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ORIGINAL INVESTIGATION

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Aminoglutethimide, a corticosteroid synthesis inhibitor, facilitates brain stimulation reward in food-restricted rats: an investigation of underlying mechanisms

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Abstract It was previously observed that the corticosteroid synthesis inhibitor, aminoglutethimide (AG), markedly facilitates lateral hypothalamic self-stimulation (LHSS) in food-restricted rats. This effect is not present 30 min after injection when plasma corticosterone levels are suppressed, but rather at 2 h when corticosterone has recovered from suppression. In experiment 1, it was confirmed that AG (50.0 mg/kg, SC) lowers the threshold for LHSS in food-restricted rats but not in control rats that have ad libitum access to food. This effect occurred independently of whether food restriction, by itself, lowered threshold. Experiment 2 examined whether the facilitation of LHSS coincides with biosynthetic rebound of corticosteroid precursors. While a pregnenolone surge was demonstrated by radioimmunoassay, dose-response testing with exogenous pregnenolone and progesterone (0.1, 1.0 and 10.0 mg/kg, SC) failed to confirm the prediction that one of these precursors facilitates reward. Therefore, a general test of the involvement of adrenocortical biosynthetic events was conducted in experiment 3 where rats were adrenalectomized (ADX) or sham-operated prior to food restriction. Surprisingly, ADX did not diminish the effect of AG. This finding raises the possibility of a CNS, rather than adrenal, site of action. AG is known to penetrate the blood-brain barrier and exert weak anticonvulsant effects. The facilitation of reward may result from central inhibitory effects of the drug and share a common basis with the enhanced reinforcing potency of other CNS depressants in food- restricted rats.

Key words Aminoglutethimide · Corticosterone · Reward · Self-stimulation · Food restriction

Introduction

An important goal in drug abuse research is the identification of biological factors that predispose certain individuals to the reinforcing and addictive properties of drugs. This has focused experimental attention on the physiological concomitants of chronic food restriction. Animals maintained on a regimen of food restriction self-administer lower doses of drugs than do free-feeding animals (Carroll and Meisch 1984), display a potentiated locomotor response to fixed doses of psychostimulants and opiates (Deroche et al. 1993, 1995), and show a shift to the left in rate-frequency curves for lateral hypothalamic self-stimulation (LHSS) (Abrahamsen et al. 1995). The likelihood that these various expressions of behavioral sensitization reflect the same underlying physiological adaptation is supported by literature that attributes the locomotor and reinforcing effects of drugs and electrical stimulation to a shared neuronal circuitry that includes dopaminergic projections from ventral tegmentum to nucleus accumbens (Wise and Bozarth 1987; Koob 1992).

A component of the neuroendocrine response to food restriction, increased corticosterone secretion, may be involved in reward sensitization, since adrenalectomy prevents the sensitizing effect of food restriction on drug-induced locomotion (Deroche et al. 1993, 1995). Yet, we have previously observed that while the corticosteroid synthesis inhibitors aminoglutethimide (AG) and metyrapone suppress circulating corticosterone in food-restricted rats, neither reverses the sensitization of LHSS (Abrahamsen and Carr 1996). In fact, when LHSS was tested 2 h following AG administration, a time point at which plasma corticosterone had recovered from suppression, food-restricted rats displayed a substantial shift to the left in their rate-frequency curves, while control rats did not. The finding that AG facilitates reward in food-restricted animals is interesting in light of the fact that enhanced rather than diminished corticosterone secretion has been correlated with the rewarding effects of drugs (Piazza and Le Moal 1996). The experiments described in this paper were conducted in an effort to determine the basis for AG facilitation of reward in food-restricted rats.

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General methods

Subjects and surgical procedures

Adult male Sprague-Dawley rats (300–350 g) were singly housed under a 12/12-h light/dark schedule and provided ad libitum access to food and water. For stereotaxic implantation of bipolar stimulating electrodes (Plastic One, Roanoke, Va., USA), subjects were anesthetized with ketamine (75.0 mg/kg, SC) and xylazine (5.0 mg/kg, SC). With the bregma and lambda suture landmarks in the same horizontal plane, coordinates for electrode implantation in the lateral hypothalamic medial forebrain bundle were: 3.0 mm posterior to bregma, 1.6 mm lateral to the sagittal suture, and 8.4 mm below skull surface. Electrodes were permanently secured to the skull by applying dental acrylic around them and four anchoring jeweler's screws.

For adrenalectomy (ADX), subjects were anesthetized with ketamine/xylazine and two dorsal incisions were made just caudal to the rib cage on each side. Fat surrounding the dorsal portions of the kidney was extracted and the adrenal gland was excised in its capsule. Sham surgery consisted of similar surgical manipulation excluding adrenal gland removal. For the duration of post-surgical survival, ADX animals had ad libitum access to 0.9% saline rather than tap water.

Drugs

Aminoglutethimide (Sigma Chemical Co., St Louis, Mo., USA) was dissolved in 50% dimethylsulfoxide (DMSO) vehicle at a concentration of 50.0 mg/ml for subcutaneous injection.

Pregnenolone (PREG) and progesterone (PROG) (Sigma) were both dissolved in DMSO in concentrations of 0.1, 1.0 and 10.0 mg/ml and administered SC in a volume of 1.0 ml/kg.

Behavioral test apparatus

Behavioral testing was conducted in four 11×8.25×8.25 inch operant test chambers, each with a retractable lever mounted on a side wall. A Med-Associates (Georgia, Vt., USA) programmable constant current stimulator was used to deliver leverpress-contingent trains of 0.1-ms cathodal pulses. Pulses were conducted from the stimulator to implanted electrodes by way of a commutator (Plastic One). Lever position, duration of test trials, delivery of "primes" and response-contingent brain stimulation, and counting of reinforced leverpresses 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.

Intracranial self-stimulation

Following recovery from stereotaxic surgery, rats were trained daily to leverpress for 1-s trains of LH stimulation. During the initial days of training, each rat was assigned a current intensity that would reliably support a maximal response rate at a stimulation frequency of 80 pulses per second (pps). The stimulation intensities thus assigned ranged from 150 to 240 μA.

Sensitivity of the brain reward system to electrical stimulation was evaluated using curveshift methodology (Gallistel and Freyd 1986; Miliaressis et al. 1986). This entails the monitoring of self-stimulation across a wide range of stimulation frequencies which generate response rates ranging from maximal levels to zero. The resultant plot of reinforcement rate as a function of frequency yields a sigmoid function that can be used to derive threshold parameters of reward potency. The most commonly used index is the M-50, defined as the brain stimulation frequency that supports half-maximal responding. Experimental treatments that increase the rewarding potency of brain stimulation produce parallel leftward shifts in this function, decreasing the M-50, while treatments that decrease rewarding potency produce parallel rightward shifts, increasing the M-50.

Each rate-frequency curve was initiated by extension of the response lever accompanied by 2 s of priming stimulation at 90 pps. All animals leverpressed at rates that resulted in more than 25 reinforcements per minute. Every 75 s, stimulation frequency was decreased by 8%. Responding during the final 60 s of each 75-s trial was used to determine rate of reinforcement for a given frequency. Trials in which a criterion number of reinforced responses (five responses/min) were not emitted were designated as negative trials and were followed by 2 s of priming stimulation at the same frequency. Following the prime, a second trial at the same frequency was conducted. 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. Baseline data collection and experimental testing were initiated only after animals displayed stable rate frequency curves over 5 consecutive training days.

Food restriction

Food restriction consisted of switching animals from ad libitum access to just a single 10-g meal of Purina rat chow per day. Control animals continued to have ad libitum access to food. All animals had ad libitum access to water. Drug testing began once the body weights of food-restricted animals had declined by at least 15% (see individual experiments below).

Radioimmunoassays

Pregnenolone

Samples of trunk blood were centrifuged at 10,000 xg for 20 min and approximately 2 ml of serum was extracted and immediately frozen. Serum pregnenolone concentrations were determined using celite chromatography and a ^{3}H pregnenolone radioimmunoassay kit (ICN Biomedicals, Costa Mesa, Calif., USA).

Corticosterone

Samples taken by pipette from the tail vein were centrifuged at 10,000 xg for 20 min and approximately 25 μl of serum was extracted. Serum corticosterone levels were measured using a 125I-RIA kit (ICN Biomedicals).

Data analysis

The two LHSS rate-frequency curves obtained each day were averaged to yield one curve per rat. Each of these curves was subsequently partitioned into two line segments; one defined the asymptotic (i.e. maximal) range of responding and was parallel to the x-axis and the other defined the ascending portion of the curve (Abrahamsen et al. 1995). Using the regression equation for the latter segment, the slope and stimulation frequency supporting half the maximum reinforcement rate (M-50) were computed. The maximum reinforcement rate for each session was defined as the mean of all sequential values within 10% of the highest reinforcement rate obtained during the session. Rats would occasionally generate a curve that did not display an asymptotic range (i.e. sequential values within 10% of the highest reinforcement rate). In such cases, the highest reinforcement rate obtained was taken as the maximum from which M-50 values would be derived.

All behavioral and biochemical data were analyzed by between groups and/or repeated measures analysis of variance (ANOVA) using SPSS/PC software. Tukey post-hoc ($P<0.05$) tests were conducted where appropriate.

Experiment 1

It was previously observed that 30 min following SC administration of aminoglutethimide (AG; 50.0 mg/kg), the elevated plasma corticosterone levels of food-restricted rats decrease by more than 70% (Abrahamsen and Carr 1996). This treatment produced a small (about 5%) but consistent decrease in the LHSS threshold of both food-restricted and control rats. Two hours following AG treatment, plasma corticosterone levels had recovered from suppression. Yet, at this time point, food-restricted animals displayed a substantial facilitation of LHSS (i.e. >20% decrease in threshold), while control rats were unaffected. The dose of 50.0 mg/kg was chosen on the basis of previous pilot work which indicated that this dose effectively suppresses steroidogenesis, yet is below threshold for producing disabling sedation and motor impairment. The first purpose of the present experiment was to replicate the unique facilitatory effect of AG on LHSS in food-restricted rats.

Prior work in this laboratory indicates that chronic food restriction facilitates LHSS (Carr and Wolinsky 1993). This effect is limited, however, to electrode sites in the medial forebrain bundle dorsal and lateral to the fornix. Self-stimulation in the zona incerta, ventral LH and extreme lateral LH is typically not affected. Thus, a second purpose of this experiment was to evaluate whether a relationship exists between the effect of food restriction on LHSS and the consequent facilitatory effect of AG. For this analysis, results from our prior AG experiment (Abrahamsen and Carr 1996) were combined with the present results to yield sample sizes large enough to conduct a meaningful analysis of this particular relationship.

Method

For the replication, nine food-restricted and seven control rats were tested. LHSS behavior was periodically monitored during the first 3 weeks of food restriction, while body weights of restricted rats declined from 457 (±8.8) to 342 (±5.8) g. During this same period body weights of control rats increased from 462 (±9.5) to 500 (±8.9) g. AG (50.0 mg/kg, SC) and vehicle were then administered 2 days apart, in counterbalanced order, 2 h prior to LHSS rate-frequency testing.

To evaluate whether a relationship exists between responsiveness to food restriction, itself, and AG, data from the present subjects were combined with those from the 24 subjects in our previous AG study (Abrahamsen and Carr 1996). Methods employed in the previous study were essentially identical to those employed in the present replication. To determine the magnitude of a subject's response to food restriction, the M-50 value obtained on the vehicle treatment day during food restriction was expressed as a percentage of the mean M-50 value obtained in the final 2 days of baseline testing prior to the implementation of food restriction. By defining responsiveness to food restriction as a greater than 5% decrease in M-50, roughly half of all food-restricted rats (n=10) qualified as "restriction responsive", with a mean (±SEM) decrease in M-50 of 16.8 (±2.8)% and half (n=11) as "restriction non-responsive", with a mean increase in M-50 of 4.1 (±2.5)%. The control rats (n=19) displayed a mean increase in M-50 of 5.0 (±3.3)%.

Results

Figure 1 displays representative rate-frequency curves for a food-restricted and control animal during vehicle and AG testing. This figure illustrates the marked facilitation of LHSS induced by AG in a food-restricted, relative to a control subject. Figure 2 displays the overall percent change in LHSS threshold for each group when testing was conducted 2 h following systemic AG administration. Here it can be seen that AG produced a greater overall decrease in LHSS threshold in food-restricted animals. A repeated-measures ANOVA confirmed this general observation, demonstrating a significant decrease in threshold produced by AG ($F_{1,14}$=21.75, P<0.001) and a significant Group × Drug interaction ($F_{1,14}$=5.06, P<0.041). A post hoc correlated t-test confirmed that AG did not significantly decrease the threshold of control rats [t(6)=2.06, P>0.05].

A comprehensive analysis of the effect of AG on LHSS in "food restriction responsive", "food restriction

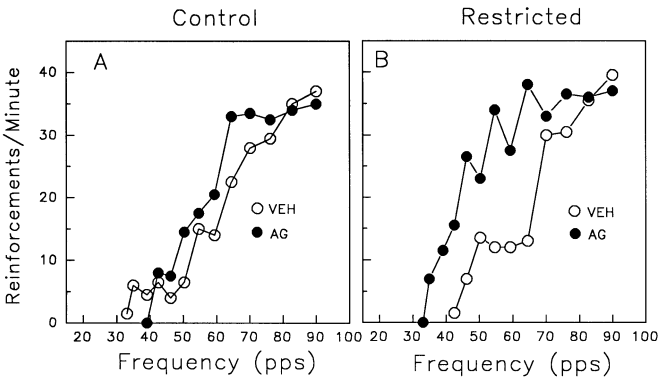

Fig. 1A, B Representative rate-frequency curves for ad libitum fed control (*left*) and food-restricted (*right*) rats 2 h following injection of vehicle and AG (50.0 mg/kg). The number of reinforced leverpresses per minute is plotted as a function of the lateral hypothalamic pulse stimulation frequency delivered

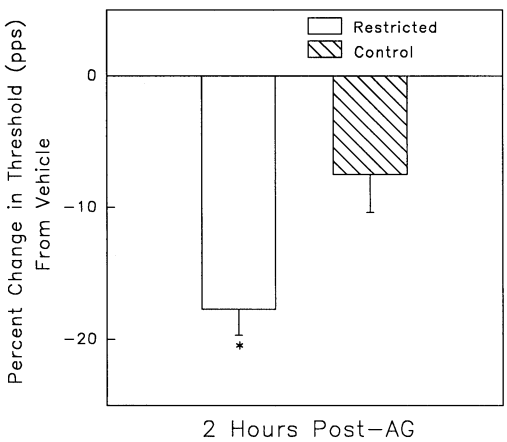

Fig. 2 Mean (±SEM) percent change in LHSS frequency threshold, relative to vehicle, 2 h following injection of AG in food-restricted (*open bars*, n=9) and ad libitum fed control (*hatched bars*, n=7) rats. *P<0.05

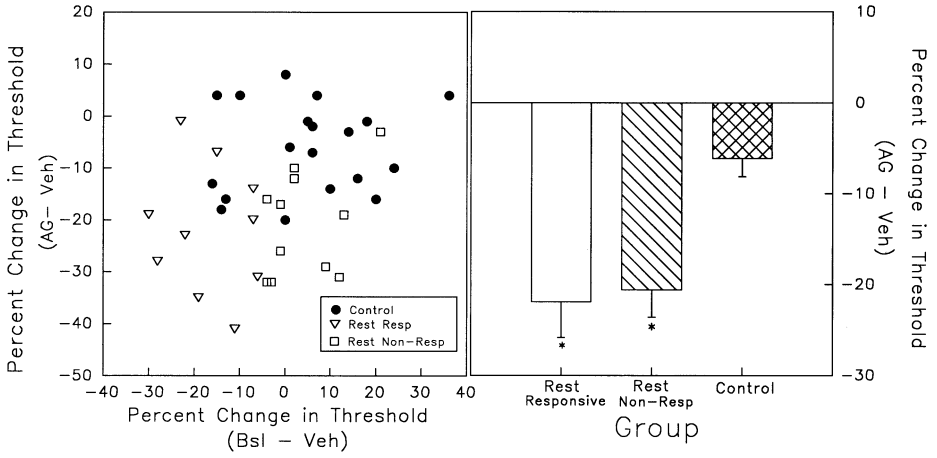

Fig. 3 *Left panel*: bivariate scatterplot displaying percent change in LHSS threshold observed during the vehicle test for AG in relation to the pre-restriction baseline value (*x-axis*) and percent change in threshold produced by AG (*y-axis*). For purposes of analysis, food-restricted subjects were categorized as restriction responsive if the percentage decrease in threshold (*x-axis*) was 5% or greater (*open triangles*); otherwise these subjects were categorized as restriction non-responsive (*open squares*). Scores on the y-axis illustrate the facilitatory effect of AG on LHSS. *Right panel*: overall percent change in LHSS threshold as a consequence of AG treatment for food restriction responsive, food restriction non-responsive, and ad libitum fed control animals

non-responsive" and control animals was conducted by pooling the present data with those of our prior study (Abrahamsen and Carr 1996). The results are displayed in Fig. 3. The left panel of Fig. 3 displays a bivariate scatterplot of each animal's change in threshold as a consequence of food restriction (x-axis) and AG treatment (y-axis). The right panel displays the mean percent change in LHSS threshold induced by AG for the three groups. Food-restricted subjects displayed a sensitized response to AG that was independent of whether their LHSS thresholds had been decreased by food restriction itself. ANOVA confirmed this general observation yielding a significant effect of group ($F_{2,37}$=11.33, P<0.001). A Tukey multiple comparison yielded significant differences between the control group and the two food-restricted groups which did not differ from one another.

Experiment 2a

AG suppresses circulating corticosterone by inhibiting cholesterol side chain cleavage and the conversion of the corticosterone precursor pregnenolone from cholesterol (Robel and Baulieu 1994). Moreover, following release of AG blockade, pregnenolone synthesis rapidly returns to normal (Hu et al. 1989) or can be potentiated by ACTH (Hall 1985). Several studies have demonstrated behavioral effects of exogenous pregnenolone, including mood elevation (Flood et al. 1992; Hu et al. 1989; Lancel et al. 1994; Roberts 1995). Thus, the purpose of experiment 2a was to determine whether the facilitation of LHSS 2 h following AG administration coincides with a biosynthetic surge of pregnenolone.

Method

Three days following the completion of behavioral testing in experiment 1, half of the rats in each group (i.e. food-restricted or control) received another SC injection of either AG (50.0 mg/kg) or vehicle. Two hours later, each animal was killed by decapitation and trunk blood was collected for pregnenolone radioimmunoassay.

Experiment 2b

As a further test of whether biosynthetic rebound of corticosterone precursors could be involved in the facilitation of reward by AG, pharmacological dose-response tests were conducted with pregnenolone and progesterone in experiment 2b. Metabolites of progesterone (PROG) and pregnenolone (PREG) are agonists and antagonists, respectively, at the $GABA_A$ receptor (Majewska 1992; Bitran et al. 1993; Robel and Baulieu 1994) and produce behavioral effects when administered as pharmacological agents (e.g. Flood et al. 1992; Bitran et al. 1993; Melchior and Ritzmann 1994; Picazo and Fernandez-Guast 1995; Robel et al. 1995). In addition, PROG has been shown to enhance dopamine release in vitro and in vivo (Dluzen and Ramirez 1990; Petitclerc et al. 1995; Cabrera and Navarro 1996). Given the critical involvement of both GABA and dopamine in brain reward circuitry (Koob 1992), the post-AG surge in corticosterone precursors seemed to merit further investigation.

Method

In this experiment, all drug testing was conducted during week 3 of food restriction. On the inital day of dose-response testing, body weights of food-restricted rats had declined to 364 (±3.6) from the baseline value of 427 (±4.6) g. By the final day of dose-response testing, weights had declined to 343 (±3.8) g. Half of the food-restricted and control subjects were administered PREG (0.1, 1.0, 10.0 mg/kg, SC) and the other half PROG (0.1, 1.0,

10.0 mg/kg, SC), with the three doses administered in random order, but matched between groups. Each drug test was preceded by a vehicle test day and followed by a rest day. LHSS testing was initiated 15 min following injection.

Results

Figure 4 displays the results of the pregnenolone (PREG) RIA on serum samples collected 2 h following the administration of AG. AG increased levels of circulating PREG in both groups. Two-way ANOVA revealed significant main effects of Group ($F_{1,12}$=5.38, P<0.05) and Drug ($F_{1,12}$=23.46, P<0.001). While food restricted animals appeared to display a greater increase in circulating pregnenolone relative to controls, the Group × Drug interaction was not significant ($F_{1,12}$=1.90, P>0.10).

Figure 5 displays the percent change, relative to vehicle, in LHSS threshold produced by the three doses of PREG and PROG. Neither drug, at any dose tested, had an appreciable effect on the LHSS threshold of food-restricted or control rats. ANOVA failed to yield significant effects of Group, Dose, or an interaction between these factors (all P>0.25).

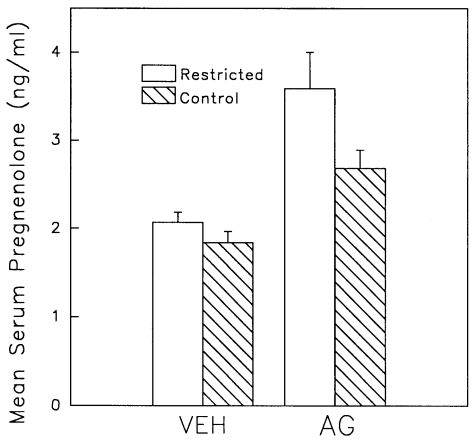

Fig. 4 Mean (±SEM) serum pregnenolone levels (ng/ml) 2 h following injection of AG or vehicle in food-restricted and ad libitum fed control rats (all n=4)

The 15-min interval between injection and behavioral testing in this experiment was intended to mimic the endogenous surge in PREG 2 h after AG suggested by the results of experiment 2a. However, because pharmacological effects of PROG on behavior have been detected 4 h after injection (Bitran et al. 1993; Bless et al. 1997), the PROG and PREG dose-response tests were repeated in the same animals with a 4-h interval between injection and testing. Neither compound significantly altered LHSS threshold, and in no case did a mean decrease in threshold, relative to vehicle, exceed 4.5%.

Experiment 3

While the results of experiment 2 support the occurrence of a biosynthetic rebound that produces supranormal levels of corticosterone precursors during recovery from AG, doubt was cast on the involvement of PROG and PREG in the facilitation of reward. Other adrenocortical mechanisms could, however, account for the facilitatory effect of AG on reward in food-restricted rats. For example, transient suppression of corticosterone may have a delayed effect on central protein synthesis (Nichols and Finch 1994) or corticosterone responsive hormones within the hypothalamic-pituitary-adrenal axis (Jacobson et al. 1989; Bray 1993; Dallman et al. 1993), such that behavioral consequences manifest after corticosterone suppression has dissipated. Alternatively, other hormones within the adrenocortical biosynthetic pathway, such as dehydroepiandrosterone (DHEA) or deoxycorticosterone (DOC), both of which exert fast actions on membrane receptors (Majewska 1992; Robel and Baulieu 1994), may mediate the effect of AG on LHSS. The third experiment therefore tested the tenability of any adrenal-based hypothesis by assessing the effect of adrenalectomy on the facilitation of LHSS by AG in food-restricted rats.

Method

Following the completion of LHSS training, nine rats underwent bilateral adrenalectomy and nine underwent a sham surgical pro-

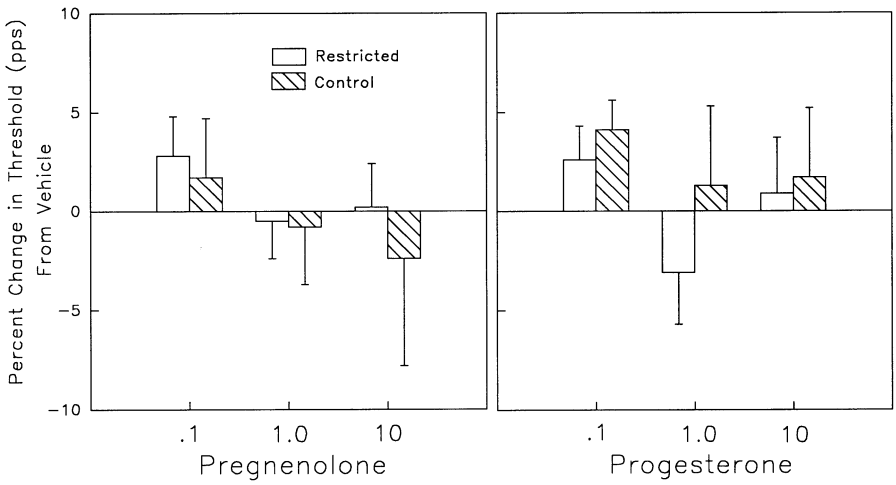

Fig. 5 Mean (±SEM) percent change in LHSS frequency threshold produced by pregenenolone (*PREG*) and progesterone (*PROG*) in food-restricted (*open bars*, n=7) and ad libitum fed control (*hatched bars*, n=7) rats

cedure. LHSS testing resumed 5 days later. Several days of post-surgical baseline data were collected and all rats were then placed on food restriction. ADX animals were maintained on 0.9% saline supplemented with 25 ng/ml corticosterone until 4 days prior to drug testing when the corticosterone supplementation was discontinued. Because of the diminished ability of ADX rats to survive sustained food restriction, testing began on day 13 of food restriction, when body weights had declined from the presurgical value of 460 (±14) to 376 (±9) g. In this same period, body weights of sham-operated rats declined from 490 (±9) to 410 (±7) g. Roughly half of the rats in each group received AG (50.0 mg/kg, SC), while the remainder received vehicle 2 h prior to LHSS testing. Two days later treatments were reversed. To confirm the success of adrenalectomy, blood for corticosterone radioimmunoassay was sampled from the tail vein on day 9 of food restriction. Samples were taken immediately prior to the scheduled daily meal (1600 hours), since plasma corticosterone levels of intact food-restricted rats peak at this time (Honma et al. 1984; Abrahamsen et al. 1995). Detectable, but low, levels of corticosterone were expected in ADX rats since sampling occurred on the final day of oral corticosterone supplementation.

Results

Relative to vehicle, AG produced virtually identical decreases in the LHSS thresholds of ADX and sham-operated food-restricted rats. The mean (±SEM) decrease in threshold was 12.7 (±4.0)% in sham-operated and 13.4 (±4.3)% in ADX rats. Two-way ANOVA with repeated measures on one factor confirmed the significant decrease in threshold produced by AG ($F_{1,16}$=19.3, P<0.001), with no significant interaction between drug treatment and surgical treatment ($F_{1,16}$=0.04). The mean pre-meal plasma corticosterone level in sham-operated animals was 209.3 (±31.2) ng/ml while the level in ADX animals was 37.6 (±12.1) ng/ml.

Discussion

The hypothesis that corticosterone modulates responsiveness of the brain reward system to drugs of abuse has potentially important implications for understanding individual differences in vulnerability to drug addiction (Piazza and Le Moal 1996). The facilitation of LHSS in food-restricted rats by a corticosteroid synthesis inhibitor appeared to offer an opportunity to uncover a novel facet of the regulatory relationship between adrenocortical hormones and the brain reward system. Experiment 1 confirmed that a dose of AG that previously produced a short-latency suppression of plasma corticosterone (Abrahamsen and Carr 1996) produces a longer-latency facilitation of LHSS in food-restricted rats. The leftward shift in rate-frequency curves produced by AG is comparable to that produced by low doses of drugs such as amphetamine, cocaine, nicotine and morphine (Wise et al. 1992). Thus, AG, or a hormonal response to its administration, excites the brain reward system.

It was also demonstrated in experiment 1 that the facilitation of LHSS by AG is independent of whether food restriction itself causes a leftward shift in a particular animal's rate-frequency curve. While facilitation of LHSS by food restriction appears to be dependent upon electrode placement (Carr and Wolinsky 1993), the facilitation of LHSS by AG, though requiring food restriction, may be independent of electrode placement. In this regard, AG may interact with LHSS in food-restricted rats as drugs of abuse interact with LHSS in free-feeding rats. Typically, site specificity is not a reported property of the facilitation of LHSS by abused drugs (Kornetsky and Esposito 1979; Wise 1996). At least one exception has, however, been reported in connection with the rate-increasing effect of D-amphetamine relative to L-amphetamine (Stephens and Herberg 1975). While some LH electrodes may lie "downstream" from the tissue that is sensitized by food restriction, systemically administered drugs may always be distributed to this tissue and produce rewarding effects that summate with electrical stimulation regardless of where within the LH the electrical stimulus is introduced.

In experiment 2, evidence was obtained for adrenocortical biosynthetic rebound coinciding with the facilitation of LHSS. A surge in PREG, and possibly other adrenal steroids that precede corticosterone in the biosynthetic pathway, represented a possible hormonal basis for reward sensitization. As a GABA antagonist and NMDA agonist, PREG would be expected to exert excitatory effects throughout the CNS (Roberts 1995). Indeed, when administered in pharmacological doses to mice, PREG produces hyperactivity, anxiety (Melchior and Ritzmann 1994), and memory enhancement (Flood et al. 1992). PROG, which is a more immediate precursor of corticosterone, is a GABA agonist with documented anxiolytic action in the rat (Picazo and Fernandez-Guast 1995). Compounds with similar neuropharmacological actions, such as muscimol and diazepam, have been reported to facilitate LHSS (Zarevics and Setler 1981; Carden and Coons 1990), though inhibition has also been reported (Backus et al. 1988). PROG could also facilitate LHSS via effects on dopaminergic transmission, since this hormone potentiates the NMDA and amphetamine-induced release of dopamine from nerve terminals (Dluzen and Ramirez 1990; Cabrera and Navarro 1996). Despite the plausibility of PREG or PROG involvement, dose-response testing in experiment 2 provided no suggestion of facilitatory effects of PREG or PROG on LHSS. These results cast some doubt on the role of adrenocortical biosynthetic rebound in the facilitation of LHSS by AG.

A final encompassing test of whether events associated with the suppression or rebound of adrenocortical biosynthesis could account for the AG effect was conducted in experiment 3 by administering AG to animals that were adrenalectomized or sham-operated prior to food restriction. Adrenalectomy was confirmed, on the final day of oral corticosterone supplementation, insofar as pre-meal plasma corticosterone levels in ADX rats were 18% of the levels in sham-operated controls. Yet, AG produced an identical leftward shift in the rate frequency

curves of both groups. The mean decrease in M-50 was, however, somewhat smaller (about 13%) than in prior studies (about 20%). This may be due to the fact that testing was conducted after fewer days of food restriction in order to avoid complications in ADX rats. The fact that ADX failed to diminish the facilitatory effect of AG strongly suggests that the adrenal cortex is not the site of action through which AG facilitates reward in food-restricted rats.

Among the remaining explanations of the AG effect in food-restricted rats is a CNS site of action with increased bioavailability of AG, or a psychoactive metabolite, consequent to a change in hepatic cytochrome P-450 activity. Starvation is known to affect microsomal enzymes (Ma et al. 1989) and thereby alter the bioavailability of hexobarbital (Sachan 1982), midazolam (Lau et al. 1996) and other drugs (Ma et al. 1989). Food restriction may also increase CNS entry of AG and metabolites by opening the blood-brain barrier. Acute stressors, such as forced swimming, have been shown to increase blood-brain barrier permeability via serotonergic and noradrenergic mechanisms (Sarmento et al. 1991; Sharma et al. 1991).

Consideration should also be given to the possibility that AG facilitates reward via direct effects on a sensitized reward substrate. AG was originally introduced as an anticonvulsant but was abandoned following reports of adrenal insufficiency in several of the early patients (Shaw et al. 1988). Clinically, use of AG therefore shifted to the treatment of adrenal carcinoma, breast cancer in postmenopausal women, and prostatic cancer. Among the characteristic side effects of AG are sedation and ataxia. While the mechanism of AG's anticonvulsant action has not, to our knowledge, been demonstrated, its reported behavioral effects are suggestive of agonist activity at the GABA/benzodiazepine receptor complex. Other compounds with anticonvulsant action and agonist activity at this receptor complex have facilitated LHSS (Zarevics and Setler 1981; Carden and Coons 1990). Furthermore, it would not be surprising that food restriction amplifies an otherwise marginal rewarding effect, since extensive research has been done with other CNS depressants, demonstrating an amplification of reinforcing potency in food-restricted rats (Carroll and Meisch 1984). This hypothesis can of course be tested by challenging AG with GABA antagonists and by comparing the effects of well-characterized GABA agonists on LHSS in food-restricted and free-feeding animals. If food restriction does prove to amplify the facilitatory effect of some or all drugs of abuse on LHSS, a useful assay for investigating an endogenous risk factor in drug abuse will be available.

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