Effect of Manganese chloride on the neurochemical profile of the rat hypothalamus

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Introduction: Manganese-Enhanced MRI (MEMRI) studies of the neuronal pathways of the hypothalamus showed that information about the regulation of food intake and energy balance circulate through specific hypothalamic nuclei (1). The dehydration-induced anorexia (DIA) model (2) demonstrated to be appropriate for studying the hypothalamus with MEMRI (3). Manganese (Mn) is involved in the normal functioning of a variety of physiological processes, plays a role in the regulation of cellular energy and is associated with enzymes contributing to neurotransmitter synthesis and metabolism. However, it is also a neurotoxicant and induces psychiatric and motor disturbances (4). The molecular mechanisms by which Mn produces alterations of the hypothalamic physiological processes are not well understood. ^{1}H-MRS measurements of the rodent hypothalamus are challenging due to the distant location of the hypothalamus resulting in limited measurement sensitivity. After the characterization of the rat hypothalamic metabolic profile with proton MRS at 14.1T, the present study proposes to investigate the effects of Mn^{2+} on the neurochemical profile the hypothalamus in normal and DIA female rats.

Materials and Methods: 20 female Wistar rats (225-250g body weight (b.w) on Day 0 (D0)) were divided into 2 groups: the Control group (CTL, n=10) and the dehydration induced anorexia group (DIA, n=10). On D0, animals from the CTL group received water and food *ad libitum;* DIA received 2.5% NaCl (SIGMA, Zofingen, Switzerland) as drinking solution (2). On D3, rats from each group were anesthetised with 2% isoflurane in a mixture of O_2 and N_2O via a facemask. 5 rats per group received an i.v infusion of 100 mg/kg at 100mM $MnCl_2$ at a rate of 0.5ml-1ml/h via the tail vein to avoid cardiac arrest. The rats were positioned in a dedicated holder with head fixation. Their body temperature was maintained at 37.5±0.5ºC using a temperature-controlled water circulation and a rectal probe. Their respiration rate was monitored throughout the experiment. MRI and MRS were performed on D4 24 hours after $MnCl_2$ infusion. During the MR experiments, rats were anesthetized with 2% isoflurane in a mixture of O_2 and N_2O. All the experiments were performed on a 14.1T/26cm horizontal bore magnet (Magnex Scientific, Oxford, UK). The magnet was equipped with 12-cm inner-diameter actively shielded gradient sets (Magnex Scientific, Oxford, UK) allowing a maximum gradient of 400mT/m in 120µs. A home-built quadrature T/R 17-mm surface coil was used. Localized proton spectroscopy was done using SPECIAL (5). Voxels of interest (VOI) of 24 µl were localized in the Paraventricular Nuclei (PVN) region and the hippocampus in for all the rats using previously acquired 3D-GRE images (Fig1A-B). All 1st and 2nd order shims were re-adjusted using FASTMAP (6) in the VOI which resulted in water linewidths of 23 ± 5 Hz. To obtain adequate signal to noise ratio (SNR), 640 scans were acquired. For absolute quantification, water signal was used as a reference. The *in -vivo* ^{1}H MR spectra were processed using LCmodel (7) combined with a simulated basis set. All data are presented as mean ± standard error of the mean (S.E.M). Unpaired Student t-tests were applied to compare data acquired in the three groups of rats.

Results: In the present study, despite challenges due to the depth of the hypothalamus in the rat, 11 metabolites were reliably measured with Cramer-Rao lower bounds under 20% (Fig1C). The neurochemical profile of the normal rat hypothalamus was characterized by lower PCr (-46%), Glu (-53%), Tau (-77%), NAA (-48%) and GABA (-65.7%) concentrations relative to the normal hippocampus (Fig1C &Fig2). In DIA rats without $MnCl_2$, most metabolite concentrations increased relative to the CTL condition: ΔTau=+106% (CTL vs DIA, p<0.05); ΔGln=+89% (CTL vs DIA, p=0.01); Δmyo-Ins=+92%(CTL vs DIA, p=0.008); ΔGABA=+160% (CTL vs DIA, p=0.0007) (Fig3A).

24 hours post-infusion of $MnCl_2$ in the rat tail vein, CTL rats demonstrated a significant increase in GABA concentrations (unpaired t-test, p=0.01) compared to CTL rats without $MnCl_2$ infusion. Moreover, $MnCl_2$-infused DIA rats had a significantly lower Tau level (unpaired t-test, p=0.013) compared to non-$MnCl_2$ –infused DIA rats. However, no significant differences were detected between CTL and DIA rats in the presence of $MnCl_2$ (Fig3B). In particular the relative difference between CTL(+$MnCl_2$) and DIA(+$MnCl_2$) was only 3.3%.

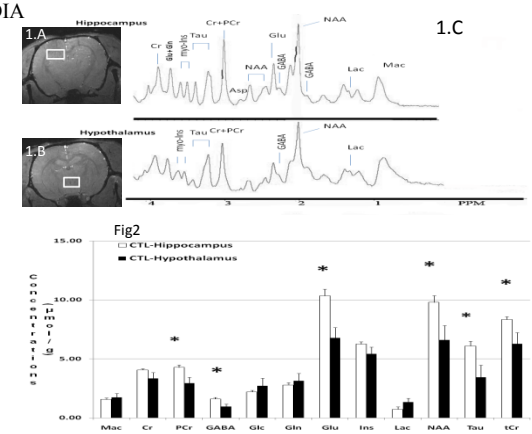


Fig3.A

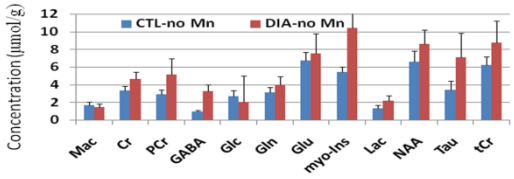
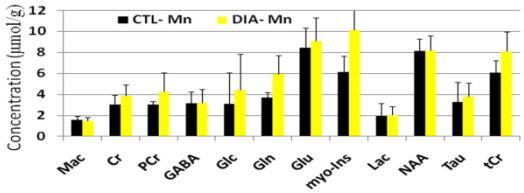


Fig3.B



Discussion: In CTL rats, regional metabolic differences between hypothalamus and hippocampus were in agreement with a previous study in mice (8). Lower hypothalamic GABA levels relative to the hippocampus are attributed to the fact that the VOI was positioned at the level of the PVN which does not contain GABAergic neurons.

However, GABA levels are significantly increased in CTL(+$MnCl_2$). An earlier study showed that $MnCl_2$ stimulates the release of luteinizing hormone releasing hormone (LHRH) from the hypothalamus but simultaneously increases GABA release that in turn inhibits LHRH release (9). This process may ultimately contribute to suppressed reproductive function in female rats (9). These results confirm the important inhibitory role played by GABAergic projections from the lateral hypothalamus (LH) in the regulation of the hypothalamic-pituitary-adrenocortical axis (HPA) (10) and especially at the level of the PVN.

Taurine has neuroprotective properties and acts as an osmoregulator (11). Increases of Taurine due to hyperosmotic conditions were previously observed (11). In the presence of $MnCl_2$, taurine levels were also either significantly decreased in (12) or not significantly changed after 18 hours of exposure in (13). In the present work, $MnCl_2$ appears to mask the effects of dehydration-induced anorexia on most metabolites. Although, the metabolic hypothalamic processes involved in anorexic and $MnCl_2$ challenges are not yet understood, a potential explanation could be found in the regulation, at the level of the PVN, of CRH and TRH hormones (2) that in turn may influence voltage –gated Ca^{2+} channels.

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Figure1: A-B: 3D $T1_w$ images (TR/TE=20/5ms, Flip angle=70º, FOV=25x25x25mm^3, matrix size=256x256x128, coronal slices, BW=25 KHz, 5 averages) with corresponding VOI in the hippocampus and hypothalamus and C. corresponding 14.1T spectra with metabolite assignments. **Figure2.** Comparison of neurochemical profiles in the hippocampus and hypothalamus of CTL rats. * represents significance level. **Figure3.** A. Comparison between CTL and DIA metabolites without $MnCl_2$. B. Comparison between CTL and DIA metabolites in the presence of $MnCl_2$.