Chronic stress hormone treatment reduces glutamine levels in the hippocampus - an in vivo MR spectroscopy study in rats at 7T.

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Introduction

Both stress and glucocorticoids (GC) were shown to increase glutamate levels and to activate glutamatergic pathways in the rat hippocampus (1-7). These findings led to the hypothesis that stress-related hippocampal damage is mediated by glutamate excitotoxicity (8, 9). In favor of this hypothesis, we found increased glutamate in the hippocampus of adrenalectomized rats treated with the exogenous glucocorticoid dexamethasone (10). It is, however, unclear in what way adrenalectomy or the stress-level exogenous steroid may have affected that result. To address this, in this study we aimed to investigate the metabolic effects of corticosterone, the endogenous glucocorticoid in rodents, on normal rats. We hypothesized that this model would more truly reflect metabolic alterations due to GC-mediated glutamate excitotoxicity.

Material and Methods

Adult male Wistar rats (n=30, Charles River, UK) were either treated with corticosterone (CORT, n=17, 218±8 g body weight; 400 µg/mL in <2% ethanol; C2505, Sigma-Aldrich, UK), or with vehicle (VEH, n=13, 217±11 g body weight; <2% ethanol) in the drinking water. Six to 9 weeks later rats underwent *in vivo* ^{1}H-MRS (7T Bruker Biospec, Germany). The amount of drinking water was monitored, and the body weight (BW) measured weekly. Plasma corticosterone levels were explored by EIA (IDS Ltd., UK). Postmortem, the thymus-to-body weight ratio (THY/BW) was determined. For ^{1}H MRS of right (RHV) and left hippocampus (LHV), a PRESS sequence (TR=5000 ms, TE=8 ms, number of points=2048, SW=4006 Hz, voxel size 3x2x2mm^{3}) was used. Metabolite concentrations were estimated using LCModel (S.W. Provencher) and metabolite ratios were calculated by dividing the metabolite of interest by the sum of estimated metabolites (Ins, NAA+NAAG, tCr, tCho, Glu+Gln). The fitting quality was found to be good to excellent for most metabolites (CRLB<13%) and acceptable for glutamine (CRLB<22%; Fig. 1). One VEH data set of the left hippocampus had to be excluded due to CRLB >30%. For group comparison, univariate ANOVA was done for plasma CORT, BW, THY/BW, and glutamate/glutamine ratio, and a MANOVA for ^{1}H-MRS data (NAA, mIns, tCr, tCho, glutamate, glutamine). Two-tailed Spearman's rank correlation was used to investigate for significant associations between metabolites and plasma CORT levels.

Results

Alterations of the corticosteroid milieu were reflected in reduced BW ($F(1,28)=70.729$, $p<0.001$), THY/BW ($F(1,28)=8.568$, $p<0.01$), and plasma CORT levels ($F(1,28)=114.226$, $p<0.001$) in CORT compared to VEH rats. MANOVA revealed a group difference in metabolic profile in the left hippocampus ($F(6,22)=6.561$, $p<0.008$) with lower Gln ($F(1,27)=15.722$, $p<0.001$), and higher NAA ($F(1,27)=8.138$, $p=0.008$) and tCr ($F(1,27)=4.743$, $p=0.038$) in rats treated with CORT in comparison with VEH rats. For the metabolites of the right hippocampus, there was a similar group effect ($F(6,23)=3.333$, $p<0.016$) due to lower Gln ($F(1,28)=5.563$, $p=0.026$) in rats treated with CORT in comparison with VEH rats. No significant group differences emerged from hippocampal glutamate. The Glu/Gln ratio was higher in CORT compared to VEH rats, both in the left ($F(1,27)=9.902$, $p=0.004$) and the right ($F(1,28)=6.590$, $p=0.016$) hippocampus. Bilateral hippocampal Gln ratios were positively correlated with plasma CORT levels ($r=0.589$, $p=0.001$; $r=0.552$, $p=0.002$; Spearman's rank correlation).

Discussion

We showed that chronic treatment of rats with high dose of endogenous stress hormone (i) reduces Gln, and (ii) increases the Glu/Gln ratio. Unexpectedly, no effect was seen for glutamate. As in vivo MRS largely reflects intracellular metabolites, an extracellular Glu increase may have remained unnoticed. Considering this, our findings accord with a microdialysis study in rats showing that intrahippocampal GC infusion led to a decrease in extracellular Gln, and an increase in Glu and Glu/Gln ratio (11). Glutamine, a precursor for glutamate, is predominantly located in glia cells (12), on which MR and GR receptors are expressed (13), possibly rendering astrocytes vulnerable to chronic elevations of GCs. Furthermore, GCs were shown to regulate the glutamine synthetase (14, 15), but not the glutaminase (16). Both glutamine synthetase (Glu-Gln conversion) and glutaminase (Gln-Glu conversion) are essential enzymes of the Glu-Gln cycle that helps maintain the supply of Glu and reduce Glu excitotoxicity. Our findings would be in line with a GC-induced impairment of this energy-dependent process either via reduced glial Glu uptake or reduced Glu-Gln conversion. This is in line with a previous report showing that GCs reduce glial Glu uptake (17). Interestingly, a decreased hippocampal Gln/Cr ratio was also found in patients with major depression, a psychiatric disorder characterized by high cortisol levels (18).

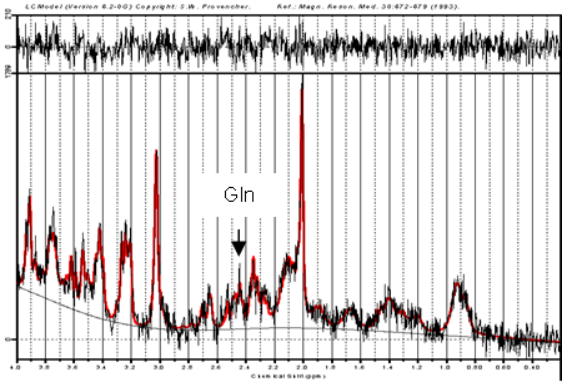

Fig. 1. Example for fitting quality (Gln (arrow), CRLB=9%).

Conclusion

Endogenous GCs at stress level were shown to induce a metabolic profile consistent with impaired Glu-Gln cycle, thus supporting the hypothesis that GCs induce hippocampal damage by glutamate excitotoxicity. Furthermore, the data suggest impaired Glu uptake and/or Glu-Gln conversion as main mechanism.

References

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