Pregnenolone sulphate potentiates NMDA-receptor mediated responses *in vitro* and *in vivo*

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Introduction

The major sources of steroids in the body are the adrenals and the gonads. However, it has been shown that some steroids can also be synthesized in the central nervous system independently of these sources. In rodents and particularly in the rat, one of the most abundant neurosteroids is pregnenolone sulphate (PS). It has been shown that PS may play an important role in memory and cognitive performances.

The aim of these experiments was to determine the *in vitro* and *in vivo* electrophysiological activity of PS. In particular, we examined the effects of PS on NMDA-induced currents in rat cortical neurones in primary cultures using whole-cell patch clamp recording techniques and a flex station assay. The effects of PS on NMDA-induced neuronal activity and synaptic responses were also examined *in vivo*.

Material & Methods

***In vitro* profiling:**

- Whole cell patch-clamp and Flex station experiments were performed in embryonic rat cultured cortical neurons (DIV 5-6) at room temperature. For flex station experiments cell were loaded with Fluo-3AM at 37°C.
- NMDA (1 µM, co-applied with 10 µM glycine; 0.1 µM TTX; 5 µM strychnine) induced intracellular calcium responses recorded with fluo-3AM using the flex station
- In whole-cell patch clamp experiments NMDA (30 µM for 1 s; co-applied with 10 µM glycine; 0.1 µM TTX; 5 µM strychnine) was applied to cortical neurones with a local application device to induce AP5-sensitive inward currents

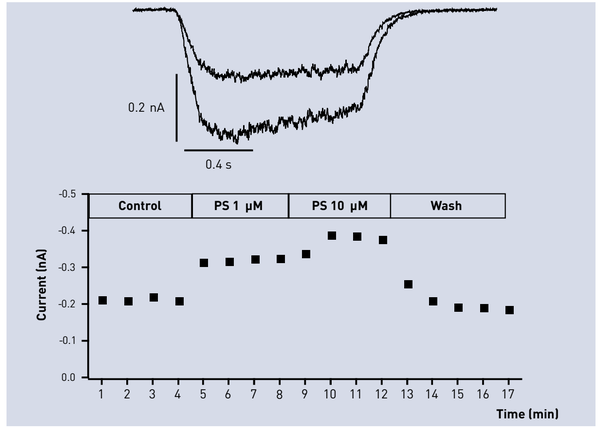
***In vivo* profiling:**

- Young adult male Sprague-Dawley rats (270-370 g) were anaesthetized by isoflurane induction followed by α-chloralose anaesthesia; anaesthetic depth was assessed by withdrawal responses to paw-pinch and the stability of measured cardiovascular variables.
- Core body temperature was monitored and maintained at 37°C.
- Recording electrodes were placed in the CA1 region of hippocampus by stereotaxic co-ordinates; Bregma -4.2 , lateral 2.4, 1.8-2.4 mm below pial surface.
- PS was admistrated i.c.v. via a stainless steel cannula descended 3.7 mm at a 20° rostro-caudal angle 0.5 mm rostral to bregma and 1.5 mm lateral to midline into the lateral ventricle on the same side as the recording electrode.
- Extracellular recordings were made from hippocampal neurones using multi-barrelled glass microelectrodes. Pipette barrels contained
  - Chicago Sky Blue (CSB, 2% dissolved in 0.5 M sodium acetate), NMDA (20 mM in 180 mM NaCl; pH 7)
  - AP-5 (50 mM in ddH_2O; pH 8)
  - The CSB solution-containing barrel provided the balancing for the current ejection.

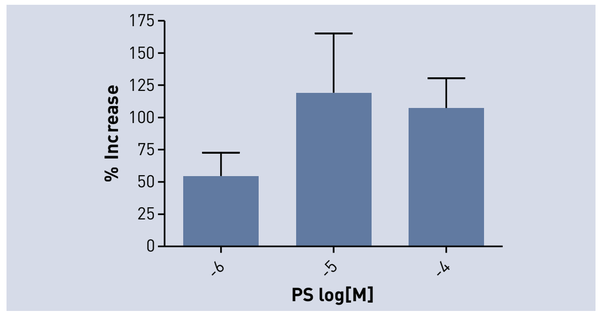
PS enhances NMDA-induced currents in cultured embryonic rat cortical neurons

- Using flex station we have shown that PS enhances NMDA (1 µM)-induced intracellular calcium responses recorded with fluo-3AM (pEC_{50}=5).
- Local application of NMDA (30 µM for 1 s) to cortical neurons resulted in AP-5-sensitive inward currents. In the presence of PS the amplitude of the NMDA currents was increased. The effects of PS were concentration-dependent (1-100 µM; n=3-5) and were reversible upon washout

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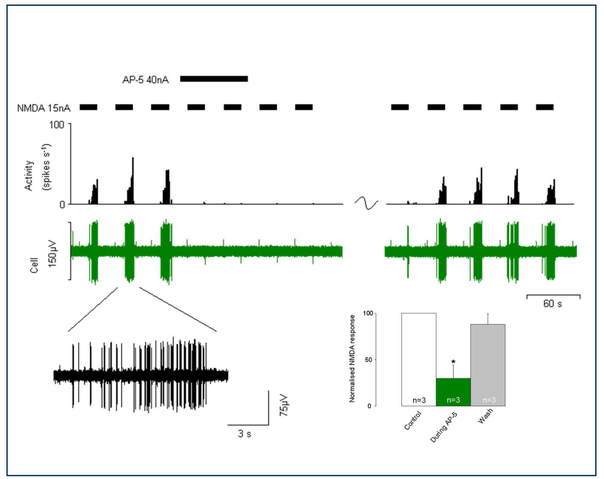
PS enhances NMDA-induced currents in cultured cortical neurons



Iontophoretic application of NMDA increases neuronal activity recorded from the CA1 region

- Iontophoretic application of NMDA (10-80 nA) evoked a mean excitation in neuronal activity of 26.6±9.0 spikes/s, recorded from the CA1 region (n=3)
- In the presence of the selective NMDA receptor antagonist AP-5 (40 nA), this mean excitation was significantly reduced to 10.4±5.3 spikes/s (29.5±14.5% of baseline activity, n=3, $P < 0.05$)
- This reduction in NMDA-evoked excitation recovered to 22.0±6.7 spikes/s (87.8±11.5% of baseline activity) after application of AP-5 was turned off, confirming that the evoked excitation was mediated via NMDA receptor activation

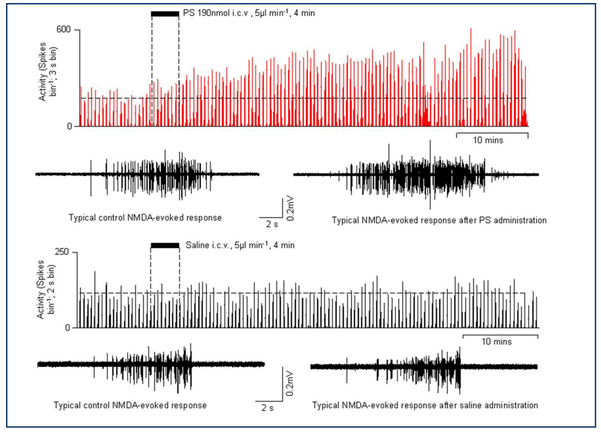
Iontophoretic application of NMDA increases neuronal activity recorded from the CA1 region



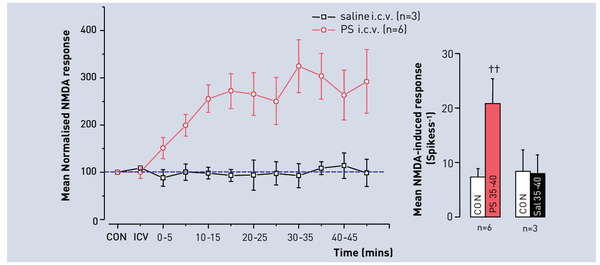
PS enhances NMDA-induced neuronal activity in the CA1 region of the rat hippocampus

- Local application of PS (190 nmol, solution; 4 mg/ml in saline, 20 µl, i.c.v.) increased the mean NMDA-evoked neuronal activity from 7.3±1.6 to a maximum of 22.5±5.4 spikes/s (324.8±77.5% of baseline activity; n=6, $P < 0.05$) 30-35 minutes after administration in the CA1 region.
- This increase was maintained for at least 50 minutes after the 4 minute i.c.v. administration of PS.
- Vehicle applications did not produce any significant effect on the NMDA-induced responses.

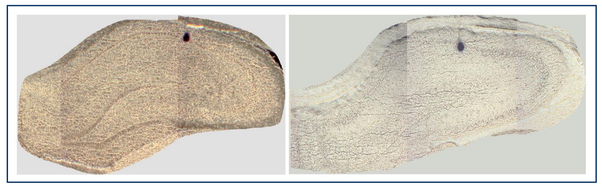
PS enhances NMDA-induced neuronal activity in the CA1 region of the rat hippocampus



PS enhances NMDA-induced neuronal activity in the CA1 region of the rat hippocampus



Some typical CSB-stained recording sites clearly located within the CA1 region of rat hippocampus



Conclusions

- **These findings demonstrate that pregnenolone sulphate positively modulates NMDA receptor-mediated neuronal activity *in vitro* and *in vivo*.**
- **These observations are consistent with a neuromodulatory role for pregnenolone sulphate.**

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