



Neuroprotective effect of PACAP against alcohol-induced toxicity in the developing rat cerebellum

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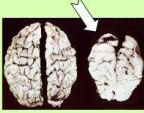


Introduction:

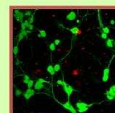
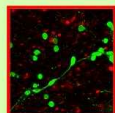
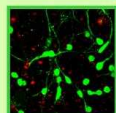
1: Alcohol consumption during pregnancy induces harmful effects leading to fetal alcohol syndrome.



2: Ethanol exposure leads to severe neuronal loss in the central nervous system. Most structures of the brain can be affected including the cerebellum.



3: Incubation of cerebellar granule cells with ethanol induces apoptosis and inhibits neuronal differentiation. Pituitary adenylate cyclase-activating polypeptide (PACAP) blocks the deleterious effects of ethanol *in vitro* but no data are available *in vivo*.



Control Ethanol Ethanol + PACAP

Methodology:

8-day-old Wistar rats



Intra-peritoneal ethanol injections



Sub-arachnoid PACAP injections

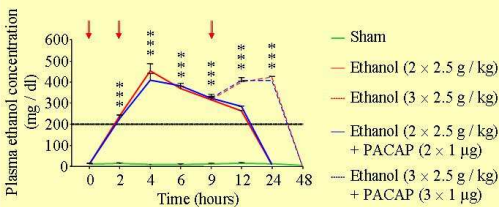
Sham

Ethanol

Ethanol + PACAP

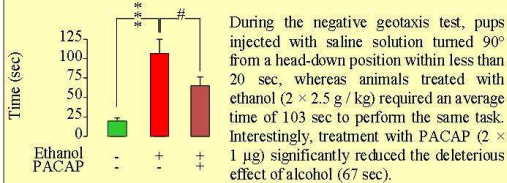
Removal of the cerebellum (enzymatic, transcriptomic, morphologic and behavioral studies)

1: Effect of PACAP on ethanol-induced plasma alcohol levels



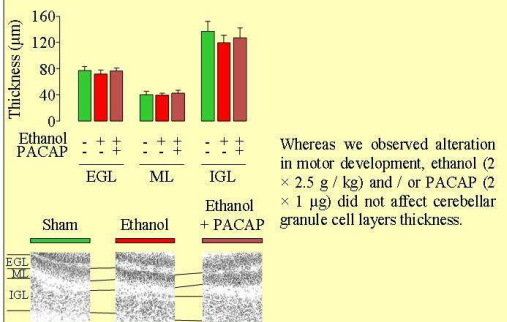
Ethanol injection (2 x 2.5 g/kg) to rats induced a strong increase of plasma alcohol concentration. The maximum induction was observed after 4 hours (453 mg/dl). The ethanol concentration could be maintained above a toxic level (Kononidou *et al.*, 2000) for more than 24 hours by a third injection 9 hours after the first administration. PACAP did not modify plasma alcohol levels.

2: Effects of ethanol and PACAP on pups behavior



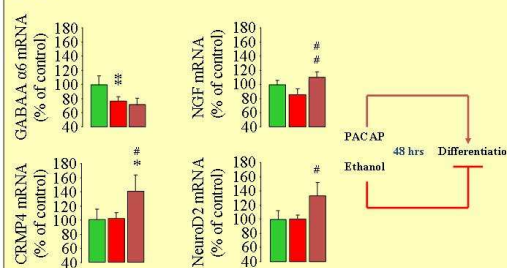
During the negative geotaxis test, pups injected with saline solution turned 90° from a head-down position within less than 20 sec, whereas animals treated with ethanol (2 x 2.5 g/kg) required an average time of 103 sec to perform the same task. Interestingly, treatment with PACAP (2 x 1 µg) significantly reduced the deleterious effect of alcohol (67 sec).

3: Effects of ethanol and PACAP on cerebellar cortex morphogenesis



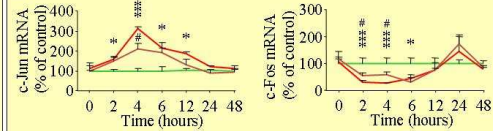
Whereas we observed alteration in motor development, ethanol (2 x 2.5 g/kg) and/or PACAP (2 x 1 µg) did not affect cerebellar granule cell layers thickness.

4: Effects of ethanol and PACAP on neuronal differentiation *in vivo*

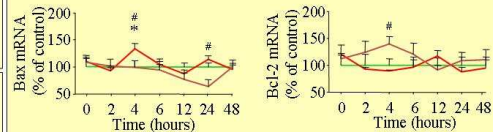


Treatment of rats with ethanol (2 x 2.5 g/kg) and/or PACAP (2 x 1 µg) resulted in differential regulation on genes known to be involved in neuronal differentiation. Ethanol seems to inhibit cell maturation whereas PACAP appears to promote cell neurogenesis.

5: Effects of ethanol and PACAP on neuronal apoptosis *in vivo*

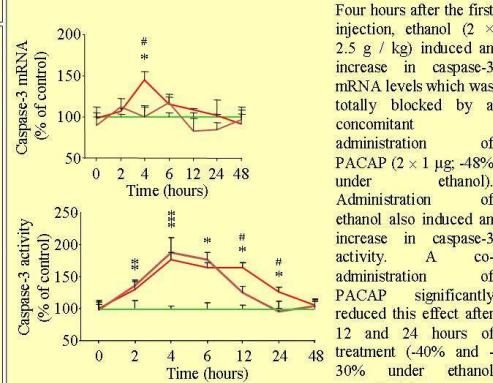


Ethanol (2 x 2.5 g/kg) injection induced a long lasting increase of c-jun mRNA expression with a maximum effect after 4 hours (+223% over control). The administration of PACAP (2 x 1 µg) reduced this effect by 38%. Ethanol also induced a rapid and strong inhibition of c-fos mRNA levels and this effect of ethanol was partially blocked by PACAP administration after 2 and 4 hours of treatment (+26% and +34% over ethanol).

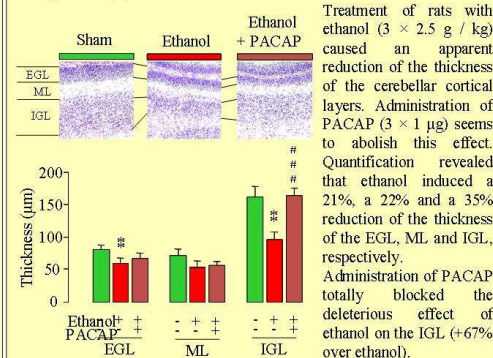


Ethanol (2 x 2.5 g/kg) increased bax mRNA expression after 4 hours (+36% over control). This induction was totally abolished by a concomitant administration of PACAP (2 x 1 µg). Only a co-administration of alcohol and PACAP significantly increased bcl-2 mRNA levels (+40% over control) after 4 hours of treatment.

6: Effects of PACAP on ethanol-induced caspase-3 activity

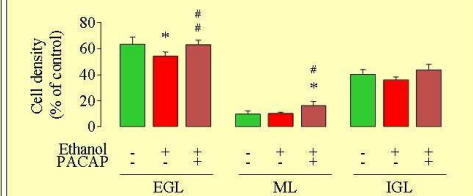


7: Effects of ethanol and PACAP on the cerebellar cortex histogenesis (1)



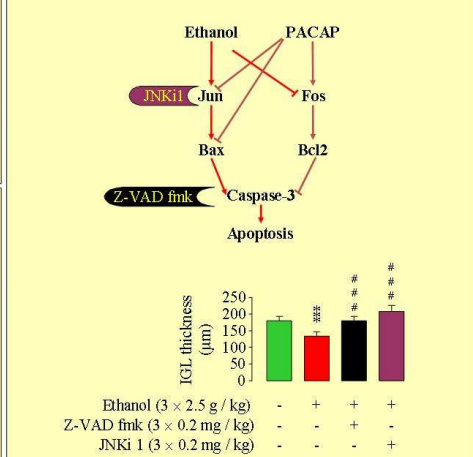
Treatment of rats with ethanol (3 x 2.5 g/kg) caused an apparent reduction of the thickness of the cerebellar cortical layers. Administration of PACAP (3 x 1 µg) seems to abolish this effect. Quantification revealed that ethanol induced a 21%, a 22% and a 35% reduction of the thickness of the EGL, ML and IGL, respectively. Administration of PACAP totally blocked the deleterious effect of ethanol on the IGL (+67% over ethanol).

7: Effects of ethanol and PACAP on cerebellar cortical histogenesis (2)



Ethanol (3 x 2.5 g/kg) induced a significant decrease in the number of cells in the EGL and this effect was blocked by PACAP (2 x 1 µg). PACAP also increased cell density in the ML.

8: Effects of inhibitors administration on ethanol-altered cerebellar cortical morphogenesis



Inhibition of jun and caspase-3 mimicked the protective effect of PACAP on ethanol-reduced IGL thickness, confirming the key role of those transduction pathways in the control of cerebellar neurons survival. Surprisingly, neither JNK1 nor Z-VAD-fmk had a protective action on ethanol-reduced cell number in the EGL.

Conclusion:

- 1/ PACAP blocks the neurotoxic effects of alcohol
- 2/ Mechanisms *in vivo* ≈ mechanisms *in vitro*

