Synthesis, Release, and Action

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9.1 The Three Steps of Hormone Regulation

To understand regulation of hormones, three steps have to be distinguished:

1. Their synthesis
2. Their release or secretion
3. Their action on one or several receptors

We described hormone synthesis while presenting the different hormones: various enzymes by which protein/peptide hormones are correctly cleaved from their precursor or modulated posttranslationally; the two classes of cytochrome P450 (CYP)-dependent monooxygenases and hydroxysteroid dehydrogenases (HSDs), both generating steroids from cholesterol and the intermediates; and those enzymes by which tyrosine or tryptophan is converted into catecholamines, melatonin, or thyroid hormones.

Only steroid release is not blocked by the cell membrane, and steroids leave the synthesizing cell by diffusion. Their release is not deferred, but immediate. The other hormones are not released directly, but are stored in intracellular vesicles and are released on demand. Nerve cells and neurosecretory cells act in a similar way: neurotransmitters and neuropeptides as well as adrenaline and glycoprotein hormones are collected in secretory granules.

These secretory granules are usually located close to the cell surface. When a messenger demands release of a hormone by acting on a particular surface receptor, this ligand binding initiates an increase in intracellular calcium level, either by

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opening calcium channels or, more often, by release of calcium from intracellular calcium stores. This enhanced calcium level triggers fusion of the granules with the membrane. It has been known for only a short time that soluble *N*-ethylmaleimide sensitive factor attachment receptors (SNAREs) are required for this membrane fusion, by which action two surfaces usually rejecting each other come into contact and fuse (Jena 2004).When the membranes of the vesicle and cell fuse, the inside of the vesicle becomes the outside of the cell and the content of the vesicle is free to leave the cell.

Hormonal release from secretory granules is thus an active process. It is triggered extracellularly by neurotransmitters at synapses, and also by other endocrine or paracrine messengers. Many hormones are released in pulses. For this several cells have to fuse their granules to the cell membrane simultaneously. The regulation of gonadotropin-releasing hormone (GnRH) pulses, for example, is age dependent: postpartum, the GnRH release, which is high in the fetus, is downregulated to almost zero; therefore, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are hardly released. At puberty, GnRH release increases again. Although the release does not yet occur in steady pulses, mood swings occur in a parallel way. Once the pulse rate has been correctly set at the level of an adult, these mood swings mostly go. During aging, the pulse rate drops again.

Pulsatile GnRH release is required not only for female fertility, it is required for male fertility as well. If by external doses of GnRH or stable agonists a constantly elevated GnRH level is established, pituitary LH and FSH release is blocked and infertility occurs. This is one method of temporary contraception. However, in men constantly elevated GnRH levels maintain elevated testosterone levels, potentially triggering prostate carcinomas.

The action of hormones occurs via receptors, as presented Chap. 8. Since hormones circulate in the blood, specificity of hormone action is exclusively generated by expression of specific receptors in specialized sensitive target cells. Cells react to hormones only when they bear receptors for the given hormone.

9.2 Effective Steroid Concentrations of the Hypothalamic–Pituitary–Gonadal Axis

If the amounts of hormones and their concentrations released in the hypothalamus, the pituitary, and the target organs are measured, an amplification cascade can be observed. Hypothalamic releasing hormones are released in picomolar concentrations: GnRH at 1 ng/l (about 0.6 pM). In the pituitary, the release is in the range of micrograms per liter. In contrast to the decapeptide GnRH, the molar LH release is 25-fold higher. The adrenal release of dehydroepiandrosterone (DHEA) is in the milligram range. Since DHEA is small, about 200,000 DHEA molecules are released per GnRH molecule.

If we assume there are 5,000 GnRH neurons, these release 6 ng GnRH into 6 l of blood—that is, 3.6 pmol, which corresponds to 21×10^{11} GnRH molecules and about 4×10^{8} GnRH molecules per neuron. Since the half-life of GnRH in blood is only

a few minutes, most of the pulsatile-released GnRH is metabolized before the next pulse. To reach a concentration of 1 ng/l in blood again, all 4×10^8 molecules have thus to be newly synthesized in the period between the two pulses. At a pulse rate of one pulse every 2 h, each cell has to synthesize and store 41,700 molecules every second. These cells are like a GnRH factory. In the immune system the plasma cells are highly specialized and can produce about 2,000 antibodies per second. With the GnRH precursor (approximately 70 amino acids) being much shorter than an immunoglobulin heavy chain (approximately 400 amino acids), the GnRH neuron synthetic capacity is, however, of the same magnitude.

When GnRH is released at only 0.1 ng/l, the number of molecules drops to 4,100. The exact number is to be treated with caution; however, the magnitude is interesting. All molecules have to undergo the different posttranslational steps described before. With exact knowledge of the enzyme kinetics, we could estimate how many enzyme molecules a GnRH neuron requires to fulfill the GnRH demand.

Figure 9.1 demonstrates additional aspects of quantitative endocrinology: steroids do not exist in their free form, but are preferentially bound to transporter proteins or are sulfated like DHEA. Since steroids are modified only intracellularly, they have to dissociate from their binding globulin before they are able to diffuse

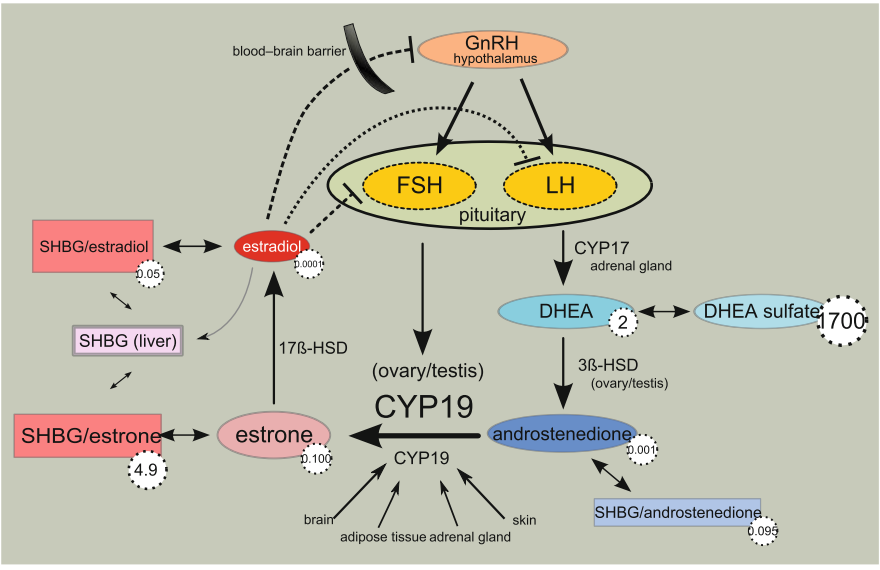

Fig. 9.1 Steroid hormone stores and relative steroid amounts of the hypothalamic–pituitary–gonadal axis. Steroid hormones are poorly water soluble. Complexed to the sex-hormone-binding globulin (*SHBG*), androgens and estrogens can be transported. Dehydroepiandrosterone (*DHEA*), however, is soluble as its sulfate derivatives. The *numbers in the dashed circles* give serum concentrations of the respective hormones or SHGB–hormone complexes in nanograms per milliliter. *CYP* cytochrome P450, *FSH* follicle-stimulating hormone, *GnRH* gonadotropin-releasing hormone, *HSD* hydroxysteroid dehydrogenase, *LH* luteinizing hormone

into reactive cells. Only then can they be modified. The reasons when and why a steroid dissociates from its binding globulin have not yet been identified in the case of androgens or estrogens. There might well be an equilibrium reaction which delivers new free steroids whenever the free steroid concentration in the surroundings of a reactive cell drops. However, from vitamin D_3 and its binding protein (vitamin D_3 binding protein), it is known that the binding protein is cleaved when vitamin D_3 is required. Such a mechanism has not yet been found for sex-hormone-binding globulin (SHBG).

Furthermore, Fig. 9.1 shows that the content of free steroids is considerably lower than that of the complexed or sulfated steroids. For estrogens and SHBG, the ratio is 1:49, for androstenedione it is 1:24, and for DHEA it is 1:1,000. The ratio of progesterone to DHEA to androstenedione to estrone to estradiol is 0.02:2:0.001:0.1:0.0001. These molecules all have similar molecular masses. Thus, DHEA is the steroid made in the largest amounts. The adrenal CYP17 is thus the dominant enzyme. The conversion of androstenedione to estrone by CYP19 in the ovaries and testes, as well as in adipose tissue, skin, brain, and (to a small degree) adrenal gland, reduces the androstenedione concentration to 1/100 that of estrone. This steroid, on its own, is not active, but has to undergo conversion by 17β-HSD into estradiol, which has the lowest serum levels of all steroids, but the highest activity.

Figure 9.1 explains that only estradiol stimulates liver SHBG synthesis. Absence of 17β-HSD leads to hyperandrogenism since there are too many free androgens in the blood.

The final step of estradiol synthesis is not restricted to granulosa or Sertoli/Leydig cells: aromatase (CYP19) is expressed in other tissues. As long as the estrone–SHBG complex is circulating in the blood, a cell with 17β-HSD might generate estradiol. 17β-HSD type 1, which preferentially reduces estrone to estradiol, is expressed in the anterior pituitary and in tumors, whereas the same enzyme in mice has been identified only in the pars intermedia (Peltoketo et al. 1999b; Green et al. 1999). Not only reducing activity has been observed converting estrone into estradiol; in other tumors, oxidative activity inactivating estradiol to estrone has been found.

It appears possible that the availability of cofactors determines whether in the pituitary estrone is converted into estradiol, whether such estradiol is readily oxidized, and whether an inhibiting action is exerted on FSH and LH biosynthesis. The simplified assumption that follicular estradiol is made to regulate pituitary FSH and LH release appears too simplistic. It is not known how the apparent estradiol level increase in the follicular phase of the menstrual cycle acts on gene expression in the pituitary, most notably in gonadotropic cells. Release inhibition by downregulating intracellular calcium and thus inhibiting membrane fusion of secretory granules to the cell membrane does not appear highly probable: estrogen receptors are preferentially transcription factors acting on gene expression and much less on signal cascades.

In Fig. 9.1 it is also shown that estradiol blocks GnRH release. This, however, appears feasible since, as described above, GnRH is freshly produced between two pulses, and estradiol while inhibiting transcription will diminish GnRH release since the stores are not renewed.