

*Supporting Information*

# The neuroactive steroid pregnanolone glutamate: anticonvulsant effect, metabolites and its effect on neurosteroid levels in developing rat brain

Eva Kudova <sup>1,\*</sup>, Pavel Mares <sup>2</sup>, Martin Hill <sup>3</sup>, Katerina Vondrakova <sup>2,4</sup>, Grygoriy Tsenov <sup>2,4,5,\*</sup>, Hana Chodounská <sup>1</sup>, Hana Kubová <sup>2</sup>, and Karel Váles <sup>2,4</sup>

<sup>1</sup> Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 2, 16000 Prague, Czech Republic; hchod@uochb.cas.cz

<sup>2</sup> Institute of Physiology, Academy of Sciences of the Czech Republic, Videnská 1083, 14220 Prague, Czech Republic; pavel.mares@fgu.cas.cz (P.M.); katavondrakova@seznam.cz (K.V.);

hana.kubova@fgu.cas.cz (H.K.); karel.vales@nudz.cz (K.V.)

<sup>3</sup> Institute of Endocrinology, Narodní 8, 11694 Prague, Czech Republic; mhill@endo.cz

<sup>4</sup> Institute of Mental Health, Topolová 748, 25067 Klecany-Prague East, Czech Republic

<sup>5</sup> Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Strada le Grazie 8, 37134 Verona, Italy

\* Correspondence: eva.kudova@uochb.cas.cz (E.K.); grygoriy.tsenov@nudz.cz (G.T.)

## Table of Contents

Table S1. Steroid levels (pM/g) in hippocampal tissue of rats in the control group of animals treated with cyclodextrin (i.p.), intact animals, and in the group treated with pregnanolone glutamate (PA-G, i.p., CDX, 1 mg/kg) 15 min after the application. ....	2
Table S2. Chemical names, common names, and abbreviations for steroids studied.....	4
References .....	5

**Table S1.** Steroid levels (pM/g) in hippocampal tissue of rats in the control group of animals treated with cyclodextrin (i.p.), intact animals, and in the group treated with pregnanolone glutamate (PA-G, i.p., CDX, 1 mg/kg) 15 min after the application. %5 $\beta$  = percentage of total significantly increased 5 $\beta$ -steroids in rat hippocampus 15 minutes after PA-G application;  $\Delta_r$  = steroid increase above the basal levels 15 minutes after the pregnanolone glutamate application; C = controls with cyclodextrin *i.p.* application, I = intact controls, P = PA-G (*i.p.*, CDX); 12, 25 = week 12 and 25 after labor, respectively. The GC-MS/MS based steroid quantification method was described in detail in our recent article.<sup>1</sup>

Steroid	Age [Day]	Age × Group		P	%5 $\beta$	$\Delta_r$
		Control	Intact			
5 $\beta$ -Dihydroprogesterone (5 $\beta$ -DHP)	12	0.0796 (0.0501, 0.123)	0.294 (0.173, 0.547)	1.51 (0.677, 5.72)	0.07%	19 (111, 856)
	25	0.157 (0.0962, 0.263)	0.154 (0.0998, 0.244)	0.299 (0.185, 0.518)	0.09%	1.9 (7.68, 22.8)
ANOVA: Age: F=1.7, p=0.21, Group: F=11.5, p<0.001, Age × Group: F=4.2, p=0.029; P25>P12, P>I, P>C, I12>C12, P12>C12						
Pregnanolone (PA)	12	0.736 (0.509, 1.06)	0.799 (0.575, 1.11)	200 (81.7, 630)	9.47%	272 (111, 856)
	25	1.23 (0.885, 1.74)	0.334 (0.221, 0.489)	15.8 (9.48, 28.2)	9.30%	12.8 (7.68, 22.8)
ANOVA: Age: F=6.9, p=0.016, Group: F=131.6, p<0.001, Age × Group: F=7.2, p=0.004; I25<I12, P25<P12, P>I, P>C, P12>I12, P12>C12, I25<C25, P25>I25, P25>C25						
Conjugated pregnanolone	12	0.643 (0.452, 0.896)	1.2 (0.834, 1.72)	128 (58.7, 345)	6.04%	199 (111, 856)
	25	1.93 (1.4, 2.7)	0.746 (0.508, 1.07)	18.9 (11.6, 32.8)	10.83%	9.76 (7.68, 22.8)
ANOVA: Age: F=0.3, p=0.581, Group: F=115.8, p<0.001, Age × Group: F=11.5, p<0.001; C25>C12, P25<P12, P>I, P>C, P12>I12, P12>C12, I25<C25, P25>I25, P25>C25						
5 $\beta$ -Pregnane-3 $\alpha$ ,20 $\alpha$ -diol	12	0.337 (0.253, 0.449)	1.06 (0.776, 1.48)	26.3 (14.3, 53.9)	1.23%	78.1 (111, 856)
	25	0.182 (0.135, 0.244)	0.0887 (0.0581, 0.128)	2.87 (1.97, 4.31)	1.72%	15.7 (7.68, 22.8)
ANOVA: Age: F=75.2, p<0.001, Group: F=129.5, p<0.001, Age × Group: F=9.5, p=0.001; C25<C12, I25<I12, P25<P12, P>I, P>C, I12>C12, P12>I12, P12>C12, P25>I25, P25>C25						
Conjugated 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol	12	0.112 (0.0705, 0.174)	0.563 (0.314, 1.05)	11.2 (5.69, 23.7)	0.53%	99.7 (111, 856)
	25	0.158 (0.101, 0.245)	0.243 (0.157, 0.379)	1.62 (0.971, 2.81)	0.93%	10.2 (7.68, 22.8)
ANOVA: Age: F=5.9, p=0.024, Group: F=53.7, p<0.001, Age × Group: F=4.3, p=0.026; P25>P12, I>C, P>I, P>C, I12>C12, P12>I12, P12>C12, P25>I25, P25>C25						
17-Hydroxypregnanolone (17-OH-PA)	12	2.76 (1.76, 4.28)	7.59 (5.04, 11.8)	1550 (341, 33000)	73.67%	562 (111, 856)
	25	3.41 (2.3, 5.06)	2.57 (1.71, 3.81)	122 (52, 390)	75.85%	35.8 (7.68, 22.8)
ANOVA: Age: F=5.8, p=0.025, Group: F=76.6, p<0.001, Age × Group: F=2.9, p=0.077; I25<I12, P>I, P>C, P12>I12, P12>C12, P25>I25, P25>C25						
Conjugated 17-hydroxypregnanolone	12	0.00972 (0.00448, 0.0203)	0.0151 (0.0078, 0.0293)	0.822 (0.262, 3.47)	0.04%	84.6 (111, 856)
	25	0.0169 (0.00807, 0.0357)	0.00837 (0.00378, 0.0175)	0.248 (0.096, 0.768)	0.15%	14.7 (7.68, 22.8)
ANOVA: Age: F=0.5, p=0.506, Group: F=26.3, p<0.001, Age × Group: F=1.1, p=0.367; P>I, P>C, P12>I12, P12>C12, P25>I25, P25>C25						
5 $\beta$ -Pregnane-3 $\alpha$ ,17 $\alpha$ ,20 $\alpha$ -triol	12	1.91 (1.13, 3.21)	7.65 (4.73, 12.5)	17.4 (10.5, 29.4)	0.74%	9.16 (111, 856)
	25	1.19 (0.735, 1.89)	0.659 (0.393, 1.07)	2.83 (1.68, 4.79)	1.05%	2.39 (7.68, 22.8)
ANOVA: Age: F=32.7, p<0.001, Group: F=10.8, p<0.001, Age × Group: F=4.5, p=0.023; I25<I12, P25<P12, P>I, P>C, I12>C12, P12>C12, P25>I25						
Etiocholanolone (ETIO)	12	0.183 (0.155, 0.217)	0.36 (0.293, 0.456)	2.25 (1.15, 26.1)	0.10%	12.3 (111, 856)
	25	0.134 (0.115, 0.157)	0.123 (0.104, 0.147)	0.263 (0.219, 0.32)	0.08%	1.96 (7.68, 22.8)

ANOVA: Age: F=69.5, p<0.001, Group: F=43.5, p<0.001, Age × Group: F=6.3, p=0.007; I25<I12, P25<P12, P>I, P>C, I12>C12, P12>I12, P12>C12, P25>I25, P25>C25					
3 $\alpha$ ,5 $\beta$ -Tetrahydrocorticosterone (3 $\alpha$ ,5 $\beta$ -THCC)	12	49.2 (28.1, 79.9)	114 (76.8, 163)	220 (157, 301)	8.12% 4.47 (111, 856)
ANOVA: Age: F=18.9, p<0.001, Group: F=3.5, p=0.047, Age × Group: F=6.8, p=0.005; I25<I12, P25<P12, P12>C12					
	25	62.2 (39.2, 94)	18.3 (8.72, 32.4)	42.8 (23.8, 70.8)	0.00% 0.688 (7.68, 22.8)

**Table S2.** Chemical names, common names, and abbreviations for steroids studied. The first column gives the chemical name, the second column indicate a trivial name accepted for this compound, and the third column shows abbreviation. N/A – abbreviations were not introduced for these compounds.

Chemical Name	Trivial Name	Abbreviation
3 $\alpha$ -Hydroxy-5 $\alpha$ -pregnan-20-one	Allo pregnanolone	ALLO
3 $\alpha$ -Hydroxy-5 $\beta$ -pregnan-20-one	Pregnanolone	PA
20-Oxo-5 $\beta$ -pregnan-3 $\alpha$ -yl 3-Sulfate	Pregnanolone sulfate	PA-S
20-Oxo-5 $\beta$ -pregnan-3 $\alpha$ -yl L-glutamyl 1-ester	Pregnanolone glutamate	PA-G
17 $\alpha$ -Hydroxy-20-oxo-5 $\beta$ -pregnan-3 $\alpha$ -yl L-glutamyl 1-ester	17-Hydroxypregnanolone glutamate	17-OH-PA-G
(20S)-20-Hydroxy-5 $\beta$ -pregnan-3 $\alpha$ -yl glutamate	(20S)-Hydroxypregnanolone glutamate	N/A
3 $\alpha$ -Hydroxy-5 $\beta$ -androstan-17-one	Etiocholanolone	ETIO
3 $\alpha$ ,17 $\alpha$ -Dihydroxy-5 $\beta$ -pregnan-20-one	17-Hydroxypregnanolone	17-OH-PA
3 $\alpha$ ,17 $\alpha$ -Dihydroxy-5 $\alpha$ -pregnan-20-one	17-Hydroxyallopregnanolone	17-OH-ALLO
3 $\alpha$ -Hydroxy-5 $\beta$ -pregnan-20-one	Pregnanolone	PA
(20S)-5 $\beta$ -Pregnane-3 $\alpha$ ,20-diol	5 $\beta$ -Pregnane-3 $\alpha$ ,20 $\alpha$ -diol	Not introduced
(20S)-5 $\beta$ -pregnan-3 $\alpha$ ,17 $\alpha$ ,20-triol	5 $\beta$ -Pregnane-3 $\alpha$ ,17,20 $\alpha$ -triol	Not introduced
5 $\beta$ -Pregnane-3,20-dione	5 $\beta$ -Dihydroprogesterone	5 $\beta$ -DHP
3 $\alpha$ ,11 $\beta$ ,21-Trihydroxy-5 $\beta$ -pregnan-20-one	3 $\alpha$ ,5 $\beta$ -Tetrahydrocorticosterone	3 $\alpha$ ,5 $\beta$ -THCC
3 $\beta$ -Hydroxy-5 $\alpha$ -pregnan-20-one	Pregnenolone	PE
20-Oxo-pregn-5-en-3 $\beta$ -yl 3-Sulfate	Pregnenolone sulfate	PE-S
20-Oxo-5 $\alpha$ -pregnan-3 $\alpha$ -yl 3-Sulfate	Allo pregnanolone sulfate	ALLO-S
3 $\beta$ -Hydroxy-androst-5-en-17-one	Dehydroepinandrosteone	DHEA
17-Oxo-androst-5-en-3 $\beta$ -yl 3-Sulfate	Dehydroepiandrosterone sulfate	DHEA-S
3 $\beta$ ,17 $\beta$ -Dihydroxy-androst-5-ene	Androstenediol	Not introduced
17 $\beta$ -Hydroxy-androst-5-ene-3 $\beta$ -yl 3-Sulfate	Androstenediol sulfate	Not introduced

## References

1. Hill, M.; Hana jr., V.; Velikova, M.; Parizek, A.; Kolatorova, L.; Vitku, J.; Skodova, T.; Simkova, M.; Simjak, P.; Kancheva, R.; Koucky, M.; Kokrdova, Z.; Adamcova, K.; Cerny, A.; Hajek, Z.; Duskova, M.; Bulant, J.; Starka, L., A method for determination of one hundred endogenous steroids in human serum by gas chromatography-tandem mass spectrometry. *Physiol. Res.* **2019**, *68* (2), 179-207.
2. Holubova, K.; Chvojkova, M.; Krausova, B. H.; Vyklicky, V.; Kudova, E.; Chodounská, H.; Vyklický, L.; Vales, K., Pitfalls of NMDA Receptor Modulation by Neuroactive Steroids. The Effect of Positive and Negative Modulation of NMDA Receptors in an Animal Model of Schizophrenia. *Biomolecules* **2021**, *11* (7).