Evolution of mice brain metabolism after a convulsive dose of soman : a HRMAS 1H-NMR study

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Purpose: Soman is an irreversible organophosphorus inhibitor of cholinesterases with severe neurotoxic effects. It induces epileptic seizures that can last for several hours leading to brain oedema and neuronal lesions in different brain areas, mainly the piriform cortex and the hippocampus [1]. The nature and time-course of the soman-induced electrophysiological and histological disturbances are now well known. Conversely, a more limited amount of data is available on the associated brain metabolic disturbances. The purpose of this study was to analyse by HRMAS ^{1}H-NMR spectroscopy the brain metabolite concentration changes in the piriform cortex region (Pir) from the initial phase (1h) to 7 days after soman intoxication, in order to assess as comprehensively as possible the modifications occurring in the brain tissue. Cerebellum (Cb) was also analysed as a pseudo-control structure since lesions were never observed in this brain area of mice.
Method: Six mouse groups (n=7) were intoxicated with soman (172 µg/kg SC + HI-6 MNA) and sacrificed 1h, 4h, 24h, 48h, 72h and 7 days after. In control animals (n=18) soman was replaced by saline. Pir and Cb biopsies were immediately stored in liquid nitrogen after dissection.
The HRMAS ^{1}H-NMR experiments were performed on a Bruker DRX Avance spectrometer at 9.4Tesla, using a 4mm 1H-13C-31P probe head. Samples were spun at 4KHz and the temperature maintained at 4°C. A spin-echo sequence with a 30 ms total echo time was used.
The signals were processed using the jMRUI-sofware [2]. Quantitation was performed with "substract-QUEST" [3] in combination with a simulated metabolite data basis set. 23 metabolites were included in the basis: acetate (Ace), alanine (Ala), aspartate (Asp), creatine (Cr), choline (Cho), ethanolamine (Eth), γ-amino-butyric acid (GABA), glutamate (Glu), glutamine (Gln), glutathione (Gsh), glycerophosphocholine (GPC), glycine (Gly), hypotaurine (Hyp), lactate (Lac), myo-inositol (Myo), N-acetylaspartate (NAA), phosphoethanolamine (PE), phosphorylcholine (PC), scyllo-inositol (Syll), serine (Ser), taurine (Tau) and valine (Val).
The total spectrum intensity was used for normalization.
Results are presented as a percentage of control values, as mean +/-SEM.

Results and discussion:

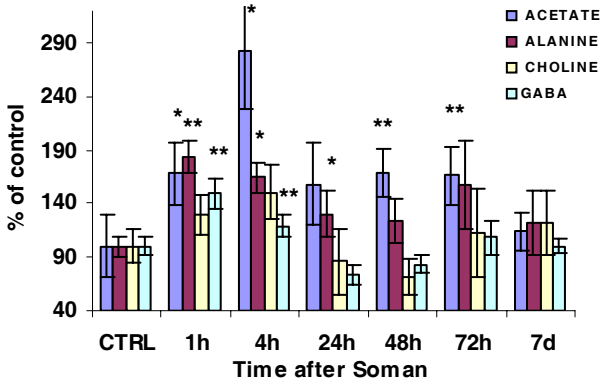

Figure1 : Time-course of concentration changes for the metabolites which exhibit the most important changes during the first hours in the Pir.

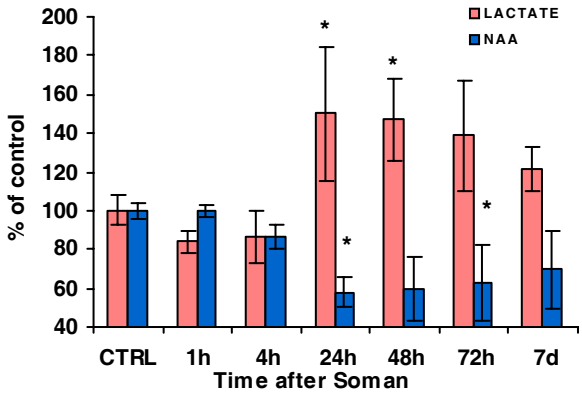

Figure2 : Time-course of concentration changes for the metabolites which exhibit the most important changes during the following days in the Pir.

- In the initial phase of intoxication (1h and 4h after soman injection), epileptic seizures have evolved into a severe status epilepticus. Ala and GABA in Pir reached their maximum level at 1h while Cho and Ace were maximum at 4h (fig 1). Surprisingly, an increase of PC (max at 1h) and GPC (max at 4h) was observed in Cb but not in Pir (not shown).
- 24h after soman injection, Seizures are not totally arrested but are now discontinuous. Histopathological lesions are maximum in Pir while no lesion can be detected in Cb. Pir biopsies were characterized by a strong increase of Lac and a strong decrease of NAA (fig 2), as classically observed in previous studies with neuronal loss or suffering [4]. Myo exhibited a significant decrease at 24h and 48h in Pir. Gln strongly increased not only in Pir but also in Cb.
- Seven days after intoxication only lactate and NAA in Pir remain at an abnormal level (Figure 2).

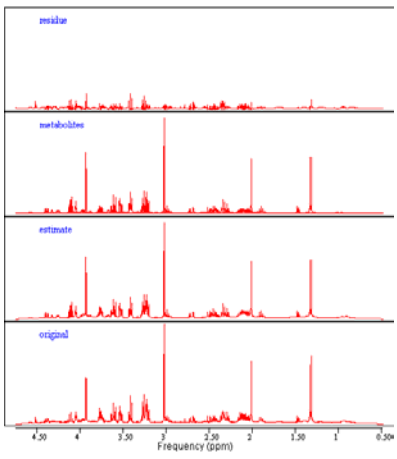

Figure 3 : Quest quantitation results. From bottom to top : original NMR spectrum in piriform cortex obtained from a mouse 4h after intoxication; estimated spectrum with background; metabolites spectrum and residue.

Conclusion

This study enabled the acute observation of metabolic disturbances at different time points after soman intoxication. Ala, GABA, Cho, Ace exhibit the most important changes during the initial status epilepticus while Lac, NAA and Myo levels were modified at a latter time point, when lesions are clearly visible histologically.
This work also shows that metabolism changes (GPC,PC, levels) could be observed in a brain area that does not appear to exhibit lesions in mice, viz. cerebellum.
A very good reproducibility of estimated concentrations in the control group (n=18) was obtained by using the 'substract'-QUEST procedure of jMRUI for quantitation. Only weakly represented metabolites (Asp, Eth, Hyp, Syll, Ser and Val) are not reliable. This algorithm is fast and efficient for quantitation of large series of highly resolved NMR spectra.

[1] G Lemercier et al., *Acta Neuropathologica* 61 (1983): 123-9.
[2] http://www.mrui.uab.es/mrui/.
[3] H. Ratiney et al, NMR in biomedicine, 18, (2005) 1.
[4] C. Cudalbu et al., Proc. ESMRM, (2006).