Summary

Reproductive processes are mainly regulated by the brain-pituitary-gonad axis (BPG-axis). Gonadotropin-releasing hormone (GnRH) neurons localized in the brain release their hormone GnRH, which allows the release of gonadotropic hormone by gonadotropic cells in the pituitary. Gonadotropic hormone, in turn, regulates the production of sex steroids and germ cells in the gonads. The steroids complete the dynamic BPG-axis by exerting feedback effects at the level of the brain, pituitary and gonads.

The transition period of a juvenile, inactive axis towards a mature, functional system is known as puberty. Several concepts deal with the question how the onset of puberty is regulated. The prevailing "missing-link" concept assumes that one or more components of the axis are absent or not functional before puberty. For our animal model, the African catfish, *Clarias gariepinus*, it has been proposed that sex steroids initiate and/or accelerate pubertal development. However, based on earlier findings, it was suggested that the "missing link" could also be localized at a higher level of the BPG-axis, i.e. at the GnRH system in the brain.

Most vertebrates express two forms of GnRH: a species-specific form and cGnRH-II. The latter is identified in all vertebrates and is well conserved throughout evolution. The species-specific GnRH with a hypophysiotropic function, varies amongst species, but represents a separate lineage in GnRH genealogy. The African catfish carries two forms of GnRH in the brain: catfishGnRH (cfGnRH) in neurons dispersed over the entire ventral forebrain, from the olfactory bulb till the pituitary and cGnRH-II expressed by cells in the midbrain.

In order to test the hypothesis that sex steroids and/or a functional GnRH system are important determinants in the onset of puberty in the African catfish, we studied the normal development of the GnRH system in the brain and the effects of certain steroids on this development. Our results revealed that the cfGnRH system in the ventral forebrain achieves its morphological adult status just at the onset of puberty, probably after a migratory route, which originates in the olfactory placode, similar as in other vertebrates. The innervation of the gonadotropic cells in the pituitary by cfGnRH fibers

is, however, not completed before the end of puberty. A second population of neurons also expressing cfGnRH was identified in the most rostral region of the telencephalon, in the terminal nerve ganglion (TN). Because of its different morphology, later appearance during development and the absence of a connection with the pituitary, the TN population was considered as a separate category of cfGnRH neurons. In modern fish species the TN population expresses a third form of GnRH, which also favors a specific functional identity.

In contrast to the cfGnRH system in the ventral forebrain, the cGnRH-II system displays an adult pattern already before the onset of puberty and, moreover, has no morphological connection with the pituitary. Nevertheless, cGnRH-II is a potent releaser of gonadotropic hormone *in vitro*. In order to understand more of the functional significance of the cGnRH-II system, we studied its structural properties and neuroanatomical environment. Microscopical studies revealed that cGnRH-II cells in the midbrain have no axons and are scarcely innervated by neurons of the nucleus fasciculus longitudinalis medialis. Furthermore, the cGnRH-II cells showed many characteristics of high metabolic activity, but clear evidence for cGnRH-II release could not be obtained, despite their close apposition to capillaries. On the other hand, cGnRH-II cells display striking contacts with dilated extracellular spaces in the subventricular reticulum, suggesting a release into the cerebrospinal fluid.

Since only the ventral forebrain cfGnRH system is directly connected with the pituitary, steroid effects on this system were investigated. For this study we selected three developmental periods: the immature, juvenile period (2-6 weeks of age), the onset of puberty (10-12 weeks of age) and the actual pubertal period (12-17 weeks of age). It was found that testosterone (T) stimulated the immunoreactivity and the peptide content of cfGnRH neurons during these three periods. In addition, in juvenile fish T treatment also resulted in a significant increase in the number of cfGnRH neurons. Other sex steroids, like 11 β-hydroxyandrostenedione (OHA) or 11-ketotestosterone (11KT), which are strong stimulators of testicular development and spermatogenesis, did not (or hardly) affect the cfGnRH system in the African catfish. The development of the cGnRH-II system in the midbrain remained unchanged after T administration.

The effects of T on the cfGnRH system are probably mediated via its aromatization product estradiol (E_2), similarly as T exerts its stimulatory effect on the gonadotropic cells. Indeed, aromatase, the enzyme responsible for the conversion of T into E_2 is present in the brain areas where cfGnRH neurons are localized.

The present thesis shows that specific sex steroid (T and E_2) are important for the recruitment of cfGnRH neurons and for the increase in the

cfGnRH content in brain and pituitary by stimulating the cfGnRH production and/or by inhibiting its release. Thus, in the African catfish, T is a serious candidate for the "missing-link", since it is required for activating the cfGnRH neurons in the preparation for the onset of puberty.

Another candidate for the "missing-link" is the actual released amount of cfGnRH that is available for the gonadotropic cells at the onset of puberty. It has been demonstrated *in vitro* that gonadotropic cells are able to respond adequately upon GnRH stimulation at this stage. Moreover, pituitary incubation studies revealed that suitable amounts of endogenous cfGnRH are present in the axon terminals. We hypothesize that functional contact between the first cfGnRH terminals in the pituitary and the gonadotropic cells is required for the initiation of puberty. Once this switch is turned on, the three levels of the BPG-axis simultaneously display their maturational processes: innervation of the pituitary by cfGnRH fibers, development of gonadotropic cells and the first wave of spermatogenesis.