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Adrenalectomy Increases Bicuculline-Induced Seizure Sensitivity in Long-Sleep and Short-Sleep Mice

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BOWERS, B. J., T. Z. BOSY AND J. M. WEHNER. *Adrenalectomy increases bicuculline-induced seizure sensitivity in long-sleep and short-sleep mice.* PHARMACOL BIOCHEM BEHAV **38**(3) 593–600, 1991.—Susceptibility to bicuculline-induced seizure onset and tonus was increased in LS and SS mice after adrenalectomy (ADX). Replacement with 10% corticosterone (CCS) in ADX animals resulted in a return to seizure latencies equal to those of sham-operated (SHAM) mice. In SS mice, dexamethasone (DEX) and cholesterol-control replacement was as effective as 10% CCS in returning seizure thresholds to SHAM values. In LS mice, DEX was only effective at a low bicuculline dose. Within the sham-operated group SS mice were more susceptible to bicuculline-induced seizure onset than LS mice; however, after ADX latencies did not differ between the two lines. These results suggest that seizure thresholds are regulated to some extent by the hypothalamic-pituitary-adrenal (HPA) axis. The effects of ADX on GABA-related seizure activity may also be influenced by genotype, such that genetic differences in $GABA_A$ receptor function and adrenocortical responses in LS and SS mice may be responsible for the differential seizure latencies observed in sham-operated mice.

Bicuculline Seizures Adrenalectomy Mice

A relationship between steroid hormones and seizure sensitivity has been suggested by several studies (9, 12, 16, 39). Cyclic exacerbations of seizure activity in epileptic women have been associated with ovulatory changes in estrogen/progesterone ratios (39,45). In addition, several steroids, or their metabolites, have been shown to be potent anticonvulsants. Pretreatment with progesterone or its metabolites will inhibit t-butylbicyclophosphorothionate (TBPS)-induced myoclonus, maximal electroshock- and metrazol-induced seizures in rodents and will reduce the frequency of interictal spikes in penicillin foci in cats (3, 9, 14). Recently, several studies have indicated that in brain a primary modulatory site for these steroids is the $GABA_A$ receptor complex (12,27). Their physiological relevance is based on their ability to potentiate GABA-regulated inhibition at nanomolar concentrations (27). Removal of endogenous glucocorticoids by adrenalectomy (ADX) can also alter GABAergic responses demonstrated by changes in $GABA_A$ receptor complex binding parameters (11,38) and reductions in concentrations of GABA in the central nervous system (10,37).

The $GABA_A$ receptor complex has been implicated in the regulation of seizure activity. Manipulations of GABAergic neurotransmission at pre- and postsynaptic sites will alter seizure thresholds such that disinhibition of GABA function results in an increase in seizure sensitivity; e.g., the $GABA_A$ antagonist, bicuculline, has been shown to be a potent convulsant in rodents (19, 22, 36). Bicuculline's convulsant properties are the results of its blockade of the $GABA_A$ receptor; administration of bicuculline does not change brain levels of GABA or the activity of the GABA synthesizing enzyme, glutamate decarboxylase (GAD) (22). In contrast, drugs that potentiate GABA inhibition are classified as anticonvulsants (21,44). It has been demonstrated that the mechanisms regulating seizure thresholds are controlled to some extent by genetic factors (30,33). Both pro- and anticonvulsant effects of drugs acting at the $GABA_A$ receptor complex are demonstrated differentially in genetically diverse stocks of mice (30,31).

The purpose of this study was to examine the effects of ADX on seizures induced by the $GABA_A$ antagonist, bicuculline, in two lines of mice, long-sleep (LS) and short-sleep (SS), that have previously been shown to differ in their sensitivity to bicuculline-induced seizures (41,42). The LS and SS mice were originally selected for their differential responses to the sedative-hypnotic effects of ethanol (23) and have been used extensively to investigate ethanol-related responses. As a consequence of these investigations, it has been determined that LS and SS mice also differ

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in several other responses including those related to $GABA_A$ receptor function (2, 29, 32, 35) and in their adrenocortical responses to ethanol administration (18, 50, 52).

Mice from the more ethanol-sensitive LS line demonstrate a greater sensitivity to muscimol-stimulated $^{36}Cl^-$ flux and to ethanol potentiation of muscimol-stimulated $^{36}Cl^-$ flux in cerebellum (2). LS mice also appear to be more sensitive to the effects of GABA mimetics on the incoordinating effects of ethanol (35). In addition, their adrenocortical response to ethanol, as measured by plasma CCS levels, is also greater than the response by SS mice (18, 50, 52). In contrast, SS mice exhibit greater enhancement by GABA of ^{3}H-flunitrazepam (FNZ) binding in cortex and cerebellum (29) and are more sensitive to the anticonvulsant effects of the benzodiazepine, flurazepam (30). The effects of stress-induced CCS release on GABA-enhanced ^{3}H-FNZ binding also differs between LS and SS mice, but is dependent on brain region (8).

It is these differences between LS and SS mice related to GABAergic function, including differential seizure susceptibility to bicuculline, and those related to CCS release, that make these lines of mice a valid genetic model to examine the influence of endogenous glucocorticoids and $GABA_A$ receptor-mediated seizure sensitivity. The responses measured in the present study may be related to genotype-dependent ethanol sensitivity; however, previous investigations have indicated that the association between bicuculline's effects at the $GABA_A$ receptor and ethanol-sensitivity are, at best, indirectly related (43), and are more likely to be genetically uncorrelated (51).

METHOD

Chemicals

Bicuculline, cholesterol, corticosterone and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]-Corticosterone was obtained from Amersham, Arlington Heights, IL (specific activity = 50 Ci/mmole). Corticosterone antiserum was purchased from Dr. Gordon Niswender, Department of Physiology and Biophysics, Colorado State University, Fort Collins, CO. Bicuculline was made fresh daily by initially dissolving the drug in 1 M HCl, neutralizing with 1 M NaOH, and diluting to proper concentrations with 0.9% saline; final pH = 6.8.

Animals

Male LS and SS mice were obtained from the Institute for Behavioral Genetics, Boulder, CO. All mice were 60–90 days of age when tested, were maintained on a 12-hour light/dark cycle (lights on at 0700 to 1900) and were allowed free access to food (Wayne Lab Blox) and water. Mice were divided into five experimental groups: 1) ADX, 2) sham-operated (SHAM), 3) CCS-replacement (ADX + CCS), 4) DEX-replacement (ADX + DEX), and 5) cholesterol-replacement controls (ADX + cholesterol). In SS mice, an additional group of SHAM animals received cholesterol pellets to examine the effect of the control vehicle on seizure latencies.

Surgery

Adrenalectomies were accomplished by removal of the adrenals through two dorsal incisions under pentobarbital (50 mg/kg) anesthesia. Sham operations were performed in a similar manner, without removal of the adrenals. ADX and SHAM animals were housed in a temperature-controlled room and allowed free access to food and water or 0.9% saline (ADX only). CCS and DEX replacement in ADX animals was accomplished by implanting pellets containing either 10% CCS or 10% DEX subcutaneously at the time of surgery. The pellets were prepared in a 10% CCS (10% DEX): 90% cholesterol weight to weight ratio and weighed approximately 80 mg. The pellets were made by heating 10% CCS (10% DEX) and 90% cholesterol over a low flame until melted. A small amount of peanut oil was added to improve the quality of the pellet. The melted CCS (DEX)/cholesterol mixture was poured into pellet molds and allowed to harden. Control pellets consisted of 100% cholesterol.

Seizure Testing

Seizure testing was done 7 days after ADX and/or pellet replacement. This 7-day period allowed the animals sufficient time to recover from surgery. In addition, it has been shown that maximal increases of GABA uptake and significant decreases in GABA synthesis occur 7 days after ADX (9,36). Doses of bicuculline (2.0–6.0 mg/kg) were injected intraperitoneally (IP) in a volume of 0.01 ml/g body weight. The number of mice receiving this range of doses in each surgical group were as follows: 1) ADX, n = 11–17; 2) SHAM, n = 8–14; 3) ADX + CCS, n = 6–10; 4) ADX + DEX, n = 6–14; and 5) ADX + cholesterol, n = 4–9. The larger number of mice in some groups was due to the additional testing of some doses to assure repeatability of the results. Dexamethasone (DEX) replacement was tested in groups of mice receiving 3.0–5.0 mg/kg bicuculline. In the ADX groups, 6 SS mice and 5 LS mice were excluded from seizure testing because they did not recover from surgery.

After IP injection, mice were individually placed in a 1.5 liter glass jar for observation. Latencies from injection to clonus, defined as seizure onset for the purposes of this study, and tonus were recorded. Clonus, or seizure onset, is identified as a loss of righting reflex with convulsive movements in all extremities. Tonus is characterized by full caudal limb extension and respiratory arrest that may result in death. Observation time was limited to 900 s after injection; any animal not seizing within this time period was given a score of 900. To determine plasma levels of CCS after pellet replacement, immediately after seizure testing 50 μl of blood was collected via retro-orbital sinus punctures into heparanized micro-hematocrit tubes. Ten microliters plasma were extracted with absolute ethanol and proteins were pelleted by centrifugation at 2100 × g for 15 min in a tabletop International Clinical Centrifuge. CCS levels from the extracted plasma were evaluated using a modification of a radioimmunoassay (RIA) developed by Gwosdon-Cohen et al. (15) and described previously (34).

Data Analysis

Analysis of variance (ANOVA) was used to evaluate the effects of line of mouse, surgical treatment and dose of bicuculline. Post hoc comparisons (Newman-Keul's) among ADX, SHAM, ADX + CCS, ADX + DEX and ADX + cholesterol groups were used to determine the rank order of seizure latencies as a function of surgical treatment. ED_{450} values representing the dose that produces seizures by 450 s ± 95% confidence intervals were calculated from the seizure onset dose-response curves of ADX and SHAM-treated mice. Linear regressions were computed from individual latency scores at each dose. Analysis of variance was also used to evaluate body weight after surgery and plasma CCS levels in 10% CCS, 10% DEX, and cholesterol control pellet-implanted animals.

RESULTS

Latency to Seizure Onset

Comparison of SHAM, ADX, 10% CCS, and cholesterol

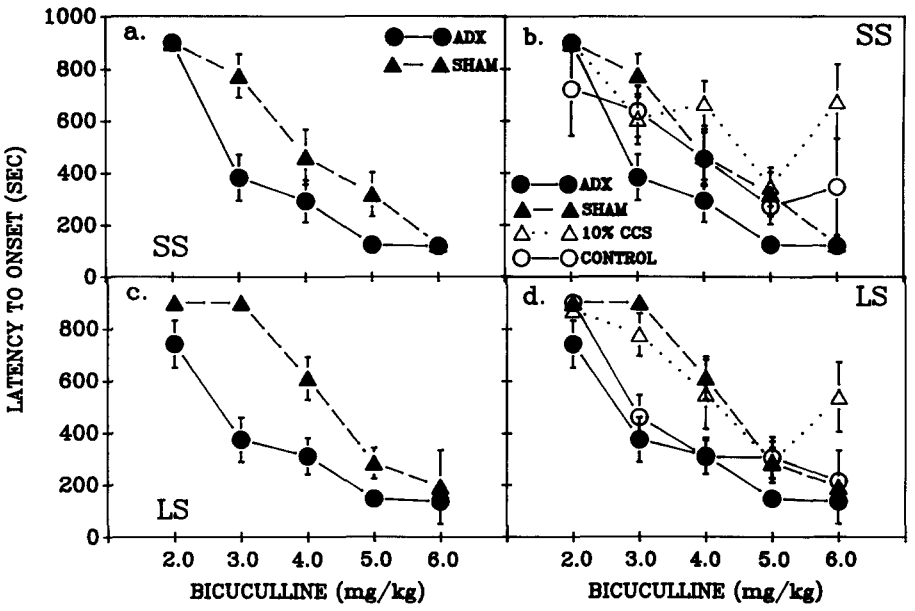

FIG. 1. Dose-response curves of latencies to bicuculline-induced seizure onset in SS and LS mice. (a and c) Effects of ADX on seizure latency. (b and d) Effects of 10% CCS and cholesterol control replacement on seizure latency. Scores are mean latencies in seconds ± SEM (n = 4–17 at each dose).

treatments in LS and SS mice showed significant main effects of surgery, $F(3,455) = 19.7$, $p<0.001$, and dose of bicuculline, $F(4,455) = 4.9$, $p<0.001$. Bicuculline decreased latency to seizures in a dose-dependent manner regardless of surgical treatment. Within the sham-operated group of animals, SS mice were more sensitive to bicuculline-induced seizures than LS mice, $F(1,116) = 4.9$, $p<0.05$, as shown in Fig. 1a and c. However, after ADX, the difference between the lines was lost and is represented by an increased susceptibility in both lines of mice. In both lines of mice, seizure thresholds were significantly reduced after ADX, $F(1,103) = 44.9$, $p<0.001$; $F(1,105) = 13.3$, $p<0.001$; LS and SS, respectively. The increased sensitivity was particularly apparent at doses between 3.0–5.0 mg/kg. The shift in the dose-response curves after ADX can also be demonstrated by a change in ED_{450} values. These values were calculated from the seizure onset dose-response curves in Fig. 1 and represent the dose that produces seizures by 450 s. In SS mice, the ED_{450} dose was decreased from 4.3 ± 0.38 mg/kg in sham-operated mice to 3.4 ± 0.37 mg/kg after ADX. A slightly greater reduction was observed in LS mice; after ADX the ED_{450} dose shifted to 3.0 ± 0.57 mg/kg from the SHAM value of 4.7 ± 0.29 mg/kg.

Figure 1b and d illustrate the effects of CCS and cholesterol-control pellets in ADX animals. In LS mice, replacement with 10% CCS returned seizure threshold latencies to SHAM values. In other words, seizure latencies in CCS-replaced and SHAM animals did not differ. Furthermore, in both lines of mice, 10% CCS replacement provided enhanced protection against the 6.0 mg/kg bicuculline dose over and above that displayed by any other treatment group ($p<0.05$). Replacement with 100% cholesterol pellets did not alleviate the effects of ADX in LS mice, but in SS mice it had a substantial effect such that animals with control-cholesterol pellets were not distinguishable from SHAM-SS mice.

Interpretation of steroid replacement experiments can be confounded because pituitary release of adrenocorticotropin (ACTH) after ADX also is altered due to changes in adrenal negative feedback mechanisms (7). Previous studies have also shown that circulating levels of ACTH modify GABA receptor binding (19). To determine whether changes in ACTH levels after ADX could alter susceptibility to bicuculline seizures, dexamethasone, a potent suppressor of ACTH release was administered to some mice. Figure 2 compares the effects of replacement with a 10% DEX, 10% CCS, or cholesterol on seizure latency at 3.0–5.0 mg/kg bicuculline in ADX mice.

Across lines of mice, there were significant effects of treatment, $F(4,378) = 12.5$, $p<0.001$, and dose, $F(2,378) = 46.7$, $p<0.0001$, on seizure onset. In SS mice, the significant effect of the various treatments, $F(4,192) = 5.3$, $p<0.001$, was due to the ADX mice having lower seizure latencies than any of the other treatment groups. DEX was as effective as CCS in returning seizure thresholds to SHAM values. This effect may not be a simple effect of DEX because all forms of replacement including the cholesterol-control pellets returned seizure latencies back to SHAM values. However, this effect of cholesterol was only observed in ADX + cholesterol mice; SHAM animals implanted with a cholesterol pellet did not show abnormal seizure values (data not shown).

In LS mice, the effects of DEX were more complicated. There were significant effects of treatment, $F(4,185) = 10.0$, $p<0.0001$, and dose, $F(2,185) = 31.5$, $p<0.0001$. Replacement with DEX raised latencies to onset toward SHAM values at the lowest 3.0 mg/kg bicuculline dose. At 4.0 mg/kg, replacement with 10% DEX did not reverse the effects of ADX in LS mice. After the 5.0 mg/kg dose of bicuculline, the difference between SHAM and ADX LS mice is very small and none of the treatments were different from the ADX condition.

Latency to Tonus

Higher doses of bicuculline were required to produce tonic seizures within the 900 s testing time in both lines of mice. Essentially the pattern of significant dose-responses, $F(4,455) =$

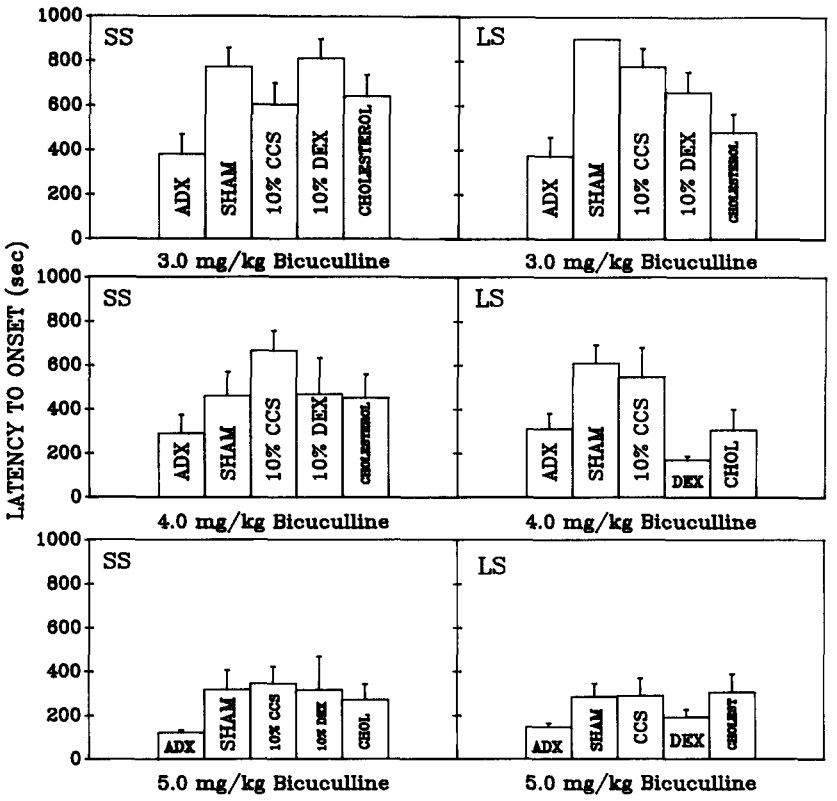

FIG. 2. Latencies to seizure onset at 3.0–5.0 mg/kg bicuculline in ADX, SHAM, 10% CCS-, 10% DEX-, and cholesterol-replaced SS and LS mice. Scores are mean latencies in seconds ± SEM (n = 6–16 at each dose).

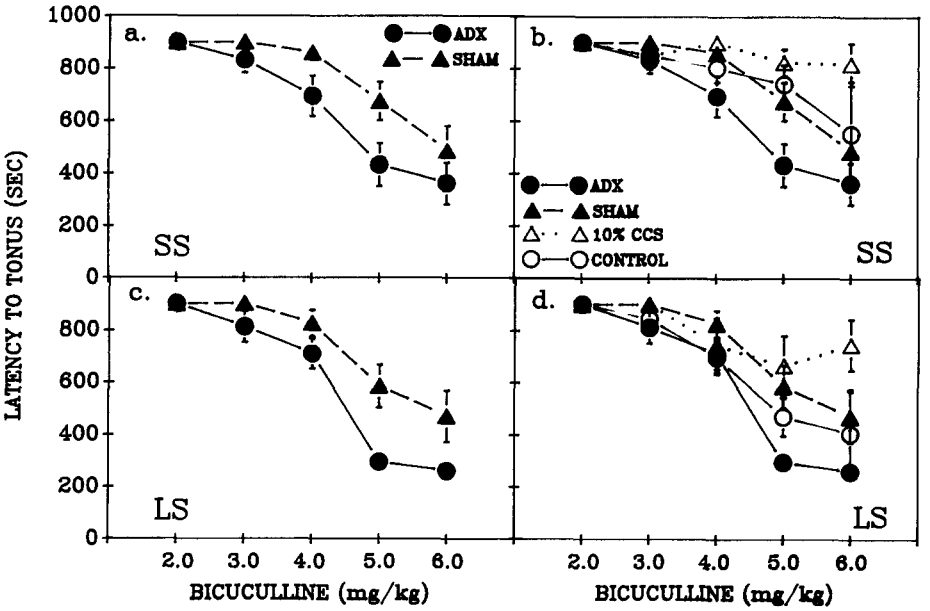

FIG. 3. Dose-response curves of latencies to bicuculline-induced tonus in SS and LS mice. (a and c) Effects of ADX on seizure latency. (b and d) Effects of 10% CCS and cholesterol control replacement on seizure latency. Scores are mean latencies in seconds ± SEM (n = 4–17 at each dose).

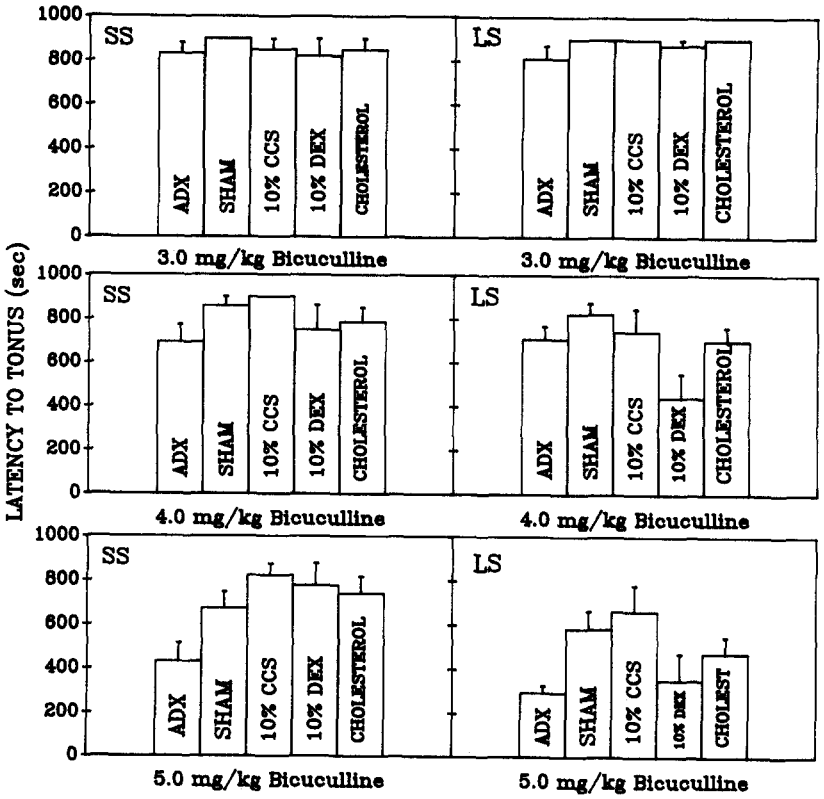

FIG. 4. Latencies to tonus at 3.0–5.0 mg/kg bicuculline in ADX, SHAM, 10% CCS-, 10% DEX-, and cholesterol-replaced SS and LS mice. Scores are mean latencies in seconds ± SEM (n = 6–16 at each dose).

45.0, $p<0.0001$, and treatment, $F(3,455)=16.5$, $p<0.0001$, demonstrated for onset was repeated for tonus with the exception that susceptibility to bicuculline-induced tonus did not differ between LS and SS mice in any treatment group. As was observed for seizure onset, ADX significantly shortened latencies to tonus in both LS and SS mice compared to SHAM animals, $F(1,103)=13.3$, $p<0.001$; $F(1,105)=7.7$, $p<0.01$, and replacement with 10% CCS in ADX animals returned latency scores to SHAM values in both lines of mice (Fig. 3). The enhanced anticonvulsant response in the CCS-replaced animals to the 6.0 mg/kg dose of bicuculline was also observed for tonus ($p<0.05$).

In comparing the effects of DEX and CCS replacement in ADX animals, the pattern for these effects was similar to that observed for seizure onset except that higher bicuculline doses were required to observe significant treatment effects (Fig. 4). At the 3.0 and 4.0 mg/kg doses, the ceiling of 900 s prohibited observation of any significant effect. In SS mice, at 5 mg/kg bicuculline, 10% DEX replacement was as effective as 10% CCS in returning latencies to tonus to SHAM values in ADX-SS mice, but again control cholesterol pellets were also effective. In ADX-LS animals, DEX was not effective in changing latency to tonus back to SHAM values at the 5.0 mg/kg dose of bicuculline.

Plasma CCS Levels and Body Weight

Plasma CCS was assayed to determine whether the various replacement treatments altered circulating CCS levels. These levels were determined in two different groups of mice, 1) those which had received bicuculline and 2) a separate group which received steroid implants that had not undergone seizures. This second group were tested totally independent of the first group, i.e., they received a different batch of steroid pellets and were tested at a completely different time. In the first group receiving bicuculline, both lines of mice showed significant treatment effects [LS mice: $F(2,108)=241$, $p<0.0001$; SS mice: $F(2,117)=47.7$, $p<0.0001$]. CCS levels in the CCS-replaced LS and SS mice were greater than in cholesterol-control and DEX-replaced animals ($p<0.01$) and were intermediate between basal and stress-induced levels observed previously in our laboratory in unoperated mice (unpublished data). In LS mice, plasma CCS levels in 10% CCS-replaced animals averaged 162.2 ± 7.6 ng/ml vs. 29.8 ± 2.7 ng/ml in cholesterol-control-treated mice and 22.2 ± 1.7 ng/ml in DEX-treated mice. The corresponding values in SS mice were 177.6 ± 14.9 vs. 30.1 ± 2.8 ng/ml in cholesterol-treated animals and 21.4 ± 3.5 ng/ml in 10% DEX-replaced mice.

In the second group of mice, replacements were performed with 10% CCS or cholesterol-control pellets. The values for circulating CCS levels were only slightly different from the first group. LS mice replaced with 10% CCS had 233 ± 20 ng/ml and those with cholesterol pellets 42 ± 5 ng/ml CCS. SS mice replaced with 10% CCS had 221 ± 15 ng/ml and those replaced with cholesterol had 37.0 ± 8.0 ng/ml. It is likely that small differences in the levels of CCS after replacement between these mice and those that had undergone seizures is due to the different batch of pellet implants.

Another group of naive animals were given vehicle injections and examined for CCS release as a function of time. The times were chosen to correspond to typical time periods for seizure latencies: immediately after saline injection, 300 s and 900 s. LS mice had 61 ± 7.8, 236 ± 28, and 221 ± 33 ng/ml CCS respec-

tively for these three time periods. SS mice had 68.5 ± 13, 91.5 ± 16, and 142 ± 30 ng/ml CCS for the same periods. LS and SS mice were significantly different for CCS release after vehicle injection with main effects of line, $F(1,89) = 16.1$, $p<0.001$, and time after injection, $F(2,89) = 15.9$, $p<0.001$, with the LS mice having a more responsive system.

Body weight decreased in LS mice, but not SS mice, after ADX, $F(3,153) = 24.9$, $p<0.001$. After ADX, the average weight in LS mice was approximately 22 grams compared to 27 grams in sham-operated animals. Corticosterone-replacement produced a slight gain in body weight in these mice (mean = 24.5 grams), but remained significantly less than SHAM animals (Newman-Keuls, $p<0.01$). DEX replacement also did not increase body weight in ADX-LS mice (mean = 21.9 grams).

DISCUSSION

After ADX, thresholds for bicuculline-induced seizures in LS and SS mice were substantially reduced. In the present study, the modifications of seizure activity by ADX and subsequent restoration toward normal values by steroid replacement indicates that susceptibility to these seizures may be regulated to a large degree by the HPA axis. Replacement with 10% CCS resulted in seizure latencies equal to those of intact (sham-operated) animals. Dexamethasone replacement provided protection against the effects of ADX at a low bicuculline dose in LS mice and also appeared to return seizure latencies in ADX-SS mice to SHAM values. This glucocorticoid analog is a powerful suppressor of ACTH and is more potent than CCS in inhibiting ACTH release from the anterior pituitary (46). Therefore, the effects of DEX pellet replacement indicates that pituitary release of ACTH may also be important in the regulation of seizure activity. After ADX, plasma ACTH levels rise and remain elevated up to 10 days postsurgery due to the removal of the adrenal negative feedback system (7). Therefore, the elevated levels of ACTH may influence seizure thresholds in general and the $GABA_A$ receptor complex more specifically. Evidence for the role of ACTH in seizure behavior is indicated by its clinical use in the treatment of myoclonic seizure disorders in infants (48). The greater effect of DEX replacement in SS mice suggests that the sensitivity to ACTH mediation of seizure latency may be more pronounced in this line of mouse.

Seizure activity is partially regulated by the GABAergic system (44); manipulations of GABA levels in brain will alter seizure sensitivity. Previous investigations have shown that adrenalectomy decreases the concentration of GABA in the central nervous system through two mechanisms. Miller et al. (37) demonstrated that ADX increases the high-affinity uptake of ^{3}H-GABA in hippocampal synaptosomes by 45%, 7 days after surgery. In addition, ADX interferes with the utilization of the cofactor, vitamin B_6, in the synthesis of GABA by the enzyme, glutamate decarboxylase (10) resulting in a 77% decrease in presynaptic levels of GABA 8 days post-ADX. The consequence of these two responses to ADX is to reduce the inhibitory actions of GABA at its postsynaptic site. Therefore, after ADX, the reduced levels of GABA may lead to enhanced binding of the $GABA_A$ antagonist, bicuculline, at the receptor thereby altering seizure thresholds.

Several steroid hormones have been shown to function as anticonvulsants and appear to influence brain excitability through modulation of the $GABA_A$ receptor complex (5, 6, 14). The rank order potency of steroids in the inhibition of ^{35}S-TBPS binding, including the ring-A reduced metabolites of progesterone and the adrenal glucocorticoid, deoxycorticosterone, coincide with their in vivo potencies as anticonvulsants and anesthetics (13). Whereas potentiation of GABA inhibition appears to be the physiologically relevant event for these steroids, modulation of the $GABA_A$ receptor by some glucocorticoids, including CCS, is more complex. In vitro responses to CCS (and its equivalent in humans, cortisol) have been reported to be biphasic; stimulation of GABAergic activity occurs at low nanomolar concentrations; however, at higher concentrations, inhibition is observed (26–28, 40). This could be due to alternate sites of action such that potentiation of GABA responses is accomplished by low concentrations of steroid acting at a putative steroid membrane-receptor site at or near the $GABA_A$ receptor complex, whereas steroids at higher concentrations interact at the membrane level perturbing receptor conformation (12). Perhaps the regulation of seizure thresholds by glucocorticoids is a steroid-receptor mediated activity. Therefore, occupation of the receptor by glucocorticoids would enhance inhibition by GABA, decreasing brain excitability and their removal by ADX would result in an increase.

Other neurotransmitter systems associated with brain excitability have also been shown to be affected by ADX and/or treatment with glucocorticoids. In hippocampal CA1 pyramidal cells, the norepinephrine-induced increase in the total number of action potentials was greater in cell slices from ADX rats. This was subsequently decreased below sham values with the addition of CCS (17). The time course of this inhibitory response suggested that genomic actions by steroids were responsible for the result and appeared to be independent of activity at the $GABA_A$ receptor. In our study the 7-day replacement with CCS would be sufficient for restoration of GABA levels in the CNS as well as changes in genomic activity mediating decreased excitability and could explain the return of seizure latencies to SHAM values.

The results observed in this investigation are inconsistent with those reported in a study by Sze and Maxson (49) in which audiogenic seizure susceptibility was blocked in ADX mice. This would suggest that glucocorticoids play a permissive or an excitatory role in the induction of seizures. However, the contradictions in our results may be due to the use of different seizure models that measure different parameters of seizure activity. Susceptibility to acoustic induction of audiogenic seizures is possible only in immature mice when the adrenocortical stress response is still developing (47). In addition, Lee et al. (20) reported a tendency for the latency to onset of kindled seizures in prepyriform cortex to be increased in ADX rats, although the rate of kindling remained unchanged.

Seizure-onset thresholds were significantly lower in SHAM-SS mice than in SHAM-LS animals. These results are in agreement with previous reports of bicuculline-induced seizure sensitivity in naive LS and SS mice (41,42). However, after ADX, the lines did not differ. This appears to be due to the fact that ADX produced a larger change in mean latency to seizure onset in LS mice than in SS mice at low doses of bicuculline. It is possible that the more reactive HPA axis in LS mice as evidenced by increased release of CCS after even a vehicle injection may be impart related to the differential susceptibility between LS and SS mice to an injection of bicuculline.

McIntyre et al. (24) showed that GABA enhancement of ^{3}H-FNZ binding in cortical membranes from LS mice was more sensitive to temperature shifts than membranes from SS mice. This study, as well as heat denaturation studies (32), have led to the suggestion that the $GABA_A$ receptor complex from the two lines of mice differ in their physical properties. In LS mice, bicuculline interactions at the GABA receptor may be more sensitive to the removal of endogenous steroids than those of SS mice.

The greater sensitivity to bicuculline-induced seizures in SHAM animals from the less ethanol-sensitive SS line, and conversely, the greater decrease in seizure susceptibility in LS mice after ADX may suggest a relationship between sensitivity to the sedative-hypnotic effects of ethanol and the effects of bicuculline at the $GABA_A$ receptor; i.e., brain excitability. However, a recent study

evaluating the genetic correlation between ethanol-induced sleep times and bicuculline-induced seizures in 26 LS × SS recombinant inbred strains indicated that these two responses are not genetically correlated; that is, they are not regulated by common genetic mechanisms (51). Other studies of the effects of bicuculline on ethanol-induced sleep times in LS and SS mice also suggest considerable complexity between the GABAergic system and genotype-dependent ethanol sensitivity (43). The fact that these responses may be regulated differently is not surprising in light of recent evidence. Aguayo (1) conducted a patch clamp study of individual mammalian GABAergic neurons and observed that some, but not all, GABAergic cells were responsive to ethanol. These results support the idea that various subtypes of GABAergic neurons have functionally different roles. Therefore, a genetic interpretation of the effects of ADX on bicuculline-induced seizures must be based on differences between LS and SS mice in $GABA_A$ receptor parameters and adrenocortical activity and not in the context of ethanol sensitivity. Definitive studies of potential LS/SS differences in GABAergic receptors, however, await better characterization of either purified receptors or the structural genes for the receptor complex in these mice.

The cholesterol effect in the SS mice was unexpected. The importance of cholesterol in stabilizing the ion channel activity of another receptor, the nicotinic cholinergic receptor, has been demonstrated in vitro (25); however, such a role for cholesterol in vivo remains to be determined. Regulation of endogenous cholesterol levels is largely a liver function, therefore, it is difficult to explain how exogenous cholesterol from the implanted pellet would make a significant contribution to overall membrane cholesterol content. Furthermore, LS and SS mice do not differ in cholesterol concentrations in synaptosomal plasma membranes (4). It seems unlikely that cholesterol replacement simply altered drug distribution of bicuculline because cholesterol implants in sham-operated mice did not alter seizure latencies or plasma CCS levels normally observed in sham-operated mice. Thus it remains unclear why seizure susceptibility in SS mice would be altered by implantation of a cholesterol pellet.

In summary, ADX significantly reduced seizure thresholds in LS and SS mice. Replacement with 10% CCS, or in some cases 10% DEX, resulted in a return of seizure latencies to SHAM values. These results suggest that GABA-related seizures are partially regulated by activity of the HPA axis, which in turn modulates GABA/BZ receptor function.

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REFERENCES

1. Aguayo, L. G. Demonstration that ethanol potentiates the GABA-activated Cl^- current in central mammalian neurons: A patch clamp study. Alcohol.: Clin. Exp. Res. 14:265; 1990.
2. Allan, A. M.; Harris, R. A. Gamma-aminobutyric acid and alcohol actions: neurochemical studies on long sleep and short sleep mice. Life Sci. 39:2005–2015; 1986.
3. Backstrom, T.; Bixo, M.; Hammarback, S. Ovarian steroid hormones: effects on mood, behavior and brain excitability. Acta Obstet. Gynecol. Scand. Suppl. 130:19–24; 1985.
4. Baker, R. C. Dissociation of cerebellar phospholipid composition and acute ethanol effects in mice selectively bred for differential sensitivity to ethanol. Alcohol. Drug Res. 7:291–299; 1987.
5. Belelli, D.; Gee, K. W. 5 alpha-pregnane-3 alpha, 20 alpha-diol behaves like a partial agonist in the modulation of GABA-stimulated chloride uptake by synaptoneurosomes. Eur. J. Pharmacol. 167:173–176; 1989.
6. Belelli, D.; Bolger, M. B.; Gee, K. W. Anticonvulsant profile of the progesterone metabolite 5 alpha-pregnane-3 alpha-ol-20-one. Eur. J. Pharmacol. 166:325–329; 1989.
7. Bohus, B.; DeKloet, E. R.; Velduis, H. D. Adrenal steroids and behavioral adaptation: relationship to brain corticoid receptors. In: Ganten, D.; Pfaff, D., eds. Current topics in neuroendocrinology. New York: Springer-Verlag; 1982:107–148.
8. Bowers, B. J.; Wehner, J. M. Interaction of ethanol and stress with the GABA/BZ receptor in LS and SS mice. Brain Res. Bull. 23:53–59; 1989.
9. Craig, C. R. Anticonvulsant activity of steroids: separability of anticonvulsant from hormonal effects. J. Pharmacol. Exp. Ther. 153: 337–343; 1966.
10. David, S.; Kalyankar, G. D. Effect of adrenalectomy on gamma-aminobutyric acid concentrations in the central nervous system. J. Neurochem. 46:161–165; 1986.
11. DeSouza, E. B.; Goeders, N. E.; Kuhar, M. J. Benzodiazepine receptors in rat brain are altered by adrenalectomy. Brain Res. 381: 176–181; 1986.
12. Gee, K. W. Steroid modulation of the GABA/benzodiazepine receptor-linked chloride ionophore. Mol. Neurobiol. 2:291–317; 1988.
13. Gee, K. W.; Chang, W. C.; Brinton, R. E.; McEwen, B. S. GABA-dependent modulation of the Cl^- ionophore by steroids in rat brain. Eur. J. Pharmacol. 136:419–423; 1987.
14. Gee, K. W.; Bolger, M. B.; Brinton, R. E.; Coirini, H.; McEwen, B. S. Steroid modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. J. Pharmacol. Exp. Ther. 246:803–812; 1988.
15. Gwosden-Cohen, A.; Chen, C. L.; Besch, E. L. Radioimmunoassay (RIA) of serum corticosterone in rats. Proc. Soc. Exp. Biol. Med. 170:29–34; 1982.
16. Holmes, G. L.; Weber, D. A.; Kloczko, N.; Zimmerman, A. W. Relationship of endocrine function to inhibition of kindling. Dev. Brain Res. 16:55–59; 1984.
17. Joels, M.; De Kloet, E. R. Effects of glucocorticoids and norepinephrine on the excitability in the hippocampus. Science 245:1502–1505; 1989.
18. Kakihana, R. Adrenocortical function in mice selectively bred for different sensitivity to ethanol. Life Sci. 18:1131–1138; 1976.
19. Kendall, D. A.; Fox, D. A.; Enna, S. J. Effect of gamma-vinyl GABA on bicuculline-induced seizures. Neuropharmacology 20:351–355; 1981.
20. Lee, P. H. K.; Zhao, D.; Mitchell, C. L.; Hong, J-S. Effects of corticosterone on shaking and seizure behavior induced by deep prepyriform cortex kindling. Neurosci. Lett. 82:337–342; 1987.
21. Loscher, W. Comparative assay of anticonvulsant and toxic potencies of sixteen GABAmimetic drugs. Neuropharmacology 21:803–810; 1982.
22. Loscher, W.; Frey, H. H. Effect of convulsant and anticonvulsant agents on level and metabolism of gamma-aminobutyric acid in mouse brain. Naunyn Schmiedebergs Arch. Pharmacol. 296:263–269; 1977.
23. McClearn, G. E.; Kakihana, R. Selective breeding for ethanol sensitivity; SS and LS mice. In: McClearn, G. E.; Deitrich, R. A.; Erwin, V. G., eds. Development of animal models as pharmacogenetic tools. Washington, DC: United States Government Printing Office; 1981: 147–159.
24. McIntyre, T. D.; Trullas, R.; Skolnick, P. Differences in the biophysical properties of the benzodiazepine/gamma-aminobutyric acid receptor chloride channel complex in the long-sleep and short-sleep mouse lines. J. Neurochem. 51:642–647; 1988.
25. McNamee, M. G.; Fong, T. M. Effects of membrane lipids and fluidity on acetylcholine receptor function. In: Aloia, R. C.; Curtain, C. C.; Gordon, L. M., eds. Lipid domains and the relationship to membrane function. New York: Alan R. Liss, Inc.; 1988:43–62.
26. Majewska, M. D. Antagonist-type interaction of glucocorticoids with the GABA receptor-coupled chloride channel. Brain Res. 418:377–382; 1987.
27. Majewska, M. D. Steroids and brain activity: essential dialogue be-

tween body and mind. Biochem. Pharmacol. 36:3781–3788; 1987.
28. Majewska, M. D.; Bisserbe, J. C.; Eskay, R. L. Glucocorticoids are modulators of $GABA_A$ receptors in brain. Brain Res. 339:178–182; 1985.
29. Marley, R. J.; Wehner, J. M. GABA enhancement of flunitrazepam binding in mice selectively bred for differential sensitivity to ethanol. Alcohol Drug Res. 7:25–32; 1986.
30. Marley, R. J.; Gaffney, D.; Wehner, J. M. Genetic influences on GABA-related seizures. Pharmacol. Biochem. Behav. 24:665–672; 1986.
31. Marley, R. J.; Freund, R. K.; Wehner, J. M. Differential response to flurazepam in long-sleep and short-sleep mice. Pharmacol. Biochem. Behav. 31:453–458; 1988.
32. Marley, R. J.; Stinchcomb, A.; Wehner, J. M. Further characterization of benzodiazepine receptor differences in long-sleep and short-sleep mice. Life Sci. 43:1223–1231; 1988.
33. Martin, B. J.; Marley, R. J.; Miner, L. L.; Wehner, J. M. Classical genetic analysis of GABA-related seizures. Pharmacol. Biochem. Behav. 29:501–507; 1988.
34. Martin, B. J.; Wehner, J. M. Influence of genotype on nicotine-induced increases of plasma corticosterone in mice as a result of acute nicotine pretreatment. Pharmacol. Biochem. Behav. 30:1065–1070; 1988.
35. Martz, A.; Deitrich, R. A.; Harris, R. A. Behavioral evidence for the involvement of gamma-aminobutyric acid in the actions of ethanol. Eur. J. Pharmacol. 89:53–62; 1983.
36. Mehta, A. K.; Ticku, M. K. Interactions of pentobarbital and phenobarbital with GABAergic drugs against chemoconvulsants in rats. Pharmacol. Biochem. Behav. 30:995–1000; 1988.
37. Miller, A. L.; Chaptal, C.; McEwen, B. S.; Peck, E. J. Modulation of high affinity GABA uptake into hippocampal synaptosomes by glucocorticoids. Psychoneuroendocrinology 3:155–164; 1978.
38. Miller, L. G.; Greenblatt, D. J.; Barnhill, J. G.; Thompson, M. L.; Shader, R. I. Modulation of benzodiazepine receptor binding in mouse brain by adrenalectomy and steroid replacement. Brain Res. 446: 314–320; 1988.
39. Newark, M. E.; Penry, J. K. Catamenial epilepsy: a review. Epilepsia 21:281–300; 1980.
40. Ong, J.; Kerr, D. I. B.; Johnston, G. A. R. Cortisol: a potent biphasic modulator at $GABA_A$-receptor complexes in the guinea pig isolated ileum. Neurosci. Lett. 82:101–106; 1987.
41. Peris, J.; Wehner, J. M.; Zahniser, N. R. [^{35}]TBPS binding sites are decreased in the colliculi of mice with a genetic predisposition to bicuculline-induced seizures. Brain Res. 503:288–295; 1989.
42. Phillips, T. J.; Dudek, B. C. Bicuculline-induced seizures in mice which differ in sensitivity to ethanol. Soc. Neurosci. Abstr. 9:1240; 1983.
43. Phillips, T. J.; Dudek, B. C. Modification of ethanol effects by bicuculline: genotype-dependent responses and inheritance. Psychopharmacology (Berlin) 98:544–548; 1989.
44. Roberts, E. GABA: the road to neurotransmitter status. In: Olsen, R. W.; Venter, J. C., eds. Benzodiazepine/GABA receptors and chloride channels: structural and functional properties. New York: Alan R. Liss, Inc.; 1986:1–39.
45. Rosciszewska, D.; Buntner, B.; Guz, I.; Zawisza, L. Ovarian hormones, anticonvulsant drugs, and seizures during the menstrual cycle in women with epilepsy. J. Neurol. Neurosurg. Psychiatry 49: 47–51; 1986.
46. Sakakura, M.; Yoshioka, M.; Kobayashi, M.; Takebe, K. The site of inhibitory action of a natural (corticosterone) and synthetic steroid (dexamethasone) in the hypothalamus-pituitary-adrenal axis. Neuroendocrinology 32:174–178; 1981.
47. Sapolsky, R. M.; Meaney, M. J. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res. Rev. 11:65–76; 1986.
48. Snead, O. C.; Benton, J. W.; Hosey, L. C.; Swann, J. W.; Spink, D.; Martin, D.; Rej, R. Treatment of infantile spasms with high-dose ACTH: efficacy and plasma levels of ACTH and cortisol. Neurology 39:1027–1031; 1989.
49. Sze, P. Y.; Maxson, S. C. Involvement of corticosteroids in acoustic induction of audiogenic seizure susceptibility in mice. Psychopharmacology (Berlin) 45:79–82; 1975.
50. Wand, G. S. Ethanol differentially regulates proadrenocorticotropin/endorphin production and cortiosterone secretion in LS and SS lines of mice. Endocrinology 124:518–526; 1989.
51. Wehner, J. M.; Pounder, J. I.; Bowers, B. J. The use of recombinant inbred strains to study mechanisms of drug action. In: Deitrich, R. A., ed. NIAAA Research Monographs. vol. 20. Washington, DC: U.S. Government Printing Office; 1990:345–380.
52. Zgombick, J. M.; Erwin, V. G. Ethanol differentially enhances adrenocortical response in LS and SS mice. Alcohol 5:287–294; 1988.