

LACK OF ANTICONVULSANT ACTIVITY OF INTRACEREBROVENTRICULAR PROGESTERONE AND ALLOPREGNANOLONE IN MALE AMYGDALA-KINDLED RATS

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ABSTRACT

The effect of intracerebroventricular administration of progesterone and its metabolite allopregnanolone (3α -hydroxy- 5α -pregnane-20-one) on the amygdala kindling model of seizures was studied in male rats. Neither progesterone (10, 50, 200 $\mu\text{g}/\text{rat}$) nor allopregnanolone (12.5, 50, 100 $\mu\text{g}/\text{rat}$) had any effect on seizure parameters at 5, 15 and 30 min. after administration. Although previous studies imply the anticonvulsant effect of progesterone and its metabolite allopregnanolone against seizures induced by GABA_A receptor antagonists, the present data do not support the anticonvulsant activity of these two steroids in amygdala-kindled seizures.

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INTRODUCTION

It has been shown that progesterone has anticonvulsant activity in some animal models of seizure.^{6,7,21-23,26} Also, clinical evidence suggests that progesterone therapy exerts a significant anti-seizure effect in women who have complex partial seizures of temporal lobe origin and inadequate luteal phases.^{1,8} The amygdala-kindling model of epilepsy which involves daily application of a brief subconvulsive electrical stimulus to the amygdaloid nucleus has been considered as a good model of human complex partial seizures with secondary generalization.¹⁴ However, the effect of progesterone or allopregnanolone on kindled seizures that are fully developed has not been studied. We

have already demonstrated that intraperitoneally administered progesterone at high and sedative doses possesses some anticonvulsant activity in male amygdala-kindled rats.²⁰ Many researchers have interpreted the effects of progesterone to be due to its bioconversion to allopregnanolone (3α -hydroxy- 5α -pregnane-20-one) which subsequently acts at the GABA_A receptor complex.^{3,13,15} Moreover, it has been reported that allopregnanolone has anticonvulsant activity against seizures induced by bicuculline, picrotoxin and PTZ.^{2,5,11} Therefore, the present study was undertaken with the aim of discovering whether direct application of progesterone and its metabolite allopregnanolone to the central nervous system can induce anticonvulsant effects in fully amygdala-kindled rats.

MATERIALS AND METHODS

Animals and surgery

Male albino rats (260-300g) were used throughout this

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Table I. Effect of progesterone and allopregnanolone on kindled seizures.

	SS	S5D (s)	ADD (s)
Control	5±0	13.0±2.2	73.0±8.8
Progesterone (10 µg)	5±0	12.0±1.9	73.2±7.5
Control	5±0	7.2±0.7	61.2±8.4
Progesterone (50 µg)	5±0	7.2±2.5	65.2±6.6
Control	5±0	16.0±2.5	55.4±4.9
Progesterone (200 µg)	5±0	16.3±2.6	63.6±7.7
Control	5±0	12.5±1.8	62.5±7.2
Allopregnanolone (12.5 µg)	5±0	12.7±1.8	64.7±6.2
Control	5±0	11.4±1.0	58.0±4.8
Allopregnanolone (50 µg)	5±0	13.4±1.5	65.0±5.4
Control	5±0	12.7±2.2	74.5±1.7
Allopregnanolone (100 µg)	5±0	18.0±1.0	74.7±6.0

Data were elicited 5 min. after intracerebroventricular injections and represent mean ±SEM (n=5). There is no statistically significant difference between the control and test groups using paired t-test. SS, seizure stage; S5D, duration of stage 5 seizure; ADD, afterdischarge duration; s, second.

investigation. The animals were fed laboratory chow and tap water and maintained on a 12h light-dark schedule. Animals were implanted with a tripolar electrode into the right basolateral amygdala using stereotaxic techniques as previously described.¹⁷

Rats were also implanted with a 23 gauge stainless steel guide cannula into the left lateral ventricle at coordinates -0.9 mm posterior, -1.4 mm lateral and -2.2 mm ventral to bregma¹⁶ which was kept open with a stainless steel stylet.

Electrical kindling and intracerebroventricular administrations

Ten days after surgery, the threshold of afterdischarges (AD) for each rat was determined by electrical stimulation as previously described.¹⁸ The animals were then stimulated daily at AD threshold until five consecutive stage 5 seizures were elicited. These animals were then considered fully kindled and ready for drug treatment. Behavioral seizure stage was monitored according to the classification of Racine¹⁹: stage 1, facial clonus; stage 2, head nodding; stage 3, forelimb clonus; stage 4, rearing and bilateral forelimb clonus; stage 5, rearing and falling. Electrical activity of the amygdala was recorded after stimulus delivery and the following parameters were recorded: seizure stage (SS), afterdischarge duration (ADD) and the duration of stage 5 seizures (S5D). For intracerebroventricular (icv) injections, progesterone (Fluka Chemie AG) and allopregnanolone (Sigma) were dissolved in propylene glycol (E. Merck) and injected (4 µL/rat over 1 min.) into the lateral ventricle through a 30 gauge cannula which extended 1mm below the tip of the guide cannula in order to reach the lateral ventricle and was connected by polyethylene tubing to a 5 µL Hamilton microsyringe.

Progesterone at doses of 10, 25, and 200 µg/rat and

allopregnanolone at doses of 12.5, 50 and 100 µg/rat were administered icv and after 5 min., seizure parameters including SS, S5D and ADD were elicited. 24 h prior to drug treatment, rats received an equal volume of vehicle and 5 min. after injection, the seizure parameters were recorded as control values.

Histology

At the end of the experiments, animals were sacrificed, their brains were removed, sectioned and examined for electrode and cannula position.

Statistical analysis

Data are presented as mean±SEM of each group and were analyzed using paired t-test. A *p* value of less than 0.05 was considered to represent a significant difference.

RESULTS

At the doses employed, neither progesterone nor allopregnanolone induced changes in any of the seizure parameters at 5, 15 and 30 min. after drug injection. Results obtained 5 min. post administration are presented in Table I. It is evident from here that progesterone and allopregnanolone up to doses of 200 and 100 µg/rat respectively, do not exhibit any anticonvulsant activity in male amygdala-kindled rats. The steroid vehicle used did not affect sensitivity to the seizures. Moreover, neither the drugs employed nor the vehicle exerted any noticeable effect on animal behavior or locomotor activity.

DISCUSSION

It has been shown that progesterone possesses some

anticonvulsant activity in male amygdala-kindled rats.²⁰ Studies on the effect of progesterone in experimental animals have suggested the effects of progesterone to be due to its bioconversion to allopregnanolone which subsequently acts at the GABA_A receptor complex.^{3,13,15} The most convincing evidence may be that the anxiolytic effect of progesterone can be blocked by administering drugs which inhibit 5 α -reduced metabolism and subsequently the formation of allopregnanolone.⁴ Therefore determination of the anticonvulsant activity of allopregnanolone in the kindling model of epilepsy could provide good insight in this regard. Results of the present study show that icv administration of progesterone and allopregnanolone up to doses of 200 and 100 μ g/rat respectively do not exhibit any anticonvulsant activity in male amygdala-kindled rats. However, we have recently reported that intraperitoneal (ip) progesterone at high and sedative dose of 75 mg/kg possesses anticonvulsant activity in male amygdala-kindled rats.²⁰ Moreover, it has been shown that icv administration of allopregnanolone up to 15 μ g/rat has protective effects against PTZ-induced convulsions.⁵ The lack of anticonvulsant activity of icv administration of progesterone and allopregnanolone observed in this study could be accounted for by either of two explanations. First, the doses applied in this study may have not been sufficient and are lower than the concentrations reached after high ip doses. Therefore higher icv doses would be required to produce anticonvulsant effects. However, due to weak solubility, icv injection of higher doses of these steroids was not possible. Second, it appears that the anticonvulsant activity of allopregnanolone may differ among different seizure models. Some investigators have shown the anti-seizure activity of progesterone and allopregnanolone against PTZ-induced seizures.^{11,21-23} It is not unlikely that the disagreement of our results with these studies may be attributable to distinct neuroanatomical pathways and/or neurochemical mechanisms that underlie these different types of seizures, as it has been shown that allopregnanolone does not exhibit any anticonvulsant activity against seizures evoked by electroshock.¹¹

It has been demonstrated that the potency of allopregnanolone in enhancing the function of GABA_A receptors appears markedly increased under pharmacological conditions in which GABAergic transmission is reduced, for example with PTZ or isoniazid treatment.⁵ However, a wealth of experimental evidence links the development of electrical kindling to changes in some properties of the GABA_A receptors including ligand binding and expression levels of its subunits in the brain.^{9,10,25} On the other hand, some studies imply steroid (including allopregnanolone) actions to be subtly influenced by GABA_A receptor subunit composition.¹² It may be that electrical kindling-induced changes in GABA_A receptor

subunit expression levels and subsequent subunit composition yield a functional diversity of GABA_A receptor mediated inhibition and/or even steroid-insensitive GABA_A receptors in different brain areas.¹² Whether this is the reason behind the lack of anticonvulsant activity of progesterone and allopregnanolone in the amygdala kindling model of seizure, or other mechanisms are involved, remains to be determined.

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