# A2A ADENOSINE RECEPTOR AND CFOS/CJUN EXPRESSION IN BRAIN OF FREE-MOVING RATS SUBMITTED TO HYPERBARIC HYPEROXIA


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## INTRODUCTION

### HYPEROXIA

| HUMAN | | RAT |
|---|---|---|
| $PPO_2$ > 0.25 MPa | « Effet Paul Bert » OXYGEN NEUROTOXICITY | $PPO_2$ = 0.5-0.6 MPa |

**Normobaric oxygen (NBO) and /or Hyperbaric oxygen (HBO):**

↗ arterial $PO_2$
↗ $O_2$ delivery to tissues, to cells
… to treat clinical disorders

### CNS OXYGEN TOXICITY

HOWEVER, exposure to increased partial pressure of oxygen induced production of radical oxygen species (ROS) leading to an unbalance between antioxidative defense systems and ROS generation, called oxidative stress.

HBO has been demonstrated to induce DNA, lipid and protein damages, and produce also alterations in cellular function and neurotransmission.

### OXIDATIVE STRESS AND BRAIN DAMAGE

- ↗ $O_2$ delivery to cells, to mitochondria : ↗ ROS production (superoxide radical, $O_2^{\bullet-}$; hydrogen peroxide, $H_2O_2$)
- Swollen mitochondria: metabolism dysfunction
- Deformed nuclei : DNA damages leading to APOPTOSIS
- Lipid peroxidation : ↘ membrane permeability
- Protein damages: ion channels, receptors, transporters, enzymes …
- Alteration of enzyme activity : synthesis, storage, degradation of neurotransmitters …
- Abnormal accumulation of vesicles at nerve terminals : ↘ release of neurotransmitters

### HYPEROXIA-INDUCED EPILEPTIC SEIZURES

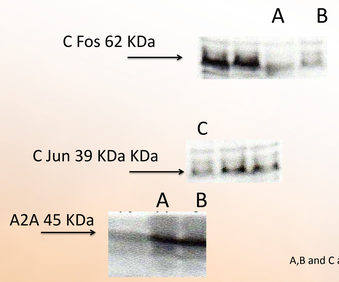
Epilepsy is a neurological disorder in which normal brain function is disrupted, as a consequence of intensive and synchroneous burst activity from neuron assemblies, inducing long-term change in brain.

There is a considerable evidence for alterations in glutamatergic and GABAergic neurotransmission in the origin of the paroxysmal depolarization shifts that initiate epileptic activity.

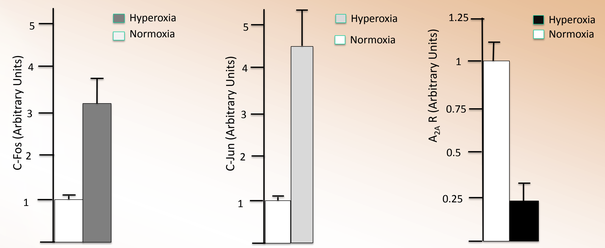
### AIM OF THIS STUDY

**c-Fos**, a cellular proto-oncogene upregulated by many extracellular signals including stress, dimerises with **C-jun** to form the AP-1 transcription factor, which upregulates transcription of a diverse range of genes involved in consequences to defence against invasion and cell damage. Upregulation of **c-fos** or **c-Jun** mRNA in a neuron indicates recent activity. Adenosine receptors ($A_{2A}R$) are implicated in cardiovascular system regulation, expressed in a brain stem area implicated in blood pressure control and in brain ganglia implicated in motor control regulation via the dopamine D2 receptors. Moreover Dipeptidyl peptidase-4 (**DPP IV** or CD26) is a cluster which is expressed at the lymphocyte cell membrane, leading to the clivage of peptides. It is also the protein carrier of adenosine deaminase, an enzyme implicated in adenosine metabolism and is implicated in stress response of lymphocytes
The aim of this study was to evaluate the influence of hyperbaric hyperoxia on **cFos**/**cJun** and $A_{2A}R$ expressions in rat brains and **DDP IV/CD26** activity.

## RESULTS

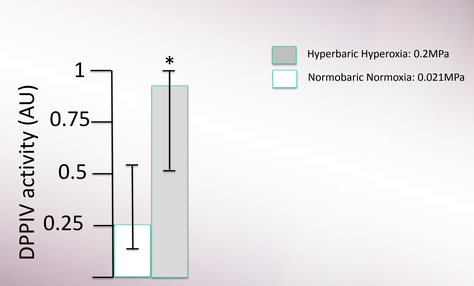



A,B and C are controls




| C-Fos | C-Jun | $A_{2A}$ |
|---|---|---|
| ↗ +320±70% | ↗ +450±89% | ↘ -76±14% |

Changes in hyperbaric hyperoxia compared to control.




Data are expressed as median and IQR. * p=0.012 compared with controls

DPP IV activity increased (more than two folds) in rats exposed to hyperbaric hyperoxia compared to controls. DPP IV is a sensitive marker of intracellular stress and red/ox status.

## MATERIALS AND METHODS

### ANIMAL MODELS

Male Sprague-Dawley rats (Weight 300g) were exposed in a pressure chamber for 4 hours at 2ATA of oxygen

Temperature: 25/26°C;

H20: 40-60%;

CO2: <300ppm).

### HBO Experimental Protocol

After habituation to experimental environment (1 week),

| EXPOSURE | EXPOSURE | EXPOSURE | EXPOSURE |
|---|---|---|---|
| 0.1 MPa 4 hours | 0.2 MPa 4 hours | 0.1 MPa 4 hours | 0.2MPa 4 hours |
| O2 : 0.021MPa | O2 : 0.021MPa | O2 : 0.1MPa | O2 : 0.2 MPa |

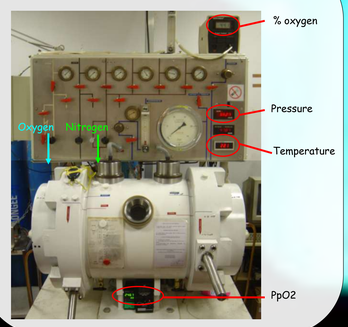
### Measurements of A2AP, c-FOS, c-Jun

After exposure, brains (n=6+6 controls) were taken and frozen at -70°C, quickly thawed at 37°C and solubilized with 4%SDS aqueous solution by 30min sonication at 47kHz. After protein quantification, 100mg of murine brain solubilisates were submitted to standard electrophoresis procedure. Separated proteins in 12%acrylamide minigel were electrotransfered onto a PVDF membrane. Blotted membrane was placed into the blot holder of the SNAP i.d. protein detection system, saturated with nonfat dried milk and incubated 20min with one of the appropriately diluted mouse monoclonal antibody, **anti-A2AR**, **anti-c-Fos** and **anti-c-Jun**. Blots were visualized by horse-radish peroxidase labeled anti-mouse IgG Fab specific antibodies and enhanced chemiluminescence substrate using a Kodak Image Station 440CF. The staining intensities of the bands were measured densitometrically using the public domain NIH Image software.

### DPP IV Activity

Cell samples were incubated with Gly-Pro-p-nitroanilide, a colorimetric substrate of DPP IV (3 mM, 30 min in glycine buffer, pH 8). Signal background was determined by incubation in acetate buffer, pH 5, a condition in which DPP IV is inactive. Optical density was measured at 405 nm. Results were expressed as arbitrary units (AU).

### HYPERBARIC CHAMBER




## DISCUSSION

The decrease in A2A R expression in brain may participate in motor control disturbances associated with hyperoxia and the change of dopaminergic activity recorded in hyperoxia. The precise consequence of the increase in cFos/cJun expression during hyperoxia needs further investigations.

CD26 is a cluster which is expressed at the lymphocyte cell membrane and possesses DPP IV activity, leading to the clivage of peptides with proline at the penultieme position on the N-terminus. CD26 is also the protein carrier of adenosine deaminase, an enzyme implicated in adenosine metabolism. CD26 is implicated also in stress response of lymphocytes. Here we observed a strong increase in CD26/DPPIV activity in response to hyperbaric hyperoxia.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