論文の内容の要旨

論文題目

Regulation of cortisol secretion by fast-acting hormones in eel osmoregulation

（ウナギの浸透圧調節における速効性ペプチドホルモンによるコルチゾルの分泌調節）

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In this thesis, I studied the secretory regulation of the long (slow)-acting hormone, cortisol, by several important osmoregulatory fast (short)-acting hormones in the eel *(Anguilla japonica)*. The interaction of fast and slow acting hormones is a crucial step in the process of osmoregulation in euryhaline fish that have the ability to acclimate to both freshwater (FW) and seawater (SW) during their life cycle. Among euryhaline fish, the eel exhibits exceptional adaptability to diverse salinity environments and considered as an excellent model for osmoregulation studies. As cortisol is considered to be a crucial for adaptation to both SW and FW, I analyzed its secretory regulation by three groups of fast-acting hormones including natriuretic peptides (NPs), adrenomedullins (AMs) and angiotensins (Angs).

Chapter 1. Effects of fast-acting hormones on cortisol secretion from eel interrenal tissue

In Chapter 1 of this thesis, I analyzed the diversified NPs present in the eel on cortisol secretion using an *in vitro* incubation system of steroidogenic interrenal cells from both FW and SW acclimated eels. A previous *in vivo* experiment from this laboratory showed that ANP increased plasma cortisol concentration in SW eels and CNP (later known as CNP1) increased it in FW eels. Only three NPs (ANP, VNP and CNP1) were identified at that time in teleost fish, but three new NPs have been identified recently in the eel. Thus I examined the effect of all 6 NPs on cortisol secretion in the newly established in vitro system in order to analyze in more detail the mechanisms of action of NPs on the interrenal. Further, I examined the newly discovered AM family and diversified Ang peptides to test their direct effect on interrenal cells.

Although steroidogenic cells are clustered as a compact tissue in tetrapods and some teleosts, the interrenal cells in the eel are scattered along the long head kidney (>10 cm in length). In order to

determine the tissue that should be used for the *in vitro* experiments, I first localized the steroidogenic and chromaffin cells in the eel head kidney by RT-PCR using steroid biosynthetic enzyme (P450scc) and tyrosine hydroxylase (TH) as markers, respectively. I found that the interrenal cells exist only in the anterior part (~2 cm) of the head kidney, but chromaffin cells are distributed more widely along the head kidney. Furthermore, I examined their tissue distribution in the head kidney by immunohistochemistry using the marker enzymes, P450scc and TH. The results indicated that both cell types exist in the close vicinity in the wall of the cardinal vein forming several clusters scattered in the hematopoietic tissue. I also found that the genes of NP receptors (NPR-A and NPR-B) are expressed in the same interrenal region of the head kidney. I also established a radioimmunoassay for cortisol myself to measure large amount of samples.

Using the in vitro incubation system of eel interrenal cells, I first examine the action of the six NPs identified in the eel (ANP, BNP, VNP, CNP1, CNP3 and CNP4) on cortisol secretion. However, none of the six eel NPs given alone produced any significant stimulation of cortisol secretion in either FW or SW eels. These results were inconsistent with the stimulatory effect in the previous *in vivo* studies. However, when NPs where incubated together with ACTH. ANP and VNP (but not BNP and CNPs) enhanced the stimulatory effect of ACTH on cortisol secretion in SW fish interrenal preparations. By contrast, CNP1 and CNP4 (but not ANP, BNP, VNP and CNP3) enhanced the ACTH action in FW fish preparations. Thus, I eventually confirmed the previous *in vivo* experiments showing that the different NPs are involved in cortisol secretion in different salinity environments, and thus play important role in the euryhalinity of the eel for adaptation to both FW and SW. In addition, these results demonstrate the importance of the modulation of the steroigogenic action of ACTH, indicating that this fast-acting hormone may be fundamental for osmoregulatory control of cortisol.

I also examined the steroidogenic action of the newly discovered AMs (AM1, AM2 and AM5) and Angs (Ang II, Ang III and Ang IV) in the eel interrenal tissue. For Ang II, I used teleost [Asn^1]-Ang II (N-Ang II) and mammalian [Asp^1]-Ang II (D-Ang II) as the N-terminal Asn of N-Ang II is known to be converted to Asn by asparaginase in the eel blood. Although AM has a steroidogenic action in mammals, none of the eel AMs caused any effects on cortisol release from either FW or SW eel interrenal preparations. By contrast, Ang II and Ang III given alone to the incubation medium increased cortisol secretion in SW eel preparations. Furthermore, the heterologous D-Ang caused a greater increase than homologous N-Ang I. Ang III induced a slight increase in cortisol secretion in FW eel preparation while N-Ang II and Ang IV had no effect in either FW or SW eel preparations. Ang II acts directly on the interrenal cells to stimulate cortisol secretion. Interestingly, heterologous

D-Ang II is more potent than homologous eel N-Ang II. Since previous studies from this lab showed that N-Ang II is more potent than D-Ang II for the vasopressor effect and Ang III is almost ineffective in the eel, a new receptor for Ang that has not been identified to date could exist in the interrenal tissue.

Chapter 2. Effect of NPs on ACTH secretion from the pituitary gland of the eel

From the data in Chapter 1, I realized that ACTH may be playing a central role in cortisol secretion. Considering the differing results from *in vitro* and *in vivo*, it is possible that the circulating ACTH may be increased by NPs for cortisol secretion. Supporting this possibility is the previoud data that hypophysectomized eels did not produce cortisol secretion after SW transfer (Hirano 1969). Based on these data, I hypothesized that NPs not only modulate the ACTH action but also stimulate ACTH secretion from the eel pituitary gland.

In Chapter 2 of this thesis, I initially established the ELISA method for ACTH measurement and an *in vitro* tissue culture system of the eel pituitary gland to measure ACTH secretion from the pituitary. As the steroidogenic action of ANP and CNP1 were demonstrated in SW and FW eel interrenal tissues, I tested the effects of ANP and CNP1 on ACTH secretion in SW and FW eel pituitaries, respectively. As a result, CNP1 significantly increased ACTH secretion in the pituitaries of FW adapted eels, while the ANP effect tended to be stimulatory but non-significant. Altogether, these data suggest a link between NPs and ACTH at the pituitary for cortisol secretion.

Conclusions

Based on the results reported in this thesis, I present a new and more complex model for regulation of cortisol secretion by several fast-acting hormones. Furthermore, it is apparent that the major hormone for cortisol synthesis and secretion is ACTH and thus its regulation becomes of vital importance as the effects of NPs are only visible in the presence of this hormone. In addition, as shown in Chapter 2, NPs seem to act on the pituitary and modulate ACTH secretion, which consequently will influences cortisol secretion in vivo. This data provides a new regulatory step of fast-acting hormones to the hypothalamo-pituitary-interrenal (HPI) axis, also demonstrating the interaction between fast-acting hormones for cortisol secretion (Fig. 1). Further, not only the local action in the interrenal tissue, but the regulation of fast-acting hormones in other glands of the HPI axis like the pituitary become of interest for a wider understanding of the process of osmoregulation that euryhaline teleosts undergo during their life cycle.

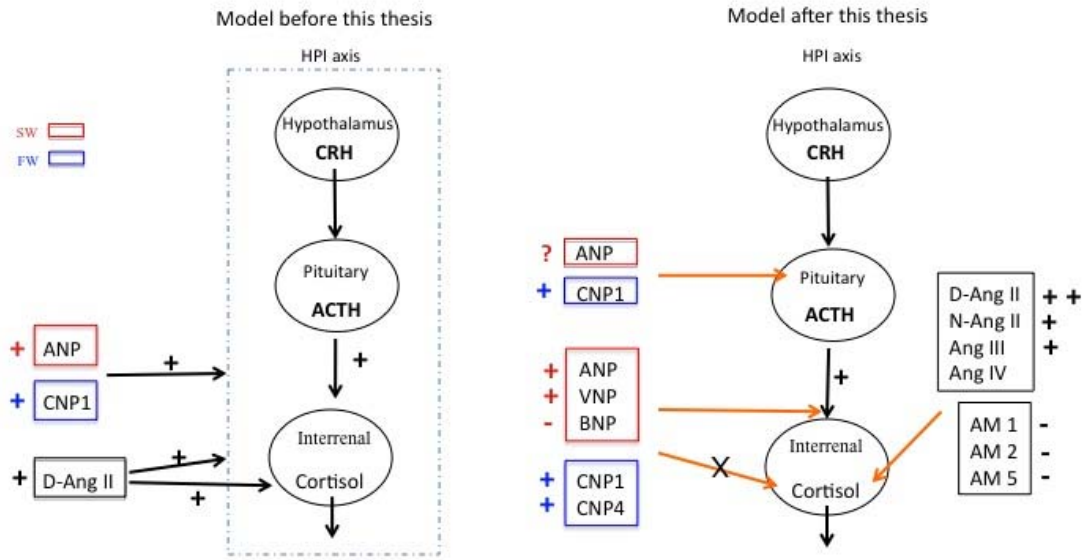

Figure 1. Model for the regulation of the hypothalamo-pituitary-interrenal (HPI) axis for cortisol secretion by natriuretic peptides (NPs), adrenomedullins (AMs) and angiotensins (Angs). Previous model based on *in vivo* results on the effects of ANP and CNP1 in the eel (Li and Takei 2003) and in in the flounder by D-Ang II (Balment *et al.* 1990).