ELSEVIER

Pharmacology, Biochemistry and Behavior 72 (2002) 987–991

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Antagonism of picrotoxin-induced changes in dopamine and serotonin metabolism by allopregnanolone and midazolam

Piotr Maciejak[a], Paweł Krząścik[b], Agnieszka I. Członkowska[b], Janusz Szyndler[b], Andrzej Bidziński[a], Jerzy Walkowiak[a], Wojciech Kostowski[b,c], Adam Plaznik[a,b,*]

[a]*Department of Neurochemistry, Institute of Psychiatry and Neurology, Al. Sobieskiego 9, 02-957 Warsaw, Poland*
[b]*Department of Experimental and Clinical Pharmacology, Medical University, Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland*
[c]*Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Al. Sobieskiego 9, 02-957 Warsaw, Poland*

Received 19 February 2002; received in revised form 4 April 2002; accepted 4 April 2002

Abstract

The effects of allopregnanolone and midazolam, given intracerebroventricularly, on the behavioral and biochemical effects of picrotoxin, were examined in a model of neurotoxin-induced seizures, in mice. After acute injections, midazolam (ED_{50} = 39.8 nmol) and allopregnanolone (ED_{50} = 11.0 nmol) produced similar and dose-dependent protection against picrotoxin-induced seizures. Picrotoxin given intraperitoneally at the ED_{85} dose decreased significantly the concentration of serotonin (5-HT), dopamine (DA), homovanilic acid (HVA) and 3,4-dihydroxyindolacetic acid (DOPAC), in the mouse striatum and the frontal cortex, in the period of time immediately preceding the onset of seizures. A single injection of allopregnanolone more potently, in comparison to midazolam, antagonized the biochemical action of picrotoxin, abolishing its effects on DA, HVA and 5-HT concentration, in the mouse striatum and the frontal cortex. These results for the first time provide a direct argument for an involvement of central dopaminergic and serotonergic systems in the seizure development. The present data add also to the accumulating evidence suggesting a favorable pharmacological profile for some neurosteroids currently considered to have a future role in the management of epilepsy.

Keywords: Seizures; Picrotoxin; Allopregnanolone; Midazolam; Dopamine; Serotonin; Intraperitoneal injections; Intracerebroventricular injections; Mice

1. Introduction

Picrotoxin, a central nervous system (CNS) convulsant, is thought to act at the so-called *t*-butylbicyclophosphorotionate/picrotoxin binding site (Huang et al., 2001; Ito et al., 1989). It is also known, along with another convulsant agent, pentylenetetrazol, to interact with overlapping but distinct domains of the $GABA_A$ receptor (Huang et al., 2001). Furthermore, by blocking GABA-activated Cl^- currents in a concentration-dependent manner, disinhibiting excitatory processes in the brain and facilitating the propagation of neuronal membranes' depolarization, picrotoxin causes the onset of convulsions. The abovementioned process is controlled by brain monoaminergic systems.

A spontaneous and experimentally induced depletion of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) have been implicated in the onset and development of many seizure disorders (Abed, 1989; Shouse et al., 2001; Zis et al., 1992). On the other hand, many experimental procedures designed to obtain an increase in a CNS monoaminergic activity have demonstrated antiepileptic properties of these compounds (Shouse et al., 2001; Yan et al., 1995). While most of these experiments focus on the effects of convulsive agent-induced seizures on the monoamine concentration, a different approach, i.e., analysis of changes in monoamine levels in the prodromal period of seizure development, before the onset of convulsions, is required. Such studies can help to characterize more deeply the nature of seizure disorders and the role that monoaminergic neurons play in this phenomenon. Since the seizures result from a continuous process of changes in brain activity after a convulsant administration, the time of an examination has to be selected in an arbitrary way. In the present experiment, the moment immediately preceding clonic–tonic convulsions was cho-

* Corresponding author. Department of Neurochemistry, Institute of Psychiatry and Neurology, 02-957 Warsaw, Al. Sobieskiego 9, Poland. Tel.: +48-22-842-45-98; fax: +48-22-642-66-11x471, +48-22-642-53-75.
E-mail address: adaplaz@yahoo.com (A. Plaznik).

0091-3057/02/$ – see front matter
PII: S0091-3057(02)00811-0

sen. This corresponds with a culmination of long-lasting central processes evoked by a picrotoxin administration.

The other objective of this study was to examine the role of a representative neurosteroid, allopreganolone, in a model of picrotoxin-associated behavioral and biochemical effects. Some neurosteroids are potent and specific endogenous modulators of $GABA_A$ receptors' activity. Moreover, they are thought to play an important role in the future management of epilepsy, anxiety and insomnia (Gąsior et al., 1999). Since the peripheral metabolism of neurosteroids to their active derivatives is very complex, all the drugs were administered intracerebroventricularly. The effects of allopregnanolone were compared with those of midazolam, a full agonist of the benzodiazepine receptor.

2. Materials and methods

2.1. Animals

The experiments were carried out on adult male albino Swiss mice weighing 20–25 g. All animals were acclimatized to their cages for 5 days before testing. They were housed under a 12-h light–dark cycle, at a controlled temperature (20 °C), with water and food ad libitum. All experiments were done between 11:00 a.m. and 4:00 p.m. 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 Local Committee for Animal Care and Use at the Medical University in Warsaw.

2.2. Convulsant test

Picrotoxin was dissolved in 0.9% NaCl and was administered intraperitoneally (10 ml/kg). The mice were placed singly in Plexiglas cages ($20 \times 25 \times 15$ cm) immediately after convulsant injection and were observed for 30 min for the occurrence of the 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 status epilepticus, loss of righting response, tonic hindlimb extension and death. The proconvulsive potency of picrotoxin was defined as the percentage of animals showing consistent seizures leading to death within 30 min after the administration of this chemoconvulsant. For subsequent experiments, the dose of picrotoxin was chosen to be within the LD_{85}–LD_{95} limits (effective lethal dose in 85–95% of mice), as determined during preliminary experiments.

2.3. Surgical procedure and microinjections

The intracerebroventricular injections of drugs were performed according to the procedure described previously (Członkowska et al., 2000; Herman, 1975). Mice were anaesthetized with ketamine (50 mg/kg/10 ml, ip) and a sagittal incision was made along the midline 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 sharp needle. The hole was made by

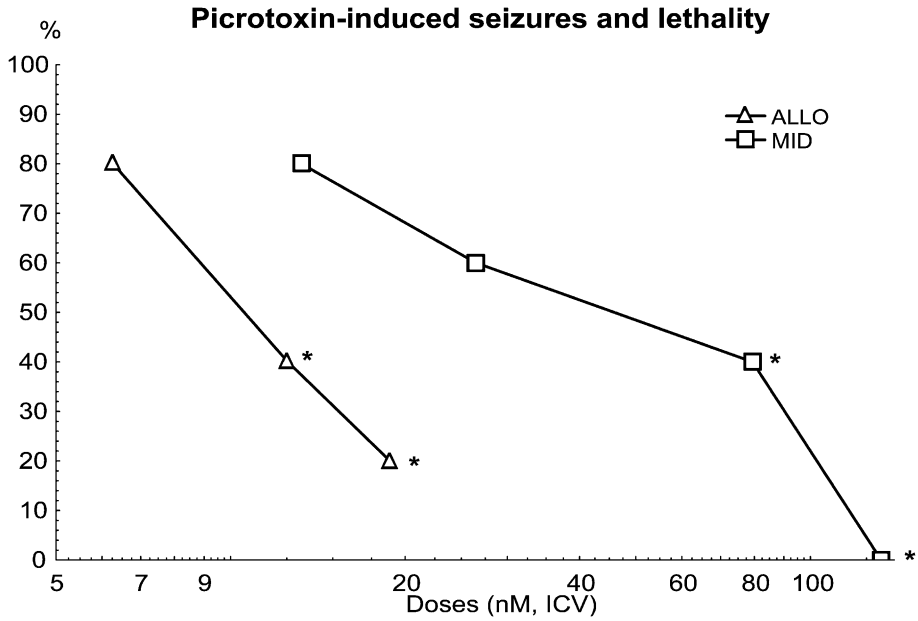

Fig. 1. Effects of acute intracerebroventricular injections of midazolam (MID) and allopregnanolone (ALLO) against seizures induced by intraperitoneal injections of picrotoxin. The dose of neurotoxin was within the LD_{85}–LD_{95} limits (see Materials and methods). Each data point indicates the percentage of animals with seizures. MID and AP were injected intracerebroventricularly 10 min before the convulsant administration. $n = 6$–8 mice per one data point. * $P < .05$ compared to the convulsant alone (Fisher's Exact Probability Test).

Table 1
The protective doses (nM) of allopregnanolone and midazolam administered intracerebroventricularly against picrotoxin (ED_{85}, ip) induced seizures and lethality in mice

Drug	ED_{16}	ED_{50}	ED_{85}
Allopregnanolone	6.6 (4.8–9.1)	11.0 (6.4–18.3)	18.5 (13.4–25.6)
Midazolam	13.8 (6.4–29.8)	39.8 (18.1–87.2)	114.9 (53.1–248.5)

The data are expressed in nanomoles with 95% CL. Both compounds were injected 10 min before picrotoxin administration.

rotating the needle. The animals were tested further 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 picrotoxin was administered intraperitoneally 10 min later. The injection site was checked by injection of methylene blue solution (5 μl/50 s) according to the above procedure on the last day of the experiment, and 10 min prior to decapitation of the animals.

2.4. Drugs

The following drugs were used: midazolam maleate (Hoffman La Roche, Switzerland), 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) (RBI, USA), picrotoxin (Sigma-Aldrich, Poland). Midazolam was dissolved in distilled water. Allopregnanolone was suspended in 45% 2-hydroxypropyl-β-cyclodextrin (CDX) (RBI, USA) and was sonificated for 30 min before administration.

2.5. Administration regimen

In the biochemical part of the experiment, the drugs were given once at their ED_{50} (the dose inhibiting convulsions in 50% of animals). The drugs and a solvent were administered intracerebroventricularly 10 min before picrotoxin injection. Mice were decapitated 5–10 min after picrotoxin administration, just before the commencement of seizures, when the prodromal symptoms of seizures could be observed: body tremor, Straub tail.

2.6. Biochemical analysis of monoamine and amino acid concentrations

The midazolam (39.8 nmol) and allopregnanolone (11.0 nmol) ED_{50} values determined in the behavioral part of the study were subsequently used for the biochemical analysis. The mouse brains were rapidly removed, and the striatum and the frontal cortex were dissected bilaterally and frozen at −70 °C. NA, DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were assayed using a fully automated high-pressure liquid chromatography system with electrochemical detection and standard biochemical methods (Stefański et al., 1993).

2.7. Data analysis

The ED_{50} values with 95% confidence limits (CL) were determined using a computerized version of the Litchfield and Wilcoxon procedure. Fisher's Exact Probability Test was used for specific comparisons between treatments. In the biochemical assay, the data are shown as means ± S.E.M., and were checked statistically by one-way analysis of variance followed by the Least Significant Difference test (LSD).

3. Results

Picrotoxin produced seizures and lethal effect at an LD_{50} = 9.15 mg/kg (95% CL = 7.91–10.59). Subsequently, the dose of 11.14 mg/kg (LD_{85}) was selected for further experiments. After acute injections, midazolam (ED_{50} = 39.8 nmol) and allopregnanolone (ED_{50} = 11.0 nmol) produced similar and dose-dependent protection against picrotoxin-induced seizures (Fig. 1, Table 1).

Analysis of variance showed differences among groups in the concentration of 5-HT, DA, HVA and DOPAC, in the mouse striatum and the frontal cortex [striatum, DA, $F(4,27) = 4.32$, $P < .01$; HVA, $F(4,27) = 4.06$, $P < .01$; DOPAC, $F(4,27) = 7.12$, $P < .01$; 5-HT, $F(4,27) = 4.07$,

Table 2
Effects of midazolam, allopregnanolone and cyclodextrin on picrotoxin-induced changes in the concentration of monoamines and their metabolites in the mouse striatum

Group	*n*	NA	DA	HVA	DOPAC	5-HT	5-HIAA
Control	6	287.8 ± 55.5	11,350 ± 968.6	2032.3 ± 142.4	590.5 ± 57.5	590.2 ± 59.9	502.3 ± 79.3
PIX	6	184.5 ± 37.2	7246 ± 253.6**	1249.2 ± 63.2**	336.3 ± 32.8**	324.0 ± 44.5**	294.0 ± 38.7
PIX + ALLO	6	174 ± 36.9	10,124 ± 1077.2	1747.5 ± 181.5	335.8 ± 20.69**	471.8 ± 56.0	370.0 ± 43.3
PIX + MID	7	192.9 ± 49.1	7964.43 ± 651**	1593.3 ± 162.9*	351.0 ± 45.0**	396.7 ± 45.2**	341.3 ± 42.6
PIX + CDX	7	255.9 ± 54.9	9269.6 ± 701.1	1565.0 ± 100.5*	360.3 ± 33.5**	444.3 ± 31.3*	351.6 ± 31.3
ANOVA		$F(4,30) = 1.02$	$F(4,27) = 4.32$, $P < .01$	$F(4,27) = 4.06$, $P < .01$	$F(4,27) = 7.12$, $P < .01$	$F(4,27) = 4.0$, $P < .01$	$F(4,27) = 2.44$

The drugs were administered acutely at the ED_{50} doses, established in the behavioral part of the experiment. The data are shown as means ± S.E.M. (ng/g tissue).

* $P < .05$ difference from control (LSD test).

** $P < .01$ difference from control (LSD test).

Table 3
Effects of midazolam, allopregnanolone and cyclodextrin on picrotoxin-induced changes in the concentration of monoamines and their metabolites in the mouse frontal cortex

Group	*n*	NA	DA	HVA	DOPAC	5-HT	5-HIAA
Control	7	272.0 ± 114.2	27.14 ± 5.58	112.0 ± 32.7	346.3 ± 45.8	376.0 ± 31.0	184.6 ± 27.6
PIX	6	95.17 ± 17.3	7.0 ± 4.69**	52.3 ± 9.0	102.7 ± 36.2**	255.16 ± 37.9	144.3 ± 12.5
PIX + ALLO	7	98.8 ± 14.8	30.67 ± 5.02	117.5 ± 21.6	230.3 ± 40.2	346.8 ± 31.8	177.0 ± 35.6
PIX + MID	6	103.8 ± 15.6	24.0 ± 5.01	102.3 ± 17.4	298.7 ± 54.4	324.2 ± 33.2	143.7 ± 11.3
PIX + CDX	6	116.0 ± 13.7	19.3 ± 3.73	83.9 ± 26.1	197.4 ± 46.8*	307.3 ± 37.6	203.7 ± 23.5
ANOVA		$F(4,27) = 1.82$	$F(4,27) = 3.38$, $P < .05$	$F(4,27) = 1.16$	$F(4,27) = 4.26$, $P < .01$	$F(4,27) = 1.71$	$F(4,27) = 1.17$

The drugs were administered acutely at the ED_{50} doses, established in the behavioral part of the experiment. The data are shown as means ± S.E.M. (ng/g tissue).

* $P < .05$ difference from control (LSD test).

** $P < .01$ difference from control (LSD test).

$P < .01$; frontal cortex, DA, $F(4,27) = 3.38$, $P < .05$; DOPAC, $F(4,27) = 4.26$, $P < .01$] (Tables 2 and 3). Post hoc test displayed that picrotoxin given intraperitoneally at the ED_{85} dose significantly decreased in the striatum concentrations of DA ($P < .01$), HVA ($P < .01$), DOPAC ($P < .01$), 5-HT ($P < .01$) and in the frontal cortex concentrations of DA ($P < .01$), and DOPAC ($P < .01$).

A single injection of allopregnanolone (ALLO) was most effective, among all other treatments, in antagonizing the influence of picrotoxin on monoamines and their metabolism, abolishing its effects on DA, HVA and 5-HT concentration, in the striatum (Table 2). Pretreatment with midazolam (MID) and cyclodextrin (CDX) was less effective in this respect, leaving the picrotoxin-induced changes in HVA, DOPAC and 5-HT levels unchanged (Table 2). In the frontal cortex, a single injection of ALLO and MID blocked all the metabolic effects of picrotoxin, in the period of time preceding the onset of seizures (Table 3).

4. Discussion

A major finding of the present study is that picrotoxin-induced changes in DA and 5-HT systems' activity, occurring immediately before the onset of seizures, were attenuated by the pretreatment of mice with positive allosteric modulators of $GABA_A$ receptors, such as a neurosteroid, allopregnanolone and a full agonist of benzodiazepine receptors, midazolam. The effect of allopregnanolone was more potent than that of midazolam. It is noteworthy that allopregnanolone and midazolam were administered at the ED_{50} dose blocking picrotoxin-induced convulsions. Thus, the present results provide a direct argument for the hypothesis of an inhibitory role of central dopaminergic and serotonergic systems in seizure development. This conclusion refers to the time point immediately preceding seizures, i.e., the moment of culmination of long-lasting central processes evoked by a picrotoxin administration.

It has been previously shown that the pentylenetetrazol clonic–tonic convulsion threshold was significantly reduced after treatment of rats with monoamine depletors: reserpine, tertrabenazine and *p*-chlorophenylalanine (Abed, 1989). Moreover, lower baseline DA and 5-HT concentrations in the amygdala and locus coeruleus (in vivo microdialysis) correlated with both increases in duration of focal and generalized afterdischarges and behavioral seizures, in a model of one day amygdala kindling paradigm in cats (Shouse et al., 2001). Our data, indicating a decrease in DA and 5-HT concentrations in the mouse striatum and frontal cortex immediately before the onset of seizures, accord with abovementioned findings.

All examined drugs including a solubilizer cyclodextrin (45% 2-hydroxypropyl-β-cyclodextrin solution), significantly attenuated some biochemical effects of picrotoxin. Moreover, repeated administration of cyclodextrin modified the action of allopreganolone in a model of picrotoxin seizures, indicating a development of tolerance (Członkowska et al., 2001). This weak but significant effect of cyclodextrin represents a novel finding as there are no reports on the pharmacological action of the substance in the literature so far. It indicates, however, that cyclodextrin may have a direct modulatory influence on $GABA_A$ receptors. Importantly, among all the treatments, a single injection of allopreagnanolone proved to be the most effective in antagonizing the influence of picrotoxin on monoamines and their metabolism by abolishing its effects on DA, HVA, 5-HT concentration in the striatum and the frontal cortex. It is noteworthy that in a pilot study, the central injections of the same concentration and volume of cyclodextrin did not show any anticonvulsant activity (data not shown). Furthermore, the biochemical effects of allopregnanolone on picrotoxin-induced biochemical changes were much more potent than those of cyclodextrin administered alone. Thus, it appeared that allopregnanolone attenuated the biochemical effects of picrotoxin in an independent way.

The present results add to the accumulating evidence suggesting a favorable pharmacological profile of some neurosteroids considered to play a promising role in the future management of epilepsy (Członkowska et al., 2000; Gąsior et al., 1999).

Acknowledgments

This work was supported by the Institute of Psychiatry and Neurology grant no. 62/2001. A.I. Członkowska was supported by grants from the Foundation for Polish Science. We would also like to thank Dr. Paulina Rok and Dr. Małgorzata Zienowicz for correcting the English in the manuscript.

References

Abed WT. The effect of monoamine depletors on metrazol induced convulsions and brain gamma-aminobutyric acid (GABA) contents in rats. J Pharm Pharmacol 1989;41:807–8.

Członkowska AI, Krząścik P, Sienkiewicz-Jarosz H, Siemiątkowski M, Szyndler J, Bidziński A, Plaznik A. The effects of neurosteroids on picrotoxin-, bicuculline-, and NMDA-induced seizures, and a hypnotic effect of ethanol. Pharmacol Biochem Behav 2000;76:345–53.

Członkowska AI, Krząścik P, Sienkiewicz-Jarosz H, Siemiątkowski M, Szyndler J, Maciejak P, Bidziński A, Plaznik A. Tolerance to the anticonvulsant activity of midazolam and allopregnanolone in a model of picrotoxin seizures. Eur J Pharmacol 2001;425:121–7.

Gąsior M, Carter R, Witkin JM. Neuroactive steroids: potential therapeutic use in neurological and psychiatric disorders. Trends Pharmacol Sci 1999;20:107–12.

Herman ZS. Behavioural changes induced in conscious mice by intracerebroventricular injection of catecholamines, acetylcholine and 5-hydroxytryptamine. Br J Pharmacol 1975;55:351–8.

Huang RQ, Bell-Horner CL, Dibas MI, Covey DF, Dreve JA, Dillon GH. Pentylenetetrazol-induced inhibition of recombinant gamma-aminobutyric acid type A (GABA(A)) receptors: mechanism and site of action. J Pharmacol Exp Ther 2001;298:986–95.

Ito Y, Lom DK, Nabeshima T, Ho IK. Effects of picrotoxin on GABA-A receptor supramolecular complexes in rat brain. J Neurochem 1989;52:1064–70.

Shouse MN, Staba RJ, Ko PY, Saquib SF, Farber PR. Monoamines and seizures: microdialysis findings in locus coeruleus and amygdala before and during amygdala kindling. Brain Res 2001;176–92.

Stefański R, Pałejko W, Bidziński A, Kostowski W, Palznik A. Serotonergic innervation of the hippocampus and mucleus accumbens septi and the anxiolytic-like action of midazolam and 5-HT1A receptor agonists. Neuropharmacology 1993;32:977–85.

Yan QS, Jobe PC, Dailey JW. Further evidence of anticonvulsant role of 5-hydroxytryptamine in genetically epilepsy-prone rats. Br J Pharmacol 1995;115:1314–8.

Zis AP, Nomkos GG, Brown EE, Damsa G, Fibiger HC. Neurochemical effects of electrically and chemically induced seizures: an in vivo microdialysis study in hippocampus. Neuropsychopharmacology 1992;7:189–95.