Neuroactive Steroids – Promising Neuroprotectants?

Neuroprotective Effects of Synthetic Structural Modifications of Neurosteroid Pregnanolone Sulfate: Structure-Activity Relationship

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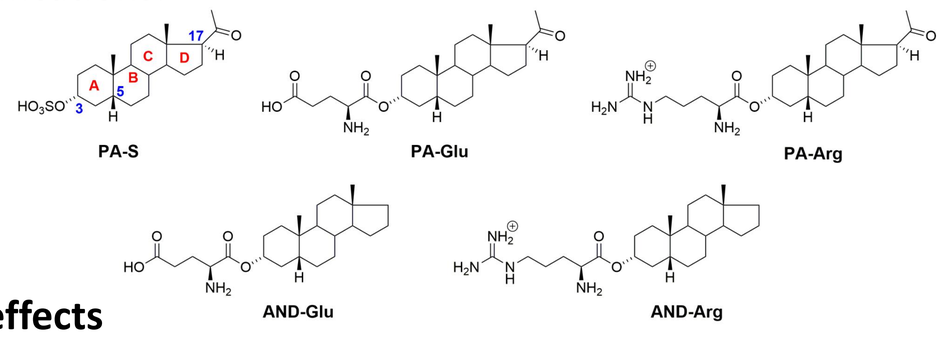
INTRODUCTION

20-oxo-5β-pregnan-3α-yl sulfate (pregnanolone sulfate, PA-S) is a naturally in the brain occurring neurosteroid inhibiting N-methyl-D-aspartate (NMDA) receptors. Its synthetic derivative 20-oxo-5β-pregnan-3α-yl L-glutamate (**pregnanolone glutamate, PA-Glu**) was proven to have neuroprotective properties and minimal side effects.

Here we tested ***in vivo*** **neuroprotective efficacy and possible behavioral side effects of selected structural modifications of the PA-S molecule**. The first modification represented a non-polar modification of the steroid D-ring (5β-androstan-3α-yl L-glutamate, **androstane glutamate, AND-Glu**), the second involved the attachment of a positively charged group to C3 (20-oxo-5β-pregnan-3α-yl L-argininate dihydrochloride salt, **pregnanolone argininate, PA-Arg**). The 5β-androstan-3α-yl L-argininate dihydrochloride salt (**androstane argininate, AND-Arg**) combined both modifications.



METHODS

Neuroprotective effects

The neuroprotective effects of steroids were tested in a model of excitotoxic hippocampal lesion, based on its behavioral consequences.

Adult male Long-Evans rats, bilateral NMDA lesion of dorsal hippocampus (1 µl of 0.05M NMDA solution; AP = - 4 mm, ML = 2.5 mm, DV = 4.6 mm). Tested steroids dissolved in (2-hydroxypropyl)-β-cyclodextrin (CDX) were applied i.p. immediately after the surgery.

Control groups (sham; NMDA+CDX), experimental groups (NMDA+PA-Glu 1, NMDA+PA-Glu 10, NMDA+PA-Arg 1, NMDA+PA-Arg 10, NMDA+AND-Glu 1, NMDA+AND-Glu 10, NMDA+AND-Arg 1, NMDA+AND-Arg 10 mg/kg).

Cognitive functions were tested in the **Carousel maze** (hippocampus-dependent task) 1 week after lesion. Four 20min sessions were performed. Mann-Whitney test was used for data evaluation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ difference from NMDA+ CDX group.

Behavioral effects

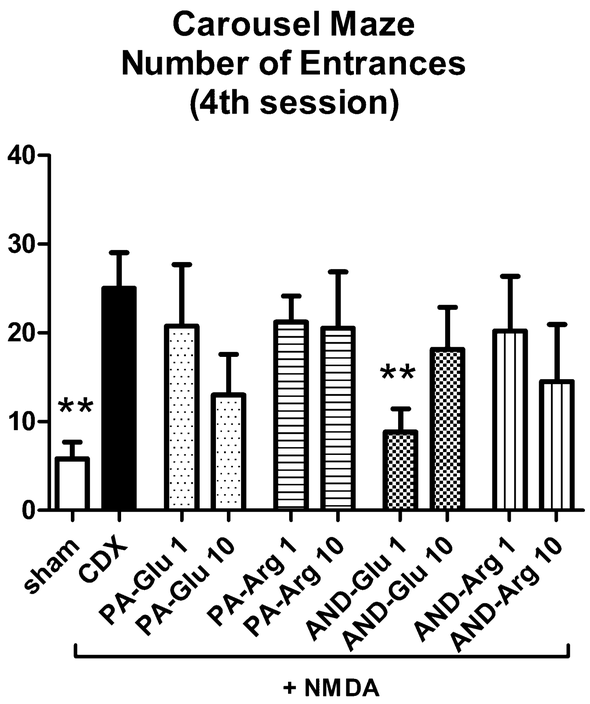
We further tested the effect of selected steroids on **locomotion (open field)** and **anxiety (elevated plus maze)**.

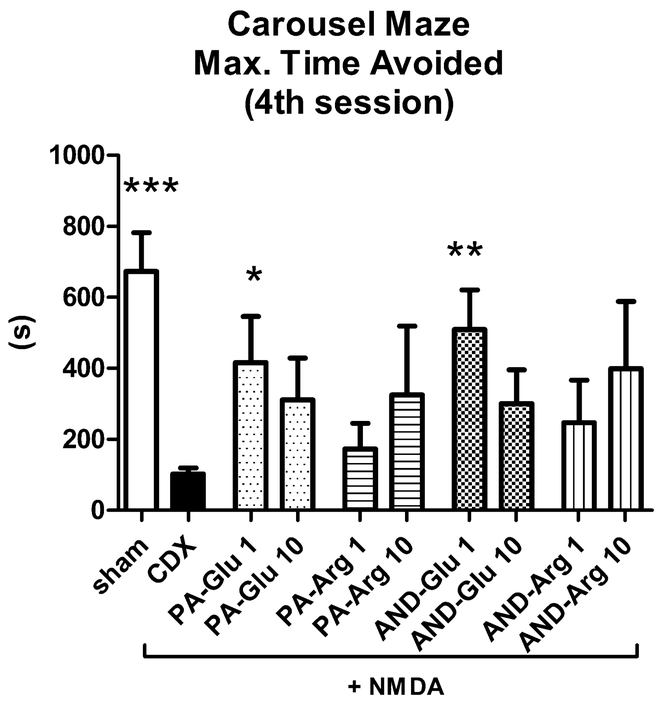
Adult male mice (30 g) of the CD-1 strain were used.

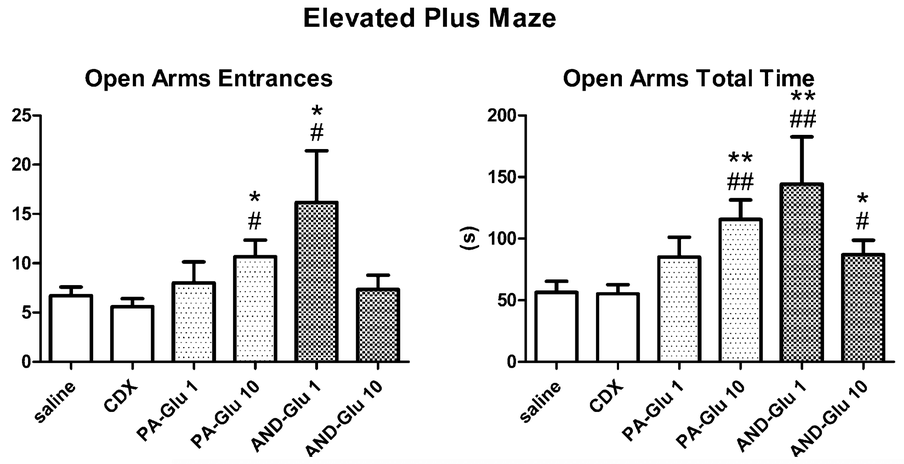
In the elevated plus maze, drugs were applied i.p. 30 min prior to the test. Control animals received saline or CDX. Mann-Whitney test was used for data evaluation.

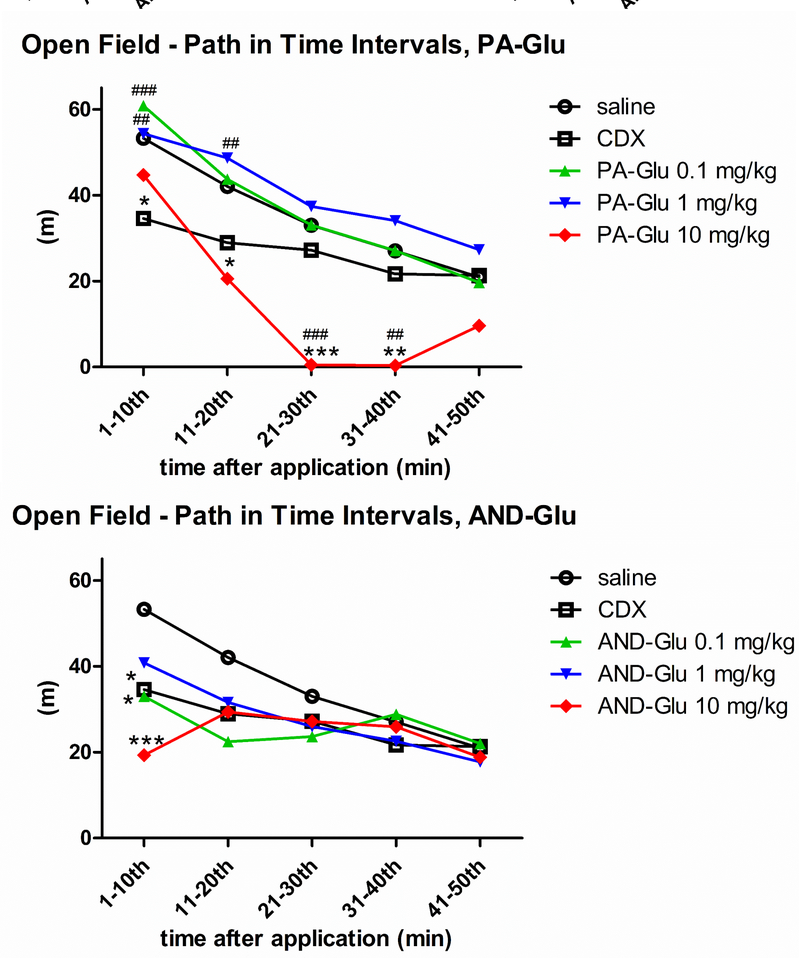
In the open field, drugs were applied immediately before test. 2way repeated measures ANOVA with Bonferroni post-tests was used. * $p < 0.05$, ** $p < 0.01$ compared to saline, # $p < 0.05$, ## $p < 0.01$ compared to CDX.

RESULTS









CONCLUSIONS

- **Neuroprotective effects of AND-Glu and PA-Glu were proven;**
- **the neuroprotective effect of AND-Glu was more pronounced than that of PA-Glu, suggesting possible benefit of the non-polar D-ring modification;**
- **positively charged molecules (PA-Arg, AND-Arg) did not have any neuroprotective effect;**
- **AND-Glu at the neuroprotective dose (1 mg/kg) did not cause hyperlocomotion, indicating minimal risk of psychotomimetic side effects;**
- **anxiolytic effects of PA-Glu, AND-Glu were shown.**

In conclusion, our results show that the non-polar D-ring modification in a molecule bearing a negatively charged group on C3 was associated with higher neuroprotective effect, whereas attachment of a positively charged group to C3 was not beneficial.

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