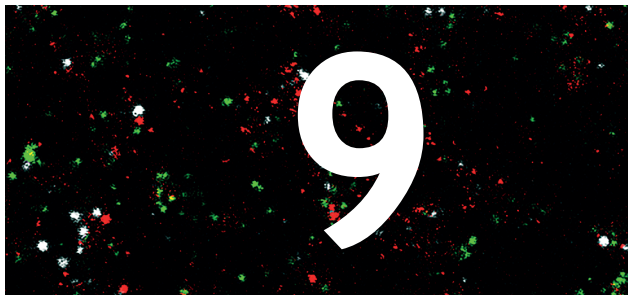
Stress, obesity and mood disorders: towards breaking a vicious cycle

Koorneef, L.L.



9

SUMMARY

Stress, obesity and stress-related mood disorders such as depression and anxiety disorders are highly prevalent in modern society. These diseases trigger and reinforce each other and, therefore, form a vicious cycle in which diseases often coincide. Although social and psychological factors play a role in this cycle, in this thesis we focused on the underlying biological mechanisms. In **chapter 1** we introduce the general principles of stress, metabolism and pharmacology. Central to this thesis is the hypothalamus-pituitary-adrenal (HPA)-axis, which regulates the release of the glucocorticoid stress hormones cortisol and corticosterone. These hormones exert their effects by binding to the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Each receptor has slightly different properties and controls different aspects of the stress response, typically by affecting gene transcription. In this thesis, we investigated how obesity may cause fear and applied novel treatment strategies that target glucocorticoid signalling to reduce obesity and its associated complications.

Glucocorticoid hormones and metabolic state both influence fear behaviour. We reasoned that hunger makes 'brave' to promote exploratory, food-seeking behaviour while satiety makes 'fearful' to prevent unnecessary risk-taking behaviour. In **chapter 2** we set out to identify which of the metabolic factors that change upon a different metabolic state may cause fear, including nutrients, hormones and components of the feeding-regulation circuitry in the brain. The potential of a metabolic factor to influence fear was assessed by its ability to enter the brain and to affect fear-related brain regions, which requires the corresponding receptor to be expressed in these regions. Fear-related brain regions include the central fear circuitry that consists of the amygdala, hippocampus, prefrontal cortex as well as regions that produce the neurotransmitters serotonin, noradrenalin and dopamine. Receptor expression was analysed using the publicly available Allen Brain Atlas database. When receptor expression was higher in fear-related brain regions than in rest of the brain, the corresponding metabolic factor was selected as a potential fear-regulating candidate. We found various metabolic receptors that were 'enriched' in these brain regions. While some of the identified metabolic factors were already known to regulate fear (e.g. glucocorticoids), we also identified novel metabolic factors that had never been associated with fear, such as glucagon and adrenocorticotrophic hormone. In addition, we identified putative sites of action for known fear-regulating metabolic factors, such as thyroid hormones action in the prefrontal cortex region. The results from this chapter provide a rich resource for new hypotheses about the metabolic factors involved in fear regulation and their sites of action in the brain.

In addition to fear regulation, glucocorticoids coordinate various metabolic processes that support the body in rest and during stress. As exemplified by patients with Cush-

ing's disease, an excess of glucocorticoids causes adverse metabolic effects such as body weight gain and elevated blood glucose and lipid levels. GR antagonists that inhibit glucocorticoid signalling may ameliorate the adverse metabolic effects of excess glucocorticoids. However, currently available GR antagonist mifepristone/RU486 lacks selectivity, as it also inhibits signalling of the sex hormones androgen and progesterone. Therefore, there is a need for a more selective GR antagonist. In **chapter 4,** we show that the novel GR antagonist CORT125281 is a full and specific antagonist for GR. In **chapter 3**, we pharmacologically characterized CORT125281 in male mice. We found that a high dose of CORT125281 fully blocks glucocorticoid signalling in the liver, partially in brown fat and muscle, but lacked effect in white fat and brain. At a low dose, CORT125281 was only effective in the liver but not in other tissues. Unlike RU486, CORT125281 did not interfere with HPA-axis activity. In a mouse model of (early) Cushing's disease, CORT125281 attenuated the increase of blood glucose and insulin levels caused by glucocorticoid treatment, although less effectively than RU486.

Since the metabolic effects of glucocorticoids are remarkably similar to the complications caused by high-calorie diets, GR antagonism may also be beneficial in diet-induced metabolic disease. In **chapter 4**, we explored the potential of CORT125281 to alleviate the metabolic effects of high-fat diet feeding in male mice. GR antagonists CORT125281 and RU486 similarly reduced body weight gain and total fat mass. CORT125281, but not RU486, additionally lowered total plasma cholesterol, triglycerides and fatty acids. The beneficial metabolic effects of CORT125281 appeared to be, at least in part, due to activation of the brown adipose tissue, which combusts lipids to generate heat. Increased brown fat activity was shown, for example, by the increased uptake of lipids into the tissue combined with a reduced intracellular lipid content. Based on **chapters 3** and **4**, we show that GR antagonism is not only effective in reducing metabolic effects caused by excess glucocorticoids, but also in diet-induced metabolic disease in which glucocorticoid levels are not severely dysregulated.

One of the metabolic complications of obesity is fatty liver disease, in which excess lipids accumulate in the liver. Fatty liver disease can also result from deregulated glucocorticoid signalling. In **chapter 5,** we investigated the efficacy of the selective GR modulator CORT118335, which combines GR agonism with antagonism, in reducing fatty liver disease in male mice. We showed that CORT118335 reduced diet-induced obesity and improved glucose tolerance. CORT118335 had remarkable lipid-lowering effects in the liver, as the compound both prevented and reversed fat accumulation in the liver. The strong reduction of liver lipids was a result from enhanced lipid efflux from the liver combined with reduced lipid influx, which likely reflects both agonistic and antagonistic glucocorticoid actions of the compound. In this chapter, we show that selective GR modulation has the potential to dissociate the beneficial from the disadvantageous

effects of glucocorticoid signalling. Therefore, treatment with CORT118335 may be an interesting therapeutic strategy to reduce fatty liver disease and obesity.

Synthetic glucocorticoid-like drugs, such as dexamethasone, can cause adverse metabolic effects comparable to those observed in Cushing's disease. Classically, the adverse metabolic effects of dexamethasone are thought to be due to GR hyperactivation. However, we propose that part of the metabolic effects of dexamethasone are due to MR hypoactivation. Dexamethasone has a high affinity for GR and therefore fully blocks endogenous glucocorticoid production. The resulting low endogenous glucocorticoid levels, along with the low affinity of dexamethasone for MR, reduce MR activation. This can simply be corrected by a low-dose corticosterone add-on treatment. In **chapter 6** we investigated the metabolic effects of corticosterone add-on during dexamethasone treatment in diet-induced obese male mice. Corticosterone add-on partially prevented the dexamethasone-induced loss of body weight, and fully counteracted the fat mass loss. Dexamethasone increased glucose and insulin levels, which was exacerbated by corticosterone add-on. However, these effects were not observed when dexamethasone was combined with aldosterone add-on, which is a specific MR agonist. We showed that corticosterone depletion during dexamethasone treatment is responsible for part of the metabolic effects of dexamethasone treatment. Although effects of corticosterone add-on are remarkably similar to known effects of MR activation, additional studies are needed to directly prove the involvement of MR in corticosterone add-on treatment.

As illustrated in **chapter 6,** MR and GR can mediate distinct activities of glucocorticoids. mRNA levels of GR and MR are frequently measured to assess the level of receptor activation. This may be especially relevant for the high-affinity MR, which is already largely occupied in the brain during non-stressed conditions. Consequently, receptor levels, and not hormone levels, may be the first limiting step for MR signalling. In contrast, ligand levels may be the first limiting step for the low-affinity GR. In **chapter 7**, we investigated how MR and GR mRNA levels relate to receptor activation, which we measured by the expression of shared MR and GR target gene *glucocorticoid-induced leucine zipper* (*Gilz*). In brains of mice pre-treated with corticosterone and vehicle, we showed that MR mRNA levels may limit *Gilz* mRNA expression at the bulk tissue level of the hippocampus and paraventricular nucleus (PVN). GR mRNA levels correlated with *Gilz* mRNA in only one subregion of the hippocampus. Correlations were much weaker at the cellular level, despite the use of two different techniques to measure gene expression. Overall, MR mRNA measurements, and GR mRNA levels to a smaller extent, may reflect receptor activation at the tissue level, but not at the cellular level. Nevertheless, mRNA levels of corticosteroid receptors should be interpreted cautiously to assess response to hormone, as many other cell- and region-specific factors eventually determine the level of corticosteroid receptor activation.

To conclude, in **chapter 8** we placed our findings in a broader context and compared them with the current knowledge from the scientific literature. We discussed the challenges of selectively targeting (cortico)steroid receptor signalling and of translating our preclinical findings to the clinic. We also discussed how the therapeutic strategies in this thesis may affect the (emotional) brain. Collectively, the results described in this thesis show that targeting glucocorticoid signalling can reduce metabolic disease, which may be a step towards breaking the vicious cycle between stress, obesity and (stress-related) mood disorders.