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The Effects of Neurosteroids on Rat Behavior and ^{3}H-Muscimol Binding in the Brain

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CZŁONKOWSKA, A. I., H. SIENKIEWICZ-JAROSZ, M. SIEMIĄTKOWSKI, A. BIDZIŃSKI, AND A. PŁAŹNIK. *The effects of neurosteroids on rat behavior and ^{3}H-muscimol binding in the brain*. PHARMACOL BIOCHEM BEHAV **63**(4)639–646, 1999.—The effects of ICV administration of metabolites of progesterone and deoxycorticosterone [i.e., neurosteroids: AP (3α-hydroxy-5α-pregnan-20-one, allopregnanolone), 5α-THDOC (3α-21-dihydroxy-5α-pregnan-20-one, 5α-tetrahydrodeoxycorticosterone), 5β-THDOC (3α-21-dihydroxy-5β-pregnan-20-one, 5β-tetrahydrodeoxycorticosterone), and PS (3β-hydroxy-5-pregnen-20-one sulfate, pregnenolone sulfate] were studied in the open-field test of neophobia and Vogel's test of conflict behavior in rats. The influence of in vivo administered 5β-THDOC, a positive allosteric modulator of the $GABA_A$ receptor complex, on ^{3}H-muscimol binding in different brain structures, was also studied with the help of quantitative autoradiography. The presented data did not reveal any anxioselective effects for a range of centrally active neurosteroids, in the ethologically orientated and conflict models of anxiety, after intracerebral drug administration. Their central effects appeared secondary to changes in rat gross behavior. It is possible that high local concentration of neurosteroids after ICV injection and production of a narrower range of behavioral effects than that of benzodiazepines, precluded manifestation of the antianxiety effects of AP, 5α-THDOC and 5β-THDOC. Autoradiography did not reveal any significant changes in the specific binding of ^{3}H-muscimol in brain structures after in vivo ICV administration of 5β-THDOC at the behaviorally active dose. Thus, the possibility that neuroactive neurosteroids may provide a novel potential site for therapeutic interventions in anxiety disorders is not supported. The part of the experiment with 5β-THDOC is interpreted as contributing to other results, suggesting the existence of a new category of neurosteroids acting as partial agonists of the $GABA_A$ receptor. © 1999 Elsevier Science Inc.

Neurosteroids Open field Vogel's test ^{3}H-Muscimol Autoradiography Rats

THE CNS concentrations of neurosteroids depend both on their peripheral and local synthesis. Neurosteroids can be further metabolized in the brain by the same enzymes that transform peripherally produced compounds. Both groups of metabolites of peripheral and central origin contribute to the development of the pool of biologically active compounds, particularly the 5-alpha–reduced derivatives, which are believed to be necessary for normal brain functioning. Neurosteroids influence the CNS function via nongenomic mechanisms, i.e., by an allosteric modulation of $GABA_A$ and, possibly, excitatory amino acid receptors (5,14). Some neuroactive steroids also inhibit voltage-operated Ca^{++} channels in neurons, and are similar to classical Ca^{++} channel blockers of the dihydropyridine class (16,27). However, the $GABA_A$ receptor complex seems to be the primary target for their action with naturally occurring metabolites of progesterone [allopregnanolone, AP, (3α-hydroxy-5α-pregnan-20-one)] and deoxycorticosterone [5α-THDOC (3α-21-dihydroxy-5α-pregnan-20-one), 5β-THDOC (3α-21-dihydroxy-5β-pregnan-20-one)] displaying high affinity for $GABA_A$ receptors, and potentiating Cl^- ion conductance (14,18,20). Some of them are synthesized in the brain (AP), while others are of peripheral

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origin (THDOC). It is now well recognized that neurosteroids can modulate GABA functions in both positive (AP, THDOC) and negative (PS 3β-hydroxy-5-pregnen-20-one sulfate, pregnenolone sulfate; DHEAS 3β-hydroxy-5-androsten-17-one sulfate, dehydroepiandrosterone sulfate) manners (19,31). Endogenously occurring neuroactive steroids have been reported to reach concentration in the brain well within the range necessary to modulate the actions of GABA (18). In vitro, most discovered neurosteroids are similar to barbiturates in enhancing benzodiazepine and GABA binding, resulting in increased Cl^- uptake (14). Molecular and biochemical experiments have demonstrated that neurosteroids affect GABAergic transmission via action at an allosteric binding site on the $GABA_A$ receptor complex linked functionally with both a GABA binding site and an ion channel (12,26). In vivo, neurosteroids are anticonvulsant, myorelaxant, sedative hypnotic, and they produce effects similar to that of benzodiazepines and barbiturates in preclinical models of anxiety (5,6,25,30). The possibility that these drugs may provide a novel site for therapeutic intervention in an anxiety disorder has attracted much attention in recent years.

Taking into consideration the complex metabolic pathways of neurosteroids and multiplicity of the central sites for their action, the purpose of the present study was to examine the anxiolytic-like effects of selected neurosteroids after their intracerebroventricular (ICV) injection, in two rat models of anxiety particularly sensitive to the $GABA_A$ receptor ligands (28). We investigated the behavioral effects of neurosteroids, positively (AP, THDOC) and negatively (PS, DHEAS) modulating the $GABA_A$ receptor complex with different efficacy, on rat neophobic and conflict behavior, in the open-field and Vogel test, respectively, in comparison to a full benzodiazepine receptor agonist (midazolam). Moreover, changes in ^{3}H-muscimol binding to $GABA_A$ receptors were studied ex vivo in different brain structures after ICV administration of a metabolite of deoxycorticosterone (5β-THDOC), at the behaviorally active dose. Thus, the purpose of this study was to measure the effect of centrally administered neurosteroids on differently evoked anxiety-related behaviors, and to correlate it with biochemical changes, thereby gaining insight into the site of their action within the CNS. The intracerebral route of drug administration and control experiments on changes in pain threshold and fluid intake might also provide new information on the specificity and selectivity of the central effects of neurosteroids.

METHOD

Animals

Male Wistar rats (200 ± 20 g) supplied by a licensed breeder were used in the study. Before the surgery rats were allowed 4 days of adaptation to the laboratory conditions. The animals were housed under a 12 L:12 D cycle (lights on at 0600h), in controlled temperature (21 ± 2°C) and 70% humidity. The rats were kept individually after ICV cannula implantation, with water and food available ad lib. The experiments were performed in compliance with the European Communities Council Directive of 24 November 1986 (86/609 EEC).

Intracerebroventricular Injections

Surgery. The rats were anesthetized with ketamine (100 mg/kg, IP) and placed in a stereotaxic apparatus (Stoelting & Co., USA). A 10 mm-long stainless steel guide cannula was implanted above the right lateral ventricle, according to the atlas of the rat brain (3.2 mm posteriorly to the bregma, 1.5 mm laterally to the sagittal suture, 2.2 mm below the dura) (22). The guide cannula was fixed to the skull with jewelry screws and dental acrylic cement. Seven days later the rats were subjected to behavioral testing.

Microinjection. Microinjections were given unilaterally using a Hamilton microsyringe connected via polyethylene tubing with an injection needle. The injection needle was inserted 2.0 mm below the tip of the guide cannula. All drugs were injected in a volume of 4 μl at a rate of 1 μl/10 s. The injection needle was removed after 30 s and the stylet replaced. The behavioral tests were started 10 min after the drug administration. First, the operated animals were tested in the open-field test, and after 7 days the rats were randomly reassigned to experimental groups and subjected to the Vogel test.

Drugs

The following drugs were used: midazolam maleate (Hoffman–La Roche, Switzerland), 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, AP) (RBI, USA), 3α-21-dihydroxy-5α-pregnan-20-one (5α-tetrahydrodeoxycorticosterone, 5α-THDOC) (Sigma–Aldrich, Poland), 3α-21-dihydroxy-5β-pregnan-20-one (5β-tetrahydrodeoxycorticosterone, 5β-THDOC) (Sigma–Aldrich) 3β-hydroxy-5-pregnen-20-one sulfate (pregnenolone sulfate, PS) (RBI). Neurosteroids were suspended in 45% 2-Hydroxypropyl-β-cyclodextrin (RBI), except for midazolam, which was dissolved in water. Drugs were sonificated for 30 min and injected ICV 10 min before experiment.

Open-Field Test

Open-field testing was performed in a soundproof chamber under dim light and white noise (65 dB), without previous habituation. The apparatus consisted of two round arenas (80-cm diameter), each equipped symmetrically with three photocells (60 cm apart, 5 cm above the floor level). General activity (number of photobeam interruptions) was scored for 10 min. Simultaneously, the rats were observed by closed-circuit television, and two additional parameters were measured—the number of entries into the central part of the open field (this parameter was defined as a movement of an animal from the wall to the central area, over a distance of approx 15 cm); and the time (in seconds) spent in the central sector (area defined as a centrally situated a 35-m diameter circle).

Vogel's Conflict Test

The method used in this test is essentially the same as in our previous article (28). Apparatus consisted of four boxes (30 × 30 × 60 cm), with a grid floor made of stainless steel bars. A water drinking tube was mounted on the wall of a cage. An electric shock generator was connected with the grid floor and the metal end piece of the drinking tube. Through a hole in the wall the rat had access to a stainless steel drinking tube connected with a shock device. The grid floor comprised the other pole to complete the shock circuit through the rat body. The rats were prehabituated for 4 days. During the first 2 days animals were deprived of water 23 h daily in the home cages. During the following 2 days the subjects were placed in the experimental cages for 15 mm without delivery of electric shocks. Subsequently, the rats were allowed to drink water in their home cages for 45 min. After training, the drinking of water for all animals was usually stabilized. On the fifth day, the animals were randomly assigned to the control groups receiving a solvent and experimental groups receiving the ap-

propriate drug solution. Two control groups were used, with and without shock stimulation. Subsequently, the rats were placed in the apparatus and the electric impulses were delivered in 4 s-long trains, with intervals lasting on an average of 5 s (4, 2, 5, 8, and 6, respectively). Shock current was set at 0.4 mA. The amount of consumed water during the 15-min test session was considered a measure of the conflict behavior.

To estimate the putative influence of drugs on shock thresholds and baseline drinking, the following control experiments were performed.

Baseline Drinking Test

A separate group of rats were water deprived and treated in the same manner as rats examined in the Vogel test. The amount of water intake (ml) during the 15-min test session (without electric shocks) was studied.

Shock Threshold Test (Flinch-Jump Test)

A separate group of animals was deprived of water before the flinch-jump test as in the Vogel test. On the testing day each rat was placed in the box used in the Vogel test. Shocks were delivered to the grid floor of the test box through a shock generator. After a 3-min period of habituation to the test box, shock titrations were continued upwards and downwards in a stepwise manner (0.05 mA, 0.05–0.85 mA range), depending upon responsiveness of the rat. The flinch threshold was defined as the lowest shock intensity that elicited any detectable response. The jump threshold was defined as the lowest shock intensity that elicited simultaneous removal of at least three paws (both hindpaws) from the grid. To avoid foot damage a cutoff equal to 1.0 mA was established. In this way the flinch and jump thresholds in mA were defined for each rat. The time gap between shocks was 10 s, and each animal was tested only once. The time between dosing and testing was the same as in the Vogel test.

Histological Analysis

After the experiments the implanted animals were sacrificed. The brains were removed and stored in a 5% formaldehyde solution. The frozen tissue was dissected into slices to establish the place of microinjection. Only data from animals with the injection site located in the lateral ventricle were taken into consideration.

Autoradiography

5β-THDOC was administered ICV (30 μg/4 μl, 1 μl/1 min) to separately prepared the group of animals. Control rats received injections of an appropriate solvent. The experimental and control groups consisted of seven and eight animals, respectively. The rats were decapitated 10 min after the microinjections had been made. The brains were rapidly removed, frozen in isopentane (−30 to −40°C) and stored at −70°C. The coronal (12 μm) sections were cut on a cryostat at −20°C, thaw mounted onto gelatinized glass slides, and stored at −20°C until use (1 to 2 days). Twenty-four slices for each structure were taken for examination. Frozen sections were brought to room temperature 30 min prior assay. Slides were preincubated in 50 mM TRIS-citrate buffer (pH 7.1) for 20 min at 4°C to remove endogenous competitors. Then they were incubated for 40 min at 4°C in the same TRIS-citrate buffer supplemented by 10 nM ^{3}H-muscimol (19.1 Ci/mmol, Amersham). Nonspecific binding was estimated in the presence of 0.2 mM GABA. The tissues were then rinsed in the cold buffer for 1 min and rapidly in–out dipped in distilled water. The slides were dried under a cold stream of air, placed in x-ray cassettes, and exposed to tritium-sensitive film (^{3}H Hyperfilm, Amersham) at 4°C together with standards (^{3}H microscale, Amersham). After an exposure of 6 weeks the films were developed using Kodak LX-24 film developer, washed in water, and then placed in Kodak fixer. The autoradiograms were analyzed with an image analysis system (Analytical Imaging Station, Imaging Research Inc., St. Catharines, Canada). Optical densities were converted into nCi/mg tissue equivalent using the standard curve. ^{3}H-muscimol nonspecific binding was negligible.

Statistical Analysis

The data are shown as the percentage of the respective control group results ± SEM, or mean ± SEM. The data involving multiple comparisons were checked statistically using a one-way ANOVA followed by post hoc Newman–Keuls test. Student's *t*-test for independent samples was used when the effect of a single dose drug treatment was compared with vehicle control. The confidence limit of $p < 0.05$ was considered statistically significant.

RESULTS

Midazolam, given at a dose of 0.5 μg/4 μl, significantly increased rats locomotor activity, $t\ (12) = 12$, $p < 0.05$, number of entries into the central arena, $t\ (12) = 2.9$, $p < 0.05$, and time spent in the central sector of the open field, $t\ (12) = 4.4$, $p < 0.01$ (Fig. 1a–c). 5α-THDOC administered ICV at the doses of 10 and 20 μg/4 μl decreased animals locomotor activity $F(2, 19) = 3.7$, $p < 0.05$ (Fig. 1a). 5β-THDOC (5 and 10 μg/4 μl) also inhibited rats locomotor activity in this test, $F(3, 25) = 9.0$, $p < 0.01$ (Fig. 1a). Allopregnanolone (5 and 10 μg/4 μl) reduced, in a significant way, the number of entries into the central part of the open-field test, $F(3, 26) = 6.0$, $p < 0.01$ (Fig. 1b). Moreover, there also appeared some tendency to decrease animals locomotor activity and time spent in the central sector. The general activity of animals was dose dependently increased after the highest dose of pregnenolone sulfate ($p < 0.05$) as well as the number of central entries ($p < 0.01$) and time spent in the central sector of the open field ($p < 0.01$) (Fig. 1a–c). The ranges of means (±SEM) of the control group results (in absolute values) were 79.2–105.6 (SEM 5.4–12.1) for activity; 3.7–8.7 (SEM 0.6–1.9) for the number of central entries, and 2.8–14.9 (SEM 0.7–4.5) for the time spend in the central sector.

Midazolam significantly disinhibited rats' behavior in the Vogel test after a dose of 10 μg/4 μl, $t\ (11) = 2.2$, $p < 0.05$ (Fig. 2). 5α- and 5β-THDOC (10, 20, and 30 μg/4 μl) also showed clear-cut and dose-dependent anticonflict effects in the Vogel test (Fig. 2). Both neurosteroids increased punished consumption of water [5α-THDOC: $F(4, 31) = 7.3$, $p < 0.01$; 5β-THDOC: $F(4, 30) = 6.12$, $p < 0.05$] (Fig. 2). Similarly, allopregnanolone, administered at the dose of 10 μg/4 μl, significantly enhanced water intake during the punished session ($p < 0.05$), whereas pregnenolone sulfate appeared ineffective after injection at a dose of 10, 20 and 30 μg/4 μl, $F(4, 30) = 1.2$ (Fig. 2). The range of means (± SEM) of the control, shocked group results (in absolute values) was 0.8–2.4 (SEM 0.2–0.7).

Autoradioradiographic studies revealed that the treatment of animals with 5β-THDOC (30 μg/4 μl) at the active behaviorally dose and at the same time interval as in the behavioral tests did not significantly change ^{3}H-muscimol binding to the occipital cor-

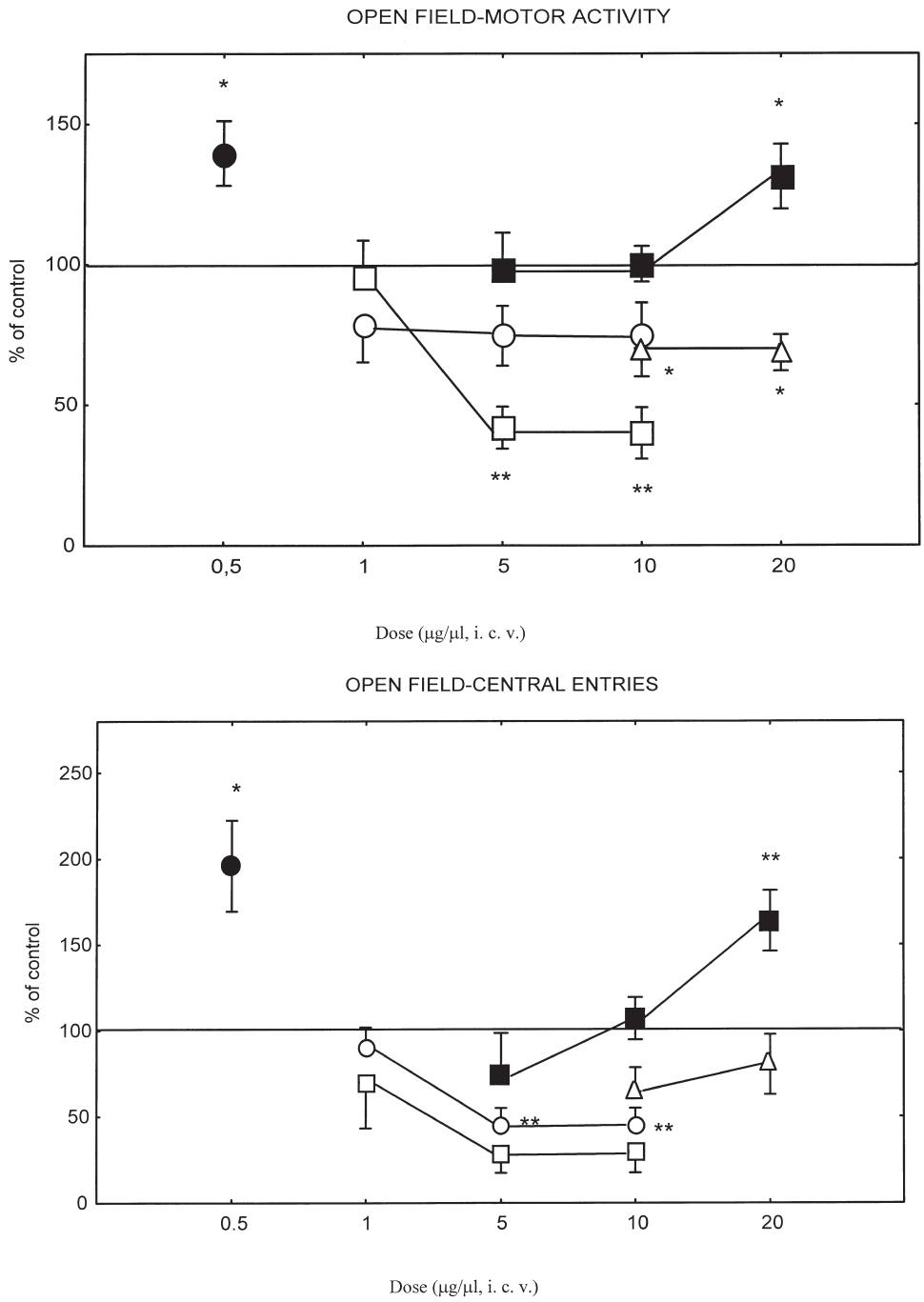

FIG. 1. The effect of ICV injections of AP, 5α-, and 5β-THDOC, PS, and midazolam on rat behavior in the open-field test: (a) motor activity; (b) the number of central entries; (c) time in the central sector of the open field. The data are shown as the percentage of the respective control group results ± SEM. *Differs from control. *$p < 0.05$; **$p < 0.01$. Asterisks after the dose of 5 and 10 μg refer to AP (b) Control ($n = 8$) ●—MIDAZOLAM ($n = 8$), ■—PS ($n = 7$), ○—AP ($n = 8$), ▽—5α-THDOC ($n = 8$), □—5β-THDOC ($n = 7$).

tex, enthorinal cortex, prefrontal cortex, nucleus accumbens, dentate gyrus, substantia nigra, amygdala, and striatum (Table 1).

5β-THDOC (30 μg/4 μl) significantly increased rats pain threshold [flinch: $t(16) = 4.1$, $p < 0.01$; jump: $t(16) = 3.8$, $p < 0.01$], and water intake in not stressed animals, $t(16) = 2.4$, $p < 0.05$ (Table 2).

DISCUSSION

The most interesting finding of the present study was the lack of a selective anxiolytic-like action of examined neurosteroids, after their ICV administration. In the open field 5α-,5β-THDOC, and AP caused a clear-cut decrease in rat

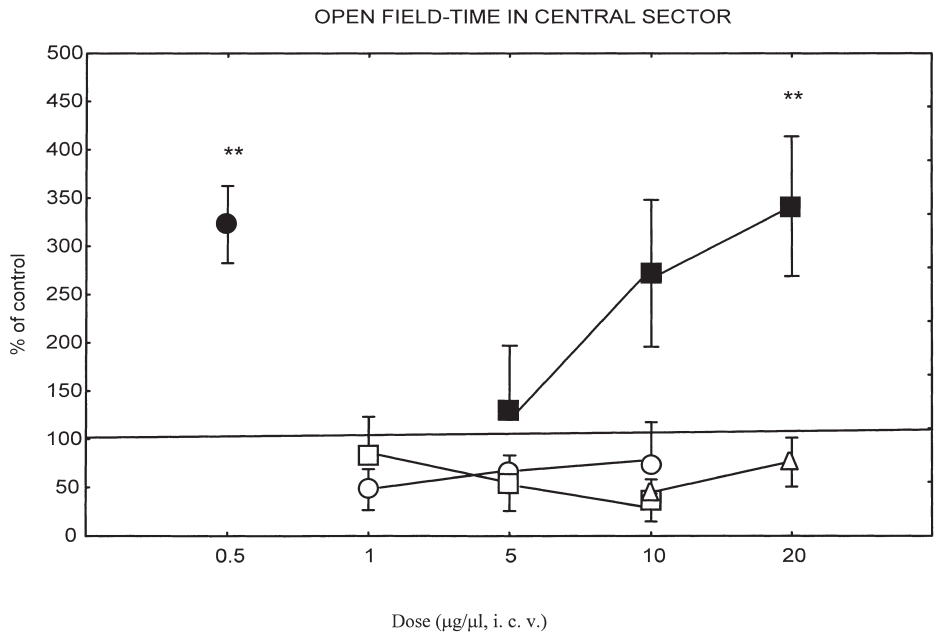

FIG. 1. Continued.

locomotor activity followed by inhibition of exploratory behavior. PS produced stimulation of animal motility with accompanying changes in other behaviors. Thus, it appeared that neurosteroids mainly modified rat gross behavior. Interestingly, the full benzodiazepine receptor agonist, midazolam, revealed in the open field a pattern of centrally mediated action similar to that of PS, with hypermotility being the most remarkable effect. Noteworthy, this reference drug was active behaviorally at the dose of at least one order of magnitude lower than that of neurosteroids. The predominantly sedative effects of 5α-, 5β-THDOC, and AP can be explained by the central action of high concentrations of positive $GABA_A$ receptor modulators, given directly to the brain. After intraperitoneal administration, peripheral metabolism and the blood–brain barrier limits the amount of neurosteroids penetrating to the brain structures. Accordingly, it was recently re-

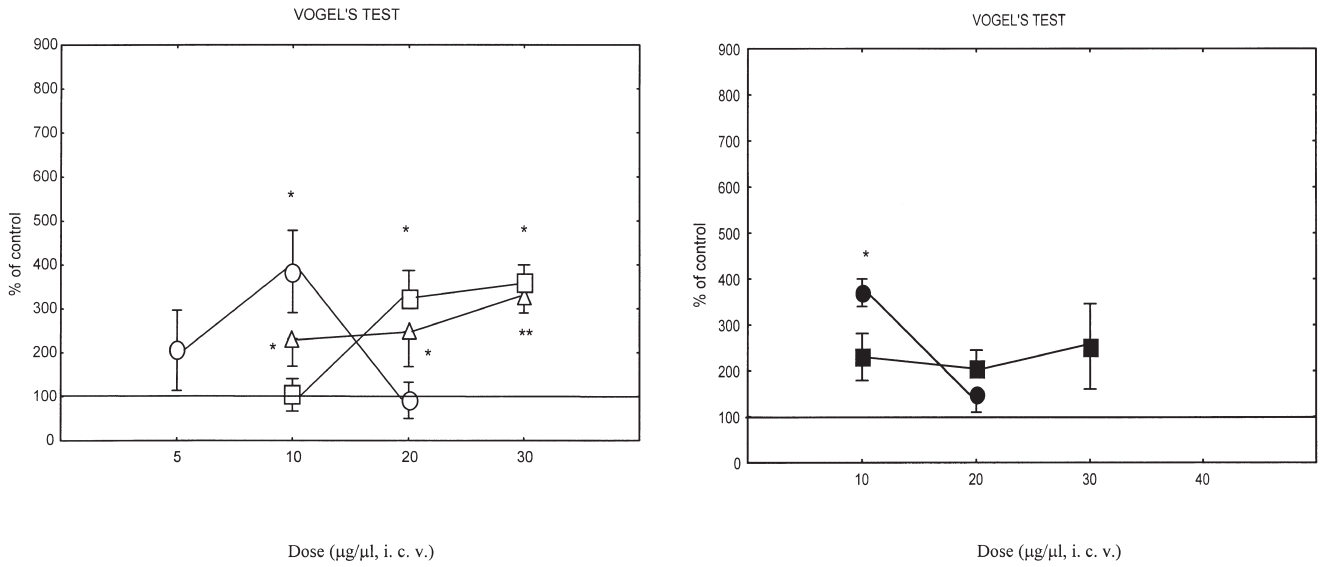

FIG. 2. The effect of AP, 5α-, and 5β-THDOC, PS, and midazolam administered ICV in the Vogel test. The data are shown as the percentage of the respective shocked control group results ± SEM. *Differs from control. *$p < 0.05$; **$p < 0.01$. Control ($n = 8$) ●—MIDAZOLAM ($n = 6$), ■—PS ($n = 8$), ○—AP ($n = 8$), ▽—5α-THDOC ($n = 8$), □—5β-THDOC ($n = 7$).

TABLE 1

AUTORADIOGRAPHIC DATA ON CHANGES IN ^{3}H-MUSCIMOL BINDING TO DIFFERENT RAT BRAIN STRUCTURES AFTER PRETREATMENT OF ANIMALS WITH 5β-THDOC (30 μg/4 μl, ICV) 10 MIN BEFORE DECAPITATION

Structure	Control	5β-THDOC	% Control	*p*-Value
Prefrontal cortex	1.85 ± 0.07	2.01 ± 0.11	108.6	0.23
Occipital cortex	4.10 ± 0.18	4.09 ± 0.23	99.7	0.99
Enthorinal cortex	3.32 ± 0.16	3.52 ± 0.24	106.0	0.52
Dentate gyrus	3.60 ± 0.16	3.54 ± 0.25	98.3	0.84
Amygdala	3.20 ± 0.14	3.12 ± 0.21	97.5	0.79
Nucleus accumbens	3.50 ± 0.10	3.67 ± 0.10	104.9	0.37
Substantia nigra	1.89 ± 0.08	1.98 ± 0.16	104.8	0.61
Striatum	1.72 ± 0.10	1.79 ± 0.14	104.1	0.67

The data are shown as mean ± SEM (nCi/mg). The number of rats in control and experimental group was seven and eight, respectively.

ported that progesterone, THDOC, and AP, administered peripherally over a wide range of doses, tended only to elevate tail-flick latencies in rats, whereas ICV infusions of the same neurosteroids significantly attenuated pain reaction (9). However, it does not seem possible that the route of administration and high local concentrations of examined compounds can fully explain the lack of anxiolytic-like effects in the open-field test. In a recently published article on the effects of neurosteroids in an ethological version of the mouse elevated plus-maze Rodgers and Johnson (25) concluded at the anxiolytic steroids tended to produce a narrower range of behavioral effects than diazepam and, in particular, did not reliably decrease measures of risk assessment. These data and present results indicate that neurosteroids and benzodiazepines have different behavioral profiles, thus indirectly pointing at different sites for their action at the $GABA_A$ receptors. Indeed, biochemical and electrophysiological studies showed neuroactive steroids to allosterically modulate GABA transmission via action at a binding site on the $GABA_A$ receptor complex different from that of benzodiazepines and barbiturates (12,13,29).

Overall, the present results confirm other authors' data showing a depressant profile of neurosteroid action in the CNS, with a narrow range of doses evoking more selective alterations in brain functioning (1,5,11,30). Stimulation of motor activity with ensuing changes in other behavioral effects of PS can be explained by its inhibitory influence on the $GABA_A$ receptor complex (19). Indeed, PS has been shown to oppositely modulate inhibitory $GABA_A$ and excitatory NMDA receptors; both types of action that might synergize to amplify excitatory transmission in the CNS (3,24,31). Stimulation of rat motility was also reported after intracerebral injection of a noncompetitive $GABA_A$ receptor antagonist, picrotoxin (23). The contrasting effects of neurosteroids adversely interacting with the $GABA_A$ receptor complex have been demonstrated, thus validating pharmacological specificity of the open-field data. Although the experimental protocol did not allow differentiation between behavioral disinhibition caused by midazolam and PS, other nonsystematized observations indicated that PS induced stimulant-like hypermotility, whereas midazolam's effect was more related to enhancement of rat exploration of the open field.

In the Vogel test, all positive modulators of the $GABA_A$ receptor complex significantly disinhibited rats conflict behavior suppressed by punishment. However, the most potently acting agent, 5β-THDOC, selected as an example for the control experiments, also enhanced pain threshold and water intake in nonstressed animals. Thus, it appeared that in this model of anxiety the action of neurosteroids might be false positive, not directly linked to emotion reduction. Interestingly, this drug was inactive in the Vogel test when administered at a dose of 10 μg, significantly inhibiting rats locomotion. This indicates that the effects of 5β-THDOC on motor activity and animal behavior in the Vogel test involve different mechanisms. Moreover, this part of the experiment points at a narrow range of behavioral effects of 5β-THDOC with a dose of 10 μg being inactive, and a dose of 20 μg potently disinhibiting punished drinking in the Vogel test. Midazolam injected to the hippocampus at a dose of 20 μg/site also increased pain threshold, but not water consumption, in thirsty rats (28). Thus, these data show some similarity between be-

TABLE 2

THE INFLUENCE OF 5β-THDOC ON BASELINE DRINKING AND SHOCK THRESHOLD

Drug	Dose (μg/4/ μl, ICV)	*n*	Spontaneous Drinking (ml)	*n*	Flinch (mA)	Jump (mA)
vehicle	—	8	5.4 ± 0.4	8	0.26 ± 0.02	0.44 ± 0.03
5β-THDOC	30.0	10	7.1 ± 0.5*	10	0.43 ± 0.03†	0.67 ± 0.05†

The data are shown as mean ± SEM.
*Differs from control. $p < 0.05$.
†$p < 0.01$.

havioral profiles of central action of neurosteroids and a benzodiazepine derivative in the Vogel test. Recently, AP was found to produce significant anxiolytic-like effect in the Geller-Seifter conflict paradigm (4). The benzodiazepine antagonist flumazenil did not block the anxiolytic-like action of AP, indicating that AP did not bind at the benzodiazepine site directly (4). However, in this experiment the pain threshold and food intake were not controlled. As it is now clear that neurosteroids can modulate pain reactions, it is evident that changes in these behavioral variables might significantly contribute to the animals' conflict behavior (9). Intracerebral administration of 5β-THDOC also increased water drinking. Likewise, it is well recognized that higher doses of benzodiazepine derivatives may modulate feeding responses, including food and water intake (8,15). Thus, the Vogel part of the experiment with 5β-THDOC points to a similar pattern of its central effects compared with that of a benzodiazepine. Altogether, the open field and Vogel test data suggest that the psychopharmacological profile of neurosteroids is less selective than benzodiazepines, and more related to regulation of rat gross behavior.

Quantitative autoradiography revealed the absence of any changes in the specific binding of ^{3}H-muscimol to different brain structures, after in vivo ICV administration of 5β-THDOC. The drug was given at the behaviorally active dose, and examined at the same time interval as in behavioral tests. This finding is unexpected, as most experiments on similar topics demonstrated stimulation by progesterone or neurosteroids of muscimol or flunitrazepam binding to the rat brain (2,7,10,17,21). This phenomenon was shown both in in vitro and in vivo experiments. For example, a single injection of 10.0 mg/kg of progesterone 5 min before rat decapitation selectively enhanced $GABA_A$ receptors density in the frontal cortex and hippocampus (17). Similarly, a single dose of 1.0 mg/kg of progesterone (IV) increased ^{3}H-muscimol binding in a number of hamster brain areas (7). These autoradiographic data indicate that progesterone, deoxycorticosterone, and some of their reduced metabolites may alter radioligand binding to the $GABA_A$ receptor complex in the rat brain in a manner that closely resembles the action of barbiturates and benzodiazepines (2,7,10,21). 5β-THDOC is also a positive modulator of the $GABA_A$ receptor complex. However, in a recently published article some important differences in potency, efficacy, and regional selectivity at the $GABA_A$ receptor complex were revealed between 5α-THDOC and its active stereoisomer 5β-THDOC (32). 5β-THDOC, but not 5α-THDOC, antagonized an AP-induced loss of the righting reflex in mice at a dose that had no effect alone. Moreover, 5β-THDOC modulated GABA-evoked Cl^- currents with low efficacy and inhibited the potentiation of GABA-evoked Cl^- currents by AP (32). These findings indicate partial agonist-like properties of 5β-THDOC. It is possible, therefore, that the efficacy of 5β-THDOC as an allosteric modulator of the $GABA_A$ receptor complex was limited in evoking any changes in ^{3}H-muscimol binding to the rat brain structures. However, the intrinsic activity of this compound was high enough to bring about significant behavioral effects. Thus, this part of the present results may contribute to other data, suggesting the existence of partial agonist neurosteroids.

In summary, the present data did not reveal any anxioselective effects for a range of centrally active neurosteroids, in the ethologically orientated and conflict models of anxiety, after intracerebral drugs administration. Their central effects appeared secondary to changes in motor activity, and most probably in pain threshold and drive for water. It is possible that high local concentration of neurosteroids after ICV injection, and production of a narrower range of behavioral effects than benzodiazepines, precluded manifestation of the anti-anxiety effects of AP, 5α-THDOC, and 5β-THDOC. Thus, the possibility that neuroactive neurosteroids may provide a novel potential site for therapeutic interventions in anxiety disorders is not supported. Moreover, the data from the part of the experiment with 5β-THDOC may contribute to other results, suggesting the existence of a new category of neurosteroids acting as partial agonists of the $GABA_A$ receptor.

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