The effects of prolonged dexamethasone treatment and single MRI procedure under anesthesia on hippocampal and prefrontal cortex volume in rats

M. I. Schubert[1,2], R. Kalisch[1], A. Wigger[3], R. Hemauer[1], O. F. Almeida[4], R. Landgraf[3], D. P. Auer[1,2]

[1]NMR Research Group, Max Planck Institute of Psychiatry, Munich, Bavaria, Germany, [2]Division of Academic Radiology, University of Nottingham, Nottingham, Nottinghamshire, United Kingdom, [3]Behavioral Neuroendocrinology Group, Max Planck Institute of Psychiatry, Munich, Bavaria, Germany, [4]Neuroadaptations Group, Max Planck Institute of Psychiatry, Munich, Bavaria, Germany

Introduction

Imbalances in the corticosteroid milieu result in hippocampal volume (HV) reductions in humans (1-3) and experimental rodents (4-6). In previous MRI studies in adrenalectomized rats, we showed a volume loss in the left anterior cingulate cortex (ACC), but not in the hippocampus in dexamethasone (DEX) treated rats (4, 7). Adrenalectomy is an interesting model of Addison's disease, but does not allow to infer possible DEX effects in healthy persons or patients with psychiatric diseases. To investigate the effects of prolonged supraphysiologic DEX treatment on the limbic system, we therefore chose to explore the DEX challenge in non-adrenalectomized rats. We hypothesized that DEX may alter the volume of the hippocampus, ACC or retrosplenial cortex (RSG) as structures involved in the control of the hypothalamo-pituitary-adrenal axis activity (8).

Material and Methods

Anesthetized and mechanically ventilated male adult Wistar rats (DEX, n=8, 364±37 g body weight) were examined by *in vivo* MRI (7T Bruker Biospec, Germany; 30 coronal slices, TR=4000 ms, TE=19.4 ms, RARE factor 4, six averages, resolution: 0.068x0.068x0.75 mm^3, 39 min 19 sec) before and six weeks after continuous substitution of DEX (0.313 µg/ml drinking water). In contrast, control rats (CON, n=8, 339±47 g) received saline. Both ACC (from approx. 1.6 to -1.4 mm from Bregma), RSG (from approx. -2.12 to -5.3 mm from Bregma) and hippocampi (from approx. -2.12 to -7.04 mm from Bregma) were manually outlined using the manufacturer's software (9, 10, FUNtool, Bruker). The subtotal brain volume (BV) was delineated on 14 to 16 consecutive slices (from approx. 4.20 mm to –8.8 mm from Bregma) excluding the olfactory bulb and the cerebellum. Volumes were calculated by multiplying the area with interslice distance and normalized to BV and HV. MANOVA with repeated measure design of normalized right and left HV, and right and left ACC, right and left RSG, each normalized to BV and HV, and BV was done. Significance was considered if $p < 0.05$.

Results

There was an overall significant time effect ($F(14,1)=465.864$, $p<0.05$) with significantly reduced volumes of the normalized right and left ACC and RSG ($p<0.05$), and increased BV ($p<0.05$), but no overall significant time by treatment effect. Univariate testing, however, revealed significantly more reduced normalized right and left ACC in CON vs. DEX rats ($p<0.05$), and a similar trend for normalized right RSG. There was neither a significant time nor time by treatment effect on HV. However, normalized right HV tended to decrease in CON whereas in DEX it tended to increase ($p=0.08$). Intrarater variability as measured in CON rats ranged from $r=0.90$ to $r=0.99$ ($p<0.01$, Pearson) at both points of time. Interrater variability for CON and DEX was 0.77 and 0.72 for RHV and LHV ($p<0.01$, Pearson, first time point).

Conclusion

This study did not show any HV loss upon six weeks DEX treatment in non-adrenalectomized rats. Thus, the prevailing assumption of hippocampal pathology induced by chronic elevated glucocorticoids could not be verified in this experimental design. While the study design proved useful to noninvasively monitor ACC, RSG and HV in rats, the deployed technique is still limited to the overall HV, such that possible changes in hippocampal subfields may go unnoticed. Nevertheless, previous claims of glucocorticoids to induce HV loss were based on total hippocampal measurements, and in fact were only directly proven by a therapeutic intervention study in Cushing's disease (11). Thus, an even longer exposure to even higher stress hormone levels may be required to induce total HV volume loss. The observed volume losses in the ACC and RSG in the follow-up scans of both untreated and DEX treated animals are noteworthy for three reasons: (i) to alert to the possible brain plasticity occurring even after a single highly stressful event (12), (ii) because of the assumed regional specificity preferentially affecting prefrontal cortex and not the hippocampus (7), and (iii) because of a potentially modifying or indeed protecting effect of prolonged DEX treatment which needs further elucidation (13).

References

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