study.

Because aminoglutethimide acts earlier in the biosynthetic pathway than metyrapone, its general facilitatory effect could alternatively be mediated by the inhibition of steroids ‘upstream’ from corticosterone. Recent studies indicate that several precursors of corticosterone can modulate physiology and behavior [22,36,44,47,50]. Particular attention has focused on pregnenolone (PREG), dehydroepiandrosterone (DHEA), progesterone (PROG) and their respective metabolites which modulate neuronal excitability through GABA_A receptors [35,47,48,50] and NMDA [7,36] receptors. Both of these receptors appear to regulate LHSS since GABA_A agonists [8] and NMDA antagonists [9] enhance LHSS. Thus, the overall facilitatory effect of aminoglutethimide could plausibly result from the inhibition of PREG and DHEA metabolites which antagonize GABA_A receptor function [35,48] and positively modulate NMDA receptors (PREG; [7]). Another possibility, not incompatible with the first, is that the effects of aminoglutethimide reflect a central site of action. Steroid synthesis has recently been demonstrated in the CNS and can occur de novo in glial cells or from circulating steroid precursors (see [27,35,50,47]). Many of these ‘neurosteroids’ are known to accumulate in CNS tissues through mechanisms independent of the hypothalamo-pituitary-adrenal (HPA) axis [44,47]. Thus, the overall short-term facilitation of LHSS produced by aminoglutethimide could reflect changes in steroid metabolism independent of those occurring in adrenal tissues.

A different pattern of results emerged when testing was conducted 2 h following the administration of aminoglutethimide. This is a time when circulating corticosterone levels in food-restricted animals return to pre-drug baseline. While aminoglutethimide still produced a modest decrease in LHSS threshold in control animals it had a considerably greater effect in food-restricted animals, essentially doubling the effect of food-restriction. While the cause of this effect is not clear, several possibilities will be discussed. The first involves the accumulation of cholesterol that occurs following aminoglutethimide administration in tissues that synthesize steroids. Upon cessation of aminoglutethimide blockade, steroid precursors (PREG) are readily converted from cholesterol [27]. This metabolic ‘rebound’ may be particularly robust in food-restricted animals, given their normally elevated corticosterone levels. If so, one or more steroid precursors of corticosterone
may mediate this effect. One possibility is that PROG metabolites, which are GABA_A receptor agonists could facilitate LHSS as do exogenous GABA_A agonists [8,58]. A second possibility concerns the central inhibition of neurosteroid biosynthesis by aminogluthethimide. It is known that several steroids are found in higher concentrations in the brain than in plasma ([44], see also [35]) and acute stressors produce elevations in brain that are independent of those observed in plasma [44]. Importantly, aminogluthethimide, which blocks the synthesis of all steroids from cholesterol, would be expected to affect neurosteroid levels while metyrapone, which only blocks the synthesis of corticosterone, would not. While aminogluthethimide was shown to have a brief suppressive effect on peripheral steroid synthesis (Expt. 1), the effect of systemic aminogluthethimide on in vivo neurosteroid synthesis remains undetermined. Therefore, it is possible that the effect of aminogluthethimide at 2 h may reflect a long-term suppressive effect of the drug on central steroid synthesis. Such a mechanism may involve the interaction between aminogluthethimide and opioid, or other CNS, mechanisms that are uniquely active in the food-restricted rat. The plausibility of an interaction with opioid mechanisms is supported by the finding that blockade of the NMDA receptor with MK-801 potentiates the threshold-lowering effect of morphine on LHSS [9]. Blockade of PREG and DHEA by aminogluthethimide may yield functional effects that are similar to administering an NMDA antagonist, and could thereby potentiate the effect of endogenous opioid activity that is uniquely present in food-restricted rat brain [5,57]. Of course, a central synthesis "rebound effect" may also mediate these effects by mechanisms similar to those hypothesized in adrenal tissues.

The final possibility to be considered concerns the metabolic consequences of suppressing corticosterone in food-restricted rats. Corticosterone normally mediates an adaptive response to undernutrition by increasing proteolysis and hepatic gluconeogenesis [38]. Corticosterone also has anti-insulin effects on peripheral glucose transport which lead to a compensatory increase in insulin secretion [15]. Pharmacological suppression of circulating corticosterone would therefore be expected to exacerbate both the hypoglycemia and hypoinsulinemia that already exist in the food-restricted rat, and it may be these indicators of metabolic need that underlie the sensitization of reward. If one of these conditions does underlie the sensitization of reward, an exacerbation that outlasts corticosterone suppression could account for the further potentiation of reward seen 2 h after aminogluthethimide treatment. In this case, metyrapone should also potentiate reward in food-restricted rats, presumably at some time point later than the 1 h post-injection test that was conducted. This prediction is of course testable as are the contributions of hypoglycemia and hypoinsulinemia to reward sensitization.

The present data demonstrate that the inhibition of circulating corticosterone fails to reverse the sensitizing effect of food restriction on LHSS. Furthermore, they suggest that other circulating steroids, possibly synthesized within the brain, may have modulatory effects on brain reward that are most apparent in the food-restricted rat. The implications of these findings for our understanding of reward and its sensitization by food-restriction, and possibly stress in general, remain to be elucidated.

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