Chapter 7

SUMMARY AND MAIN CONCLUSIONS
The actions of glucocorticoid hormones are essential for healthy functioning of brain and body, particularly during stress. Secretion of these hormones by the adrenals is tightly controlled through the Hypothalamus-Pituitary-Adrenal-axis (HPA-axis). Activity of the HPA-axis follows a circadian rhythm with highest plasma glucocorticoid levels just prior to awakening. Furthermore, secretion of glucocorticoids is stimulated after stress. This stress response plays an important role in the adaptation to a changing environment.

Glucocorticoids affect a wide range of processes in both periphery and brain. They regulate storage and mobilisation of energy, affect immune and inflammatory responses and act on diverse endocrine systems including the HPA-axis. Their effects in the central nervous system are particularly potent. Within the brain they modulate synaptic plasticity and regulate neuronal function, which likely underlie their effects on behavioural adaptation to stress, learning and memory processes and mood.

Glucocorticoids mainly exert their actions through genomic mechanisms altering gene expression. The genomic actions are mediated by two types of receptors, the high affinity mineralocorticoid receptors (MR) and the lower affinity glucocorticoid receptors (GR). In the periphery glucocorticoid actions are mostly mediated by GR, which is abundantly expressed in almost any cell in the body. Within the brain both types of corticosteroid receptors are present although with a different localisation pattern. MR and GR mediate different but partly overlapping aspects of central glucocorticoid action.

To reach their central target areas glucocorticoid hormones have to enter the brain by passing the blood-brain barrier (BBB). The BBB is a dynamic physical and metabolic barrier consisting of specialised endothelial cells that protects the brain from blood-borne compounds, and plays a role in maintaining brain homeostasis. The BBB can strongly interfere with distribution to the brain of endogenous and exogenous compounds. Generally, hydrophilic and large lipophilic compounds are not able to penetrate the brain, as they are not able to pass cell membranes, whereas small lipophilic compounds can easily cross the BBB by passive diffusion through the endothelial cells. However, for a number of highly lipophilic compounds including the synthetic glucocorticoid dexamethasone BBB permeability is unexpectedly low. The multidrug transporter P-glycoprotein (Pgp) is an important functional component of the BBB and various other tissues with a barrier function. It acts like a ‘gatekeeper’ at the BBB actively keeping a wide variety of lipophilic, potentially neurotoxic substances out of the brain. This transmembrane protein is encoded by the multidrug resistance (MDR) genes, mdr1a in rodents and the highly homologous MDR1 in humans. Studies using mdr1a (-/-) knockout mice which lack functional Pgp at the BBB, have shown that this efflux transporter is responsible for the apparent low permeation of dexamethasone and a wide range of other compounds that should easily penetrate the BBB as expected based on their size and their sufficient high lipid solubility.

Although the importance of the actions of glucocorticoids in brain is commonly accepted, modulation of glucocorticoid access at the BBB level has hardly been a subject of research as
these compounds are considered to readily pass this barrier. Now that it has been demonstrated that transmembrane proteins are able to transport these hormones, this issue becomes an increasingly interesting subject to study. Any process at the BBB that can influence the endothelial crossing of glucocorticoids would directly affect central corticosteroid receptor occupancy and the magnitude of the central response to corticosteroids.

The aim of the studies described in this thesis was to examine the interaction of glucocorticoids and the efflux transporter P-glycoprotein expressed at the BBB as a possibly new level at which access to the brain and thus central corticosteroid receptor function may be controlled. Modulation of access of glucocorticoids to the brain may provide a new way to restore aberrant corticosteroid signalling associated with hypercortisolemia, glucocorticoid feedback resistance or MR/GR imbalance.

In chapter 2, findings regarding the expression of Pgp in the brain at the level of mRNA are presented. In situ hybridisation with a riboprobe recognising both murine mdr1 genes (mdr1a and mdr1b) demonstrates presence of mRNA in the endothelial cells of brain capillaries throughout the rat brain confirming presence of Pgp at the BBB. Surprisingly, expression of mdr1 mRNA was also found in granule cells of the dentate gyrus, a subfield of the hippocampus highly expressing both MR and GR. Its function at this site has yet to be resolved.

In chapters 3 and 4, the interactions of various naturally occurring glucocorticoids and synthetic glucocorticoids with Pgp are described particularly with regard to the uptake of these hormones into the brain. The hypothesis that endogenous glucocorticoids would easily reach the central glucocorticoid target areas, whereas Pgp would protect the brain against exogenous synthetic glucocorticoids, was rejected. Autoradiographic studies in adrenalectomised mice with a disrupted mdr1a gene confirmed that the synthetic glucocorticoid prednisolone like dexamethasone is hampered to enter the mouse brain and to reach glucocorticoid receptors. Presence of Pgp did not affect the uptake of the endogenous rodent glucocorticoid corticosterone into mouse brain. In sharp contrast to corticosterone, the main human glucocorticoid hormone cortisol was hampered to enter the brain of mice expressing Pgp at the BBB, but not of mice lacking Pgp. Although the absence of cortisol in the normal physiology of the mouse might explain this differential interaction with mouse Pgp, the remarkable difference between the cortisol and corticosterone transport capability of Pgp was also demonstrated for human Pgp using an in vitro model. Pig kidney epithelial cells form monolayers when seeded on filters and when stably transfected with human Pgp MDR1 cDNA these monolayers express high levels of Pgp at their apical side. Polar translocation of Pgp substrates to the apical side of these monolayers is reminiscent of Pgp function at the human BBB. Human Pgp-mediated polar transport of cortisol, prednisolone and dexamethasone, but also of the naturally occurring corticosteroids
cortisone, 11-deoxycortisol and, although to a lesser extent, aldosterone was demonstrated with these monolayers. Inhibition of transport by a selective Pgp blocker confirmed involvement of Pgp. In contrast, Pgp did not transport corticosterone in this system.

To further corroborate the differential uptake of corticosterone and cortisol in human brain as suggested by the mouse and monolayer findings, cortisol and corticosterone levels were measured in extracts of human post-mortem brain samples and plasma using liquid chromatography-mass spectrometry (LC-MS) to determine the ratio of corticosterone over cortisol (chapter 3). In contrast to rodents, both cortisol and corticosterone are circulating in human plasma, although corticosterone is present at ten- to twentyfold lower levels than cortisol. While in human plasma corticosterone concentrations are only 5% of cortisol levels, in the brain corticosterone levels are 30% of those of cortisol as determined using LC-MS.

Thus, in contrast to corticosterone, which readily enters rodent and human brain, cortisol, the main endogenous glucocorticoid in human, appears to be partially excluded from rodent and human brain.

The differential uptake of corticosterone and cortisol may have important implications for glucocorticoid feedback to human brain. The LC-MS data suggest that cortisol levels in human brain are 6 times lower than those in human blood. The total glucocorticoid levels in human brain may thus be lower than assumed based upon plasma levels.

Furthermore, the preferential uptake of corticosterone in human brain suggests that this endogenous glucocorticoid may play a more prominent role in human brain function than hitherto recognised. Due to the differential uptake of cortisol and corticosterone, the human glucocorticoid feedback system might be more complex than the rodent system. Whether this is actually the case remains to be resolved, but literature data indicate that corticosterone may be the more effective glucocorticoid at the human MR, suggesting that corticosterone might have a potentially different role than cortisol in brain.

In chapter 5 the data showing the functional consequences of Pgp-mediated exclusion of dexamethasone from the brain on central gene expression are presented. It was hypothesised that glucocorticoid feedback to the brain would be reduced as a consequence of depletion of glucocorticoids from the brain induced by treatment with low-dose dexamethasone.

Dexamethasone acting at the level of the pituitary potently suppresses corticosterone secretion. As dexamethasone poorly enters the brain due to the presence of Pgp at the BBB, administration of low amounts of dexamethasone depletes the brain from endogenous glucocorticoids, for which dexamethasone does not appropriately substitute. Peripherally, dexamethasone replaces corticosterone at the GR. The resulting condition is a brain-selective hypocorticoid state.

To confirm the working hypothesis, dexamethasone was chronically administered to rats for 6 days or three weeks. As expected, dexamethasone circulating at low concentrations did not feed back at several glucocorticoid responsive genes expressed in the hypothalamic
paraventricular nucleus (PVN). Stress-induced responses of c-fos mRNA and CRH hnRNA were not reduced, whereas CRH mRNA expression was even increased after three weeks of treatment. After high-dose treatment dexamethasone turned out to be able to enter the brain in sufficient amounts to activate GR. Expression of glucocorticoid responsive genes was strongly reduced indicating that the barrier formed by Pgp is not able to completely exclude dexamethasone from entering the brain. In contrast, various peripheral glucocorticoid targets were strongly affected by dexamethasone independent of the amount administered.

After low-dose treatment, dexamethasone-induced effects on brain function should probably be ascribed to decreased rather than increased central glucocorticoid action.

Other glucocorticoids that are substrates of Pgp are less likely to create this central adrenalectomy-like condition, as the high affinity to GR, lack of plasma binding and long-lasting activity favours the potency of dexamethasone to completely inhibit pituitary-adrenal secretion at low plasma levels. To suppress pituitary-adrenal secretion to the same extent high doses of cortisol (about 70-fold those of dexamethasone) are needed, which will likely reach the brain as well activating both MR and GR.

In conclusion, the findings presented in this thesis have made clear the importance of glucocorticoid transport at the BBB in controlling glucocorticoid access to the brain. The data show that Pgp is able to hamper penetration of various corticosteroids into the brain, particularly when these hormones are circulating at low plasma levels. Efflux transporters like Pgp may play a crucial role as an intermediate between brain and periphery by controlling transport of corticosteroids at the BBB. As exemplified by the dexamethasone study, impaired uptake of synthetic glucocorticoids, but also of the naturally occurring glucocorticoid cortisol, likely results in a reduced occupation of central corticosteroid receptors and thus in a diminished response to these glucocorticoids. Intriguingly, both mouse and human Pgp do not transport corticosterone in contrast to cortisol. Future investigations will reveal whether corticosterone rather than cortisol may be the major endogenous corticosteroi in mediating corticosteroid actions, particularly via MR, on human brain function, as suggested by the preferential uptake of endogenous corticosterone into human brain.

The brain-selective low-corticosteroid state created by administration of low-dose dexamethasone to rats might be used as an animal model to specifically study central roles of corticosterone without the potentially confounding effects of reduced peripheral glucocorticoid effects.