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Long-term social isolation enhances picrotoxin seizure susceptibility in mice: up-regulatory role of endogenous brain allopregnanolone in GABAergic systems

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Abstract

Allopregnanolone (ALLO, 3α,5α-tetrahydroprogesterone), a positive allosteric modulator of actions of γ-aminobutyric acid GABA) at $GABA_A$ receptors, is synthesized in the brain from progesterone by the sequential action of two enzymes: a type I 5α-reductase and a 3α-hydroxysteroid oxidoreductase. We previously demonstrated that long-term social isolation of mice caused a significant decrease in brain ALLO content via suppression of type I 5α-reductase and its mRNA expression. In this study, to clarify a physiological role of endogenous brain ALLO, we investigated changes in seizure susceptibility of mice following protracted social isolation and compared with those of mice treated with SKF105111 (SKF), an inhibitor of types I and II 5α-reductase. Social isolation of mice for 7 weeks prior to the experiments caused a significant increase of seizure susceptibility to the $GABA_A$ receptor antagonist picrotoxin but not to the glycine receptor antagonist strychnine or the glutamate receptor agonist kainic acid. The change in the seizure susceptibility was completely reversed by 2.5 mg/kg ip ALLO, a dose that per se had no effect on picrotoxin-induced seizure. Treatment of mice with SKF (20 mg/kg ip) also reduced a threshold dose of picrotoxin, but not that of strychnine or kainic acid, which was required to elicit seizure in group-housed mice. The effect of SKF was attenuated by ALLO (2.5 mg/kg ip). In contrast, SKF treatment had no effect on picrotoxin-induced seizure in socially isolated mice. These findings suggest that endogenous brain ALLO plays a suppressive role in seizure susceptibility via a positive modulation of $GABA_A$ receptor function and that social isolation enhances seizure susceptibility in mice via reduction of $GABA_A$ receptor function caused by a decrease of endogenous ALLO.

Keywords: Social isolation; Allopregnanolone (3α,5α-tetrahydroprogesterone); Type I 5α-reductase inhibitor; SKF105111; Seizure susceptibility

1. Introduction

Allopregnanolone (ALLO, 3α,5α-tetrahydroprogesterone) is a neuroactive steroid with a potent positive allosteric modulatory action on γ-aminobutyric acid (GABA)-gated Cl^- current responses at $GABA_A$ receptors (Harrison and Simmonds, 1984; Majewska et al., 1986; Puia et al., 1993). This steroid is synthesized in the brain from progesterone by two enzymes: a type I 5α-reductase that reduces progesterone to 5α-dihydroprogesterone (5α-DHP) and a 3α-hydroxysteroid oxidoreductase that reduces 5α-DHP to ALLO or oxidize ALLO to 5α-DHP, depending on the cofactors present in the environment (Rupprecht, 1997; Guidotti et al., 2001). Evidence indicates that exogenously applied ALLO regulates GABAergic neurotransmission in a nanomolar concentration range in vitro (Puia et al., 1990), and that when given exogenously to experimental animals, ALLO exerts anticonvulsant, anxiolytic, and sedative hypnotic activities (Belelli et al., 1989; Bitran et al., 1991). Based on these pharmacological properties of exogenous ALLO, a close relationship between endogenous brain ALLO and $GABA_A$ receptor function has been proposed, but a physiological and neuropathological role of endogenously synthesized brain ALLO has not been fully elucidated yet.

We have previously demonstrated that social isolation stress causes resistance to the sedative action of indirect or

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direct $GABA_A$ mimetic drugs, such as pentobarbital in mice (Matsumoto et al., 1996, 1999), and that the effect of social isolation on GABAergic systems is, in part, due to a marked decrease in endogenous ALLO content in the brain via suppressed expression of type I 5α-reductase and its mRNA expression (Dong et al., 2001). These findings raise a possibility that an endogenous brain ALLO may play an important role in keeping GABAergic tone under physiological conditions and that its decrease may reduce GABAergic function in the brain. In fact, a similar decrease in pharmacological activities of the GABAergic drugs was observed in the animals in which brain ALLO biosynthesis was inhibited by systemic administration of SKF105111 (SKF), a potent 5α-reductase inhibitor (Holt et al., 1990; Cheney et al., 1995; Pinna et al., 2000).

It has been considered that down-regulation of $GABA_A$ receptor function is responsible for an increase in seizure susceptibility since drugs with a negative allosteric modulatory activity at the $GABA_A$ receptor; for instance, benzodiazepine receptor inverse agonists and pregnenolone sulfate exert convulsant or proconvulsant actions in rodents (Novas et al., 1988; Nutt and Lister, 1988; Reddy and Kulkarni, 1998), while positive allosteric modulation of the receptors by benzodiazepines, barbiturates, and ALLO, as aforementioned, exert protective action against seizure (Belelli et al., 1989; Kokate et al., 1999). Taken together, it can be hypothesized that endogenous brain ALLO may play a suppressive role in seizure susceptibility by providing facilitatory influence on $GABA_A$ receptor function and that protracted social isolation may enhance seizure susceptibility in mice via a decrease in $GABA_A$ receptor function caused by a decrease of endogenous brain ALLO. In the present study, to test this hypothesis, we investigated changes in seizure susceptibility of mice following protracted social isolation and compared with those of mice treated with a 5α-reductase inhibitor.

2. Materials and methods

2.1. Animals

Male ddY mice, weighing 18–20 g (Japan SLC, Shizuoka, Japan) were obtained at the age of 28 days. Animals were either housed in groups of five to six per cage (24 × 17 × 12 cm) or socially isolated by being housed individually in a same-size cage for 7 weeks before the start of experiments (Matsumoto et al., 1999; Dong et al., 2001). To test the effect of SKF on seizure susceptibility, mice were housed in groups as described above for at least 1 week before the start of the experiments. Food and water were given ad libitum. Housing condition was thermostatically maintained at 24 ± 1 °C with a constant humidity (65%) and a 12-h light–dark cycle (lights on: 0700–1900 h). The present study was conducted in accordance with the standards established by the *Guide for the Care and Use of Laboratory Animals* of Toyama Medical and Pharmaceutical University.

2.2. Convulsion testing

Each mouse was taken from its home cage, placed individually in a holding cage, and transferred to a procedure room where seizure tests were conducted. The mouse was placed in a Plexiglas restrainer (10 × 10 × 10 cm) with its tail free. The tail was immersed in warm water (40 °C) for 30 s to dilate the tail veins. A butterfly needle (27-gauge × 1/2) was inserted into one of the two lateral tail veins. Upon correct placement, a mouse was placed in a Plexiglas observation cage and infusion of a convulsant was performed at a constant rate of 0.23 ml/min using a syringe pump (Model CMA/100, Carnegie Medicine, Stockholm). The mouse was observed for seizure signs (twitch, clonic convulsion, and tonic convulsion) and latencies of each seizure sign were recorded. Threshold doses of each convulsant drug for onset of seizure signs were calculated by the following equation.

$$\text{Threshold dose (mg/kg)} = [\text{total infusion volume (ml)}] \times [\text{convulsant concentration} \times (\text{mg/ml})] / [\text{body weight (kg)}]$$

Picrotoxin (1.5 mg/ml), strychnine (0.056 mg/ml), or kainic acid (6 mg/ml) was infused into the tail vein at a constant rate of 0.23 ml/min. Each convulsant was dissolved in saline. To examine the effect of ALLO on picrotoxin-induced seizure, ALLO was dissolved in 7% (2-hydroxypropyl)-β-cyclodextrin-containing saline and injected intraperitoneally 15 min before the start of picrotoxin infusion.

2.3. SKF105111 treatment

SKF105111 (SKF, 17β-(*N*,*N*-diisopropylcarbamoyl)androst-3,5-diene-3-carboxylic acid) was synthesized according to the method described by Holt et al. (1990). SKF was dissolved in 100% methanol and then mixed with equimole of NaOH, yielding SKF-Na salt. SKF-Na was dissolved in distilled water just before the start of the experiment and injected intraperitoneally.

2.4. Drugs

Drugs used were as follows: picrotoxin (Nacalai Tesque, Kyoto, Japan), strychnine and allopregnanolone (Sigma, St. Louis, MO), and kainic acid (Tocris, Bristol, UK).

2.5. Statistics

The data were analyzed with one- or two-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test for multiple comparisons among different groups.

Student's t test was used to analyze the difference between the two groups. Differences with $P<.05$ were considered significant.

3. Results

3.1. Social isolation stress enhances susceptibility to picrotoxin—but not strychnine- or kainic acid-induced convulsion in mice

As shown in Fig. 1A, socially isolated mice showed significantly reduced threshold doses for onset of picrotoxin-induced myoclonic twitch and clonic convulsion compared with the animals housed in groups for the same period. In contrast, no significant differences in the threshold doses of strychnine or kainic acid to elicit clonic convulsion were found between socially isolated and group-housed animal groups. The systemic administration of ALLO at a dose of 2.5 mg/kg ip per se did not affect the picrotoxin-induced convulsion in group-housed mice, but it completely recovered the threshold dose of picrotoxin in socially isolated mice to the level in group-housed control animals (Fig. 1B).

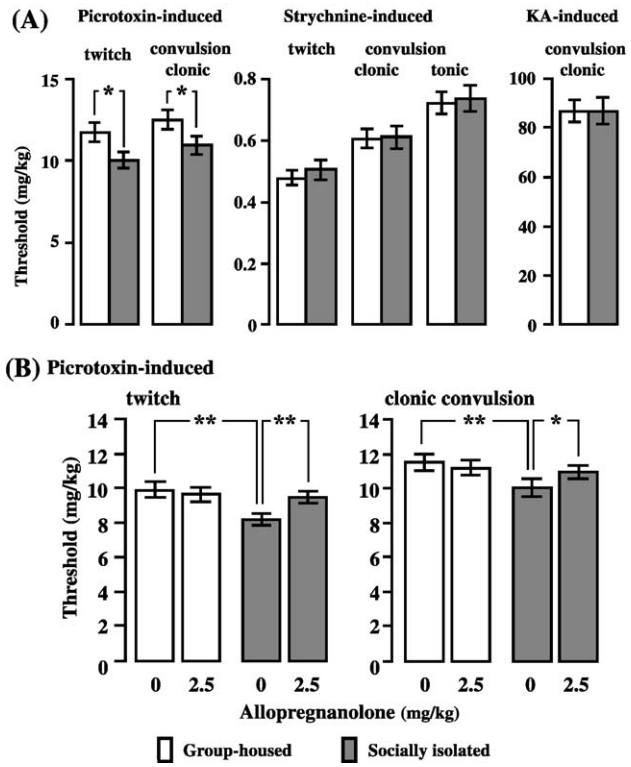

Fig. 1. Changes in threshold doses of picrotoxin-, strychnine-, and kainic acid-induced seizure following protracted social isolation in mice. Animals were either group-housed (white column) or socially isolated (black column) for 7 weeks before the start of the experiments. (A) Comparison of seizure threshold between socially isolated and group-housed mice. Picrotoxin, strychnine, or kainic acid dissolved in saline was infused into the tail vein at a constant rate of 0.23 ml/min. The threshold dose (mg/kg) for onset of seizure indices (myoclonic twitch, clonic convulsion, and tonic convulsion) was calculated as described in the text. Each column represents the mean ± S.E.M. ($n=7–10$). $*P<.05$, $**P<.01$ (Student's t test). (B) Effect of ALLO on the change in picrotoxin-seizure susceptibility caused by protracted social isolation in mice. ALLO (2.5 mg/kg) was given intraperitoneally 15 min before the start of picrotoxin infusion. $*P<.05$, $**P<.01$ [housing condition: $F(1,32)=15.880$, $P<.001$; ALLO: $F(1,32)=4.715$, $P<.05$; Housing Condition × ALLO: $F(1,32)=9.092$, $P<.01$ for twitch; housing condition: $F(1,32)=8.103$, $P<.01$; ALLO: $F(1,32)=0.889$, $P=.353$; Housing Condition × ALLO: $F(1,32)=3.99$, $P<.05$ for clonic convulsion] (a two-way ANOVA followed by Student–Newman–Keuls test).

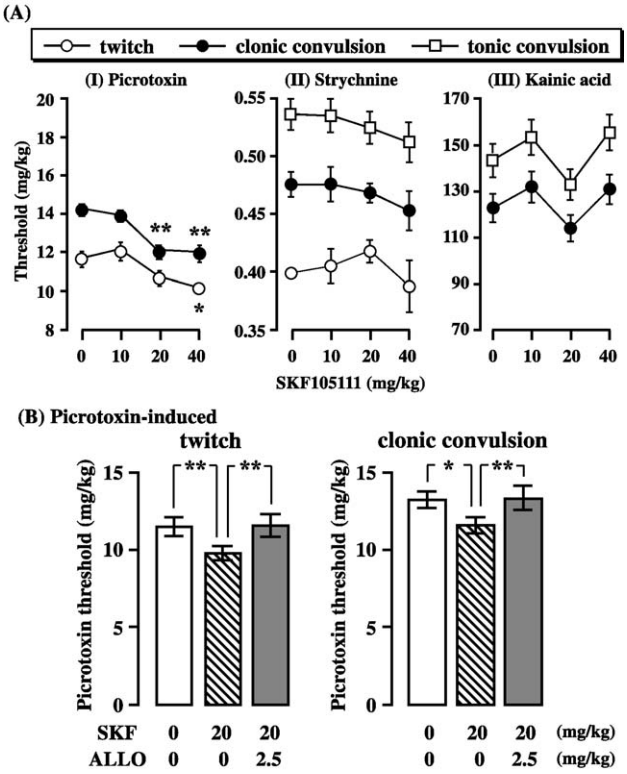

Fig. 2. Changes in threshold dose of picrotoxin-, strychnine-, and kainic acid-induced convulsion following SKF105111 (SKF) treatment in mice. (A) Comparison of seizure threshold between vehicle- and SKF-treated mice. Picrotoxin, strychnine, or kainic acid was infused into the tail vein at a constant rate of 0.23 ml/min. Mice were injected intraperitoneally with SKF (10–40 mg/kg) or vehicle 3 h before intravenous infusion of a convulsant drug. The threshold dose for onset of seizure indices was calculated as described in the text. Each data point is the mean ± S.E.M. of 8–12 mice. $*P<.05$ and $**P<.01$ compared with respective vehicle control [(I) twitch: $F(3,41)=5.019$, $P<.01$; clonic convulsion: $F(3,41)=10.507$, $P<.01$; (II) twitch $F(3,34)=0.830$, $P=.487$; clonic convulsion: $F(3,34)=0.772$, $P=.518$; tonic convulsion: $F(3,34)=0.601$, $P=.619$; (III) clonic convulsion: $F(3,31)=3.034$, $P<.05$; tonic convulsion: $F(3,31)=3.542$, $P=.027$] (a one-way ANOVA followed by Student–Newman–Keuls test). (B) Effect of ALLO on the change in picrotoxin-seizure susceptibility caused by SKF treatment in mice. SKF (20 mg/kg ip) or vehicle was administered 3 h before the start of picrotoxin infusion. SKF-treated mice were administered vehicle or ALLO (2.5 mg/kg ip) 15 min before picrotoxin. Each data column represents the mean ± S.E.M. ($n=9–10$). $*P<.05$ and $**P<.01$ [twitch: $F(2,26)=5.212$, $P<.05$; clonic convulsion: $F(2,26)=4.400$, $P<.05$] (a one-way ANOVA followed by Student–Newman–Keuls test).

3.2. SKF105111 treatment enhances susceptibility to picrotoxin-induced seizure in group-housed normal mice

In a previous study, systemic administration of SKF (20 mg/kg) significantly reduced brain ALLO levels in group-housed control mice and the brain ALLO level reached minimum (~ 80% decrease) around 3–6 h after SKF administration (Puia et al., 2003). Therefore, in this study, we used the same condition to reduce brain ALLO and examined if SKF treatment modifies seizure susceptibility of mice in a similar manner to that of protracted social isolation. As depicted in Fig. 2, when examined at 3 h after SKF injection, SKF-treated animals became more susceptible to picrotoxin-induced myoclonic twitch and clonic convulsion than the vehicle-treated control, indicating pro-convulsant activity of SKF against a $GABA_A$ antagonist-induced convulsion. In contrast, SKF at doses of 10–40 mg/kg did not affect susceptibility to strychnine-induced seizures in group-housed mice. A one-way ANOVA revealed significant effects of SKF treatment on kainic acid-induced seizure but a post hoc test did not show significant difference between vehicle- and SKF-treated animals. The increase in picrotoxin-seizure susceptibility following SKF treatment of group-housed mice was significantly attenuated by systemic administration of 2.5 mg/kg ALLO (Fig. 2B). In contrast to the effect observed in group-housed animals, SKF (20 mg/kg ip) treatment failed to alter threshold doses of picrotoxin to elicit each seizure sign in socially isolated mice (Fig. 3).

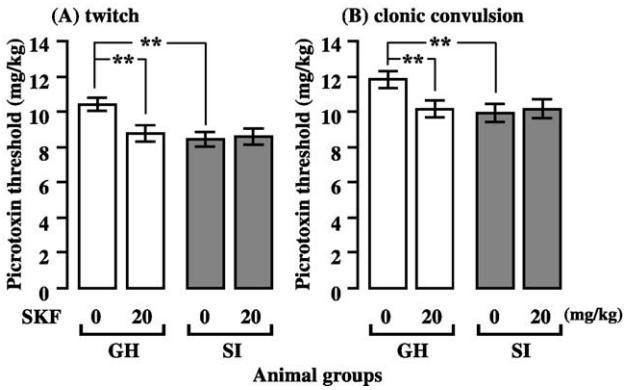

Fig. 3. Effects of SKF105111 (SKF) on picrotoxin-induced seizures in group-housed (G) and isolated (I) mice. Animals were either group-housed or isolated for 7 weeks before the start of the experiments. SKF (20 mg/kg ip) or vehicle was administered 30 min before the experiments. Picrotoxin dissolved in saline was infused into the tail vein at a rate of 0.23 ml/min. The threshold dose for onset of seizure indices was calculated as described in the text. Each data column represents the mean ± S.E.M. ($n = 9–10$). ** $P < .01$ [(A) housing condition: $F(1,35) = 11.476$, $P < .01$; SKF treatment: $F(1,35) = 4.910$, $P < .05$; Housing Condition × SKF treatment: $F(1,35) = 7.791$, $P < .01$; (B) housing condition: $F(1,35) = 6.803$, $P < .05$; SKF treatment: $F(1,35) = 4.198$, $P < .05$; Housing Condition × SKF treatment: $F(1,35) = 7.773$, $P < .01$] (a two-way ANOVA followed by Student–Newman–Keuls test).

4. Discussion

Evidence indicates a positive allosteric modulatory effect of ALLO on $GABA_A$ receptor function in the brain (for a review, see Paul and Purdy, 1992; Costa et al., 1994; Guidotti and Costa, 1998). However, a physiological role of endogenous brain ALLO has been inferred mainly from the pharmacological actions of exogenously administered ALLO or its congeners. In this study, we demonstrated that long-term social isolation increased seizure susceptibility to a $GABA_A$ antagonist picrotoxin probably via a decrease of endogenous brain ALLO in a similar manner as the types I and II 5α-reductase-inhibitor SKF. The present findings provide a further support for the hypothesis that endogenous level of brain ALLO plays a tonic facilitatory role in $GABA_A$ receptor function and that decreased endogenous brain ALLO level reduces GABAergic function in the brain.

Long-term social isolation of mice caused a significant decrease in seizure threshold of picrotoxin but this housing condition failed to change seizure thresholds of the glycine receptor antagonist strychnine and the glutamate receptor agonist kainic acid. Together with mechanisms by which these convulsants elicit seizure, the increase in susceptibility to picrotoxin-induced seizure following long-term social isolation suggests occurrence of functional changes in GABAergic systems in the brain. It should be noted that the increase in picrotoxin-seizure susceptibility by social isolation was reversed to the control level by systemic administration of ALLO (2.5 mg/kg) at a dose that per se had no effect on the picrotoxin-induced seizure in group-housed animals. In previous studies, mice exposed to social isolation for 4–10 weeks exhibited a decreased behavioral response to systemically administered GABA-mimetic drugs compared to the animals that were housed in group for the same period (Matsumoto et al., 1996, 1999). In addition, such behavioral abnormalities were in part, due to a decrease in endogenous brain ALLO via a decrease of type I 5α-reductase expression (Matsumoto et al., 1999; Dong et al., 2001). Thus, the increased susceptibility of socially isolated animals to picrotoxin seizure is likely to be due to down-regulation of brain ALLO biosynthesis in this animal group.

An important role of endogenous brain ALLO in the regulation of seizure susceptibility can be further substantiated by the present findings that systemic administration of SKF, an inhibitor of types I and II 5α-reductase, reduced a seizure threshold of picrotoxin without affecting seizure-threshold doses of strychnine and kainic acid to induce seizure in group-housed animals. In this study, we elucidated changes in seizure threshold at 3 h after SKF (20 mg/kg ip) injection, since SKF, under the same experimental condition, produced a marked decrease in mouse brain ALLO content that reached minimum (~ 80% reduction) within 3–6 h after the administration (Matsumoto et al., 1999; Pinna et al., 2000; Puia et al., 2003). Moreover, it has also been demonstrated that SKF per se failed to interact directly with $GABA_A$ receptors (Matsumoto et al., 1999; Pinna et al.,

2000). Considering a selective positive modulatory effect of ALLO on the $GABA_A$ receptor (Belelli et al., 1989; Kokate et al., 1994) and the aforementioned pharmacological properties of SKF, the increase in seizure susceptibility to picrotoxin following SKF treatment is likely to be due to a decrease in endogenous brain ALLO via inhibition of 5α-reductase rather than via antagonistic interaction between SKF and endogenous GABA and that the $GABA_A$ receptor function may receive tonically up-regulatory influence of endogenous level of brain ALLO. This idea seems to be supported by the present results that administration of ALLO (2.5 mg/kg) reversed the picrotoxin-induced seizure threshold in SKF-treated mice as well as in socially isolated mice.

In contrast to group-housed mice, socially isolated animals were insensitive to SKF treatment in terms of picrotoxin-seizure susceptibility. In previous studies, protracted social isolation of mice resulted in an approximately 50% decrease in brain ALLO content via a decrease of type I 5α-reductase (Matsumoto et al., 1999; Dong et al., 2001). Moreover, an approximately 80% decrease of brain ALLO was found between 3 and 6 h after SKF injection as aforementioned (Puia et al., 2003). Thus, a plausible explanation for the insensitivity of socially isolated mice to SKF treatment is that the decrease of $GABA_A$ receptor function could reach a maximum in socially isolated animals (Serra et al., 2000) and that an additional decrease of brain ALLO by SKF might be unable to reduce the GABAergic function in this animal group.

In conclusion, the present results indicate that endogenous ALLO synthesized in the brain plays a suppressive role in seizure susceptibility via positive allosteric modulation of $GABA_A$ receptor function and that a decrease in endogenous brain ALLO by social isolation increases seizure susceptibility of mice to a $GABA_A$ antagonist.

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References

Belelli D, Bolger MB, Gee KW. Anticonvulsant profile of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Eur J Pharmacol 1989;166:325–9.

Bitran D, Hilvers RJ, Kellogg CK. Anxiolytic effects of 3α-hydroxy-5α[β]-pregnan-20-one: endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. Brain Res 1991;561:157–61.

Cheney DL, Uzunov DP, Costa E, Guidotti A. Gas chromatographic-mass fragmentographic quantitation of 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) and its precursors in blood and brain of adrenalectomized and castrated rats. J Neurosci 1995;15:4641–50.

Costa E, Auta J, Guidotti A, Korneyev A, Romeo E. The pharmacology of neurosteroidogenesis. J Steroid Biochem Mol Biol 1994;49:385–9.

Dong E, Matsumoto K, Uzunova V, Sugaya I, Takahata H, Nomura H, et al. Brain 5α-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. Proc Natl Acad Sci USA 2001;98:2849–54.

Guidotti A, Costa E. Can the antidysphoric and anxiolytic profiles of selective serotonin reuptake inhibitors be related to their ability to increase brain 3α,5α-tetrahydroprogesterone (allopregnanolone) availability? Biol Psychiatry 1998;44:865–73.

Guidotti A, Dong E, Matsumoto K, Pinna G, Rasmusson AM, Costa E. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5α-dihydroprogesterone in psychiatric disorders. Brain Res Rev 2001;37:110–5.

Harrison NJ, Simmonds MA. Modulation of the GABA receptor complex by a steroid anaesthetic. Brain Res 1984;232:287–92.

Holt DA, Levy MA, Oh HJ, Erb JM, Heaslip JI, Brandt M, et al. Inhibition of steroid 5α-reductase by unsaturated 3-carboxysteroids. J Med Chem 1990;33:943–50.

Kokate TG, Svensson BE, Rogawski MA. Anticonvulsant activity of neurosteroids: correlation with γ-aminobutyric acid-evoked chloride current potentiation. J Pharmacol Exp Ther 1994;270:1223–9.

Kokate TG, Banks MK, Magee T, Yamaguchi S-I, Rogawski MA. Finasteride, a 5α-reductase inhibitor, blocks the anticonvulsant activity of progesterone in mice. J Pharmacol Exp Ther 1999;288:679–84.

Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 1986;232:1004–7.

Matsumoto K, Ojima K, Watanabe H. Neurosteroidal modulation of social isolation-induced decrease in pentobarbital sleep in mice. Brain Res 1996;708:1–6.

Matsumoto K, Uzunova V, Pinna G, Taki K, Uzunov DP, Watanabe H, et al. Permissive role of brain allopregnanolone content in the regulation of pentobarbital-induced righting reflex loss. Neuropharmacology 1999;38:955–63.

Novas ML, Wolfman C, Medina JH, de Robertis E. Proconvulsant and 'anxiogenic' effects of *N*-butyl beta carboline-3-carboxylate, an endogenous benzodiazepine binding inhibitor from brain. Pharmacol Biochem Behav 1988;30:331–6.

Nutt DJ, Lister RG. Antagonizing the anticonvulsant effect of ethanol using drugs acting at the benzodiazepine/GABA receptor complex. Pharmacol Biochem Behav 1988;31:751–5.

Paul SM, Purdy RH. Neuroactive steroids. FASEB J 1992;6:2311–22.

Pinna G, Uzunova V, Matsumoto K, Puia G, Mienville J-M, Costa E, et al. Brain allopregnanolone regulates the potency of the $GABA_A$ receptor agonist muscimol. Neuropharmacology 2000;39:440–8.

Puia G, Santi MR, Vicini S, Pritchett DB, Purdy RH, Paul SM, et al. Neurosteroids act on recombinant human $GABA_A$ receptors. Neuron 1990;4:759–65.

Puia G, Ducic I, Vicini S, Costa E. Does neurosteroid modulatory efficacy depend on $GABA_A$ receptor subunit composition? Recept Channels 1993;1:135–42.

Puia G, Mienville J-M, Matsumoto K, Takahata H, Watanabe H, Costa E, et al. On the putative physiological role of allopregnanolone on $GABA_A$ receptor function. Neuropharmacology 2003;44:49–55.

Reddy DS, Kulkarni SK. Proconvulsant effects of neurosteroids pregnenolone sulfate and dehydroepiandrosterone sulfate in mice. Eur J Pharmacol 1998;345:55–9.

Rupprecht R. The neuropsychopharmacological potential of neuroactive steroids. J Psychiat Res 1997;31:297–314.

Serra M, Pisu M, Littera M, Papi G, Sanna E, Tuveri F, et al. Social isolation-induced decreases in both the abundance of neuroactive steroids and $GABA_A$ receptor function in rat brain. J Neurochem 2000;75:732–40.