Chapter 1

Aims and outline of the thesis

The pulsatile secretion of growth hormone (GH) by pituitary somatotrophs is regulated by two antagonistic hypothalamic peptides: GH-releasing hormone (GHRH) and somatostatin. In addition, GH release can be stimulated by synthetic GH secretagogues (GHSs), such as growth hormone-releasing peptide-6 (GHRP-6), by acting through receptors different from those for GHRH. In 1999, the endogenous ligand for this GHS-receptor was purified and characterized from rat and human stomach and was called 'ghrelin'. Ghrelin has also been identified in the fundus of the canine stomach. The general aim of **Part I** of this thesis was to document spontaneous, GHS-, and ghrelin-induced GH release in healthy dogs and dogs with a pituitary disorder. In addition, ghrelin secretion was studied in healthy dogs.

The pituitary gland is not the only site of GH production. Under the influence of endogenous progesterone or the administration of progestins, the canine mammary gland is also able to secrete considerable amounts of GH into the systemic circulation. This mammary-derived GH is identical to pituitary GH. **Part II** of this thesis concentrates on several aspects of this progestin-induced mammary-derived GH in dogs.

The first part of the general introduction is an overview of pituitary GH secretion and its regulation, and of the diverse endocrine and nonendocrine effects of synthetic GHSs and ghrelin **(Chapter 2, part I)**. The second part of the general introduction **(Chapter 2, part II)** concentrates on the effects of progesterone and synthetic progestins in the bitch.

Besides the physiological effects of several hormones on pituitary GH secretion, the secretion pattern of GH may also change as a result of pathological hypersecretion of hormones such as, for example, cortisol. In **Chapter 3** and **Chapter 4** the effects of pituitary-dependent hyperadrenocorticism on the plasma GH profile and the GH response to various GHSs (ghrelin, GHRP-6, and GHRH) are reported.

In humans, not only diseases such as hypercortisolism, but also ageing and obesity affect pituitary GH secretion and cause a reduced response to GH stimulating factors. In dogs, little is known about the effect of age on the plasma GH response to GH-releasing stimuli. **Chapter 5** reports on the effects of several GHSs (ghrelin, GHRP-6, and GHRH) on the release of GH in young and old healthy Beagle dogs. In a search for the specificity of these stimulations, the effects of GHRP-6 and ghrelin administration on plasma adrenocorticotrophic hormone (ACTH), cortisol, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and prolactin (PRL) release were also studied.

Ghrelin is a potent stimulator of GH release. The option of using ghrelin in the diagnosis of congenital GH deficiency was studied by measuring the effect of ghrelin administration on the plasma GH concentration in German shepherd dogs with pituitary

dwarfism. The dwarfism in German shepherd dogs is a combined pituitary hormone deficiency. Therefore, also the plasma concentrations of ACTH, cortisol, TSH, LH, and PRL were determined before and after ghrelin administration **(Chapter 6)**.

Through activation of pathways distinct from those involved in the stimulation of GH secretion, ghrelin also functions as a potent orexigenic peptide. Ghrelin induces weight gain by increasing food intake and reducing fat utilization. In several mammalian species it also plays a role in meal initiation. **Chapter 7** reports on the physiological effects of food intake and fasting on the circulating concentrations of ghrelin, GH, glucose, insulin, and insulin-like growth factor-I (IGF-I) in healthy Beagle dogs.

In **Part II** of this thesis several aspects of progestin-induced mammary-derived GH in dogs are presented. Cystic endometrial hyperplasia (CEH) is frequently seen in bitches treated repeatedly with progestins for prevention of oestrus. The condition may also develop spontaneously in the luteal phase of the oestrous cycle of middle-aged or elderly bitches, i.e. bitches that have gone through several luteal phases. Because of the similarity of the progestin-induced epithelial changes in both the mammary gland and the uterus, it was hypothesized that mammary GH is involved in the pathogenesis of progestin-induced CEH. Therefore, the effect of chronic administration of a synthetic progestin on the development of CEH was investigated in bitches with surgically excised mammary glands and in healthy control bitches **(Chapter 8)**.

It is not clear whether the oestrus-preventing properties of progestins in the bitch are due to effects at the level of the hypothalamus, the pituitary gland, or the ovary. In **Chapter 9** the effects of chronic administration of a synthetic progestin on adenohypophyseal function are reported, including the effects on the GH-IGF-I axis.

The presence of progesterone receptors in mammary gland tissue of dogs allows for a targeted endocrine therapy with progesterone receptor blockers in dogs with progestin-induced mammary-derived GH overproduction. The effects of treatment with the progesterone receptor blocker aglépristone in Beagle dogs with progestin-induced mammary-derived GH excess are reported in **Chapter 10**.

In Chapter 11 the results of the studies are summarized and discussed.