

In vivo MR assessment of hippocampal volume and neurochemical changes in rats with altered corticosteroid milieu

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Introduction

Hippocampal pathology seems to be a hallmark of various mental diseases. Over the last decade, high-resolution MRI enabled ready assessment of such volume changes in extensive patient databases. These studies together with histological evidence of glucocorticoid-induced neurotoxicity in rats have led to the hypothesis that hippocampal volume loss in major depression may result from elevated levels of glucocorticoids (1-6), but direct evidence is lacking. To further characterize the nature of these hippocampal volume changes, this combined ultrahigh-field MRI and MRS study was performed in rats with long-term alterations of the corticosteroid milieu.

Animals were pretreated in analogy to previous histological studies (7, 8) to (i) analyze whether *in vivo* MR-morphometry is sensitive to known neurodegenerative, neurotoxic and neuroprotective effects of adrenalectomy, glucocorticoid and mineralocorticoid substitution respectively (9, 10), and to (ii) characterize and possibly distinguish associated neurochemical alterations by proton MRS.

Material and Methods

Male Wistar rats were studied at 4.5 months; 3/4 groups were adrenalectomized (ADX) at 2 months and subsequently treated as follows: controls (CON, n= 5), saline for 10 weeks (ADX, n=5), corticosterone 7.5 µg/ml drinking water (CORT, n=6) and dexamethasone 0.313 µg/ml drinking water (DEX, n=7).

MRI Protocol and Animal Preparation

Rats were anesthetized with isoflurane and mechanically ventilated, monitored continuously and kept warm by a heated water pad. Triplanar pilot images were used for reproducible orientation of 20 coronal images (TR=4000 ms, TE=19.4 ms, RARE factor 4, six averages, resolution: 0.068x0.068x0.75 mm³, 39 min 19 sec scan duration). ¹H MRS of the left hippocampus was acquired using a PRESS sequence (TR=5000 ms, TE=17 ms, NA=256, voxel size 3x2x2mm³, 21 min 20 sec scan duration). After the MR experiment animals were decapitated for histology.

Data Analysis

Both hippocampi were manually outlined on six consecutive slices using the manufacturer's software (FUNtool, Bruker) (11). Volumes were calculated for both sides and normalized to the total intracranial volumes (TICV). Left hippocampal volumes were correlated to stereological histology-based volume calculation. Metabolite concentrations were estimated using LCModel (S.W. Provencher) and normalized to tissue water. For group comparison, multivariate analysis of variance was done. All values are given as means +/-SD.

Results

Morphometry: Normalized hippocampal volumes (nHV) and TICV differed between groups (Wilks, p=0.003) mainly due to altered TICV and right nHV. LSD post hoc testing revealed that the right nHV was significantly reduced in the unsubstituted (ADX) and dexamethasone (DEX) substituted adrenalectomized rats compared to control rats. Corticosterone substituted rats (CORT) had significantly larger right nHV compared with ADX alone. All groups showed consistently larger right than left nHV (p<0.001). Left hippocampal volumes obtained from MR analysis correlated well with those from histological volume analysis (Pearson's correlation coefficient, r=0.67 to r=0.83).

Proton spectroscopy: The groups also differed in the metabolic profiles (N-acetyl-aspartate [NAA], total creatine [Cr], myo-inositol [Ino], glucose [Glc], glutamate [Glu] and taurine [Tau] as dependent variables (Wilks, p<0.01)), mainly due to significant changes in Glu, Glc and Ino and marginal effects from Tau. Post hoc LSD testing demonstrated significant differences between DEX and all other groups, namely elevated Glu and reduced Glc.

Conclusion

This multimodal MR study proved useful to longitudinally and non-invasively explore structural and biochemical effects of altered corticosteroid milieu, namely ADX-related neurodegeneration as well as the neuroprotective effect of low-dose corticoid substitution. The failure to demonstrate further volume loss in the dexamethasone treated group may be due to limited sample size or possibly indicates the need to separately analyze hippocampal subfields. Proton spectroscopy, on the other hand, only showed metabolic changes in the DEX group with elevated glutamate and reduced glucose concentrations, well in line with steroid-induced excitotoxicity.

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