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Neuroactive Steroids Protect Against Pilocarpine- and Kainic Acid-induced Limbic Seizures and Status Epilepticus in Mice

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Summary—Several structurally related metabolites of progesterone (3α-hydroxy pregnane-20-ones) and deoxycorticosterone (3α-hydroxy pregnane-21-diol-20-ones) and their 3β-epimers were evaluated for protective activity against pilocarpine-, kainic acid- and *N*-methyl-D-aspartate (NMDA)-induced seizures in mice. Steroids with the 3-hydroxy group in the α-position and 5-H in the α- or β-configurations were highly effective in protecting against pilocarpine (416 mg/kg, s.c.)-induced limbic motor seizures and status epilepticus (ED_{50} values, 7.0–18.7 mg/kg, i.p.). The corresponding epimers with the 3-hydroxy group in the β-position were also effective but less potent (ED_{50} values, 33.8–63.5, i.p.). Although the neuroactive steroids were considerably less potent than the benzodiazepine clonazepam in protecting against pilocarpine seizures, steroids with the 5α,3α-configuration had comparable or higher protective index values (TD_{50} for motor impairment ÷ ED_{50} for seizure protection) than clonazepam, indicating that some neuroactive steroids may have lower relative toxicity. Steroids with the 5α,3α- or 5β,3α-configurations also produced a dose-dependent delay in the onset of limbic seizures induced by kainic acid (32 mg/kg, s.c.), but did not completely protect against the seizures. However, when a second dose of the steroid was administered 1 hr after the first dose, complete protection from the kainic acid-induced limbic seizures and status epilepticus was obtained. The steroids also caused a dose-dependent delay in NMDA (257 mg/kg, s.c.)-induced lethality, but did not completely protect against NMDA seizures or lethality. We conclude that neuroactive steroids are highly effective in protecting against pilocarpine- and kainic acid-induced seizures and status epilepticus in mice, and may be of utility in the treatment of some forms of status epilepticus in humans. Published by Elsevier Science Ltd.

Keywords—Neuroactive steroid, pilocarpine, kainic acid, *N*-methyl-D-aspartate (NMDA), status epilepticus, seizure.

Neuroactive steroids are endogenous metabolites of certain steroid hormones (and their synthetic analogs) that rapidly alter the excitability of neurons by direct actions on ligand-gated ion channels (for reviews, see Majewska, 1992; Paul and Purdy, 1992). The γ-aminobutyric acid ($GABA_A$) receptor Cl^- channel complex is a major target for the action of neuroactive steroids in the central nervous system, and it is now well recognized that certain neuroactive steroids can potentiate GABA-mediated inhibitory responses (Majewska, 1992; Paul and Purdy, 1992). In particular, endogenous 3α-hydroxy ring-A reduced metabolites of progesterone (5α,3α-pregnanolone and 5β,3α-pregnanolone), produce a powerful enhancement of $GABA_A$ receptor responses *in vitro* (Majewska *et al.*, 1986; Harrison *et al.*, 1987; Gee *et al.*, 1988; Peters *et al.*, 1988) and have potent anticonvulsant, anxiolytic and sedative activity when administered *in vivo* (Hogskilde *et al.*, 1988; Belelli *et al.*, 1989; Bitran *et al.*, 1991; Wieland *et al.*, 1991).

We have recently reported that several structurally related metabolites of progesterone and deoxycorticosterone are potent anticonvulsants against pentylenetetrazol (PTZ)-induced seizures in mice and their potencies in this model are well correlated with their potencies for GABA-activated Cl^- current potentiation (Kokate *et al.*, 1994). Although the protective activity of neuroactive steroids against seizures induced by $GABA_A$ receptor

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antagonists is now well recognized, there is no information regarding the effects of neuroactive steroids in seizure models produced by other chemoconvulsants, such as pilocarpine and kainic acid. However, drugs that potentiate $GABA_A$ receptor responses, such as benzodiazepines, are highly effective in these models (Zaczek *et al.*, 1981; Heggli and Malthe-Sorenssen, 1982; Turski *et al.*, 1987, 1989, 1990), and we therefore hypothesized that the neuroactive steroids might also have protective activity. Consequently, in the present study, we evaluated a series of metabolites of progesterone and deoxycorticosterone and their isomers for protection against pilocarpine- and kainic acid-induced limbic seizures and status epilepticus in mice. For comparison, we also investigated the effects of the neuroactive steroids on NMDA-induced seizures and lethality.

MATERIALS AND METHODS

Animals

Male National Institutes of Health (NIH) Swiss mice (25–30 g) were obtained from the NIH animal program. Animals were allowed to acclimatize with free access to food and water for a 24-hr period before testing. All procedures were carried out under strict compliance with the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the NIH Animal Use Committee.

Pilocarpine seizure test

Mice were injected intraperitoneally (i.p.) with a test compound or vehicle and 20 min later received a subcutaneous (s.c.) injection of a CD_{97} dose (i.e. dose producing seizures in 97% of animals) of pilocarpine (416 mg/kg; see Results). At the time of injection of the test compound, the animals also received 1 mg/kg scopolamine methyl bromide to block the peripheral cholinergic effects of pilocarpine (Turski *et al.*, 1983). In control experiments, scopolamine alone at this dose did not protect animals against pilocarpine-induced limbic motor seizures and status epilepticus. The interval between the injection of the test compound and the injection of pilocarpine was chosen to correspond with the time of maximal anticonvulsant effect of neuroactive steroids which occurs from 10 to 20 min after administration (Kokate *et al.*, 1994). Animals were observed for at least 2 hr after the pilocarpine injection. All control mice had severe clonic–tonic seizures followed in most cases by death (see Results). Mice failing to show generalized clonic seizures lasting longer than 10 sec were scored as protected.

Kainic acid seizure test

Mice were injected i.p. with a test compound or vehicle and 10 min later received a s.c. injection of kainic acid at a dose of 32 mg/kg (previously determined CD_{97} value; Yamaguchi *et al.*, 1993). In some animals, a second injection of the test compound or vehicle was administered 1 hr after the first dose (i.e. 50 min after the kainic acid injection). Control animals exhibited typical limbic seizures characterized by repetitive rearing and falling, whole body jerks, facial clonus, severe forelimb clonus, occasional opisthotonos and Straub tail, and infrequent explosive running. In most cases the repetitive limbic seizures progressed into status epilepticus which lasted for hours and was associated with lethality in many animals. (In a series of 50 control animals, 39 expressed periods of continuous limbic seizures lasting for at least 10 min and 28 died.) Animals were observed for at least 2 hr and were scored as protected if they failed to show forelimb clonus lasting more than 10 sec (as exhibited by 49 of the 50 control animals).

NMDA-induced seizures and lethality

Mice were injected i.p. with a test compound or vehicle and 10 min later received a s.c. injection of NMDA at a dose of 257 mg/kg, s.c. (previously determined CD_{97} value; Yamaguchi *et al.*, 1993). Animals were observed for at least 2 hr and were scored as protected if they survived this period. NMDA alone produced explosive running and jumping, followed by posturing (opisthotonos and Straub tail) and clonic movements of the forelimbs and hindlimbs, and ultimately tonic extension and death. Lethality was obtained in all 78 control animals tested (usually within 5–10 min).

Drugs

Isomeric 3-hydroxy pregnane-20-ones and 3-hydroxy pregnane-21-diol-20-ones are identified using the nomenclature 5*x*,3*y*-pregnanolone (5*x*-pregnane-3*y*-ol-20-one) and 5*x*,3*y*-THDOC (5*x*-pregnane-3*y*,21-diol-20-one) where *x* and *y* is α or β (see Kokate *et al.*, 1994 for structures). Stock solutions of the steroids were prepared in 45% hydroxypropyl-γ-cyclodextrin (γ-cyclodextrin) in water (Research Biochemicals Inc., Natick, MA) and further dilutions were made using 0.9% saline. In most experiments the final concentration of γ-cyclodextrin was less than 10%. All drug solutions were administered i.p. in a volume equalling 1% of the animal's body weight. By itself, γ-cyclodextrin at concentrations as high as 45% failed to protect any animal from pilocarpine-, kainic acid- or NMDA-induced seizures and lethality. Steroids, clonazepam and pilocarpine were obtained from Sigma Chemical Co. (St Louis, MO). (−)-Scopolamine methyl bromide was from Research Biochemicals and NMDA was from Bachem Bioscience Inc. (King of Prussia, PA).

Data analysis

To construct dose–effect curves, each drug was tested at several doses spanning the dose producing 50% protection (ED_{50}). At least 8 mice were tested at each dose. ED_{50} values and their 95% confidence limits were determined by log-probit analysis using the computer programs accompanying Tallarida and Murray (1987). Drugs failing to protect >50% of animals at the maxi-

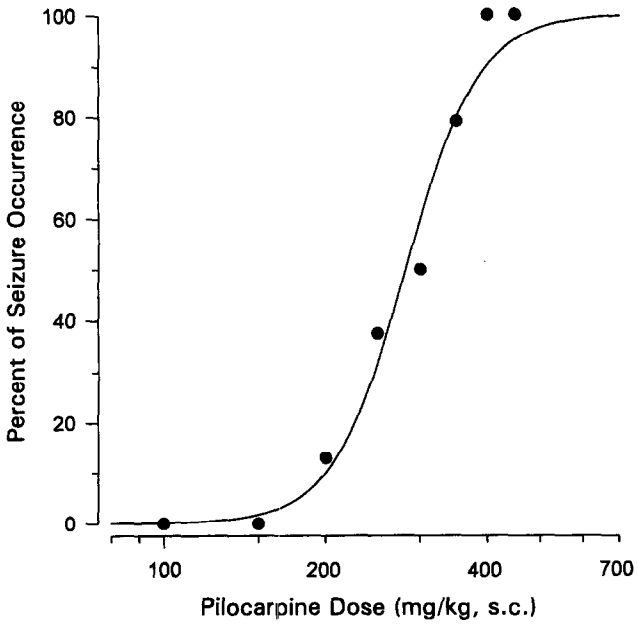

Fig. 1. Dose–response relationship for the induction of limbic motor seizures and status epilepticus by s.c. pilocarpine in mice. Each point represents data from between 8 and 32 mice. The data points were fit to a logistic function with CD_{50} value of 283 mg/kg.

mum dose tested were considered ineffective. Log-probit analysis was also used to determine the CD_{97} value from the data in Fig. 1. Data are presented as the mean ± S.E.M. The protective index (PI), a measure of relative toxicity, is the ratio of the TD_{50} and ED_{50} values, where the TD_{50} is the motor toxicity in the horizontal screen test as determined by Kokate *et al.* (1994).

RESULTS

Pilocarpine-induced limbic motor seizures and status epilepticus

Injection of pilocarpine induced intermittent limbic motor seizures consisting of myoclonic jerking of the whole body or forelimbs, rearing, severe upper extremity and forelimb clonus, and falling. These behavioral effects of pilocarpine increased in severity and frequency with time and eventually progressed into "status epilepticus" (continuous limbic motor seizure activity as defined in Materials and Methods lasting at least 10 min). The proportion of animals exhibiting intermittent limbic motor seizures followed by status epilepticus increased as the dose of pilocarpine was increased within the range of 200–350 mg/kg. With higher doses ($\geq$350 mg/kg) there was a brief period of limbic motor seizures followed by severe generalized clonic–tonic seizures and usually death. The frequency of occurrence of limbic motor seizures (lasting more than 10 sec) for various doses of pilocarpine in the range 100–450 mg/kg is plotted in Fig. 1. The CD_{97} value derived from these data is 416 mg/kg. This dose was used in subsequent experiments examining the protective activity of neuroactive steroids and clonazepam. In 40 control mice tested with the CD_{97} dose of pilocarpine, 39 exhibited severe clonic–tonic seizures and 37 died.

Protective activity of steroids and clonazepam in pilocarpine seizure test

Four pregnanolone isomers and three corresponding 21-hydroxy (tetrahydrodeoxycorticosterone; THDOC) analogs were examined for protective activity in the pilocarpine seizure test. As illustrated in Fig. 2, pretreatment with the steroids produced a dose-dependent protective effect against pilocarpine-induced limbic motor seizures and generalized status epilepticus. The potencies (ED_{50} values) of the steroids and the reference compound clonazepam in the pilocarpine seizure test are summarized in Table 1. (Clonazepam was selected as the reference compound to allow comparison with the results of our prior study; Kokate *et al.*, 1994.) Steroids with the 5α,3α- or 5β,3α-configurations were much more potent (ED_{50} values, 7.0–18.7 mg/kg) than the corresponding 3β-epimers (ED_{50} values, 33.8–63.5 mg/kg). Also shown for comparison are the potencies for protection against pentylenetetrazol (PTZ)-induced clonic seizures as reported by Kokate *et al.* (1994). Overall, the steroids were more potent in the pilocarpine model than in the PTZ test.

Steroid effects in ongoing pilocarpine-induced status epilepticus

Having demonstrated the effectiveness of the steroids in protecting against the development of status epilepticus by a subsequent dose of pilocarpine, we next sought to determine whether neuroactive steroids can interrupt ongoing pilocarpine-induced seizure activity. Animals were injected with a moderate dose of pilocarpine (325 mg/kg) that induced prolonged status epilepticus and ultimately lethality (unlike higher doses that were rapidly lethal). Fifteen min after initiation of status epilepticus, the animals received a 15 mg/kg dose of 5β,3α-pregnanolone. In 3 of 6 mice, the ongoing status epilepticus was aborted completely. The behavioral effects in these mice were limited to diminished locomotion (and in many cases motionlessness), mild tremors of the head and body, and infrequent brief myoclonic movements of the forelimbs. In the remaining 3 mice, 5β,3α-pregnanolone reduced the severity of the seizures, but did not abort them completely. A higher dose of 5β,3α-pregnanolone (30 mg/kg) completely abolished ongoing status epilepticus in all 8 of 8 mice tested, but produced marked sedation in 5 of the animals.

Steroid effects on kainic acid-induced seizures and status epilepticus

As shown in Table 2, a single injection of each of the neuroactive steroids produced a statistically significant dose-dependent delay in the onset of limbic seizures induced by kainic acid (32 mg/kg, s.c.). Steroids with the

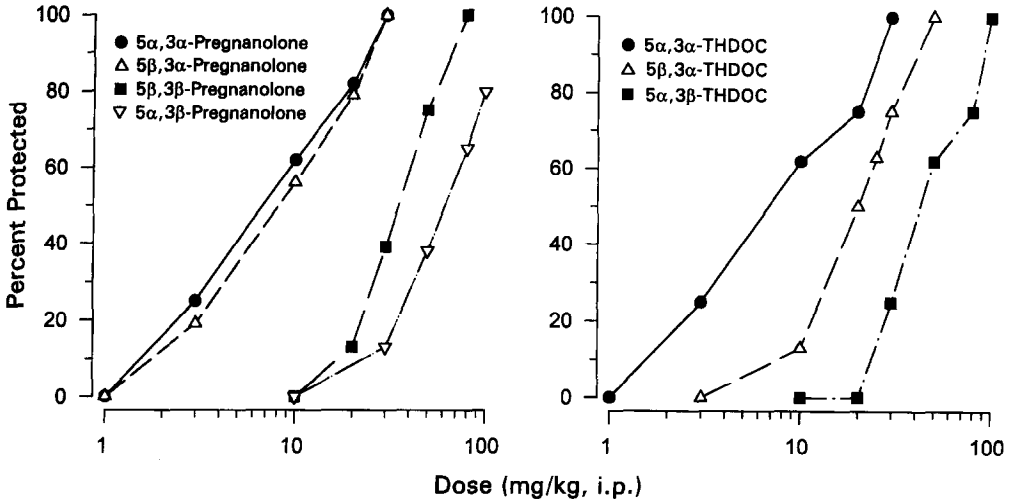

Fig. 2. Dose–response relationships of neuroactive steroids for the protection against pilocarpine (416 mg/kg, s.c.)-induced seizures and subsequent lethality in mice. Each point represents data from between 8 and 24 mice.

Table 1. Anticonvulsant activity of neuroactive steroids and clonazepam against pilocarpine- and PTZ-induced seizures

Test compound	Pilocarpine test (ED_{50})	PTZ test* (ED_{50})	Motor toxicity* (TD_{50})	PI for PTZ test* (TD_{50}/ED_{50})	PI for pilocarpine test (TD_{50}/ED_{50})
5α,3α-Pregnanolone	7.0 (3.9–11.4)	13.7 (10.1–18.7)	42.0 (31.7–55.6)	3.1	6.0
5β,3α-Pregnanolone	7.5 (4.1–13.7)	18.2 (14.7–22.6)	22.2 (16.0–30.9)	1.2	3.0
5α,3β-Pregnanolone	63.5 (43.5–92.0)	>100	>100		
5β,3β-Pregnanolone	33.8 (26.4–43.2)	>100	>100		
5α,3α-THDOC	7.3 (3.9–12.5)	15.0 (10.7–20.9)	57.1 (36.5–89.6)	3.8	7.8
5β,3α-THDOC	18.7 (14.1–24.1)	26.2 (18.9–36.3)	68.9 (56.6–83.8)	2.6	3.7
5α,3β-THDOC	45.9 (33.5–60.1)	>100	>100		
Clonazepam	0.07 (0.05–0.14)	0.044 (0.02–0.11)	0.39 (0.23–0.51)	8.9	5.6

PI, protective index. TD_{50} is the dose producing motor impairment in 50% of animals as assessed with the horizontal screen test. Values in parentheses are 95% confidence limits.
*Taken from Kokate *et al.* (1994).

Table 2. Delay in the onset of kainic acid-induced limbic seizures by a single injection of neuroactive steroids and clonazepam

	Time to seizure onset (min)				
	Dose (mg/kg)				
Test compound	3	10	30	50	100
5α,3α-Pregnanolone	42.1 ± 3.8 (0/8)	65.7 ± 9.4* (0/8)	89.2 ± 10.9* (1/8)	96.5 ± 14.4* (3/8)	N.T.
5β,3α-Pregnanolone	39.5 ± 3.6 (0/8)	61.6 ± 4.4* (1/16)	76.3 ± 7.0* (2/16)	86.8 ± 6.2* (4/16)	N.T.
5α,3β-Pregnanolone	N.T.	N.T.	34.5 ± 3.1 (0/8)	45.4 ± 5.7 (0/8)	51.2 ± 6.3* (0/8)
5α,3α-THDOC	40.0 ± 4.0 (0/8)	51.1 ± 4.3* (1/16)	79.0 ± 10.4* (2/8)	91.6 ± 8.7* (2/8)	N.T.
5β,3α-THDOC	N.T.	55.2 ± 6.0* (0/8)	66.3 ± 6.2* (0/8)	88.4 ± 9.5* (1/8)	95.6 ± 12.3*† (2/8)
5α,3β-THDOC	N.T.	N.T.	42.1 ± 4.7 (0/8)	54.9 ± 3.6* (0/8)	64.6 ± 9.1* (0/8)
	Dose (mg/kg)				
	0.02	0.05	0.1	0.3	0.5
Clonazepam	44.3 ± 7.0 (0/8)	57.9 ± 9.8* (1/8)	72.4 ± 7.6* (4/8)	55.9 (6/8)	30.5 (7/8)

Time to seizure onset for control animals was 30.2 ± 1.6 min (n = 50). Values reported are the mean ± S.E.M. of times to seizure onset. Animals were observed for 150 min after kainic acid injection. N.T., not tested. Values in parentheses indicate number of animals protected/total number of animals tested; *$p < 0.01$ (one-way analysis of variance with Newman–Keuls test).
†Test dose, 80 mg/kg.

5α,3α- or 5β,3α-configurations were much more potent in delaying the onset of kainic acid-induced seizures than the corresponding 3β-isomers. Single injections of 3α-hydroxy (but not 3β-hydroxy) steroids also partially protected against the occurrence of kainic acid-induced seizures and status epilepticus, but none of the steroids effectively protected all animals even at the highest dose tested (Fig. 3). However, when a second dose of steroid

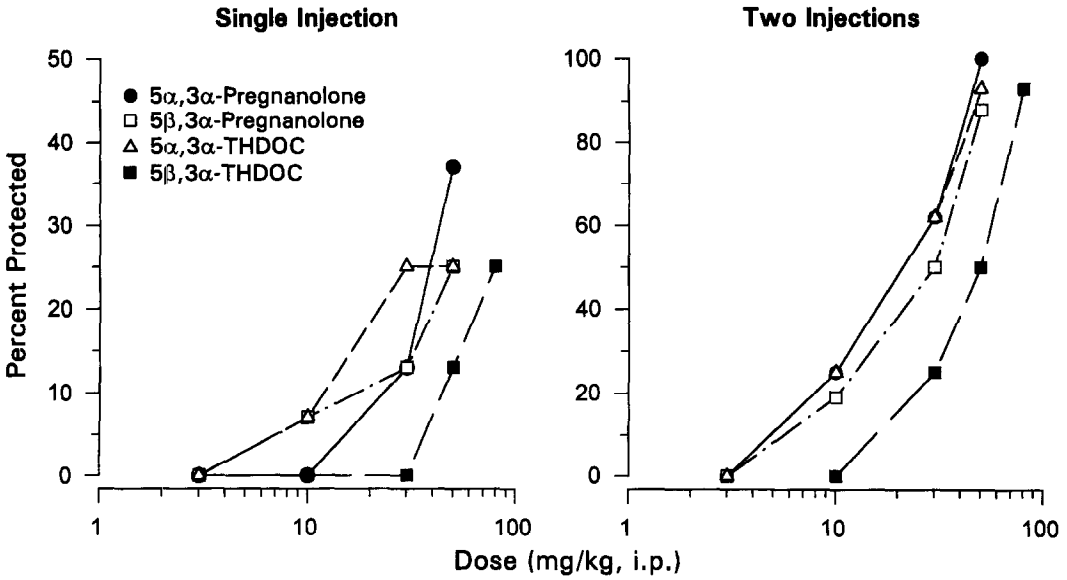

Fig. 3. Dose–response relationships of neuroactive steroids for protection against kainic acid (32 mg/kg, s.c.)-induced limbic seizures and status epilepticus in mice. Graph to the *left* shows percentage of animals protected after administration of a single dose of steroid and graph on *right* shows corresponding data when a second dose was administered 1 hr after the first dose. Each point represents data from between 8 and 16 mice.

Table 3. Delay in the onset of kainic acid-induced limbic seizures by two injections of neuroactive steroids

	Time to seizure onset (min)				
	Dose (mg/kg)				
Test Compound	3	10	30	50	80
5α,3α-Pregnanolone	44.3 ± 3.6 (0/8)	76.2 ± 7.1* (2/8)	79.5 ± 7.1* (5/8)	—† (8/8)	N.T.
5β,3α-Pregnanolone	36.8 ± 4.0 (0/8)	75.2 ± 7.2* (3/16)	81.4 ± 9.1* (8/16)	76.9 (14/16)	N.T.
5α,3α-THDOC	49.1 ± 4.7* (0/8)	60.8 ± 7.3* (4/16)	98.5 ± 1.6* (5/8)	39.0 (15/16)	N.T.
5β,3α-THDOC	N.T.	65.3 ± 5.9* (0/8)	82.2 ± 12.5* (2/8)	92.8 ± 13.1* (4/8)	49.5 (15/16)

Second dose of steroid was administered 1 hr after first dose. Time to seizure onset for control animals was 30.2 ± 1.6 min (n = 50). Values reported are the mean ± S.E.M. of times to seizure onset. Animals were observed for 150 min after kainic acid injection. N.T., not tested. Values in parentheses are number of animals protected/total number of animals tested; *$p < 0.01$ (one-way analysis of variance with Newman–Keuls test).
†All animals protected.

was administered (1 hr after first dose), all of the 3α-hydroxy compounds produced a dose-dependent protective effect and, at high doses, complete or nearly complete protection of all animals (Fig. 3). Thus, complete protection was obtained against kainic acid-induced repetitive forelimb clonus and subsequent status epilepticus. The behavioral effects in the protected animals were limited to infrequent myoclonus of the limbs and face. However, protective doses of the steroids (>30 mg/kg) also caused sedation in most animals. Table 3 shows the dose-dependent delay in the onset and protection against kainic acid seizures caused by two injections of neuroactive steroids. Since two doses of the steroids were required to produce complete protection against kainic acid-induced seizures, ED_{50} values could not be determined. Clonazepam was considerably more potent than any of the neuroactive steroids tested and a single injection was sufficient to produce complete protection against kainic acid-induced seizures (Table 2).

Steroid effects on NMDA-induced seizures and lethality

At doses that were highly effective in the pilocarpine test, none of the neuroactive steroids protected completely against NMDA-induced behavioral effects and seizures, and the subsequent lethality. However, as summarized in Table 4, neuroactive steroids with the 5α,3α- or 5β,3α-configurations produced a significant dose-dependent delay in NMDA-induced lethality. In contrast, the corresponding 3β-epimers and clonazepam produced only a modest delay in NMDA-induced lethality.

DISCUSSION

In this study, we show for the first time that GABA potentiating neuroactive steroids (metabolites of progesterone and deoxycorticosterone and their isomers) are highly effective in protecting against pilocarpine-induced limbic motor seizures and status epilepticus in mice. In

Table 4. Neuroactive steroid-induced delay in NMDA lethality

	Time to NMDA-induced lethality (min)			
	Dose (mg/kg)			
Test Compound	10	30	50	100
5α,3α-Pregnanolone	12.5 ± 2.2 (0/8)	25.7 ± 4.0* (0/8)	36.2 ± 3.6* (1/8)	N.T.
5β,3α-Pregnanolone	10.9 ± 1.9 (0/8)	24.6 ± 5.2* (1/8)	33.4 ± 2.2* (2/16)	N.T.
5α,3β-Pregnanolone	N.T.	5.4 ± 1.1 (0/8)	8.7 ± 1.4 (0/16)	9.1 ± 1.6 (0/8)
5α,3α-THDOC	12.9 ± 3.8 (0/8)	21.4 ± 3.7* (0/8)	27.2 ± 7.1* (2/8)	N.T.
5β,3α-THDOC	N.T.	13.7 ± 2.7* (0/8)	20.2 ± 2.3* (0/8)	31.1 ± 3.2* (0/8)
5α,3β-THDOC	N.T.	6.5 ± 1.1 (0/8)	8.3 ± 1.9 (0/8)	11.6 ± 1.9 (0/8)
	Dose (mg/kg)			
	0.5	1	2	3
Clonazepam	5.8 ± 0.6 (0/8)	5.7 ± 0.6 (0/8)	7.3 ± 1.0 (0/8)	11.8 ± 3.0 (0/8)

Time to lethality in control animals was 6.3 ± 0.6 min (n = 78). Values reported are the mean ± S.E.M. of times to lethality. Animals were observed for 120 min after the NMDA injection. N.T., not tested. Values in the parentheses are number of animals protected/total number of animals tested; *$p < 0.01$ (one-way analysis of variance with Newman–Keuls test).

addition, these neuroactive steroids had protective activity against kainic acid-induced limbic seizures and status epilepticus in mice, and delayed, but did not completely protect against, NMDA seizures and their subsequent lethality.

Pilocarpine is well known to produce severe limbic motor seizures and generalized status epilepticus as a result of its potent central muscarinic receptor agonist activity (Turski *et al.*, 1983, 1984, 1989). Several studies have shown that drugs which enhance $GABA_A$ receptor responses such as benzodiazepines are highly effective in protecting against pilocarpine-induced status epilepticus and lethality (Turski *et al.*, 1983, 1984, 1987, 1989). The neuroactive steroids tested in the present study are known to enhance GABA-evoked Cl^- current and have anticonvulsant activity in animals treated with GABA receptor antagonists such as PTZ (Hogskilde *et al.*, 1988; Belelli *et al.*, 1989; Kokate *et al.*, 1994). As is the case with other GABA potentiating drugs, we found that pretreatment with neuroactive steroids was highly effective in protecting against pilocarpine-induced status epilepticus and subsequent lethality in mice. In addition, as demonstrated with 5β,3α-pregnanolone, neuroactive steroids may also be effective in aborting ongoing status epilepticus. The rank order of the potencies in the pilocarpine model was identical to that previously reported for the PTZ test (Table 1). This rank ordering also matched that of the efficacies of the steroids for potentiation of GABA-activated Cl^- current in cultured hippocampal neurons [$P_{(1\ \mu M)}$ values; Kokate *et al.*, 1994]. However, the neuroactive steroids were quantitatively more potent against pilocarpine seizures than in the PTZ test. Indeed, the 3β-epimers which were inactive in the PTZ test (but which weakly potentiate GABA responses), were protective in the pilocarpine model, albeit with low potencies. We previously observed that steroids with the 5α,3α-configuration (5α,3α-THDOC and 5α,3α-pregnanolone) had higher protective index values (TD_{50} in test of motor impairment ÷ ED_{50} in PTZ test) than the corresponding 5β,3α-analogs. Since the steroids were more potent in the pilocarpine model, the calculated protective index values were higher than in the PTZ test. In fact, the protective indices for the two 5α,3α-analogs were higher than that of the reference compound clonazepam (Table 1). The observation that these neuroactive steroids have equivalent efficacy, but lower relative toxicity than this widely used benzodiazepine, suggests that the clinical potential of the steroids may be worthy of further investigation.

Neuroactive steroids with the 5α,3α- or 5β,3α-configurations, like other GABA potentiating drugs (Zaczek *et al.*, 1981; Heggli and Malthe-Sorenssen, 1982; Turski *et al.*, 1990), were also effective in protecting against kainic acid-induced limbic seizures and status epilepticus. Single injections of the steroids produced a significant dose-dependent delay in the onset of the seizures, but did not completely protect against their later development (Table 2). However, when a second dose of steroid was administered (1 hr after first dose), complete protection was obtained (Fig. 2, Table 3). The requirement for two doses is likely due to the more prolonged duration of kainic acid-induced status epilepticus than that produced by pilocarpine. Since the steroids have a relatively short duration of action ($t_{1/2}$ < 90 min for 5α,3α-pregnanolone; Kokate *et al.*, 1994), a second dose is necessary to protect against the delayed effects of kainic acid which can persist for several hours. Overall, the neuroactive steroids were less potent in the kainic acid seizure test and maximal protection could only be obtained with doses producing sedation. However, the rank ordering of potencies was similar to that obtained in the pilocarpine and PTZ tests (Table 1), indicating that protection in all three models likely occurs by a similar mechanism.

The neuroactive steroids also delayed the onset of the behavioral effects of NMDA, but did not completely protect against NMDA seizures or their subsequent

lethality (Table 4). Again, steroids with the hydroxy group in the 3α-configuration were much more potent than steroids with the 3β-configuration. We previously reported that the neuroactive steroids have no effect on NMDA receptor-mediated currents (Kokate *et al.*, 1994). Thus, the prolongation of the delay to NMDA-induced lethality is presumably not due to direct effects on NMDA receptors. Rather, the phenomenon likely reflects potentiation of GABAergic inhibitory mechanisms, as is the case for the other seizure models.

The synthetic neuroactive steroid alphaxolone has been shown to be effective in the treatment of some forms of drug resistant status epilepticus (Chin *et al.*, 1979; Munari *et al.*, 1979; Saady *et al.*, 1979). However, the anticonvulsant activity of alphaxolone only occurs at doses associated with neurological toxicity (Peterson, 1989). In contrast, the neuroactive steroids tested in this study protect against PTZ and, particularly, pilocarpine seizures at doses that are substantially below those that induce neurological (motor) impairment. Consequently, the neuroactive steroids may be useful alternatives in the management of certain forms of status epilepticus. Indeed, the 5α,3α-isomers of pregnanolone and THDOC had greater protective index values than clonazepam, a representative of the benzodiazepine class of drugs that is widely used in the therapy of status epileptics (Walsh and Delgado-Escueta, 1993). However, short duration of action is a potential disadvantage in the use of neuroactive steroids in the clinical management of status epilepticus.

In summary, certain neuroactive steroids are highly effective in protecting against pilocarpine- and kainic acid-induced limbic seizures and status epilepticus in mice, and this activity is well correlated with their GABA potentiating effects. The protective index values for certain of the neuroactive steroids were higher than that of clonazepam in the pilocarpine seizure model, indicating that the neuroactive steroids have lower relative toxicity. Thus, it may be worthwhile to evaluate the potential of neuroactive steroids for the treatment of status epilepticus in humans.

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