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Anticonvulsive Effect of Swim Stress in Mice

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PERIČIĆ, D., D. ŠVOB, M. JAZVINŠĆAK AND K. MIRKOVIĆ. *Anticonvulsive effect of swim stress in mice.* PHARMACOL BIOCHEM BEHAV **66**(4) 879–886, 2000. —To explore the possible involvement of glucocorticoids in the previously observed anticonvulsive effect of swim stress, mice were, prior to administration of convulsants, subjected to treatments that diminish or enhance plasma corticosterone levels. Aminoglutethimide, the inhibitor of steroid synthesis, failed to modify convulsant doses of picrotoxin, but enhanced threshold doses of pentylenetetrazole producing myoclonus and death, both in unstressed and stressed animals. The same drug prevented the effect of stress on pentylenetetrazole-induced running bouncing clonus (RB clonus) and abolished the appearance of tonic hindlimb extension (THE). Doses of kainic acid producing convulsions and death were not affected by stress, but they were enhanced by aminoglutethimide. Corticosterone administration could not imitate the effect of swim stress. Finasteride, a 5α-reductase inhibitor, did not interfere with the effect of stress on picrotoxin-induced convulsions. Swim stress failed to modify the binding of the convulsant t[^{3}H]-butylbicycloorthobenzoate [^{3}H]TBOB, to washed mouse forebrain membranes. The results confirmed an anticonvulsant effect of swim stress against convulsions produced by GABA-related convulsants, but they do not support the hypothesis suggesting the involvement of glucocorticoids or neurosteroids in this effect. © 2000 Elsevier Science Inc.

Swim stress Convulsions Corticosterone Aminoglutethimide Finasteride Picrotoxin Pentylenetetrazole Kainic acid t-[^{3}H]butylbicycloorthobenzoate ([^{3}H]TBOB) binding

IT has been shown that corticosteroids produce various alterations in the excitability of the central nervous system (41). Following changes in the glucocorticoids levels, both excitatory and inhibitory effects have been described. Although low concentrations of corticosterone tended to be excitatory (6,16,36), high concentrations of the same hormone tended to be inhibitory (16,21). Deprivation of adrenal steroids by surgical (10) or pharmacological manipulations (40,41) has also been reported to affect the onset of convulsions produced by different convulsants. However, the data appear to be contradictory. Whereas Bowers et al. (10) obtained decreased seizure latencies following bicuculline administration after adrenalectomy in mice, Roberts et al. (38) and Roberts and Keith (40,41) reported increased latencies to pentylenetetrazole-induced tonic hindlimb extensor convulsion following administration of aminoglutethimide, a steroid synthesis inhibitor (12,15). Aminoglutethimide has also attenuated the convulsive potency of kainic acid (40), the convulsant that binds to kainate receptors (13), a subtype of receptors for the excitatory amino acid glutamate. Stressful manipulations that are known to elevate plasma concentrations of glucocorticoids, interfere with the effects of convulsants as well. Thus, in our recent study (29) we have demonstrated that swim stress attenuates in rats the convulsive potency of bicuculline, a competitive antagonist of GABA binding sites, and we presumed that stress-induced release of glucocorticoids from the adrenal glands might be responsible for the observed anticonvulsive effect of stress.

Hence, the aim of this study was to check the previous hypothesis by using treatments known to enhance or deplete plasma corticosterone levels, and also by using another species—mice and two other GABA-related convulsants—picrotoxin and pentylenetetrazole. Kainic acid, previously described to be affected by mentioned changes in the plasma corticosterone levels (40,41) was taken for comparison. Because swim stress is known to enhance brain concentrations of $GABA_A$ receptor active neurosteroids (32), their possible involvement in stress-induced anticonvulsive activity was tested by using finasteride, the competitive 5α-reductase inhibitor (37). To evaluate whether swim stress-induced changes in the seizure threshold are associated with the changes in the characteristics of the convulsant binding site within the $GABA_A$ receptor complex, the effect of swim stress on the binding of another cage convulsant, [^{3}H]TBOB (t-[^{3}H]butylbicycloorthobenzoate), was studied.

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METHOD

Animals

Male CBA mice (25–30 g), 3 months old, were used. They were housed at a constant temperature (22°C) and with a light cycle of 12 h light/12 h darkness (lights on at 0700 h). They were caged in groups of 10. Food and water were freely available. Prior to experiment, the animals were not habituated either to IP or IV drug administration. The procedures used in the study were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Stress Procedure

Mice were subjected to swim stress (10-min swimming at 18–19°C). After swimming, the animals were dried by a towel and placed near the heater. The IV injection of convulsants started 15 min after termination of stress. Some animals were killed by cervical dislocation 15 min after stress, their forebrains were homogenized and taken for the analysis of [^{3}H]TBOB binding. Nonstressed control animals were taken for comparison.

Drugs

Picrotoxin, kainic acid, pentylenetetrazole, aminoglutethimide, and corticosterone acetate (all from Sigma, St. Louis, MO) were used. Picrotoxin and pentylenetetrazole were dissolved in saline. Kainic acid was dissolved in distilled water and pH was adjusted with 0.1 N NaOH. Aminoglutethimide and corticosterone acetate were dissolved in dimethyl sulfoxide (DMSO) and diluted in sesame oil, so that the final concentration of DMSO was 7%. Finasteride (Merck Sharp & Dohme) was dissolved in ethanol and diluted in sesame oil, so that the final concentration of ethanol was 10%. The later drugs were administered subcutaneously (SC) in a volume 1 ml/100 g body weight, 1 hr before starting the constant intravenous (IV) infusion of convulsants into the tail vein. Control animals received the corresponding vehicles.

Convulsive Activity

For determination of convulsive activity the animal was taken from its home cage and placed in a glass cylinder (20 × 7 cm) with numerous holes for ventilation. The tail of the animal was drawn through a hole of the plastic cover, and warmed for 1 min under a tensor lamp. A butterfly infusion needle (0.3 mm) was inserted into the tail vein, and correct placement was verified by the appearance of blood in the infusion tubing. During the infusion the animal was held tightly by the tip of the tail to allow free movement. The concentrations of drugs were: 0.75 mg/ml for picrotoxin, 5 mg/ml for kainic acid, and 4 mg/ml for pentylenetetrazole. The infusion rates were 0.55 ml/min for picrotoxin, 1.1 ml/min for kainic acid, and 0.275 ml/min for pentylenetetrazole. The animal was observed throughout infusion, and the time between the start of infusion and the onset of convulsive signs, mainly as described by Kosobud and Crabbe (23), was measured. In the case of picrotoxin, the convulsive signs were: running/bouncing clonus (RB clonus, violent whole-body clonus, including running and explosive jumps), and tonic hindlimb extension (THE, characterized by extreme rigidity, with forelimbs and hindlimbs extended caudally). For pentylenetetrazole, the first convulsive sign was myoclonus (a sudden involuntary muscle jerk, usually accompanied by a head twitch) followed by RB clonus and THE, while kainic acid convulsions started with writhing clonus (WR clonus, a brief episode of clonus characterized by writhing movements of the head and neck and clonic forelimbs movements) followed by THE (23). For each animal the threshold dose of convulsant (mg/kg of body weight) required to elicit a particular convulsant sign was calculated from the time of infusion, the infusion rate, the concentration of convulsant, and body weight. The time of death was also measured. All experiment were carried out between 0900 and 1300 h.

Preparation of the Membranes

Mouse forebrain membranes were homogenized in 40 vol. Tris HCl (50 mM, pH 7.4), and the membranes were prepared as described by Bitran and Dowd (9). Briefly, the homogenates were centrifuged at 20,000 × *g* for 20 min at 4°C. The pellet was washed in 50 mM Tris citrate buffer, pH 7.4, containing 100 mM NaCl, resuspended, and centrifuged four more times. The tissue was washed one final time with Tris citrate without NaCl. After a final centrifugation the resulting pellet was resuspended in 40 vol. Tris citrate and processed for [^{3}H]TBOB binding. The binding was determined in duplicate in a volume of 0.5 ml.

[^{3}H]TBOB Binding Assay

Aliquots of the cell membrane preparation (~90 μg protein) were incubated in 50 mM Tris-citrate buffer (pH = 7.4) containing 200 mM NaCl at 25°C for 90 min with [^{3}H]TBOB (Amersham, specific activity 24Ci/mmol). Data for Scatchard plots were obtained by adding varying concentrations (0–392 nM) of nonradioactive TBOB (a gift from Prof. H. I. Yamamura; dissolved at a concentration of 1 mM in dimethyl sulfoxide) to a fixed concentration (8 nM) of [^{3}H]TBOB. The total assay volume was 0.5 ml. Non-specific [^{3}H]TBOB binding, defined in the presence of 100 μM picrotoxin, was <23% of the total binding.

Protein Determination

Protein concentration was determined in 10 μl of membrane suspension according to Lowry et al. (25), using bovine serum albumin as standard.

Statistical Analysis

Results are expressed as mean values ± standard error of the mean (SEM). Statistical analysis of the results was by one-way analysis of variance (ANOVA), followed by a Newman–Keuls test and by two-way ANOVA when in the same experiment the factors stress and drug were studied. Student's *t*-test and Fisher's exact probability test were also used where suitable. *p*-Values of less than 0.05 were considered significant.

The binding data were analyzed by using a computer-based equilibrium binding data analysis (EBDA) program (28). EBDA calculates the apparent dissociation constant (K_d) and the maximum density of binding sites by Scatchard transformation of the saturation binding data.

RESULTS

As shown in Fig. 1, 15 min after exposure to swim stress (10 min swimming in water at 18–19°C) doses of picrotoxin producing RB clonus, THE, and death were approximately doubled, i.e., swim stress produced a clear anticonvulsive effect. One-way ANOVA [(RB clonus: $F(3, 25) = 57.27$; THE: $F(3, 25) = 49.92$; death: $F(3, 25) = 43.31$, $p < 0.001$ for all

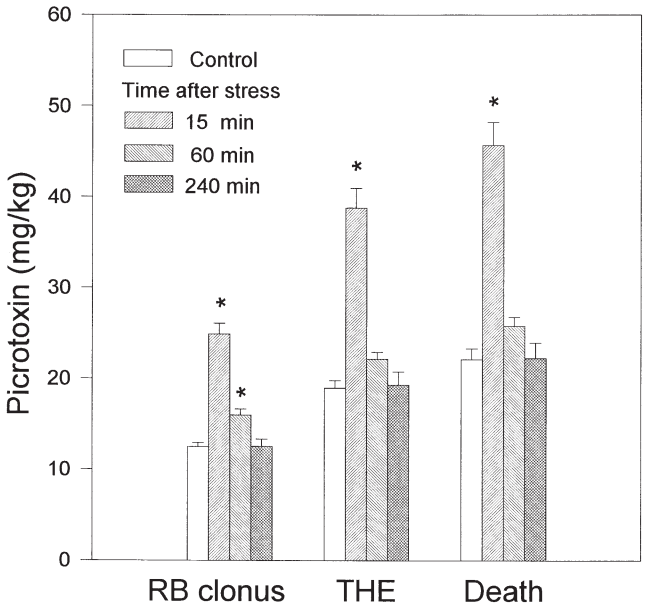

FIG. 1. Time course of the effect of swim stress on doses of picrotoxin producing RB clonus (running bouncing clonus), THE (tonic hindlimb extensor convulsion), and death in male CBA mice. Swim stress (10-min swimming in water at 18–19°C) finished 15, 60, or 240 min before the beginning of IV infusion of picrotoxin. The bars are means ± SEM from 6 (time points) or 11 (control) animals in the group. *$p < 0.01$ vs. the corresponding control (ANOVA followed by Newman–Keuls test).

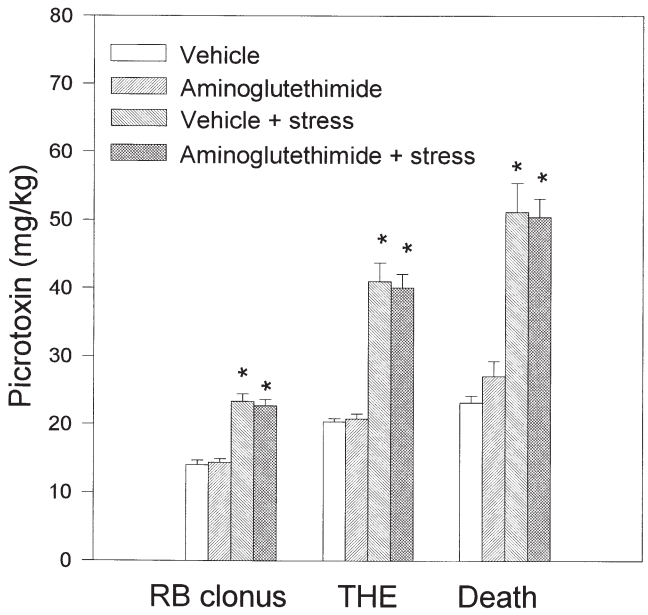

FIG. 2. Lack of effect of aminoglutethimide on swim stress-induced enhancement of the seizure threshold for picrotoxin in CBA mice. Aminoglutethimide (50 mg/kg) was administered SC 35 min prior to beginning of swim stress (10-min swimming in water at 18–19°C) and 1 h prior to beginning of IV infusion of picrotoxin. The bars are means ± SEM from (six to seven) animals in the group. *$p < 0.01$ vs. the corresponding unstressed group (ANOVA followed by Newman–Keuls test).

analyses] followed by Newman–Keuls test has confirmed a high significance ($p < 0.01$) of this effect. The effect on the RB clonus, but not on THE and death, was still present 60 min after stress. Two hundred and forty minutes after exposure to swim stress, all values returned to the control levels.

To test whether the effect of stress was due to an enhanced release of glucocoticoids, we pretreated the animals with aminoglutethimide, a steroid synthesis inhibitor (12,15), or with doses of corticosterone, known to produce in unstressed animals several fold increases in the plasma corticosterone levels (40).

Aminoglutethimide, given in a dose (50 mg/kg), which depletes adrenocortical hormones (39), failed to affect in both unstressed and stressed mice, the threshold doses of picrotoxin producing RB clonus, THE, and death (Fig. 2). In the same experiment, swim stress produced a previously shown anticonvulsive effect, resulting in, as demonstrated by one-way ANOVA, big differences between experimental groups [RB clonus: $F(3, 23) = 35.01$; THE: $F(3, 21) = 35.94$; death: $F(3, 23) = 26.69$; $p < 0.001$ for all analyses]. Two-way ANOVA has also demonstrated a lack of effect of drug, and a highly significant ($p = 0.0001$) effect of stress [RB clonus: $F(1, 23) = 104.36$; THE: $F(1, 21) = 107.61$; death: $F(1, 23) = 79.39$]. The interaction between drug and stress was in neither of these cases significant.

As indicated by the two-way ANOVA, aminoglutethimide, given in the same dose (50 mg/kg), enhanced the threshold doses of pentylenetetrazole, another GABA-related convulsant (26,34), producing myoclonus, $F(1, 40) = 11.69$, $p < 0.001$, RB clonus, $F(1, 41) = 21.32$, $p < 0.0001$, and death, $F(1, 41) = 80.34$, $p < 0.0001$, but abolished ($p < 0.01$, Fisher's test) in unstressed and stressed animals the appearance of THE

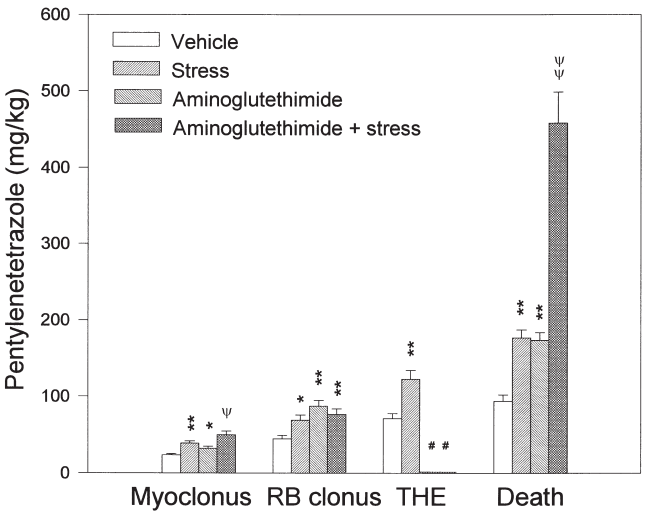

FIG. 3. Effect of aminoglutethimide and swim stress on threshold doses of pentylenetetrazole producing convulsive signs and death in male CBA mice. Aminoglutethimide (50 mg/kg) was administered SC 35 min prior to beginning of swim stress and 1 h prior to beginning of IV infusion of pentylenetetrazole. The bars are means ± SEM from 8–15 animals in the group. *$p < 0.05$; **$p < 0.01$ vs. vehicle-treated group (ANOVA followed by Newman–Keuls test, or Student's t-test in the case of THE). $^{\psi}p < 0.01$ vs. vehicle treated and $p < 0.05$ vs. two other groups; $^{\psi\psi}p < 0.01$ vs. all other groups (ANOVA followed by Newman–Keuls test). In amnoglutethimide-treated groups the appearance of THE (tonic hindlimb extensor convulsion) was absent. $^{\#}p < 0.01$ vs. vehicle-treated groups (Fisher's exact probability test).

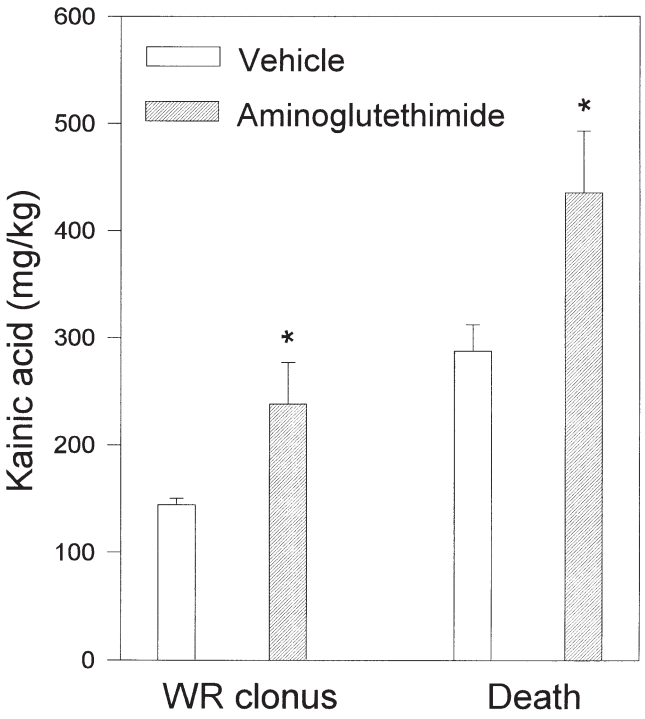

FIG. 4. Effect of aminoglutethimide on the convulsive activity induced in mice by IV infusion of kainic acid. Aminoglutethimide (50 mg/kg) was administered SC 1 h prior to beginning of IV infusion of kainic acid. The bars are means ± SEM from seven animals in the group. *$p < 0.05$ vs. vehicle-treated group (Student's *t*-test).

(Fig. 3). In the same experiment stress enhanced the threshold doses of pentylenetetrazole producing myoclonus, $F(1, 40) = 31.99$, $p < 0.0001$, and death, $F(1, 41) = 107.02$, $p < 0.0001$. However, as indicated by two-way ANOVA, stress failed to affect significantly threshold doses of pentylenetetrazole producing RB clonus, $F(1, 41) = 0.97$, NS. Namely, whereas the effect of stress was present in vehicle-treated (53% enhancement of the seizure threshold for this convulsive sign), it was not present in aminoglutethimide-treated animals, indicating that aminoglutethimide prevented the effect of stress on pentylenetetrazole-induced RB clonus. Differences in the effect of stress between vehicle-treated and drug-treated mice were also reflected in a significant drug × stress interaction, $F(1, 41) = 6.46$, $p < 0.01$. Opposite to this effect, the influence of stress on threshold doses of pentylenetetrazole producing death was greater in aminoglutethimide (163% increase) than in vehicle-treated (88.6% increase) mice. Again, this was reflected in a significant drug × stress interaction, $F(1, 41) = 32.36$, $p < 0.0001$.

TABLE 1

LACK OF EFFECT OF SWIM STRESS ON KAINIC ACID-INDUCED CONVULSIONS IN MALE CBA MICE

	Kainic Acid (mg/kg)			
	WR Clonus	THE	Death	
Control	170.18 ± 11.85	271.04 ± 11.14	292.98 ± 11.53	(7)
Swim stress	164.05 ± 9.83	264.05 ± 12.77	300.38 ± 6.15	(6)

Swim stress (10-min swimming in water at 18–19°C) finished 15 min before the beginning of IV infusion of kainic acid. The values are means ± SEM. Numbers in parentheses denote number of animals in the group.

Aminoglutethimide also enhanced ($p < 0.05$; Student's *t*-test) the threshold doses of kainic acid producing WR clonus and death (Fig. 4), but swim stress failed to affect doses of kainic acid producing convulsions and death (Table 1).

Unlike aminoglutethimide, corticosterone given in a dose (1 mg/kg), previously shown to lower the seizure threshold for pentylenetetrazole and kainic acid (45), in our study proved to be inactive when given prior to two mentioned convulsants (Table 2).

Corticosterone (0.33, 1.0, and 3.0 mg/kg) administered 15 (results not shown) or 60 min prior to IV infusion of picrotoxin, also failed to affect the threshold doses of picrotoxin producing two convulsive signs and death in male CBA mice (Table 3).

Despite stress-induced elevations of threshold doses of picrotoxin and pentylenetetrazole producing convulsive signs and death, swim stress failed to affect the binding of another convulsant, [^{3}H]TBOB, to washed mouse forebrain membranes (Fig. 5). Namely, both the affinity and the maximum number of binding sites remained unchanged in mice killed 15 min after exposure to swim stress.

The possible role of neuroactive steroids in the anticonvulsant effect of swim stress was tested by using finasteride, a 5α-reductase inhibitor (37), in combination with picrotoxin. As indicated by the two-way ANOVA, the effects of stress on threshold doses of picrotoxin producing two convulsive signs and death were again very pronounced [RB clonus: $F(1, 27) = 164.95$; THE: $F(1, 26) = 201.69$; death: $F(1, 26) = 233.28$; $p < 0.0001$ for all analyses], but finasteride failed to produce an effect and the interaction between drug and stress was also insignificant (Table 4).

DISCUSSION

This study has extended our previous study (29) and confirmed that swim stress has an anticonvulsive effect. This was demonstrated previously on rats by using bicuculline, a competitive antagonist of GABA binding site, and in this study on mice by using picrotoxin and pentylenetetrazole, two convulsants supposed to inhibit competitively the binding of [^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS) to $GABA_A$ receptor (33,34). Because swimming at room temperature also enhanced the threshold doses of picrotoxin producing convulsive signs and death, and immobilization stress enhanced the latency of convulsions produced by IP administration of the same convulsant (Perićić et al., unpublished results), one might presume that the anticonvulsant effect of swim stress, described in this study, could not be explained by cold-induced changes in hemodynamic parameters and, therefore, in the brain distribution of GABA-related convulsants.

The anticonvulsive effect of stress was absent when convulsions were produced by kainic acid, a GABA nonrelated convulsant. Soubrie et al. (45) have also demonstrated a decreased convulsant potency of picrotoxin and pentylenetetrazole following stressful manipulations in rats, while Drugan et al. (17) reported that control over stress has an anticonvulsive effect. Namely, these authors have demonstrated that a single session of escapable shock enhanced the latency to picro-

TABLE 2

LACK OF EFFECT OF CORTICOSTERONE ON THE CONVULSIONS PRODUCED IN MALE CBA MICE BY IV INFUSION OF PENTYLENETETRAZOLE OR KAINIC ACID

	Clonus*	THE	Death	
Pentylenetetrazole (mg/kg)				
Vehicle	20.70 ± 0.44	75.31 ± 3.42	90.05 ± 3.21	(6)
Corticosterone	20.81 ± 0.49	71.07 ± 1.53	85.35 ± 2.09	(7)
Kainic acid (mg/kg)				
Vehicle	117.48 ± 3.05	263.08 ± 5.99	278.50 ± 6.35	(13)
Corticosterone	110.46 ± 5.46	257.92 ± 7.78	276.07 ± 7.75	(16)

Corticosterone (1 mg/kg) was administered SC 1 h prior to IV infusion of convulsant. The values are means ± SEM. Numbers in parentheses denote number of animals in the group.

*In the case when convulsions were produced by pentylenetetrazole, the first convulsant sign was myoclonus, and in the case when they were produced by kainic acid, this symptom was writhing clonus (WR clonus).

toxin-induced seizures, while inescapable shock failed to show this effect. Changes in the response to picrotoxin were accompanied with the reduction in [^{35}S]TBPS binding. In our study, presumably due to the fact that we used well-washed membrane preparation, swim stress-induced changes in the convulsant potency of picrotoxin and pentylenetetrazole were not accompanied by concomitant changes in the binding of [^{3}H]TBOB, another radioligand for GABA-regulated chloride ionophore (24,48). Accordingly, unlike the studies that suggest an inhibitory effect of stress on the brain GABA system (7,8,14,19), these results support other studies that have shown that stressful manipulations in rats decrease convulsant potency of bicuculline (18,29), picrotoxin, and pentylenetetrazole (1,45), as well as the studies that suggested an augmented function of the brain GABA system following acute swim stress (3,4,44,45). These contradictory reports might presumably suggest that the response of the GABA system depends on the type of stressor. Besides, one cannot always exclude the possibility that the animals could have been previously (during the prenatal and/or postnatal period) stressed, which may also interfere with the response of an animal in a given experiment (11,27).

Furthermore, this study suggested that glucocorticoids are not involved in the anticonvulsive effect of swim stress. This was demonstrated by two different treatments shown in earlier studies to produce extensive changes in the plasma corticosterone levels. Namely, the administration of aminoglutethimide, a steroid synthesis inhibitor (12,15), in a dose previously described to inhibit stress-induced corticosterone release (39), failed to affect the seizure threshold for picrotoxin, either in nonstressed or in stressed animals, suggesting that enhanced glucocorticoids are not responsible for the observed anticonvulsive effect of stress. However, in accordance with the previous studies (40,41), aminoglutethimide displayed its anticonvulsant effectiveness against kainic acid and pentylenetetrazole-induced convulsions. Because swim stress, which elevates, and aminoglutethimide, which depletes, plasma corticosterone levels displayed antipentylenetetrazole

TABLE 3

LACK OF EFFECT OF CORTICOSTERONE ON PICROTOXIN-INDUCED CONVULSIONS IN MICE

	Picrotoxin (mg/kg)			
	RB clonus	THE	Death	
Vehicle	13.22 ± 0.46	18.29 ± 0.46	20.85 ± 0.63	(16)
Corticosterone (mg/kg)				
0.33	12.84 ± 0.75	18.45 ± 0.97	21.30 ± 1.07	(7)
1.0	14.28 ± 0.57	19.07 ± 0.63	21.98 ± 0.77	(15)
3.0	13.56 ± 0.72	18.13 ± 0.67	20.64 ± 0.94	(6)

Different doses of corticosterone were given SC 1 h prior to IV infusion of picrotoxin. Data are means ± SEM. Numbers in parentheses denote number of animals in the group.

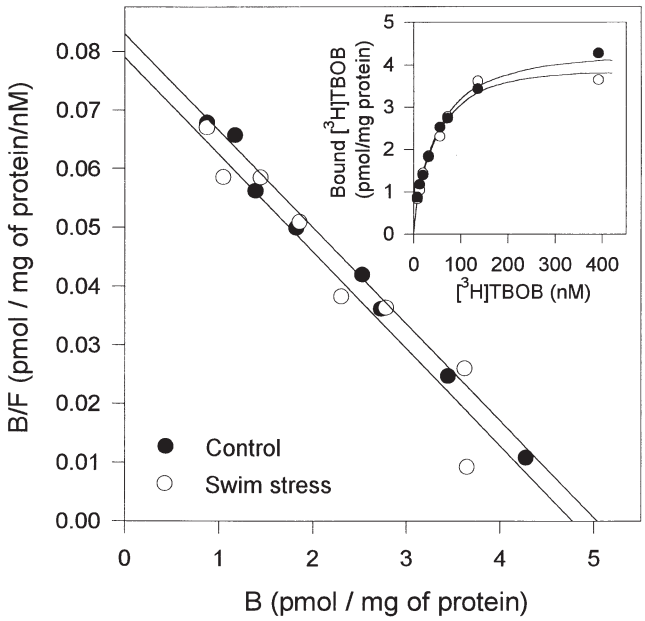

FIG. 5. Scatchard analysis of [^{3}H]TBOB binding to mouse forebrain membranes. The animals were killed by cervical dislocation 15 min after stress. Membranes were incubated with increasing concentrations of nonradioactive TBOB and a fixed concentration (8 nM) of [^{3}H]TBOB so that eight final concentrations (8–400 nM) were achieved. Radioactivity bound to membranes was determined after rapid filtration on Whatman GF/C filters. Binding in the presence of picrotoxin (100 μM) was subtracted from total [^{3}H]TBOB) binding to give specific [^{3}H]TBOB binding. Data were subjected to Scatchard analysis. Inset: saturation isotherms. The values represent one typical experiment. Two other experiments gave similar results.

TABLE 4
LACK OF EFFECT OF FINASTERIDE ON SWIM STRESS-INDUCED ENHANCEMENT OF THRESHOLD DOSES OF PICROTOXIN PRODUCING CONVULSIVE SIGNS AND DEATH IN MALE CBA MICE

	Picrotoxin (mg/kg)			
	RB Clonus	THE	Death	
Vehicle	14.89 ± 0.71	24.36 ± 0.69	29.02 ± 0.92	(8)
Swim stress	26.56 ± 1.13*	51.20 ± 2.10*	60.93 ± 2.04*	(7)
Finasteride	16.21 ± 0.69	25.16 ± 2.49	30.76 ± 3.09	(7)
Finasteride + swim stress	26.16 ± 0.83*	50.21 ± 1.78*	60.69 ± 1.76*	(8)

Finasteride (125 mg/kg) was administered SC 65 min prior to beginning of stress and 90 min prior to IV infusion of convulsant. The values are means ± SEM. Numbers in parentheses denote number of animals in the group.

*$p < 0.01$ vs. the corresponding unstressed group (ANOVA followed by Newman–Keuls test).

activity, it is obvious that this anticonvulsant activity cannot be explained by alterations in the plasma corticosterone levels. Although swim stress and aminoglutethimide produced similar enhancements of threshold doses of pentylenetetrazole producing RB clonus (82 and 85%, respectively), the effect of swim stress was greater than the effect of drug on the onset of myoclonic twitch (66 vs. 38%), and smaller than the effect of drug on the onset of death (53 vs. 95%). However, the main difference was in the appearance of the third convulsant sign. Namely, aminoglutethimide prevented the appearance of THE, suggesting that brain circuits responsible for this convulsant sign were completely protected by aminoglutethimide. The significant interactions between aminoglutethimide and stress in threshold doses of pentylenetetrazole producing RB clonus and death also suggest different mechanisms of action for the two treatments, but also for the two signs. The effects of stress and drug were additive only in the case of myoclonus. In the case of RB clonus, aminoglutethimide pretreatment prevented, and in the case of death it potentiated, the anticonvulsive effect of swim stress. The fact that the same treatment affects differently different convulsive sign is in accordance with the suggestion that the convulsive responses that appear following the administration of convulsants represent three qualitatively distinct seizure components mediated by separable and independent anatomical circuits located in forebrain and hindbrain (20). The fact that aminoglutethimide has some central effects even in adrenalectomized animals (2) suggests that its protective effect against convulsions and death produced by pentylenetetrazole and kainic acid could also be explained by the mechanisms other than the inhibition of corticosteroid synthesis. However, it remains unanswered why aminoglutethimide is ineffective against picrotoxin, the cage convulsant that like pentylenetetrazole inhibits competitively the binding of [^{35}S]TBPS (33,34). This radioligand binds at or near the picrotoxin-sensitive anion recognition sites of the $GABA_A$ receptor (49). It has been reported that the convulsant potencies of pentylenetetrazole and other tetrazoles are highly correlated with actions on $GABA_A$ receptors (34,47). However, they were four to eight times more potent in displacing specific [^{35}S]TBPS than [^{3}H]diazepam binding from rat brain membranes (34,35,47). Picrotoxin chemically does not belong to the same group of convulsants as pentylenetetrazole, and its potency to displace 50% of [^{35}S]TBPS bound to rat brain membranes (46), or [^{3}H]TBOB bound to recombinant $\alpha_1\beta_2\gamma_{2s}$ $GABA_A$ receptors (31) is more than 2000 times greater than the potency of pentylenetetrazole (IC_{50}: 251:37 ± 21.06 nM for picrotoxin vs. 537.19 ± 23.85 μM for pentylenetetrazole; our unpublished results). On the other hand, the potency of picrotoxin in producing convulsions or death is in comparison with pentylenetetrazole, after IV administration of both convulsants, greater by a factor less than 20. However, it has been reported that brain levels of pentylenetetrazole and related tetrazoles measured at the onset of generalized seizures are well within the range of the IC_{50} concentrations for displacement of specific [^{35}S]TBPS binding [see ref. (47)].

If stress would produce its anticonvulsive effect by enhanced release of glucocorticoids from the adrenal glands, than adrenalectomy would prevent this effect, while the administration of exogenous corticosterone would produce the same anticonvulsive effect as the swim stress. Although an earlier study has demonstrated that adrenalectomy prevented the effect of swim stress on the activity of the $GABA_A$ receptor/CI ion channel (43), in our recent study stress produced gender-specific anticonvulsive effect even in the animals completely deprived of steroid hormones on the peripheral origin (30). Besides, corticosterone given in doses producing severalfold increases in the plasma corticosterone levels, as well as a proconvulsant activity in combination with pentylenetetrazole and kainic acid (40), failed to affect in our study the onset not only of picrotoxin, but also of pentylenetetrazole and kainic acid-induced convulsions. However, a lack of effect of corticosterone on pentylenetetrazole-induced clonic convulsions has also been described (5). As far as we know, the only difference between our study and the study of Roberts and Keith (40) is in the strain of mice. Although these authors have used genetically heterogeneous mice, our study was done on CBA mice. Strain-related differences in the sensitivity to various convulsants have already been described (23,42). Hence, one cannot exclude the possibility that the response to corticosterone and the interaction of corticosterone with convulsants, might also be strain dependent.

Because swim stress provokes an increase of $GABA_A$ receptor-active steroids (32), some of which have anticonvulsant properties (22), it could have been presumed that the anticonvulsive effects of stress observed in our study might be due to stress-induced elevation of brain neurosteroids. However, our results with finasteride, which blocks the conversion of progesterone to its neuroactive steroid metabolite allopregnanolone (37), failed to support this presumption.

Namely, finasteride affected neither control nor stress-elevated threshold doses of picrotoxin producing two convulsant signs and death in mice.

In conclusion, swim stress enhanced, presumably by a glucocorticoid and neurosteroid unrelated mechanism, the threshold doses of picrotoxin and pentylenetetrazole, producing convulsant sign and death in male CBA mice, suggesting the possibility that this kind of stress activates the brain GABA system. Swim stress failed to affect the convulsions and death produced by kainic acid, a GABA-unrelated convulsant. The possible implications of this finding and the mechanisms modulating GABAergic neurotransmission in response to swim stress should be studied further.

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