Network: Systematic Gene Identification and Functional Analyses in Common CNS Disorders

Project: The Role of GR Pathway in Alcohol Drinking, Stress-induced Alcohol Drinking and Relapse

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Introduction

Corticosteroids are able to activate two closely related nuclear receptors, the glucocorticoid (GR) and the mineralocorticoid (MR) receptor. In the absence of their ligands these receptor molecules reside in the cytoplasm. Upon binding of the ligand the receptor-ligand complex migrates into the nucleus where it binds to specific glucocorticoid and mineralocorticoid receptor sequences. Upon binding to the response element the associated gene is activated at the transcriptional level. In response to various stimuli, i.e. stress, corticosteroids coordinate metabolic, endocrine, immune and nervous system responses and ensure an adequate profile of transcription. The mineralocorticoid receptor is expressed in the central nervous system at sites similar to the glucocorticoid receptor, e.g. the hippocampus, but is not strongly expressed in regions important for addiction. The glucocorticoid receptor is functioning in the brain reward pathway and this predicts its expression in the N. accumbens in the ventral striatum and the medial prefortal cortex. The receptor is also strongly expressed in the ventral tegmental area (VTA) leading to release of dopamine in the nucleus accumbens.

To better understand the function of these two receptors important in the nervous system, including the addictive process, we have generated mice with a floxed allele of the glucocorticoid and mineralocorticoid receptor [1, 2]. Loss of the mineralocorticoid receptor in the forebrain by expressing the Cre recombinase under the CaMKIIα promoter show impaired learning of the watermaze tasks and deficits in working memory as evidenced from the radial maze. This genetic model provides important information about the consequences of altered balance between the mineralocorticoid receptor and the glucocorticoid receptor. The mineralocorticoid receptor is not strongly expressed in areas considered important for the addictive process. In contrast, the glucocorticoid receptor is strongly expressed in neurons of the ventral tegmental area (VTA) as well as in the N. accumbens. Inactivation of the glucocorticoid receptor in the forebrain with a strategy similar to the inactivation strategy of the mineralocorticoid receptor gene leads to early lethality. This is caused by the dramatic increase in the level of glucocorticoid hormones (up to 1000 fold) which explains this early lethality. To define the role of glucocorticoid receptor function in dopaminergic neurons and in the N. accumbens, it was important to generate new GR mutant mice. Two different strategies have been used for expression of the recombinase in the forebrain and the N. accumbens.

Results/Project Status

To avoid lethality of mice with loss of GR in the forebrain, we have attempted to inactivate the GR gene in an inducible fashion. We therefore generated mice which express a Cre recombinase fusion gene with the ligand binding domain of a modified estradiol receptor. This should generate a fusion protein which becomes active only once the ligand tamoxifen has bound to it. To establish this methodology this fusion protein was expressed via a bacterial artificial chromosome (BAC) containing the CamKIIα gene. Mice with different copy numbers, 1, 2 and 4 copies, were generated. To test the efficiency of these transgenic mice they were crossed with mice containing a floxed indicator gene, the β-galactosidase gene. We could demonstrate that without induction of the fusion protein by tamoxifen little recombination took place. Upon treatment with tamoxifen recombination occurred and the extent of recombination could be quantified. Since previously inducible recombination in the central nervous system has not been achieved, we had to find optimal conditions for its execution. Dependent on the copy number, complete removal of the glucocorticoid receptor could be achieved. Using mice with different copy numbers of the transgene the extent of recombination could be chosen for different regions within the forebrain depending on the level of the Cre fusion protein.

In order to inactivate the glucocorticoid receptor gene in the N. accumbens a D1 receptor Cre line was used [3]. In these experiments we could show that Cre under expression of the D1 dopamine receptor gene leads to inactivation of the CREB gene in the striatum including the N. accumbens. To also inactivate the glucocorticoid receptor in a temporal-dependent manner we generate mice now in which the Cre recombinase fusion protein with the ligand binding domain of the estradiol receptor is expressed from a bacterial artificial chromosome containing the D1 promoter. Previous experiments using a yeast artificial chromosome (YAC)-derived DNA segment had indicated expression of the gene in D1 receptor-positive neurons. Assuming that this specificity will also be maintained for expression of the Cre fusion gene we should be able to develop mouse mutants which have lost the glucocorticoid receptor gene only in adults after inducing Cre activity with tamoxifen.

Outlook

These mice with specific mutations in the glucocorticoid receptor in the forebrain region as well as the striatum will be used for analysis of altered behaviour following drug treatment. These studies will be done in collaboration with R. Spanagel´s group at the Central Institute for Mental Health, Mannheim. In order to define genes which are controlled by the glucocorticoid receptor and are of importance in alcohol drinking, stress-induced alcohol drinking and relapse, mRNA expression analysis will be performed after these treatments. We use the Affymetrix technology as previously used for the characterization of CREB mutants in the forebrain region and in the striatum (T. Lemberger, to be submitted to Nat. Neurosci.). Comparing the level of expression of glucocorticoid-dependent genes in wildtype and mutant mice will allow to characterize GR function in the addictive state. The overall goal will be to define genes which are altered in their expression following drug treatment. The importance of these changes for the addictive states will then be followed by RNAi-dependent inactivation of these genes in neurons in culture as well as in the animal.

*Lit.: **1.** Tronche F et al. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nat Genet. 1999 Sep; 23(1):99-103. **2.** Berger S et al. Loss of the limbic mineralocorticoid receptor impairs behavioral plasticity. Proc. Natl. Acad. Sci. USA. 2005; (in press). **3.** Mantamadiotis T et al. Disruption of CREB function in brain leads to neurodegeneration. Nat. Genet. 2002 May;31(1):47-54.*