The role of estrogen in hypothalamic regulation of hypothalamus-pituitary-adrenal axis activity, energy homeostasis and bone metabolism
Liu, Ji

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General discussion

In the present thesis we examined the effects of estrogen, mediated via the central nervous system (CNS), on hypothalamo-pituitary-adrenal (HPA) axis activity, glucose homeostasis, and lipid and bone metabolism. We found that local estradiol synthesis in the paraventricular nucleus of the hypothalamus (PVN) is rapidly elevated by restraint stress, indicating that changes in central E2 concentrations may be involved in the modulation of the stress response. Additional studies in this thesis revealed that within the PVN, E2 modulates the activity of the HPA-axis both in basal (by ER\textsubscript{α}) and stress conditions (by ER\textsubscript{α} and ER\textsubscript{β}). In addition, we demonstrated that hypothalamic estrogen signaling, independently of circulating estradiol, is crucial for several aspects of energy homeostasis. First, hypothalamic estradiol was found to regulate hepatic glucose production, as well as hepatic and peripheral insulin sensitivity. Second, we showed that hypothalamic estradiol regulates UCP gene expression in brown adipose tissue as well as the expression level of a number of lipolytic genes in white adipose tissue. Third, we provided the first evidence that central E2 affects bone formation rate. In part of these studies the hypothalamic effects of E2 could be characterized further with regard to the specific estrogen receptors or hypothalamic nuclei involved, using local administration of ER subtype specific (ant) agonists via microdialysis.

Part 1 Estrogen regulation of HPA axis activity

1.1. Crosstalk between the HPA and HPG axis

It is well known that sex hormones interact with the HPA axis at several levels. In Chapter 2, we demonstrated that acute stress increased hypothalamic GnRH mRNA expression, indicating activation of the HPG axis. Indeed, we found an increase of plasma estradiol concentrations during the stress condition. On the other hand, in vitro studies showed that corticosterone blocks the E2-induced LH release in primary cultures of rat pituitary cell\textsuperscript{(1)} \textsuperscript{(2, 3)}. In Chapter 2, we also found an increase of the local estradiol concentration in the hypothalamic PVN during the stress condition. These results were supported by the local increase in PVN aromatase gene expression during acute stress. The in vivo microdialysis studies in Chapter 3 extended the observations in Chapter 2 and revealed how local changes in PVN estradiol concentrations may modulate HPA-axis activity during basal and stress conditions (Figure 7.1).
1.2 The neuronal pathway and ER subtypes involved in the effects of estrogen on the HPA axis

Previous studies in our group revealed that the ER-mediated regulation of CRH promoter activity results from the two ERE half sites, in which the -316 ERE site contributes more to the constitutive CRH expression than to the -480 ERE site (4). Based on these in vitro studies, we investigated the effects of an infusion of ER subtype specific agonists directly in the PVN, where most CRH neurons are localized, on HPA-axis activity. The results indicated that both ERα and ERβ are involved. During basal conditions ERα activation increased HPA activity. During stress conditions ERα also increased HPA-axis activity, but ERβ activation decreased HPA-axis activity. We did not investigate whether estradiol is involved in the regulation of the negative feedback via the GR, but previous studies have shown that treatment with estradiol or a selective ERα agonist diminishes the suppressive effect of dexamethasone (DEX) on the stress-induced release of corticosterone, whereas treatment with an ERβ agonist enhances the suppressive effect of DEX on the stress-induced corticosterone release (5). Together, these data indicate that ERα inhibits, whereas ERβ enhances the negative feedback of glucocorticoids by elevating or suppressing CRH expression in the PVN.

The positive effect of the ERα agonist on the HPA-axis seems somewhat paradoxical since very few ERα positive neurons can be found within the PVN. On the other hand, the peri-PVN region contains many GABA-immunoreactive neurons that express ERα and project to the PVN (5, 6), exerting an inhibitory effect on the hypophysiotropic CRH neurons (7). Moreover, there is evidence showing that E2 attenuates GABA-B responses in hypothalamic neurons (8) and that E2 suppresses the GABA-A-mediated inhibition (9).
Thus we hypothesize that estradiol may increase CRH expression via the (peri)PVN by inhibiting, via ERα, the GABAergic neurons that surround the PVN (Figure 7.2). Most likely the inhibitory effect of E2 via the ERβ only becomes apparent during stress conditions because at this time the CRH neurons are activated and thus an inhibitory effect can be observed more clearly.

**Figure 7.2.** A schematic pathway for estrogen regulation of the HPA axis via the (peri)PVN. 1) E2 activates the CRH neurons by suppressing the inhibitory effect of GABAergic neurons on CRH release through ERα in GABAergic neurons in the peri-PVN. 2) E2 can directly affect CRH neurons within the PVN via its effect on ERβ in CRH neurons. E2, estradiol; ER, estrogen receptor; CRH, corticotrophin release hormone; GABA, GABAergic neurons; ACTH, Adrenocorticotropic hormone; GR, glucocorticoid receptor; CORT, corticosterone.

### Part 2 Regulation of glucose, lipid and bone metabolism by hypothalamic estrogen and the autonomic nervous system

Estrogen receptors are expressed in many areas of the hypothalamus and colocalize not only with insulin (10) and leptin receptors (11), but also with NPY (12) and POMC (13), i.e., neurotransmitters that are known to be involved in energy metabolism. As indicated by previous studies by our group and others (14, 15), pre-autonomic neurons in the hypothalamus are likely to play an important role in the regulation of lipid, glucose and bone metabolism.

#### 2.1.1 Hypothalamic estrogen and glucose metabolism

It has been well established that the autonomic innervation of the liver is involved in the control of hepatic glucose metabolism (16, 17). However, only recently it has become clear how the hypothalamus controls peripheral glucose metabolism through the autonomic nervous system. Surgical denervation of the sympathetic nerves innervating the liver severely affects the regulatory influences of many hypothalamic signals on...
hepatic glucose production (14, 15, 18). In chapter 4 we showed that the stimulatory effect of E2 via the VMH on endogenous glucose production (EGP) and plasma glucose concentrations is abolished by a sympathetic, but not a parasympathetic, denervation of the liver. On the other hand, autonomic denervation of the liver (either sympathetic or parasympathetic) had no effect on the stimulatory effect of E2 on plasma glucose concentrations via the PVN. There is no evidence for a direct neural connection between the VMH and autonomic nuclei in the brainstem or spinal cord, contrary to the PVN. On the other hand, the VMH has pronounced projections to the PVN, which functions as the key hypothalamic integration center for autonomic and endocrine information, serving as the final neuro-endocrine and autonomic output nucleus from the hypothalamus (19, 20). ERα is expressed in the majority of glutamatergic neurons in the VMH (21) and the PVN is known to receive a strong glutamatergic input from the VMH (22). Therefore, we propose that the ERα-containing glutamatergic neurons in the VMH that project to the PVN are activated by local administration of E2. This may excite sympathetic pre-autonomic neurons in the PVN that in turn stimulate the hepatic sympathetic tone. In order to explain the opposite effect of the VMH on muscle-dedicated and liver-dedicated pre-autonomic neurons we propose that the glutamatergic projection of the VMH to the muscle-dedicated pre-autonomic neurons involves a GABAergic interneuron in the subPVN which contacts and inhibits the muscle-dedicated pre-autonomic neurons directly (Figure 7.3). Clearly this hypothesis needs to be investigated further in future studies.

Figure 7.3 Hypothetical pathway to explain the hypothalamic effects of E2 on glucose production and glucose uptake. In this scheme 2 separate pre-autonomic PVN neurons for the control of muscle glucose uptake have been indicated, but of course, it is also possible that these are one and the same neuron, i.e., that the PVN neurons that are contacted via the VMH – subPVN projection also express the ERβ.
The autonomic nervous system not only affects hepatic glucose production, but also peripheral glucose uptake. Activation of adrenergic receptors was reported to inhibit insulin-stimulated glucose uptake by 2T3-L1 adipocytes (23) and to stimulate glucose uptake in brown adipocytes (24). It is not possible to perform similar surgical denervations of muscle as we did for the liver, but beta-adrenergic antagonists and knock-down of skeletal beta adrenergic receptors attenuated the modulatory effects of VMH signaling on glucose uptake (25). In Chapter 4, we observed that both PVN and VMH E2 signaling decreased insulin-dependent glucose uptake, taking into account the studies mentioned above, this indicates that E2 signaling in the PVN and VMH may also regulate glucose uptake by changing autonomic nervous activity. The effect of E2 in the PVN on glucose uptake indicates that the pre-autonomic neurons in the PVN that control the autonomic nervous input to the muscle probably contain ERs, in contrast to the pre-autonomic PVN neurons that control hepatic glucose production (Figure 7.3).

2.1.2 Hypothalamic estrogen and adipose tissue metabolism
In Chapter 5, we found both β-adrenergic receptor (AR) and uncoupling protein (UCP) gene expression in BAT to be increased after hypothalamic E2 administration, which indicates that hypothalamic E2 signaling may also play an important role in the modulation of energy metabolism via its effect on the autonomic input into brown adipose tissue. The autonomic nervous system is not only involved in the control of BAT activity, but also in that of the activity of white adipose tissue (WAT). PVN E2 treatment increased both LPL

Figure 7.4 Hypothetical pathway to explain the hypothalamic effects of E2 on UCP gene expression in BAT and the expression of lipolysis genes in abdominal WAT.
Chapter 7

and HSL expression. This is in line with previous studies showing that activation of the sympathetic nervous system increases lipolysis in WAT (26, 27) (Figure 7.4).

2.1.3 Hypothalamic estrogen and bone metabolism

In Chapter 6, we showed that central estrogen signaling decreases bone formation rate, suggesting the presence of a functional brain - bone metabolism pathway. As we discussed before (see general introduction), hypothalamic NPY and POMC neurons are involved in the central regulation of bone metabolism (28). Whether the central effects of E2 on bone metabolism are also mediated via the NPY/POMC arcuate and the autonomic nervous system will have to be the subject of future studies.

Part 3 General perspective and future work

3.1 Perspective

Taken together, estrogen acts within the hypothalamus on different neural pathways via its two nuclear receptors, ERα and ERβ. The effects of estrogen are mediated via interactions with both neuro-endocrine and pre-autonomic neurons, as well as with interneurons. Within the hypothalamus, estrogen up- or down-regulates the activity of pre-autonomic neurons that are involved in the control of ANS projections to liver, muscle, BAT, WAT and possibly bone. In addition to directly affecting the activity of the pre-autonomic neurons, estrogen also modulates the activity of the pre-autonomic neurons via its action on other neurons, such as the GABA-containing neurons in the periPVN region (chapter 2), the glutamate containing neurons in the VMH (chapter 4), and the NPY-containing neurons in the arcuate nucleus (29). Varying parts of this neural network may also be involved in the effects of estrogen on appetite, body weight and insulin sensitivity (30). Future studies are needed to further unravel the neural pathways involved in the different effects of estrogen, as well its interaction with other hormones such as leptin and insulin that may use very much similar neural pathways to impose their effect.

Part 4 Clinical relevance

Menopause is associated with a shift towards a more masculine body fat distribution, metabolic syndrome and osteoporosis. Although estrogen hormone replacement therapy is one of the most efficient ways to relieve these symptoms, the clinical treatment is challenging since systemic estrogen replacement increases the risk of breast cancer and venous thrombosis (31, 32). Based on recent studies, including studies in the present thesis indicating central pathways for effects of estrogen that occur independently of estrogen concentrations in the circulation, it may be feasible to develop new and more specific
selective estrogen receptor modulators that primarily target estrogen signaling in the brain (33). In view of the selectivity of estrogen’s central effects, this may be an alternative way to prevent or at least relieve the systemic side effects, i.e. the risk of breast cancer. Our data provide a strong argument to further explore potential estrogen drug targets in the hypothalamus.
Chapter 7

Reference


