

## NRP Endocrine Disruptors

### Final Summary

Original project title <b>Disruption of Glucocorticoid- and Mineralocorticoid Receptor-Mediated Responses by Environmental Chemicals</b>
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### Disruption of Corticosteroid Action by Environmental Chemicals

*The corticosteroid hormones cortisol and aldosterone modulate many physiological processes, including energy metabolism, cell growth and differentiation, maintenance of blood pressure and immune responses. The potential interference of environmentally relevant pollutants with corticosteroid action has not been well studied. In this project, we identified environmentally relevant chemicals that disturb the function of the receptors and metabolizing enzymes of corticosteroid hormones.*

#### Research questions

Are there widely used industrial chemicals and compounds in natural products that interfere with the activation of mineralocorticoid receptors (MR), glucocorticoid receptors (GR), 11beta-hydroxysteroid dehydrogenase (11bHSD) type 1 or 11bHSD2?

What are the molecular mechanisms by which such compounds alter the function of these proteins?

Can we develop suitable tools to predict and rapidly analyse chemicals interfering with the function of these proteins?

#### Results

We identified two classes of industrial chemicals that block the local inactivation of glucocorticoids by 11b-HSD2. The inhibition of 11b-HSD2 by dithiocarbamates was irreversible, whereas that by organotin compounds was reversible. The reducing dithiol reagent dithiothreitol protected 11b-HSD2 from inactivation by both classes of chemicals. The endogenous reducing monothiol glutathione

was able to prevent dithiocarbamate-induced inhibition but not that by organotins, indicating different inhibitory mechanisms of these two chemicals. Mutational analysis suggested interference of these chemicals with the function of residues Cys-90, Cys-228 and Cys-264. The glucocorticoid activating enzyme 11b-HSD1 was not inhibited by these chemicals.

We identified flavanone and some hydroxylated derivatives, but not flavones, as selective inhibitors of 11b-HSD1. We also found some triterpenoids that inhibited 11b-HSD1 but not 11b-HSD2 or 17b-HSD1 and 17b-HSD2. These compounds may contribute to the beneficial anti-glucocorticoid effects of the regular consumption of fruits and vegetable.

We described a novel function of 11b-HSD1 in the metabolism of 7-ketocholesterol, the major oxidation product upon processing cholesterol-rich food. We demonstrated the accumulation of orally administered 7-ketocholesterol in rats treated with the inhibitor carbenoxolone. Moreover, recent results show that 11b-HSD1 metabolizes several 7-ketosteroids, suggesting a role in the detoxification of oxidation products.

Our most recent studies demonstrate that the organotin dibutyltin, but not tributyltin or phenyltins, blocks GR function and, to a lesser extent, MR function. Important, a reduced glucocorticoid-dependent suppression of the production of inflammatory cytokines was found in macrophage exposed to dibutyltin. This might explain some of the immunotoxic effects of this organotin.

Structure-based homology models of MR, GR and 11b-HSDs, constructed by Dr. M. Baker in San Diego, were applied to study the binding of identified chemicals. Moreover, a pharmacophore model of 11b-HSD1 was constructed by Dr. T. Langer in Innsbruck and applied in a screen of a large in silico chemical library. Hits are currently screened by newly developed cells stably expressing 11b-HSDs or 17b-HSDs.

## Perspectives

After the initial proof of concept that environmentally relevant chemicals exist that disrupt corticosteroid hormone-mediated function, we aim at applying structure-based pharmacophore models to screen a library of chemicals with reported endocrine effects. The hits will be subjected to cell-based tests and the most critical compounds will be further investigated in animals.

An ongoing study in rats addresses the relevance of GR blockade by dibutyltin for its toxicity. In addition to the assessment of glucocorticoid disruption, the toxicity of dibutyltin will be investigated by a proteomics approach in cells treated with this organotin.

The present project emphasized on the fact that many chemicals disrupt endocrine function not directly by acting on the hormone receptors but rather by altering the function of hormone metabolizing enzymes. Based on this observation, cell-based bioassays for several key hormone metabolizing enzymes, including 17b-HSDs, will be established and applied in future experiments. The combination of predictive in silico screening tools and cell-based bioassays should allow to rapidly identify chemicals interfering with endocrine function. Numerous diseases such as hypertension and heart failure, or osteoporosis, weight gain, diabetes and cataracts have been associated with disturbed mineralo- and glucocorticoid-mediated responses. The resulting abnormal MR- and GR-dependent gene regulation induces functional disturbances with enormous socioeconomic consequences. The contribution of environmental chemicals influencing these diseases is largely unknown. Therefore, the identification and characterization of chemicals interfering with corticosteroid-mediated responses is of pivotal importance.