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Short communication

Proconvulsant effects of neurosteroids pregnenolone sulfate and dehydroepiandrosterone sulfate in mice

Doodipala S. Reddy, Shrinivas K. Kulkarni *

Department of Pharmacology, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160 014, India

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Abstract

We have investigated the effects of chronic treatment with the neurosteroids, pregnenolone sulfate and dehydroepiandrosterone sulfate, on the potential neurotoxicity in pentylenetetrazol seizure sensitivity test in mice. Four weeks of subcutaneous treatment with pregnenolone sulfate and dehydroepiandrosterone sulfate, at a dose of 10 mg kg^{-1} day^{-1}, significantly shifted the pentylenetetrazol dose-percent convulsions and latency curves to the left, and markedly decreased the ED_{50} of pentylenetetrazol for tonic convulsions, indicating the increased sensitivity of mice to seizures. Chronic neurosteroid treatment significantly decreased the body weight of the animals. However, acute treatment of neurosteroids did not modify the seizure reactivity of mice to pentylenetetrazol. Furthermore, the dehydroepiandrosterone sulfate (10 mg kg^{-1}, s.c.)-induced proconvulsant effect was significantly prevented by chronic pretreatment with progesterone (5 mg kg^{-1}, s.c.), a precursor for $GABA_A$ receptor active neurosteroid, allopregnanolone, and dizocilpine (0.1 mg kg^{-1}, i.p.), a non-competitive NMDA receptor antagonist. These results suggest that long-term administration of neurosteroids pregnenolone sulfate or dehydroepiandrosterone sulfate produces proconvulsant effects. © 1998 Elsevier Science B.V.

Keywords: Neurosteroid; Dehydroepiandrosterone sulfate; Pregnenolone sulfate; GABA (γ-aminobutyric acid); NMDA (*N*-methyl-D-aspartate); Proconvulsant; Seizure

1. Introduction

Research over the past decade has characterized various neurosteroids within both the central and peripheral nervous systems (Robel and Baulieu, 1994; Kulkarni and Reddy, 1995) and a role for mitochondrial diazepam-binding inhibitor receptors in the regulation of neurosteroidogenesis has been elucidated (Costa et al., 1994). Neurosteroids have been shown to affect the activity of various neurotransmitter systems, including that involved in regulating the balance between excitation and inhibition in the brain. Allopregnanolone, progesterone and allotetrahydrodeoxycorticosterone are the positive allosteric modulators of $GABA_A$ receptor acting through a unique site termed 'epalon' (Gee et al., 1995). Pregnenolone sulfate and dehydroepiandrosterone sulfate are both allosteric antagonists of $GABA_A$ receptor (Majewska and Schwartz, 1987; Majewska et al., 1990) and positive modulators of *N*-methyl-D-aspartate (NMDA) receptor-mediated responses (Wu et al., 1991; Irwin et al., 1994). Recently, these neurosteroids have also been shown to exert a facilitatory action on NMDA-mediated glutamatergic neurotransmission through central σ receptors (Maurice et al., 1996).

Neurosteroids have shown promising therapeutic potentials in a variety of neuropsychiatric and cognitive disorders. The 3α-hydroxylated pregnane steroids have been shown to be potent anticonvulsants (Carter et al., 1997), anxiolytics (Bitran et al., 1995; Reddy and Kulkarni, 1997a), neurotrophics (Reddy and Kulkarni, 1997b), antiaddictives (Reddy and Kulkarni, 1997c) and antistress agents (Reddy and Kulkarni, 1996). Pregnenolone sulfate and dehydroepiandrosterone sulfate enhances learning and memory processes (Flood et al., 1995), and also completely counteracts the amnesic effects of scopolamine and dizocilpine (Maurice and Lockhart, 1997). However, limited studies have been performed on the chronic effect of these neurosteroids on potential neuroendocrine toxicity. We have previously reported that acute administration of these neurosteroids produces anxiogenic (Reddy and Kulkarni, 1997a) and provoking effects in hypoxic/ischemic seizure models (Reddy and Kulkarni, 1997b). The present study was performed to investigate whether

* Corresponding author. Fax: +91-172-541142.

chronic treatment with pregnenolone sulfate or dehydroepiandrosterone sulfate produces direct proconvulsant and neurotoxic effects in mice.

2. Materials and methods

2.1. Animals

Male albino mice of Laka strain (Central Animal House, Panjab University, Chandigarh), weighing 20–25 g, were housed five per cage with free access to food and water at the laboratory conditions. The animals were used following at least a 2-day period of adaptation to the laboratory conditions. The experiments were carried out between 0900 to 1700 h at the ambient temperature. All the experimental protocols have been approved by the Local Ethical Committee and conducted according to the Indian National Science Academy Guidelines on the Use and Care of Experimental Animals.

2.2. Experimental protocol

In acute studies, mice received pregnenolone sulfate (10 mg kg^{-1}, s.c.) or dehydroepiandrosterone sulfate (10 mg kg^{-1}, s.c.) or vehicle followed 30 min by one of the three doses of pentylenetetrazol (40, 60 and 80 mg kg^{-1}, i.p). After pentylenetetrazol injection, the latency for the onset of tonic convulsions was observed for a 40-min period.

In chronic studies, mice were divided randomly into several experimental groups each comprising of 18 animals. In the first series of experiments, mice received subcutaneous injections of pregnenolone sulfate (10 mg kg^{-1} day^{-1}) or dehydroepiandrosterone sulfate (10 mg kg^{-1} day^{-1}) or their vehicle once daily for 4 weeks. Around 30 min after the end of the chronic treatment, each treatment group was divided into three subgroups and the seizure reactivity of the animals was assessed by injecting one of the three doses of pentylenetetrazol (20–80 mg kg^{-1}, i.p.) as shown in Fig. 1. Mice were observed for 40 min after the pentylenetetrazol injection in order to determine the incidence of tonic convulsions. In the second series of experiments, mice received progesterone (5 mg kg^{-1} day^{-1}, s.c.), dizocilpine (0.1 mg kg^{-1}, i.p.) or vehicle daily for 4 weeks. At the end of chronic treatment, each group was divided into three subgroups. Pentylenetetrazol (60, 80 or 120 mg kg^{-1}, i.p.) was administered 30 min after last injection to evaluate the seizure reactivity of the animals. Mice were observed for 40 min after the pentylenetetrazol injection in order to determine the incidence of tonic convulsions. If no tonic seizures appeared during this time, the animals were considered protected and were given a maximum latency score of 40 min. In the interaction experiments, progesterone (5 mg kg^{-1} day^{-1}, s.c.) or dizocilpine (0.1 mg kg^{-1} day^{-1}, i.p.) was administered 30 and 15 min, respectively, before each dose of dehydroepiandrosterone sulfate (10 mg kg^{-1} day^{-1}, s.c.) throughout 4 weeks of treatment. Seizure reactivity of the animals was assessed by injecting one of the three doses of pentylenetetrazol (40, 60 and 80 mg kg^{-1}, i.p.) similarly as described above. The body weight of the animals was monitored weekly.

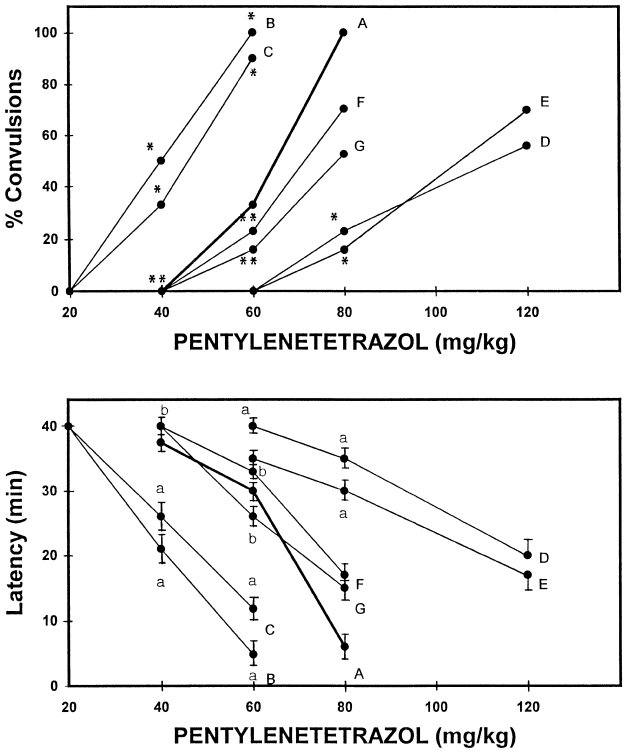

Fig. 1. Long-term effects of neurosteroids on the sensitivity of mice to pentylenetetrazol-induced convulsions (upper panel) and latency to the appearance of convulsions (lower panel). Mice were treated with pregnenolone sulfate (10 mg kg^{-1} day^{-1}, s.c.) (B), dehydroepiandrosterone sulfate (10 mg kg^{-1} day^{-1}, s.c.) (C), dizocilpine (0.1 mg kg^{-1} day^{-1}, i.p.) (D), progesterone (5 mg kg^{-1} day^{-1}, s.c.) (E) or vehicle (A) once daily for 4 weeks. In the interaction studies, progesterone (5 mg kg^{-1} day^{-1}, s.c.) (F) and dizocilpine (0.1 mg kg^{-1} day^{-1}, i.p.) (G) was administered 30 and 15 min, respectively, before each dose of dehydroepiandrosterone sulfate (10 mg kg^{-1} day^{-1}, s.c.) for 4 weeks. Pentylenetetrazol (20–120 mg kg^{-1}) was administered i.p. 30 min after the last injection of neurosteroids, and the percentage of convulsing animals and latency was recorded. Latency is expressed as mean ± S.E.M. ($n = 6$ mice per group) and analyzed by one-way ANOVA. $^{a}P < 0.05$ vs. vehicle-treated group; $^{b}P < 0.05$ vs. dehydroepiandrosterone sulfate-treated group (Duncan's multiple range test). $*\ P < 0.05$ vs. vehicle-treated group; $**\ P < 0.05$ vs. dehydroepiandrosterone sulfate-treated group (Fisher's exact probability test).

2.3. Drugs

Dehydroepiandrosterone sulfate, pregnenolone sulfate and pentylenetetrazol (Sigma, MO, USA), and dizocilpine (MSD, UK) were dissolved in 0.1% Tween 80 and diluted with saline. Progesterone (Unichem, Mumbai, India) was suspended in the refined vegetable oil and injected subcutaneously. All drugs were given at a dose of 1 ml 100 g^{-1}

body weight. Control groups received the respective vehicle treatment and all the control observations were pooled since no significant differences were found in control studies with animals which received different vehicles. To exclude the bias of the large subject size, the number of observations were kept similar to drug-treated groups rejecting three subjects from each vehicle.

2.4. Statistical analysis

The latency for convulsions and body weights are expressed as mean ± S.E.M. The ED_{50} values for convulsions and their 95% confidence limits were performed by the probit analysis (Lichtfield and Wilcoxon, 1949), with the aid of a computerized program. Body weight and seizure latency data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Percentage seizure data were analysed using Fisher's exact probability test. In all tests, $P < 0.05$ was used as the criterion for statistical significance.

3. Results

Neither pregnenolone sulfate (10 mg kg^{-1}, s.c.) nor dehydroepiandrosterone sulfate (10 mg kg^{-1}, s.c.) in acute administration modified the seizure reactivity of animals to pentylenetetrazol (40–80 mg kg^{-1}, i.p.) relative to vehicle control (data not shown). In the chronic vehicle (0.2 ml day^{-1} for 4 weeks)-treated group, pentylenetetrazol (40–80 mg kg^{-1}, i.p.) induced a dose-dependent increase in convulsions (Fig. 1, upper panel) and decreased the latency to tonic seizures (Fig. 1, lower panel), where the ED_{50} for tonic convulsions was 67.6 mg kg^{-1} (Table 1). Chronic s.c. treatment with neurosteroid pregnenolone sulfate (10 mg kg^{-1} day^{-1} for 4 weeks) or dehydroepiandrosterone sulfate (10 mg kg^{-1} day^{-1} for 4 weeks) significantly shifted the pentylenetrazol dose–response curves for convulsions and latency to the left (Fig. 1), and considerably decreased the ED_{50} of pentylenetetrazol for convulsions (Table 1). To investigate the possible mechanisms of neurosteroid-induced proconvulsant effects, we examined the effects of pretreatment of progesterone, a precursor for $GABA_A$ receptor active neurosteroid, allopregnanolone, or the non-competitive NMDA receptor antagonist, dizocilpine. Four weeks of treatment with the progesterone (5 mg kg^{-1} day^{-1}, s.c.) or dizocilpine (0.1 mg kg^{-1} day^{-1}, i.p.) alone markedly shifted to the right of pentylenetetrazol dose-convulsions and latency curves (Fig. 1), and increased the ED_{50} of pentylenetetrazol for tonic seizures 50–70% above control (Table 1). The dehydroepiandrosterone sulfate (10 mg kg^{-1} day^{-1}, s.c.)-induced proconvulsant effect was significantly prevented by chronic pre-administration of progesterone (5 mg kg^{-1} day^{-1}, s.c.) or dizocilpine (0.1 mg kg^{-1} day^{-1}, i.p.). The dose-response and latency curves of pentylenetetrazol convulsions were shifted significantly to the right (Fig. 1) and the ED_{50} of pentylenetetrazol for tonic convulsions were increased 50–60% above the neurosteroid group (Table 1), respectively.

Neurosteroid treatment failed to show any observable effect on general behavior of the animals before they were challenged with pentylenetetrazol except for an increased vigilance and running. As shown in Table 1, chronic treatment of neurosteroids for 4 weeks influenced profoundly the body weight of the animals (ANOVA: $F(13,238) = 10.494$, $P < 0.01$). While mice treated with pregnenolone sulfate and dehydroepiandrosterone sulfate had significantly decreased body weights than vehicle controls, mice treated with progesterone were heavier than vehicle treated controls. The dehydroepiandrosterone sulfate-induced decrease in the body weight of the animals

Table 1
ED_{50} values of pentylenetetrazol-induced convulsions and body weight of mice chronically-treated (4 weeks) with different neurosteroids

Treatment (mg kg^{-1})	ED_{50} (mg kg^{-1})	Body weight (g ± S.E.M.)	
		1st Day	28th Day
Vehicle	67.6 (52.6–82.6)	23.9 + 0.3	24.2 ± 0.3
PS (10)	43.6 (30.3–56.9)	23.9 ± 0.6	20.0 ± 0.5[a]
DHEAS (10)	48.6 (34.3–62.9)	24.5 ± 0.3	21.3 ± 0.3[a]
Progesterone (5)	105.9 (87.9–123.9)	24.8 ± 0.5	28.3 ± 0.7[a]
Dizocilpine (0.1)	112.2 (94.6–129.7)	24.3 ± 0.5	25.3 ± 0.6
DHEAS (10) + progesterone (5)	66.0 (78.1–53.9)	23.6 ± 0.6	24.3 ± 0.6[b]
DHEAS (10) + dizocilpine (0.1)	72.8 (60.1–85.2)	23.8 ± 0.5	24.5 ± 0.4[b]

Pentylenetetrazol (20–120 mg kg^{-1}) was administered i.p. 30 min after the last injection of neurosteroids either alone or in combination. The ED_{50} values were determined according to graphical method of Litchfield and Wilcoxon using a computer program. Numbers in parentheses denote 95% confidence limits for ED_{50} values. Each treatment group consists of 18 mice equally divided into three subgroups. Values for body weight are expressed as mean ± S.E.M. and were analyzed using one-way ANOVA.

[a] $P < 0.05$ vs. vehicle-treated group.

[b] $P < 0.05$ vs. DHEAS-treated group (Duncan's multiple range test).

PS, pregnenolone sulfate.

DHEAS, dehydroepiandrosterone sulfate.

was significantly prevented by chronic pre-administration of the progesterone or dizocilpine.

4. Discussion

The results of the present study provide experimental evidence on the proconvulsant effects of neurosteroids, pregnenolone sulfate and dehydroepiandrosterone sulfate in mice. In line with a previous study (Maione et al., 1992) showing that the pregnenolone sulfate increases the convulsant potency of NMDA, but not pentylenetetrazol convulsions, our findings suggest that acute treatment with either neurosteroid failed to potentiate the pentylenetetrazol-induced convulsions. Neurosteroids pregnenolone sulfate and dehydroepiandrosterone sulfate, up to 10 mg kg^{-1}, do not affect the occurrence of convulsions following pentylenetetrazol. Thus, a preliminary 4-week pretreatment period was selected for the study based on the premise that such a treatment protocol may be useful in predicting the potential proconvulsant neurotoxicity. Four weeks following administration of neurosteroids, mice exhibited increased sensitivity to tonic convulsions with subconvulsant doses of pentylenetetrazol and showed shorter latencies to the appearance of pentylenetetrazol-induced convulsions, and had significantly decreased body weights, consistent with activity as proconvulsant agents. These observations confirm our previous reports (Reddy and Kulkarni, 1997a,b) showing the anxiogenic and provoking effects of these neurosteroids in hypoxic seizure models. Pregnenolone sulfate and dehydroepiandrosterone sulfate are both allosteric antagonists of $GABA_A$ receptor (Majewska and Schwartz, 1987; Majewska et al., 1990) and positive modulators of NMDA receptor-mediated responses (Wu et al., 1991; Irwin et al., 1994). The NMDA and $GABA_A$ receptors play an important role in the regulation of neuronal excitability and seizures. Therefore, it is interesting to speculate that by decreasing GABAergic function and increasing glutamatergic transmission, neurosteroid sulfates may produce proconvulsant effects.

The results of our studies are consistent with above hypothesis. Chronic treatment with progesterone produced marked anticonvulsant effects, and prevented the proconvulsant effect of dehydroepiandrosterone sulfate. Progesterone exerts its behavioral actions through its possible in vivo conversion to allopregnanolone (Bitran et al., 1995; Reddy and Kulkarni, 1996), a positive allosteric modulator of the $GABA_A$ receptor complex. These observations indicate that progesterone prevents the proconvulsant effect of dehydroepiandrosterone sulfate by indirectly potentiating the $GABA_A$ receptor through conversion into allopregnanolone. Although it is difficult to explain at this juncture whether the shift in seizure threshold in the chronic progesterone animals involve residual allopregnanolone or change in receptor sensitivity, we speculate that both these effects may contribute to its activity on pentylenetetrazol test. The rapid anticonvulsant actions of progesterone may be mediated by its reduced metabolite allopregnanolone (Belelli et al., 1989; Bitran et al., 1995). Since different enzyme systems of GABA and $GABA_A$ receptor levels may be affected by progesterone's intracellular actions at the progestin receptor (Canonaco et al., 1989; Schumacher et al., 1989), its possible influence on the sensitivity of the animals at the receptor level upon long-term treatment cannot be excluded. Additional studies using specific 5α-reductase inhibitor, finasteride (which block in vivo conversion of progesterone to allopregnanolone), and the progestin receptor antagonist, mifepristone (which prevent the intracellular effects of progesterone), might help us to clarify this question.

As expected, our results have shown that chronic treatment with the non-competitive antagonist of NMDA receptor, dizocilpine, elicited potent anticonvulsant effect in the pentylenetetrazol seizure test. Further, repeated administration of dizocilpine exerted marked protective effect against dehydroepiandrosterone sulfate-induced proconvulsant activity. Brain areas which are critical for seizure activity, such as the cerebral cortex and the hippocampus, contain a high density of both GABA and NMDA receptors. On the basis of the above data, it may be speculated that the repeated administration of neurosteroid sulfates causes an enhancement of NMDA receptor-mediated neurotransmission, and/or decrease of GABAergic neurotransmission, which in turn, may induce proconvulsant neurotoxicity. However, the precise mechanism involved in the proconvulsant effect of neurosteroids remains unknown. Furthermore, since both $GABA_A$ receptor agonist and NMDA receptor antagonist prevents the neurosteroid-induced proconvulsant effects, the activity may represent a generalized decrease in the seizure threshold and/or neuronal damage, suggesting the potential to induce the generalized myoclonic seizures in human.

Though the prospect that neurosteroids could be used in the symptomatic treatment of specific neuropsychiatric and cognitive disorders has generated great enthusiasm, potential neuroendocrine neurotoxicity may be an impediment to their therapeutic use. Systemic administration of $3\alpha 5\alpha$-reduced derivatives of progesterone or androstenedione acting as positive allosteric modulators at $GABA_A$ receptors may produce profound sedation, motor impairment and ataxia (Kulkarni and Reddy, 1995; Reddy and Kulkarni, 1996). In addition, prolonged administration of neurosteroids may trigger complex genomic modulations in neuronal and glial cells (Rupprecht et al., 1993), increase in membrane peroxidation (Swierczynski and Mayer, 1996), induce peroxisome proliferation (Prough et al., 1994) and produce proconvulsant effects (Maione et al., 1992). Further, the sulfated neurosteroids, at nootropic and antiamnesic doses, may produce anxiogenic and provoking effects in hypoxic/ischemic seizure models (Reddy and Kulkarni, 1997a,b). However, clinical studies have not yet indicated such potential neurotoxic manifestations. Thus,

further studies are clearly warranted to establish the safety of neurosteroids.

In conclusion, these results suggest that chronic administration of neurosteroids pregnenolone sulfate and dehydroepiandrosterone sulfate produces proconvulsant effects in mice. The proconvulsant effect of dehydroepiandrosterone sulfate could be prevented by pre-treatment with progesterone or dizocilpine, and thus, may involve a $GABA_A$ and NMDA receptor mechanism.

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