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The effects of neurosteroids on picrotoxin-, bicuculline- and NMDA-induced seizures, and a hypnotic effect of ethanol

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Abstract

The effects of intraperitoneally (IP) or intracerebroventricularly (ICV) administered neurosteroids [allopregnanolone (AP); 5β-tetrahydrodeoxycorticosterone (5β-THDOC); dehydroepiandrosterone sulfate (DHEAS); pregnenolone sulfate (PS)] and their precursors [progesterone (PROG), pregnanedione (PREG)] on *N*-methyl-D-aspartic acid (NMDA)-, picrotoxin (PTX)- and bicuculline (BIC)-induced seizures and ethanol-induced sleep were studied in mice. It was found that IP injections of (+)MK-801 most potently antagonized NMDA-, PTX- and BIC-induced seizures, as compared to diazepam (DZP), PROG and PREG. Both precursors of neurosteroids appeared only marginally active in the applied models of convulsions. ICV injections of AP selectively blocked PTX- and BIC-induced seizures, whereas 5β-THDOC and (+)MK-801 also antagonized NMDA-induced convulsions. ICV administered DHEAS induced seizures in a dose-dependent way. ICV injections of AP and midazolam shortened the latency and prolonged the duration of sleep induced by IP injections of ethanol (5.0 g/kg). On the contrary, DHEAS and PS significantly reduced the hypnotic-like effect of ethanol. The obtained results suggest that neurosteroids may modulate in an agonistic (AP, 5β-THDOC), or antagonistic way (PS, DHEAS), the $GABA_A$ receptor complex functions. Some of them (5β-THDOC) also interact with NMDA receptors. AP appeared to be the most selectively acting compound, with its profile of action fully comparable to that of midazolam. AP also enhanced the hypnotic effect of ethanol, pointing out to the propensity to interact with centrally depressant agents. These findings, together with the possibility of conversion of some neurosteroids in the brain to other steroid hormones (testosterone, estradiol and aldosterone), indicate the limitations of their use for the treatment of neurological and psychiatric disorders.

Keywords: Neurosteroids; Intraperitoneal injections; Intracerebroventricular injections; Seizures; Ethanol-induced sleep; Mice

Neurosteroids belong to the family of naturally occurring compounds synthesized in the CNS and in the peripheral glands from steroid precursors (Fig. 1). Thus, brain concentration of neurosteroids depends both on their peripheral and local production. Pharmacological, biochemical and electrophysiological studies demonstrated that neurosteroids exert direct effects on neuronal membranes permeability and thereby rapidly affect CNS excitability [21]. This influence on neuronal excitability is largely mediated via $GABA_A$ receptor complex. Moreover, neuroactive steroids are able to influence NMDA receptors [5,7,16,31]. They can also inhibit voltage-operated Ca^{++} channels in neurons, in a way similar to classical Ca^{++} channels blockers of the dihydropyridine class [16,28]. Several lines of evidence suggest that neurosteroids, particularly 3α-hydroxy ring-A-reduced metabolites of progesterone (PROG) [3α-hydroxy-5α-pregnan-20-one, allopregnanolone (AP)] and deoxycorticosterone [3α-21-dihydroxy-5α-pregnan-20-one, tetrahydrodeoxycorticosterone (THDOC)], can act as positive modulators at the $GABA_A$ receptor, whereas sulfate esters of steroid hormone precursors — 3β-hydroxy-5-pregnen-20-one sulfate [pregnenolone sulfate (PS)] and 5-androsten-3β-ol-17-one sulfate [dehydroepiandrosterone sulfate (DHEAS)] — as

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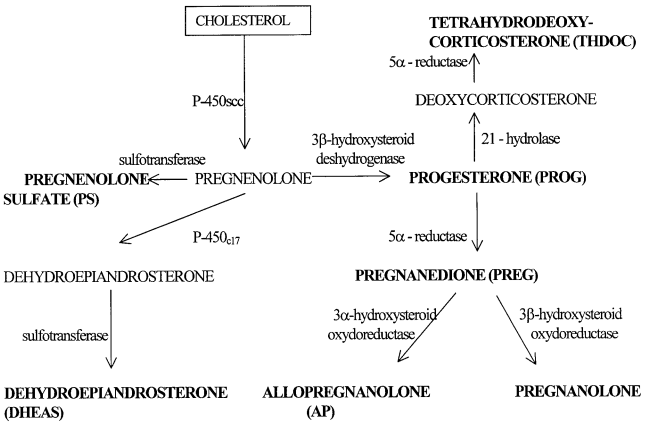

Fig. 1. Some pathways of neurosteroid biosynthesis and metabolism in the CNS. The agents used in the present experiment are shown in bold.

negative modulators [5,13–15,17,22,23,27]. In vivo, effects of neurosteroids are similar to those produced by other positive modulators of $GABA_A$ receptors such as benzodiazepines and barbiturates. Not surprisingly, many of these neuroactive steroids have anticonvulsant, myorelaxant, anesthetic and anxiolytic effects when administered to laboratory animals [6,10,12,27]. Additionally, in vitro neurosteroids potentiate ^{36}Cl-flux stimulated by $GABA_A$ receptor agonists, increase binding of [^{3}H]-muscimol and [^{3}H]-flunitrazepam and inhibit [^{35}S]-*t*-butylbicyclophosphorothionate ([^{35}S]-TBPS) binding to the $GABA_A$ receptor complex [3,11,24,27].

In spite of the many years of research, the central effects of neurosteroids are not yet fully elucidated. The majority of experimental data on the mechanisms of action of neurosteroids come from in vitro biochemical and electrophysiological studies. Moreover, behavioral experiments on the central mechanisms of neurosteroids are not homogenous, as they originate from many different laboratories, using different experimental approaches. For example, it is not clear to which extent the complex pharmacological profile of PS and DHEAS, shown to act as antagonists of the $GABA_A$ receptor complex and as positive modulators of the NMDA subtype of glutamate receptors, contribute to their central effects. Having in mind all the above facts, we have decided to re-evaluate the problem of an involvement of neurosteroids in regulation of brain excitability. One of the main objectives of this study was to investigate in one laboratory, in a battery of tests, the effects of neurosteroids given IP and ICV, in three models of epilepsy, and in a model of central depressant activity. Such pharmacological analysis of potentiation and inhibition of the behavioral effects of well-recognized and selective receptor- or receptor channel-ligands could help to better characterize the mechanisms of central action of neurosteroids. A trial was undertaken to integrate in a functional study some biochemical and electrophysiological findings on the central mechanisms of action of these naturally occurring compounds.

To this end, we evaluated the pro-convulsive properties of DHEAS, and the influence of ICV administered neurosteroids on ethanol-induced sleep, in comparison to midazolam. The effects of AP, THDOC and their precursors [PROG, pregnanedione (PREG)] (Fig. 1) on picrotoxin (PTX)-, bicuculline (BIC)- and NMDA-induced seizures were studied after intraperitoneal (IP) and intracerebroventricular (ICV) drug injections. In this way, the influence of peripheral and central administration of drugs could be compared, indicating to what extent and which metabolites of PROG and PREG are more active and responsible for the central effects of examined compounds. As most data on this topic come from peripheral drug administration, and the complex peripheral metabolism of some neurosteroids (e.g. PS and DHEAS) may involve even synthesis of testosterone, estradiol and aldosterone [1], such comparison could provide us with new important information.

1. Materials and methods

1.1. Subjects

The experiments were carried out on male albino Swiss mice weighing 20–25 g. All animals were acclimated to their home cages for 5 days before testing. They were housed with water and food ad libitum under a 12-h light–dark cycle, and at a controlled temperature (20°C). All experiments were done between 11 AM and 4 PM. The experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC). All experimental procedures using animal subjects were approved by the Committee for Animal Care and Use at the Institute of Psychiatry and Neurology.

1.2. Convulsant tests

The chemoconvulsants PTX, BIC and *N*-methyl-D-aspartic acid (NMDA) were administered intraperitoneally (IP; PTX, BIC, NMDA) or intracerebroventricularly (ICV; BIC, DHEAS). PTX and NMDA were dissolved in 0.9% NaCl and water, respectively, and BIC was suspended in 2% Tween solution for IP injections. In case of ICV injections, BIC and DHEAS were suspended in 45% 2-hydroxypropyl-β-cyclodextrin and sonificated for 30 min before central administration. The mice were placed singly in Plexiglas cages ($20 \times 25 \times 15$ cm) immediately after convulsant injection (IP or ICV) and observed for 30 min for the occurrence of following signs: wild running and jumping; posturing (Straub tail); clonic convulsions (repetitive movements involving all limbs simultaneously). The seizures increased in severity and

frequency and eventually progressed to the status epilepticus, loss of righting response, tonic hindlimb extension and death. Pro-convulsive potency of chemoconvulsants was defined as a percentage of animals showing seizures consistently leading to death within 30 min after their administrations. For the interactive experiments, the doses of convulsants were chosen to be within their LD_{85}–LD_{95} limits (effective dose necessary to induce a death in 85–95% of mice) as determined during preliminary experiments. The LD_{85}–LD_{95} doses were used in the experiments with anticonvulsants.

1.3. Loss of the righting reflex (LRR)

The mice received IP injections of ethanol at the dose of 5.0 g/kg, which induced a LRR (mouse was placed on its back and was unable to right itself three times within 30 s). The duration of the LRR (min) was defined as the interval between the loss and regaining of the righting response, and it was used as an index of central nervous system depression. The latency of the ethanol-induced LRR was recorded in minutes and referred to as time to the onset of the LRR. The end of LRR refers to the ability of the mouse to stand three times within 30 s. Drugs were administered 10 min prior to ethanol injection.

1.4. Surgical procedure and microinjections

Mice were anesthetized with ketamine (50 mg/kg/10 ml, IP) and sagittal incision was made along the mid-line of the skull. The bones were cleaned of connective tissue and the superior and transverse venous sinuses were identified. A small hole was made 2 mm caudal to the bregma and 2 mm lateral to the sagittal suture using a needle with the sharp end. The hole was made by rotary movements of the needle. Animals were used after a minimum of 4 days of recovery. Microinjections were given unilaterally using a Hamilton microsyringe through a 3-mm-long injection needle. All compounds were injected in a volume of 5 μl/50 s. The injection needle was removed after 30 s, and 10 min later, chemoconvulsants or ethanol was administered IP. The injection site was checked by injection of methylene blue solution (5 μl/50 s) accordingly to the above described procedure on the last day of experiment, 10 min prior to decapitation of animals.

1.5. Drugs

The following drugs were used: midazolam maleate (Hoffman La Roche, Switzerland), diazepam (DZP) (Polfa, Poland), dizocilpine [(+)MK-801] (RBI, USA), 4-pregnen-3,20-dione [progesterone (PROG)] (Sigma-Aldrich, Poland), 5α-pregnan-3,20-dione [pregnanedione (PREG)] (Sigma-Aldrich), 3α-hydroxy-5α-pregnan-20-one [allopregnanolone (AP)] (RBI), 3α-21-dihydroxy-5β-pregnan-20-one [5β-tetrahydrodeoxycorticosterone (5β-THDOC)] (Sigma-Aldrich), 3β-hydroxy-5-pregnen-20-one sulfate [pregnenolone sulfate (PS)] (RBI), DHEAS (RBI), *N*-methyl-D-aspartate (NMDA) (Sigma-Aldrich), PTX (Sigma-Aldrich) and (+)-bicuculline (BIC) (RBI).

1.6. Administration regimen

1.6.1. Anticonvulsant screening

DZP, (+)MK-801, PROG and PREG were administered IP in a volume equivalent to 10 ml/kg. DZP, (+)MK-801

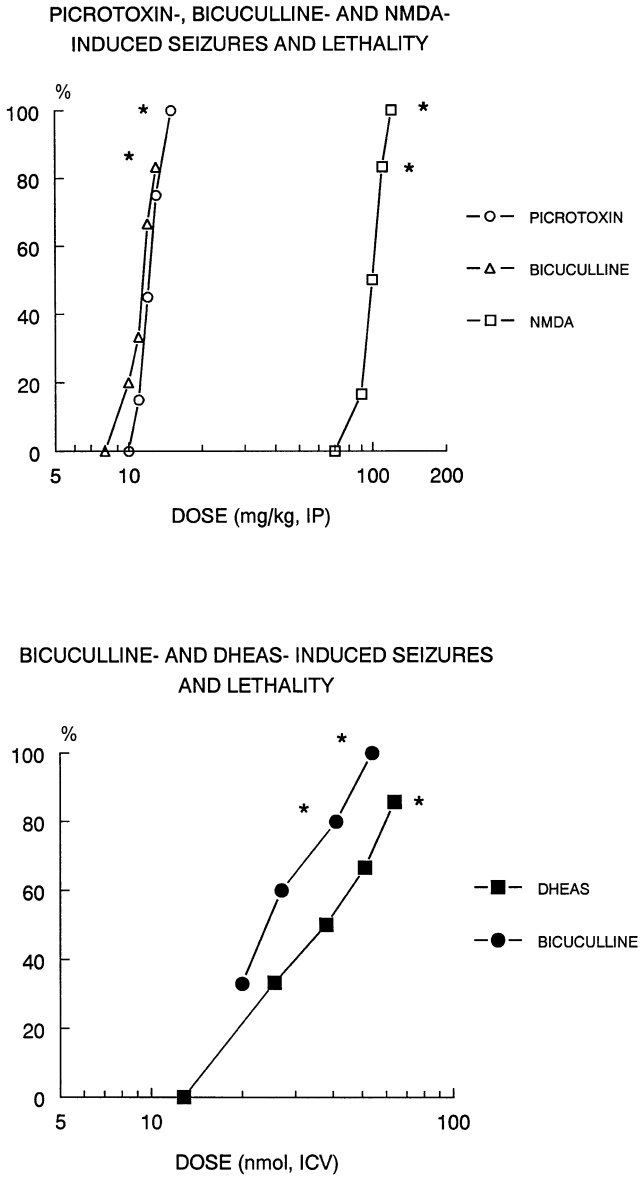

Fig. 2. Upper part of the figure. Dose–response relationships for the induction of seizures and lethality by IP administered PTX, BIC and NMDA in mice. Each data point indicates the percentage of mice showing seizures leading to death, and represents the data from 8 to 10 mice. Lower part of the figure. Dose–response relationships for the induction of seizures and lethality by ICV administered BIC and DHEAS, in mice. Each point represents the data from 8 to 10 mice. $*p<0.05$ (Fisher's exact probability test).

and PREG were given 30 min prior to chemoconvulsant injection, whereas PROG was administered 60 min earlier. All compounds were suspended in 2% Tween, except for (+)MK-801, which was dissolved in 0.9% NaCl. Midazolam, (+)MK-801, AP and 5β-THDOC were injected ICV 10 min before IP or ICV (BIC) convulsant injection. In case of BIC administration, the neurotoxin was given ICV, i.e. this group of animals received two central microinjections. AP, PS, DHEAS and 5β-THDOC were suspended in 45% 2-hydroxypropyl-β-cyclodextrin (RBI) and were sonificated for 30 min before administration. Midazolam and (+)MK-801 were dissolved in water. Control animals received an appropriate volume of a respective solvent.

1.6.2. LRR

Midazolam, PROG, AP, DHEAS, PS and 5β-THDOC were given ICV 10 min before IP injection of ethanol. All drugs administered into the brain ventricle were suspended in 45% 2-hydroxypropyl-β-cyclodextrin (RBI) and were sonificated for 30 min before injection. Ethanol was prepared as a 20% solution from 95% ethanol and injected at the dose of 5.0 g/kg.

1.7. Data analysis

Assessment of the LD (convulsant drugs) and ED (protective drugs) values with 95% confidence limits (CL) was done using a computerized version of the method of Litchfield and Wilcoxon procedure. For specific comparisons between treatments, Fisher's exact probability test was used. In the LRR assay, the data are shown as means ± SEM, and they were checked statistically using one-way ANOVA followed by post hoc Newman–Keuls test. The CL of $p < 0.05$ was considered statistically significant.

2. Results

2.1. Chemoconvulsants

PTX produced seizures and the lethal effect with a $LD_{50} = 9.92$ mg/kg (95% CL, CL = 9.05–10.87). Subsequently, the dose of 12 mg/kg (LD_{85}) was selected for further experiments (Fig. 2). In mice, IP and ICV injections of BIC produced convulsions and lethal effect with a respective LD_{50} of 11.41 mg/kg (CL = 10.48–12.43) and 28.91 nmol (CL = 18.97–43.99) (Fig. 2). The doses of 13 mg/kg and 54.44 nmol (LD_{85}) were selected for further experiments. In case of NMDA, LD_{50} was found to be 100.72 mg/kg

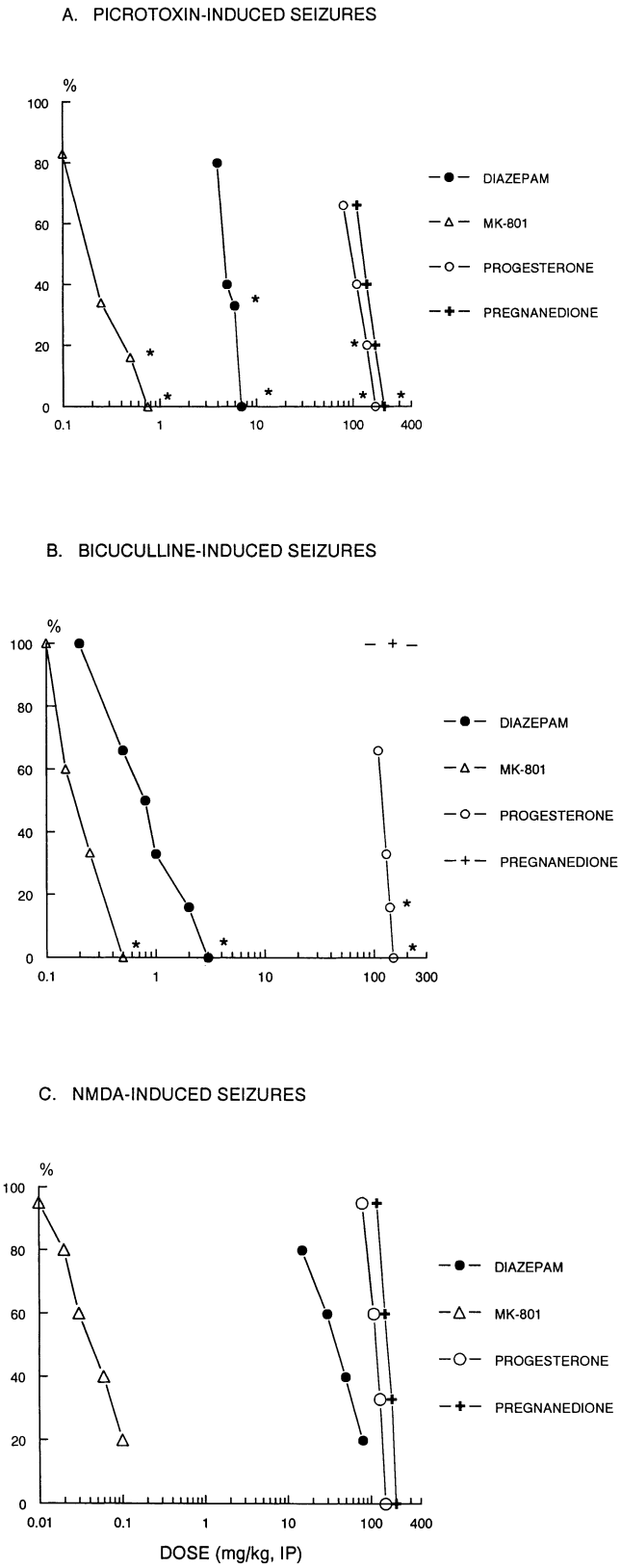

Fig. 3. Effects of PROG, PREG, DZP and (+)MK-801 against seizures and lethality induced by IP injections of PTX (12 mg/kg) (A), BIC (13 mg/kg) (B) and NMDA (120 mg/kg) (C) in mice. The doses of neurotoxins were within their LD_{85}–LD_{95} limits (see Materials and methods). Each data point indicates the percentage of mice showing seizures leading to death. PREG, DZP and (+)MK-801 were injected IP 30 min prior to, and PROG 60 min before the convulsant injection. $n \geq 6$ mice per one data point. * $p < 0.05$ compared to convulsant alone (Fisher's exact probability test).

Table 1
Protective doses of (+)MK-801, DZP, PROG and PREG after IP administration against PTX (12 mg/kg)-, BIC (13 mg/kg)- and NMDA (120 mg/kg)-induced lethality in mice

Drug	PTX	BIC	NMDA
(+)MK-801	0.21 (0.09–0.44)	0.15 (0.078–0.29)	0.037 (0.015–0.068)
DZP	4.98 (4.14–5.99)	0.70 (0.41–1.19)	36.63 (19.73–67.99)
PROG	142.36 (125.89–160.97)	116.68 (104.76–129.96)	165.85 (146.94–187.20)
PREG	149.10 (121.73–182.62)	>150	160.38 (146.65–175.39)

The data represent the ED_{50} (the dose preventing convulsions leading to death in 50% of animals) values expressed in mg/kg with 95% CL. The pretreatment time before a convulsant injection was 30 min for (+)MK-801, DZP and PREG, and 60 min for PROG.

(CL = 92.88–109.22), and the dose of 120 mg/kg (LD_{85}) was selected for subsequent tests (Fig. 2). ICV injections of DHEAS led to clonic–tonic seizures and lethal effect with a LD_{50} = 37.38 nmol (CL = 26.50–52.75) (Fig. 2).

2.2. IP administrations

2.2.1. PTX-induced seizures

(+)MK-801 produced full and dose-dependent antagonism of PTX-induced seizures (ED_{50} = 0.21 mg/kg) (Fig. 3). DZP, examined as a reference compound, was less effective against seizures and lethality produced by PTX (ED_{50} = 4.98 mg/kg), in comparison to (+)MK-801. PROG (ED_{50} = 142.36 mg/kg) and PREG (ED_{50} = 149.10 mg/kg) were much less potent in blocking PTX effect. Potencies of these neuroactive steroids were similar, and both drugs were least effective in this test. The rank order of potency in this test was (+)MK-801 > DZP > PROG = PREG.

2.2.2. BIC-induced seizures

(+)MK-801 was the most effective as protective agent in this model of seizures (ED_{50} = 0.15 mg/kg). DZP (ED_{50} = 0.70 mg/kg) was more potent than PROG (ED_{50} = 116.68 mg/kg), and PREG was found to be ineffective. The rank order of potency in this test was (+)MK-801 > DZP > PROG.

2.2.3. NMDA-induced seizures

(+)MK-801 produced the most potent and dose-dependent anti-convulsant effect (ED_{50} = 0.037 mg/kg). DZP also suppressed seizures produced by NMDA (ED_{50} = 36.63 mg/kg). The protective effects of PROG (ED_{50} = 165.85 mg/kg) and PREG (ED_{50} = 160.38 mg/kg) were similar and less potent. The rank order of potency in this test was (+)MK-801 > DZP > PROG = PREG. The relative potencies of all tested compounds (ED_{50}s with 95% CL) against PTX-, BIC- and NMDA-induced seizures, after IP drugs administration, are summarized in Table 1.

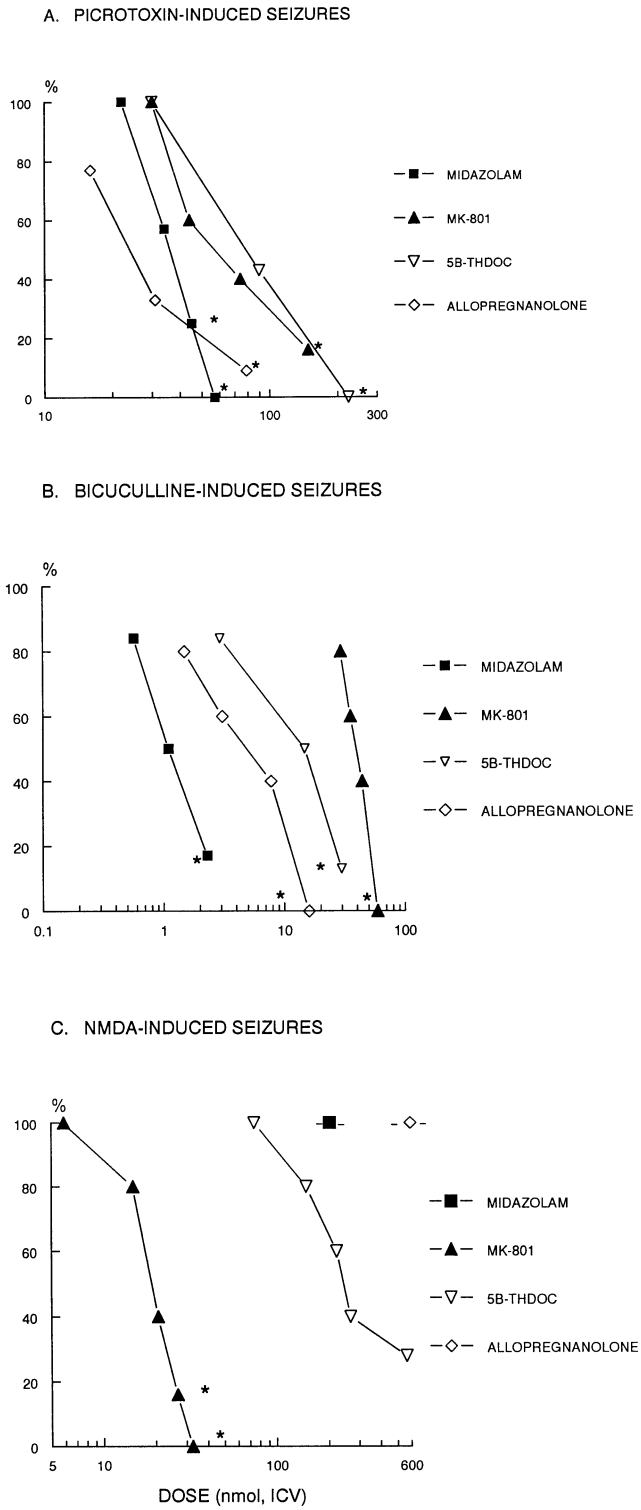

Fig. 4. Effects of AP, 5β-THDOC, midazolam and (+)MK-801 given ICV against seizures and lethality induced by IP injections of PTX (12 mg/kg) (A), NMDA (120 mg/kg) (C) and ICV administered BIC (54.44 nmol) (B). The doses of neurotoxins were within their LD_{85}–LD_{95} limits (see Materials and methods). Each data point indicates the percentage of mice showing seizures leading to death. The compounds were injected ICV 10 min before the convulsant administration. $n \geq 6$ mice per one data point. * $p < 0.05$ compared to convulsant alone (Fisher's exact probability test).

Table 2
Protective doses of (+)MK-801, midazolam, AP and 5β-THDOC administered ICV against picrotoxin (IP)-, NMDA (IP)- and BIC (ICV)-induced lethality in mice

Drug	PTX	BIC	NMDA
(+)MK-801	70.66 (44.87–111.27)	38.50 (31.50–47.04)	17.66 (12.83–24.30)
Midazolam	38.25 (30.58–43.41)	1.13 (0.72–1.76)	>226
5β-THDOC	83.47 (49.80–139.80)	10.25 (4.36–24.07)	307.02 (199.46–472.55)
AP	26.34 (13.03–53.28)	4.18 (1.98–8.82)	>314

The data represent the ED_{50} values expressed in nmol with 95% CL. The pre-treatment time before a convulsant injection was 10 min for all compounds.

2.3. ICV injections

2.3.1. PTX-induced seizures

AP (ED_{50} = 26.34 nmol) was more potent than midazolam (ED_{50} = 38.25 nmol) in antagonizing seizures (Fig. 4). (+)MK-801 (ED_{50} = 70.66 nmol) and 5β-THDOC (ED_{50} = 83.47 nmol) also produced full and dose-dependent protection against PTX-induced seizures with similar potency. The rank order of potency in this test was AP > midazolam > (+)MK-801 ≥ 5β-THDOC.

2.3.2. BIC-induced seizures

Midazolam (ED_{50} = 1.13 nmol) was more potent than AP (ED_{50} = 4.18 nmol) and 5β-THDOC (ED_{50} = 10.25 nmol). (+)MK-801 revealed the weakest activity in this test (ED_{50} = 38.50 nmol). The rank order of potency in this test was midazolam > AP > 5β-THDOC > (+)MK-801.

2.3.3. NMDA-induced seizures

Protective effect of (+)MK-801 (ED_{50} = 17.66 nmol) was stronger than that of 5β-THDOC (ED_{50} = 307.02 nmol). AP and midazolam appeared completely ineffective in this test. The rank order of potency in this test was (+)MK-801 > 5β-THDOC. The relative potencies of all tested compounds (ED_{50} s with 95% CL) against PTX-, BIC- and NMDA-induced seizures, after ICV drugs administration, are summarized in Table 2.

After intraventricular injections, AP and 5β-THDOC induced motor disturbances and ataxia-like symptoms in mice in a dose-dependent manner. These symptoms could be observed, but were not systemically followed, at the time period preceding chemoconvulsant administration.

2.4. LRR

Midazolam given as a reference compound, significantly prolonged duration of the ethanol-induced LRR after a dose of 45.3 nmol [$t(13) = 3.47$, $p < 0.005$] (Table 3). Pretreat-

Table 3
Effects of ICV injections of midazolam, 5β-THDOC, AP, PROG, PS and DHEAS on ethanol-induced LRR in mice

Drug	Dose (nmol)	*N*	Latency of LRR (min)	Duration of LRR (min)
Vehicle	0	7	2.31 ± 0.27	48.22 ± 3.70
Midazolam	27.1	6	2.36 ± 0.21	32.70 ± 6.32
	36.2	6	1.52 ± 0.10	47.95 ± 6.97
	45.3	6	1.98 ± 0.25	98.80 ± 15.21**
Vehicle	0	7	1.42 ± 0.14	42.00 ± 2.56
PROG	159.0	6	1.91 ± 0.11*	57.73 ± 8.86
	238.5	6	1.40 ± 0.15	61.89 ± 9.22
	318.0	6	1.17 ± 0.03	60.56 ± 12.77
Vehicle	0	6	1.68 ± 0.37	52.00 ± 10.04
5β-THDOC	89.7	8	1.29 ± 0.06	36.50 ± 5.68
	149.5	9	1.47 ± 0.15	50.50 ± 10.51
	209.3	7	0.77 ± 0.14*	57.50 ± 10.47
Vehicle	0	8	1.42 ± 0.02	52.35 ± 11.02
AP	31.4	7	0.91 ± 0.13**	69.39 ± 14.02
	62.8	7	1.25 ± 0.60	100.71 ± 14.60
	94.2	8	0.77 ± 0.16**	126.17 ± 25.70**
Vehicle	0	14	1.48 ± 0.09	48.09 ± 4.97
PS	59.7	5	1.40 ± 0.23	42.63 ± 4.40
	119.5	7	1.31 ± 0.04	26.06 ± 4.65
	179.2	6	1.36 ± 0.14	18.57 ± 4.25**
DHEAS	5.1	5	1.20 ± 0.03	47.65 ± 7.18
	10.2	6	1.48 ± 0.15	34.67 ± 5.46
	15.3	7	1.18 ± 0.05	35.76 ± 5.27
Vehicle	0	7	1.40 ± 0.13	55.86 ± 8.17
DHEAS	20.5	8	1.26 ± 0.05	34.00 ± 4.07*

The data are shown as means ± SEM. *N* = number of mice.
* Differs from control, vehicle-treated animals; $p < 0.05$.
** $p < 0.01$.

ment of mice with PROG did not significantly influence duration of sleep induced by ethanol, but the drug at the dose of 159 nmol prolonged the latency of the LRR [$t(13) = 2.63$, $p < 0.05$]. 5β-THDOC did not affect ethanol-induced sleep time [$f(3, 25) = 0.9$, $p < 0.5$], however, the latency of sleep was significantly shortened after the dose of 209.3 nmol of this drug [$t(13) = 2.60$, $p < 0.05$]. AP dose-dependently decreased the latency of sleep [$f(3, 26) = 7.68$, $p < 0.01$] at the dose of 31.4 [$t(13) = 4.00$, $p > 0.001$] and 94.2 nmol [$t(14) = 4.03$, $p > 0.001$], and increased sleep time after the highest dose administered (94.2 nmol) [$t(16) = 2.90$, $p < 0.01$]. PS did not affect the latency of sleep [$f(4,32) = 1.58$, $p < 0.2$], but significantly decreased duration of the ethanol-induced LRR after a dose of 179.2 nmol [$t(20) = 3.17$, $p < 0.001$]. Similarly, DHEAS administered at the dose of 20.5 nmol shortened the duration of ethanol-induced sleep [$t(13) = 2.49$, $p < 0.05$].

3. Discussion

Both precursors of neurosteroids, PROG and PREG, appeared only marginally active in all examined models of seizures. The potencies of PROG and PREG in inhibiting convulsions were at least two orders of magnitude lower than those of DZP and (+)MK-801. Several factors may contribute to this finding, e.g. peripheral drugs metabolism and limited penetration of their active metabolites to the brain. It is noteworthy that PROG and PREG do not show any appreciable affinity towards $GABA_A$ receptors by themselves [4,13,14]. This means that some anti-epileptic activity, evident after extremely high doses of both compounds, is due to the action of their derivatives formed either peripherally and/or centrally. The weak effect of PROG administered peripherally, and suspended in a medium known to prolong the absorption time of suspended drugs, indicates that the centrally but not peripherally synthesized neurosteroids, may play more important role. In contrast to these findings, Kokate et al. [17,19] reported recently that PROG (200.0 mg/kg) produced a sustained anticonvulsive effect against PTZ-induced seizures for 2 h after administration. It is conceivable that the mechanism of pentylenetetrazol-induced convulsions, and therefore the mode of its interaction with PROG, may be different from that of PTX and BIC. However, there still remains possibility that the weak effects of neurosteroids precursors given 60 min before the test, are due to their fast conversion to the derivatives with a short half-life time. The most likely candidate is AP. This neurosteroid constitutes the final step of a metabolic pathway of PROG, it binds with high affinity to the $GABA_A$ receptor complex, and shows very potent neuroprotective and anti-convulsive activity [2,6,9,18,20]. The potency and selectivity of ICV administered AP against PTX- and BIC-induced convulsions, was fully comparable to that of midazolam, a full nonselective agonist at the benzodiazepine receptors. This finding indicates, that the mechanism by which AP affects the $GABA_A$ receptors may be very similar to that of benzodiazepines. Indeed, it has been recently found that AP synthesized in brain plays an important physiological permissive role in the modulation of $GABA_A$-gated Cl^- channel function [25].

5β-THDOC, a derivative of PROG, potently inhibited PTX and BIC seizures, however, with much lower selectivity. This drug antagonized also NMDA-induced convulsions; with the ED_{50} much higher than that of (+)MK-801, a selective uncompetitive antagonist of NMDA receptors [30]. Several neurophysiological experiments showed modulatory influence of some neurosteroids on the NMDA receptor functions [16,19,31]. Such effects may be due to a direct action of neurosteroids on NMDA receptors via allosteric binding site [31]. Neurosteroids might also attenuate the pro-convulsive activity of NMDA by blocking the spreading of neuronal depolarization, due to enhancement of $GABA_A$ receptor mediated hyperpolarization of neuronal membranes, along the pathways in the brain involved in the initiation, propagation and maintenance of a seizure activity. However, the absence of a similar action of ICV administered AP and midazolam stands at odds with such interpretation of the effects of 5β-THDOC. This part of data indicates that 5β-THDOC may interfere in a more direct way with NMDA receptors, confirming other authors' findings [7,12].

It seems that the effects of (+)MK-801 may be interpreted almost solely in terms of a blockade of NMDA receptors. This drug does not show any appreciable affinity towards $GABA_A$ receptors [8,12,30], and it has appeared to be the most potent anticonvulsant agent after peripheral administration. Both peripheral and central injections of (+)MK-801 blocked all types of convulsions to a similar extent. Given the facts that NMDA receptors play a fundamental role in regulating the excitability of neuronal membranes, and glutamic acid occurs at the high concentrations in all brain structures, the strong and multilateral effects of (+)MK-801 observed in the present experiment can be easily explained.

The modulatory influence of neurosteroids on the sensitivity of neurons to the depolarizing stimuli was confirmed in the part of experiment with DHEAS. After ICV administration, DHEAS induced seizures in a dose-dependent way, in the dose-range similar to that of BIC. DHEAS, derivative of pregnenolone, is known to inhibit the $GABA_A$ receptor function via allosteric binding site located on this receptor complex [26,27]. This part of the experiment confirmed the corollary that neurosteroids belong to the family of centrally acting agents, which can potently and in a diversified way modify the susceptibility of the brain to the seizure-evoking stimuli. Moreover, some protective effects of IP administered PROG indicate that fluctuation of its levels in hormonally cycling women with epilepsy may be one of the important determinants of their illness.

The potency and selectivity of central actions of neurosteroids, in comparison to midazolam, were also

analyzed in a model of ethanol-induced LRR after ICV drugs administration. The reaction of mice to a high dose of ethanol is considered to reflect predominantly the hypnotic influence of alcohol, due to a nonselective stimulation of the $GABA_A$ receptor-related inhibition of the central nervous system functions [29]. The short latency of action, and an appearance of other signs of sleep (a decrease in body temperature and closed eyes — not systematized observations) indicate that this is a real hypnotic-like reaction, with minor, if any, contribution of other factors (e.g. muscle relaxation). It was found that central injections of PROG did not consistently change the latency and duration of ethanol sleep. AP appeared to be the most potently acting agent in this model, as it both decreased the latency and increased the duration of sleep, in a dose-dependent manner. 5β-THDOC decreased the latency only, and midazolam significantly prolonged sleep time. In contrast, PS and DHEAS shortened, at some doses, the duration of ethanol sleep. Thus, the profiles of central action of different neurosteroids, revealed in the models of epileptic activity, were also confirmed in this experiment. The lack of effects of ICV administered PROG indicates that this drug has to be metabolized peripherally to neuroactive steroids, which can enhance the GABA-related actions of ethanol, after penetration to the brain. The data on the potentiation or antagonism of ethanol-induced LRR show once again, that neurosteroids synthesized centrally but not peripherally, may play more important role in the modulation of the nervous system functions. ICV route of drugs administration excludes the possibility of a pharmacokinetic interpretation of the obtained results. This new finding also indicates that the profiles of central effects of some neurosteroids (AP, 5β-THDOC), including drug interactions, resemble those of other positive modulators of the $GABA_A$/benzodiazepine receptor complex (e.g. benzodiazepines).

Neurosteroids were found to modulate neuronal excitability due to interaction with the $GABA_A$- and NMDA-receptor functions. Centrally synthesized neurosteroids regulate in an agonistic (AP, 5β-THDOC) and antagonistic manner (PS, DHEAS) the $GABA_A$ receptor complex activity. Some of them (5β-THDOC) also exhibit functional interaction with NMDA receptors. AP appeared to be the most selectively acting compound, with its selectivity and potency fully comparable to that of midazolam, a full nonselective agonist at the $GABA_A$/benzodiazepine receptor complex. AP also enhanced the hypnotic effect of ethanol, confirming its propensity to interact with the centrally depressant agents. These findings, together with the possibility of conversion of some neurosteroids in the brain to other steroid hormones (testosterone, estradiol and aldosterone), point to the limitations of their use for the treatment of neurological and psychiatric disorders. Moreover, the applied functional analysis appeared sensitive and selective enough to be useful for comparing the pharmacological profiles of examined drugs.

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