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Protective efficacy of neuroactive steroids against cocaine kindled-seizures in mice

Rafal M. Kaminski[a,*], Maciej Gasior[a,1], Richard B. Carter[b,2], Jeffrey M. Witkin[a,3]

[a] Drug Development Group, NIDA Addiction Research Center, 5500 Nathan Shock Drive, Baltimore, MD 21224, USA
[b] CoCensys Inc., 201 Technology Drive, Irvine, CA 92618, USA

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Abstract

Neuroactive steroids demonstrate pharmacological actions that have relevance for a host of neurological and psychiatric disorders. They offer protection against seizures in a range of models and seem to inhibit certain stages of drug dependence in preclinical assessments. The present study was designed to evaluate two endogenous and one synthetic neuroactive steroid that positively modulate the γ-aminobutyric acid ($GABA_A$) receptor against the increase in sensitivity to the convulsant effects of cocaine engendered by repeated cocaine administration (seizure kindling). Allopregnanolone (3α-hydroxy-5α-pregnan-20-one), pregnanolone (3α-hydroxy-5β-pregnan-20-one) and ganaxolone (a synthetic derivative of allopregnanolone 3α-hydroxy-3β-methyl-5α-pregnan-20-one) were tested for their ability to suppress the expression (anticonvulsant effect) and development (antiepileptogenic effect) of cocaine-kindled seizures in male, Swiss-Webster mice. Kindled seizures were induced by daily administration of 60 mg/kg cocaine for 5 days. All of these positive $GABA_A$ modulators suppressed the expression of kindled seizures, whereas only allopregnanolone and ganaxolone inhibited the development of kindling. Allopregnanolone and pregnanolone, but not ganaxolone, also reduced cumulative lethality associated with kindling. These findings demonstrate that some neuroactive steroids attenuate convulsant and sensitizing properties of cocaine and add to a growing literature on their potential use in the modulation of effects of drugs of abuse.

Keywords: Neuroactive steroid; Cocaine kindling; Sensitization; (mouse)

1. Introduction

Neuroactive steroids are both endogenous and synthetic molecules with potential therapeutic utility in a host of neurological and psychiatric conditions (for review, see Gasior et al., 1999a). Endogenous neuroactive steroids are synthesized from cholesterol in peripheral endocrine glands and in the central nervous system (CNS) as metabolites of progesterone or desoxycorticosterone (Mensah-Nyagan et al., 1999). In contrast to steroid hormones that regulate gene transcription through interactions with intracellular receptors, neuroactive steroids can alter the excitability of membrane-bound receptors in the nervous system. Although neuroactive steroids are capable of modulating a variety of neurotransmitter systems in the CNS (for reviews, see Baulieu, 1998; Rupprecht et al., 2001), the γ-aminobutyric acid ($GABA_A$) receptor is their primary molecular target (Majewska et al., 1986). Neuroactive steroids positively modulate the $GABA_A$ receptor function through a putative recognition site that is distinct from the benzodiazepine and barbiturate binding sites (Gee et al., 1995). Thus, neuroactive steroids may be particularly useful for the treatment of epilepsy, anxiety and migraine. Indeed, some neuroactive steroids are currently under clinical evaluation for these disorders and under preclinical consideration for others (Gasior et al., 1999a).

Neuroactive steroids, positively modulating the $GABA_A$ receptor, demonstrate anticonvulsant activity in a number of experimental seizure models including pentylenetetrazol-, picrotoxin-, bicuculline-, *N*-methyl-D-aspartic acid-

* Corresponding author. Epilepsy Research Section, NINDS/NIH, 49 Convent Drive, MSC 4457, Bldg. 49 Rm. 5A75, Bethesda, MD 20892-4457, USA. Tel.: +1-301-402-1315; fax: +1-301-480-1978.
E-mail address: kaminskr@ninds.nih.gov (R.M. Kaminski).
[1] Present address: Behavioral Pharmacology Program, Department of Psychiatry, Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, USA.
[2] Present address: AlgoRx Pharmaceuticals, 101 Interchange Plaza, Suite 102, Cranberry, NJ 08512, USA.
[3] Present address: Neuroscience Discovery Research, Lilly Research Labs, Lilly Corporate Center, Indianapolis, IN 46285-0510, USA.

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(NMDA), kainate- and pilocarpine-induced convulsions (Belelli et al., 1989; Budziszewska et al., 1998; Kokate et al., 1994, 1996; Leskiewicz et al., 1997). Moreover, neuroactive steroids are efficacious against different types of kindled seizures (Holmes and Weber, 1984; Carter et al., 1997; Gasior et al., 1998). They also display anticonvulsant efficacy against clonic seizures induced by acute doses of cocaine (Gasior et al., 1997). The latter effect of neuroactive steroids is of particular interest as cocaine-induced seizures under these conditions are refractory to a host of conventional and newly developed anticonvulsants including those that also increase GABA-mediated neural inhibition (Gasior et al., 1999b; Witkin et al., 1999). The anticonvulsant effects of neuroactive steroids against cocaine are additionally intriguing in light of the fact that this class of compounds has been suggested to have therapeutic potential in certain stages of drug dependence such as in sensitization, tolerance and withdrawal (Budziszewska et al., 1996; Grobin et al., 1998; Reddy and Kulkarni, 1997).

Repeated administration of cocaine can induce sensitization to its convulsant effects, a phenomenon that has been termed "kindling", which is analogous to the kindling of epileptic seizures engendered by electrical stimulation of specific brain sites (cf., Goddard et al., 1969). Cocaine-induced kindling has been recognized as an advantageous model for studying the psychopathology and toxicity associated with cocaine abuse (Miller et al., 2000). In fact, prolonged cocaine abuse is not only associated with a potential increase in seizure probability (cf., Alldredge et al., 1989; Dhuna et al., 1991), but also violent behaviors (Richards et al., 1998; Ruttenber et al., 1997) and panic disorders (Louie et al., 1989) have been reported. Interestingly, overlapping pharmacological mechanisms have been proposed to underlie these neurological and behavioral disorders (Post, 2002).

Anticonvulsant and antiepileptogenic properties of neuroactive steroids have been already established in other kindling models (Carter et al., 1997; Gasior et al., 1998, 2000a). There are no data, however, to address whether this class of compounds is efficacious against the seizure sensitizing effects of repeated cocaine exposure. The present study was designed to explore the effects of selected neuroactive steroids on the expression and development of cocaine-kindled seizures. The identification of compounds that can attenuate cocaine kindling could provide clues as to the underlying neural changes that result from repeated cocaine exposure and suggest treatment strategies for the neurological and psychiatric consequences associated with repetitive cocaine use. For this purpose, we used three structurally different endogenous or synthetic neuroactive steroids: allopregnanolone, pregnanolone and ganaxolone. Allopregnanolone and pregnanolone are endogenous neuroactive steroids with an α-OH at the C3 position of the steroid A ring and 5α- or 5β-reduced pregnane skeleton, respectively. Ganaxolone is a 3β-methylated synthetic analog of allopregnanolone with similar anticonvulsant activity and improved bioavailability (Carter et al., 1997).

2. Materials and methods

2.1. Subjects

Male Swiss Webster mice (Taconic Farms, Germantown, NY), weighing 25–35 g, were housed five per cage with free access to food and water. Mice were kept in a vivarium under controlled laboratory conditions (temperature 22–26 °C, humidity 40–50%) with an artificial 12-h light/dark cycle. All animals were allowed to acclimate for at least 5 days before testing. The experimental groups consisted of 10–12 animals. The experiments were performed during the light cycle after at least 30-min acclimation to the experimental room. Mice were put back into their home cage after daily testing and returned to the housing vivarium. At the completion of the full kindling experiments, the mice were euthanized.

Animals used in these studies were maintained in facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care and were tested under approved protocols from the Animal Care and Use Committee of the National Institute of Drug Abuse, under guidance by the Guide for Care and Use of Laboratory Animals (National Research Council, 1996, National Academy Press, Washington, DC).

2.2. Drugs

The following neuroactive steroids were studied: allopregnanolone (3α-hydroxy-5α-pregnan-20-one), pregnanolone (3α-hydroxy-5β-pregnan-20-one) purchased from Sigma, St. Louis, MO and ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one; also known as CCD 1042) obtained from CoCensys, Irvine, CA. Stock solutions of these compounds were prepared in 40% w/v solution of hydroxypropyl-γ-cyclodextrin (Research Biochemical In-

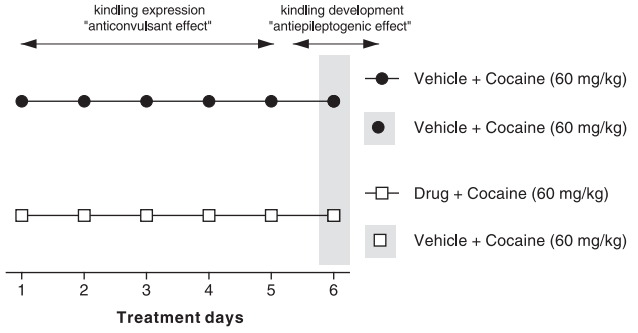

Fig. 1. Scheme illustrating the treatment protocol. All animals received daily injections of cocaine (60 mg/kg; i.p.; days 1–6). The control group (●) was pretreated with vehicle, whereas experimental groups (□) was pretreated with neuroactive steroids (s.c.; days 1–5), which allowed assessment of the effects of neuroactive steroids upon the expression of cocaine-kindled seizures (anticonvulsant effect). On day 6 (shaded area), both groups were pretreated with vehicle and challenged with cocaine to assess the ability of previous neuroactive steroid administration to prevent the development of cocaine kindling (antiepileptogenic effect).

ternational, Natick, MA) in saline after mild heat and sonication. (−)-Cocaine HCl (National Institute on Drug Abuse, Baltimore, MD) was dissolved in sterile saline. All neuroactive steroids were given subcutaneously, whereas cocaine was administered intraperitoneally. The injection volume for all drugs was 0.01 ml/g body weight. The pretreatment time (15 min) and the administered doses of the neuroactive steroids were based on previous experiments showing acute anticonvulsant activity in mice (Gasior et al., 1997).

2.3. Cocaine kindling and treatment protocol

The animals were kindled with a dose of 60 mg/kg of cocaine for 5 days sequentially as described by (Miller et al., 2000). The effects of neuroactive steroids against the expression of seizures and development of cocaine kindling were examined in this paradigm as previously described (Gasior et al., 2000b). Briefly, animals were pretreated with either vehicle or neuroactive steroid (with different groups of mice receiving different doses) and then given cocaine (60 mg/kg) for 5 consecutive days (Fig. 1). Such treatment allowed assessment of the effects of neuroactive steroids upon the expression of cocaine-kindled seizures (anticonvulsant effect; Fig. 1). On day 6, mice were challenged with 60 mg/kg of cocaine after receiving vehicle instead of neuroactive steroid to assess the ability of neuroactive steroids to prevent the development of cocaine kindling (antiepileptogenic effect; Fig. 1).

The endpoint of cocaine-induced convulsions was defined as the occurrence of clonic seizures accompanied by the loss of righting reflex lasting at least 5 s. The development of kindling was reflected by monotonic and statistically significant increases in the number of mice exhibiting seizures

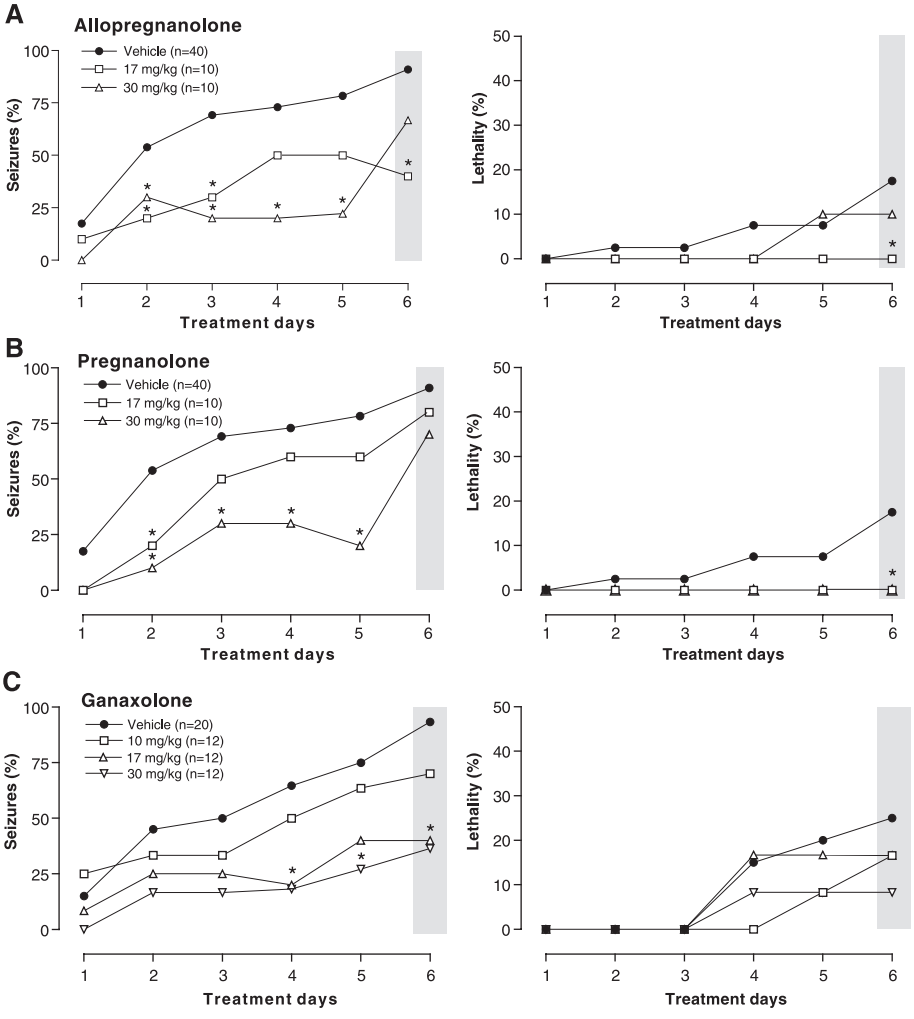

Fig. 2. Effects of allopregnanolone (A), pregnanolone (B) and ganaxolone (C) upon seizures and cumulative lethality during cocaine kindling. The control groups (closed symbols) were given vehicle 15 min before cocaine (60 mg/kg) injection on days 1 to 6. Experimental groups (open symbols) were pretreated with neuroactive steroids (doses in legends) 15 min prior to cocaine (60 mg/kg) administration on days 1 to 5. On day 6, the animals received vehicle instead of the neuroactive steroid (open symbols on gray background) before the last cocaine administration. Lethality was assessed 60 min following cocaine injection every day. *$P < 0.05$ (Fisher's exact probability test). Separate control groups treated with vehicle were used for each neuroactive steroid, but the generated data were pooled into a common control group (A and B); please see Materials and methods.

upon repeated treatments with cocaine (Gasior et al., 2000b; Miller et al., 2000). Lethal effects occasionally occurred after repeated injections of cocaine at 60 mg/kg; therefore, lethality within a 60-min post-injection time was noted. Based on these observations, cumulative lethality was calculated.

2.4. Data analysis and presentation

The results obtained from cocaine kindling experiments were expressed as the percentage of animals showing seizures or the cumulative percentage of mice dying on each day of cocaine administration. The conservative Fisher's exact probability test was used for statistical comparisons between data obtained in neuroactive steroid- and vehicle-treated groups. Statistical significance was considered when the P value was less than 0.05.

Separate control groups treated with vehicle were used for each neuroactive steroid. The generated data were pooled into a common control group ($n = 20–40$) for statistical analysis since there were no significant differences across the individual control groups ($P>0.05$; Fisher's exact test).

3. Results

Daily administration of cocaine at 60 mg/kg resulted in injection-dependent increases in the incidence of seizures and lethality. For mice given cocaine alone, the mean percentage of animals exhibiting seizures on day 1 was less than 20% and this value increased to about 90% on day 6 (Fig. 2A, B, C; left side). Cumulative lethality under these conditions increased from 0% on day 1 to approximately 20% on day 6 (Fig. 2A, B, C; right side).

Daily treatment with allopregnanolone (30 mg/kg) decreased the percentage of mice exhibiting clonic seizures after successive cocaine injections. This effect was significant on days 2 through 5 (Fig. 2A; left side). A lower dose of allopregnanolone (17 mg/kg) was effective in this respect only on days 2 and 3. Allopregnanolone (17 mg/kg) attenuated sensitization to the convulsive effects of cocaine in cocaine-kindled mice. This effect was seen on day 6 when the animals were challenged with cocaine after substituting vehicle for allopregnanolone pretreatment. The higher dose of 30 mg/kg did not significantly attenuate kindling development as evidenced on day 6. Mice pretreated for 5 days with 17 mg/kg also displayed a significant reduction in cumulative lethality on day 6 compared to vehicle control mice (Fig. 2A; right side).

As with allopregnanolone, pregnanolone (30 mg/kg) suppressed the expression of cocaine-induced seizures on days 2 through 5 (Fig. 2B; left side). The lower dose of pregnanolone (17 mg/kg) was effective only on day 2. Pregnanolone (17 and 30 mg/kg) did not block the development of cocaine kindling as evidenced on day 6. Nonetheless, both doses of pregnanolone completely blocked the increase in cumulative lethality resulting from repeated cocaine exposure (Fig. 2B; right side).

Groups of mice pretreated daily with 17 or 30 mg/kg ganaxolone but not 10 mg/kg showed a significant decrease in seizure incidence on days 4, 5 (30 mg/kg only) and 6 demonstrating the efficacy of ganaxolone to attenuate seizure expression and development (Fig. 2C; left side). In the presence of ganaxolone, fewer mice died during the kindling process; however, this effect did not reach statistical significance nor was it dose-dependent (Fig. 2C; right side).

4. Discussion

The present study shows that both endogenous and synthetic neuroactive steroids, which are positive modulators of the $GABA_A$ receptor, are capable of decreasing the cumulative lethality of mice kindled with cocaine, and both prevent the expression of seizure kindling as well as inhibit the development of cocaine-kindled seizures.

Recently, we have shown that neuroactive steroids posses anticonvulsant activity against cocaine seizures when administered acutely prior to cocaine (cf., Gasior et al., 1997). Although efficacy against cocaine kindling was evidenced in this study with the neuroactive steroids, their protective effects were not uniformly dose-dependent as observed in other kindling models (Carter et al., 1997; Gasior et al., 1998, 2000a). On the other hand, neuroactive steroids are fully efficacious against cocaine-kindled seizures, while the commonly used antiepileptic drugs are only partially effective under these conditions (Kaminski et al., 2001; Gasior et al., 2000b). Similarly, these drugs do not alter the epileptogenic process (kindling development) whereas ganaxolone, for instance, is fully efficacious against pentylenetetrazol (Gasior et al., 1998) or cocaine-induced kindling (present study). This is an important finding, because it has been suggested that acute anticonvulsant activity or the ability to protect against kindling expression does not necessarily predict antiepileptogenic response (Löscher and Schmidt, 1988).

In the present study, ganaxolone inhibited both the expression and development of cocaine kindling. This compound belongs to the family of synthetic neuroactive steroids developed to overcome pharmacokinetic limitations of endogenous neuroactive steroids while retaining the same pharmacological properties (Carter et al., 1997; Gasior et al., 1999a). Ganaxolone is a promising compound showing efficacy in various models of experimental epilepsy and the clinical treatment of childhood epilepsies (for reviews see Gasior et al., 1999a; Monaghan et al., 1999). As it was mentioned, ganaxolone suppresses the expression and development of pentylenetetrazol-induced kindling (Gasior et al., 1998). Further experiments revealed that ganaxolone was highly effective against clonic and tonic seizures as well as lethality induced by a high dose of pentylenetetrazol in pentylenetetrazol-kindled mice (Gasior et al., 2000a). In addition, ganaxolone, unlike established antiepileptics, al-

most completely reverses behavioral symptoms associated with pentylenetetrazol administration in mice using a locomotor activity suppression measure (Beekman et al., 1998); similar effects were observed in bicuculline- or picrotoxin-treated animals (Ungard et al., 2000). Furthermore, ganaxolone not only prevents but also partially reverses depression in locomotor activity induced by pentylenetetrazol administration (Ungard et al., 2000). Ganaxolone and other neuroactive steroids that are positive modulators of the $GABA_A$ receptor have been consistently reported to have features distinct from other well-established ligands of this receptor, e.g. diazepam or phenobarbital (Beekman et al., 1998; Gasior et al., 1997; Ungard et al., 2000; Vanover et al., 2000). With regard to cocaine, neuroactive steroids attenuate both convulsant and behavioral effects of cocaine, whereas diazepam or phenobarbital is ineffective (Gasior et al., 1997; Vanover et al., 2000).

The mechanisms underlying cocaine kindling are not fully understood, but down regulation of the GABA-ergic system after repeated cocaine administration has been reported (Goeders, 1991; Pecins-Thompson and Peris, 1993; Peris, 1996). On the other hand, pharmacological evidence shows that GABA-mimetic drugs (e.g. benzodiazepines, barbiturates) are not fully effective against cocaine kindling (Gasior et al., 2000b; Kaminski et al., 2001). Interestingly, chlormethiazole, a drug that positively modulates GABA-ergic neurotransmission in a manner different form benzodiazepines or barbiturates, also shows high efficacy against both expression or development of cocaine kindled seizures (Gasior et al., 2000b). Although the mechanism of modulation of the $GABA_A$ receptor by chlormethiazole and neuroactive steroids is different, these drugs show similar beneficial effects against cocaine kindling.

Protective effects of ganaxolone against the expression and development of cocaine kindling are also shared by allopregnanolone. In the case of allopregnanolone, however, the effects observed in the present study were not dose-dependent. It is possible that the same neuroactive steroid may differentially modulate the $GABA_A$ receptors depending upon its concentration (Majewska et al., 1988). Furthermore, high concentrations of neuroactive steroids are capable of producing nonspecific actions upon various ligand-gated ion channels (Rupprecht and Holsboer, 1999). Pregnanolone, unlike allopregnanolone and ganaxolone, did not prevent the development of cocaine kindling. Although only two doses of pregnanolone were tested in the present study, it should be underlined that the highest dose was effective against the expression of cocaine kindling. Pregnanolone has also been shown to have a different anticonvulsant profile as compared to allopregnanolone and ganaxolone (Gasior et al., 1997). Moreover, allopregnanolone, but not pregnanolone, is effective against kainate-induced convulsions (Leskiewicz et al., 1997). The above-mentioned observations could be explained, at least in part, by differences in the structural assembly of these neuroactive steroids. Both, allopregnanolone and ganaxolone possess a 5α-reduced pregnane skeleton with α-OH at the C3 position of the steroid A ring (5α,3α configuration), whereas pregnanolone has a 5β,3α configuration. It has been suggested that the 5α,3α configuration of neuroactive steroids yields higher anticonvulsant potency and potentiation of GABA receptor currents than the 5β,3α configuration (Kokate et al., 1994, 1996).

Another potential explanation for the differential effects of the neuroactive steroids on the development of cocaine kindling is their differential accumulation and activity when tested with cocaine alone on day six. Recall, mice are treated on days 1 to 5 with cocaine plus neuroactive steroid. The activity of the neuroactive steroid on day 6 could dampen cocaine-induced convulsions by way of its anticonvulsant effects. Several pieces of data argue against this possibility and point instead to pharmacodynamic explanations. First of all, both the susceptibility of animals to convulsant effects of pentylenetetrazol (Gasior et al., 2000a) and the efficacy of ganaxolone remain unchanged after its chronic administration (Reddy and Rogawski, 2000). As such, the observed effects of ganaxolone on the development of either pentylenetetrazol (Gasior et al., 1998, 2000a) or cocaine kindling (present study) do not seem to be of cumulative nature. Further, despite greater metabolic stability than natural neuroactive steroids, ganaxolone still has a relatively short half-life that would suggest complete clearance by 24 h (Carter et al., 1997). Thirdly, although all of the neuroactive steroids tested attenuated the expression of cocaine kindling, pregnanolone with generally similar kinetics to allopregnanolone, did not attenuate the development of sensitization as measured on day six. Although these data cast doubt upon pharmacokinetic factors influencing day 6 results, kinetic analyses and in vivo anticonvulsant assessments in mice treated as in the present study are necessary to provide definitive closure on this question.

The neuroactive steroids examined in the present study proved effective against the expression and development of cocaine kindling. As reported recently, they were also highly effective against acute cocaine-induced seizures (Gasior et al., 1997). In this light, neuroactive steroids appear to represent a novel class of drugs with potential for treating sensitizing and convulsant effects of cocaine, a feature that may join well with data suggesting their efficacy against sensitization, tolerance and withdrawal effects of drugs of abuse.

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