II. Examining endocrine disruptors

1. Actions of hormones in the human body

This chapter is designed to make understanding of subsequent chapters easier by providing essential information. It describes the physiological roles of endocrine organs and hormones and the possible mechanisms, that is how the inherent actions of hormones could be disrupted by exogenous chemicals.

(1) What is “endocrine”?

Among the various cell groups within the body, many cells create proteins, polypeptides, amines and lipids, and retain them in the cytoplasm in the form of secretory granules. Secretion generally refers to extracellular excretion of the products in these secretory granules. The secretion can be divided into exocrine and endocrine according to the secretory pathway. For example, exocrine cells of the pancreas produce substances that become digestive enzymes and contents of digestive juices, and secrete them into the duodenum through the pancreatic duct. This process is considered to be a typical external secretion or exocrine.

On the other hand, the pancreas has a cell group called Islet of Langerhans. Although these cells have secretory granules, they do not have an excretory duct to guide the secreted materials to the place in which they play a role. These secreted materials are transported by the circulation to the target organs, where they stimulate and switch on the functions. As they are secreted into the circulation and are not connected via an external conduit, this mode of secretion is called internal secretion or endocrine. In contrast to substances that are carried far to target organs, substances that act on cells neighboring them can be classified as paracrine and those acting on the secretory cells themselves can be classified as autocrine.

Substances which are secreted from endocrine glands and act on target organs, tissues, and cells are classically called "hormones". The word "hormone" was coined by W.M. Bayliss and E.H Starling in 1902 and comes from the Greek "to awaken". Currently it is used to include substances capable of transferring information. However, among information transferring substances, those secreted by cells of the immune system are specifically called "cytokines". The following are the classical endocrine organs of humans:

1. Thyroid gland

   Thyroid gland located in the frontal portion of the neck, under the cricoid cartilage. This is a butterfly-shaped organ that can be palpated from outside. It produces thyroid hormones, which play an important role in the growth and development, in particular, in mental and physical development during the prenatal and infant periods. Its absence brings about severe mental and growth retardation called "cretinism". In adults, it plays an important role in metabolism and heat production. An excess of thyroid hormone results in tachycardia, tremor, heat intolerance and weight reduction which are typical signs and symptoms for "hyperthyroidism".

2. Parathyroid gland

   This organ, located next to the thyroid glands, consists of four rice grain-sized tissues. It secretes parathyroid hormone that mobilize the calcium from the bone to maintain normal calcium concentrations in the blood. Its absence causes cramp and tetany and is coined as "hypoparathyroidism". An excess of parathyroid hormone causes an elevation of serum calcium, urinary tract stone, or decaleificafion of bone and is coined as "hyperparathyroidism".

3. Adrenal gland

   Located on the upper part of the kidney, it produces steroid hormones, among which are glucocorticoids, mineral corticoids and sex steroids, as well as their precursors.

1) Glucocorticoids are essential and permissive for vital metabolism and are particularly necessary for the body to cope with stress such as shock and infection; the lack of adrenocortical activity causes Addison's disease.
2) Mineral corticoids are necessary to control the body electrolytes and fluid balance as well as to maintain blood pressure.
3) Sex steroids have been shown to be basic not only to sexual development and behavior, but also to the maintenance of various tissues such as bone.

4. Ovary

   The ovary consists of ovarian follicles, luteal body and connective tissues.

Ovarian follicles contain ovarian cells and granulosa cells surrounding them. During ovarian follicle development, the granulosa cells synthesize estrogen. By the effect of estrogen, one of the follicles grows to the mature ovum and releases an ovum into Fallopian duct connecting to uterus. After ovulation, the luteal body is formed and progesterone is secreted. The ovarian follicles also secrete inhibin which suppresses pituitary FSH secretion. If a woman has normal menstrual cycle, it is warranted that the hormone secretion of the ovary is normally controlled by dominating pituitary and hypothalamus and ovarial hormones normally act on the endometrium.

5. Testicle

The testicle consists of seminiferous tubules and interstitial tissues. The seminiferous tubules have Sertoli cells, and various spermatogenic cells of different stages ranging from spermatocytes to sperm. Sertoli cells also secrete inhibin which suppresses FSH. Interstitial Leydig cells secrete testosterone.

6. Pituitary gland

This bean-sized organ protrudes from the base of the brain. It regulates and integrates the above mentioned hormone secretion. From the hypothalamus, which is part of the brain, it is connected with the nervous system and the special vascular system (pituitary portal system). The hypothalamus functions as commanding center where neuronal information from the brain and endocrine information from each part of the body are integrated; some of the newly integrated information is transmitted to the pituitary glands. In response, the pituitary gland secretes hormones that stimulate the thyroid gland (thyroid stimulating hormone: TSH), adrenal cortex (adenocorticotropic hormone: ACTH) and gonads (luteinizing hormone: LH, and follicle stimulating hormone: FSH) in needs and automatically control the amount of hormones secreted from each endocrine organ. The pituitary gland also secretes growth hormone to secure normal height development; lack of this hormone causes short stature, while their excess by the neoplasm causes acromegaly. Pituitary gland also secretes prolactine which stimulates lactation. Excess production of this hormone by neoplasma is relatively frequent and inhibit the ovullatory surge of LH causing amenorrhea in women and also inhibit LH-responsiveness of Leydig cells resulting in testosterone deficiency in man. The posterior lobe of pituitary secretes antidiuretic hormone (ADH) that reabsorbs water from the kidney. Lack of ADH causes diabetes insipidus, in which as much as 10 liters of urine is produced a day.

7. Hypothalamus

Pituitary gland function is also controlled by a higher regulatory center, namely, hypothalamus. The hypothalamus secretes growth hormone-releasing hormones (GRH) that simulate growth hormone secretion, thyrotropin-releasing hormones (TRH) that stimulate thyrotropin secretion, corticotropin releasing hormone (CRH), and gonadotropin-releasing hormones (GnRH) that stimulate LH and FSH secretion from the pituitary gland. GnRH is secreted at a pulse of once every two hours. This pulse frequency in itself is important for pituitary LH secretion and the continuous secretion is known to suppress LH secretion.

In this way, the thyroid, adrenal and gonad hormones are dominated by the pituitary glands, which in turn are dominated by the hypothalamus. On the other hand, the hormones secreted by these endocrine organs act on the pituitary gland and on the hypothalamus in such a way that thyroid hormones suppress TSH secretion, glucocorticoids suppress ACTH secretion, and estrogen suppresses LH in women, and testosterone suppresses LH in men, adequately controlling the secretion of hormones from the endocrine organs. In addition, from the ovary follicles and testicular Sertoli cells, a hormone called inhibin is secreted and acts to suppress the secretion of FSH. Follistatine, a protein that binds with inhibin, is present in the blood and acts to control the actions of inhibin. Through this control network, adult human is able to control hormone concentrations within a limited range, even if a certain amount of exogenous substance mimicking or inhibiting the hormonal action enters the body.

8. Others

Besides these classically known endocrine organs, adipose tissues are known to secrete a hormonal substance, leptin, to control appetite and energy metabolism; and the heart and vascular system secrete atrial natriuretic polypeptides (hANP) and endotherine, while the central nervous system secretes many peptide hormones, including neuropeptide Y (NPY), CCK and TRF, etc. Steroid hormones such as dehydroepiandrosterone sulfate (DEHA-S) are also shown to be secreted from nervous system.

(2) Differentiation and formation of gonads

The differentiation and development of gonads proceed as follows: Both male and female primoridal gonads are produced from the area called the genital ridge, which is formed in the initial stage of fetal development. In women, the Fallopian tubes and uterus are formed from the Muellerian tubes; in men, under the dominance of androgen, the gonads that become the testicles descend and the semiferous tubules and external genitalia are induced from the Wolffian duct.

In the formation of external genitalia, a symmetrical portion of skin extends to wrap the urinary tract and form the penis under the effect of androgen and the interruption of this process results in retentio testis and hypospadias. This process is dominated by the effects of androgen. Besides androgen itself, transcription factors induced by androgen is required for this process. When the transcription factors, a functional protein bind with a specific sequence of DNA directly or indirectly and induce organ development, may be not formed due either to 1) lack of androgen, 2) lack of receptors to receive the actions of androgen, 3) abnormalities in the collaborative proteins (co-activators, co-repressors, etc.) that control the actions of the hormone receptors complex and 4) other causes, various grade of androgen unresponsiveness or insensitivity is resulted. In its extreme from, the external genitalia and the body characteristics are female in the presence of male sex chromatin (XY) and male gonads (testicles). If the degree of abnormality is slight, abnormalities such as hypospadias or retentio testis are observed.

At the onset of puberty, the secretion of gonadotropic hormones is induced and the development of gonads is promoted; in women, ovulation and menstruation begin, and in men, sperm formation starts. In childhood, the secretion of gonadotropic hormones is suppressed by complex nervous and endocrine mechanisms. When this suppression is removed at the onset of puberty, sexual maturity is brought about.

(3) Chemistry of hormones

Hormones can be roughly divided into water-soluble and fat-soluble categories.

Water-soluble hormones are either proteins, polypeptides, amines or amino acids; hormones secreted from the hypothalamus, pituitary and thyroid glands are either proteins, polypeptides, amines, or amino acids, while adrenal cortex and gonads secrete steroids which is fat-soluble hydrocarbons. As hormones consisting of proteins and polypeptides have high molecular weight, they cannot pass through the cell membrane. They exert their actions by binding with receptor proteins on the cell surface membrane, and initiate a specific signal transduction process. For example, adrenaline and noradrenaline are low molecular size amines but exert their actions by binding with the receptors on the cell membrane. Thyroid hormones are a kind of amino acid, but reach the cell nucleus and bind with the nuclear receptors. The structure of these receptors is also very similar to that of steroid hormone receptors. On the other hand, steroid hormones can easily pass through the cell membrane and reach the nucleus, exerting their actions by binding with the nuclear receptor. In any event, a hormone does not exert its action until it binds with the receptor protein peculiar to the hormone.

(4) Regulation of blood hormone concentration

As a hormone acts in proportion to its concentration in the blood, blood hormonal concentrations are often kept constant and controlled by negative feedback mechanism. However, a positive feedback mechanism also works to quickly achieve a high concentration if required. For example, to cause ovulation, pituitary LH secretion must be increased to a higher level. In this instance, positive feedback occurs in which estrogen secreted from the ovarian follicles increases blood concentration, and the latter, in turn, inducing LH secretion. In addition, information that the hormones give to the target organs is not only hormone concentrations; in some cases, information regarding the number of secretory pulse within a certain time is given as well. For example, GnRH has a pulsatile secretion every two hours. This pulsatile secretion stimulate LH secretion. In contrast, when GnRH is secreted at a constant rate, LH secretion is suppressed. In some cases, various hormone-binding proteins present in the blood and tissues regulate the fraction of hormones reached to the target cell.

(5) Binding of hormones with hormone receptors

Hormones exert their actions by binding with the receptor specific to each hormone. The actions of hormones after binding with hormone receptor proteins are to start the existing response already programmed in the cells, not to produce a new response.

For example, when insulin binds with a receptor, phosphoric acid binds with the receptor which itself becomes an enzyme that phosphorylates proteins called IRS. This IRS, in turn, phosphorylates various other functional protein and changing their shapes and functions, for example, accelerating the shuffling of glucose transporting protein and enhancing the incorporation of glucose into the cells, such as of the muscles. In addition, insulin promotes cell proliferation. As exemplified by insulin, the signal transduction mechanism is sometimes due to phosphorylation of intracellular proteins. The mechanism is also known in which transcription of a target protein is enhanced as the phosphorylated proteins migrate into the nucleus and bind with its DNA.

On the other hand, fat-soluble hormones directly pass through the cell membrane and enter the nucleus to bind with the receptor. Some hormones bind with the receptor in the cytoplasm. The hormone-cytoplasm receptor protein complex migrates to the nucleus and bind to the specific sequence of DNA of the target gene. In some cases, the receptor protein is bound with a variety of chaperon protein (for example, estrogen binds with heat shock protein) in the cytoplasm. When the hormone binds with the receptor, the protective chaperon protein is separated, and hormone-receptor complex migrates into the nucleus. The receptor protein possesses a domain which bind with hormones and another domain that binds with a specific DNA sequence (in many receptor proteins, the latter has a digital structure containing zinc coined as "Zinc finger"). In many cases, the receptor associated with the hormone (=hormone receptor complex) binds with DNA in pairs (homodimer), but there are cases in which the combination of a receptor specific to the hormone and a receptor of a different substance (for example, one receptor for thyroid hormone and the other for retinoic acid) binds with the specific part of the gene (heterodimer). Many collaborative proteins, such as co-activators and co-repressors, bind with these hormone-receptor complex. The process modulates the efficiency of transcription of a target gene, thereby promoting and suppressing synthesis of a target protein, respectively. There are cases in which several nucleus receptors have been found for one hormone (alpha, beta, isomers). For example, thyroid hormone receptors have alpha and beta isomers with different tissue distribution and different actions. Estrogen receptors also have alpha and beta isomers and may also have different actions.

Although many hormone receptors for known hormones have been identified there are some receptors whose binding hormones (ligand) in the body have not yet been elucidated; they are called "orphan receptors." Orphan receptors have been found among membrane receptors and also among nucleus receptors; this is one of the fields that most rigorous investigation is concentrated at present; this may lead to the discovery of new hormones. For example, from research on an unknown ligand of the membrane receptor that binds with G-proteins, many new hormones have been discovered, such as Ohlexin, which is a new appetite controlling substance, and a prolactin-secreting hormone different from TRH, which had been unknown and which has not yet been properly named. The aryl hydrocarbon (Ah) receptor, which is present in the nucleus, binds with dioxins; however, it is not yet clear which endogenous substance in the body binds with this receptor, or what it does. The investigation of the endogenous ligand of Ah-receptor would give us a clue in clarifying the mechanism of action of dioxin in animal and man.

The following are also examples in which endogenous factors that do not normally exist in the body affect normal hormone actions through receptors and the signal transduction mechanism. For example, hyperthyroidism of Graves' disease is caused when the TSH receptor antibody produced as a result of autoimmune abnormality. The antibody bound with TSH receptor mimics TSH in effect and exerts its actions. Pertussis toxins and cholera toxins bind to G-proteins, which normally binds hormone-cell membrane receptor complex and initiate signal transduction, and thus mimics the effect of hormone. It is also known that tamoxifen (anti-estrogen, breast cancer treatment drug) binds with estrogen receptor and that pharmaceutical agent RU486, binds with progesterone receptors, to exert their inhibitory actions.

(6) Manifestation of hormone actions

The cells in the body each have unique characteristics and functions, and all have a copy of the same DNA in their gene. The characteristics and functions of the cells differ because each cell translates the necessary parts the DNA design and synthesizes the necessary proteins and enzymes. A hormone may often promote protein synthesis de novo, but in these cases, the hormone receptor complex binds with a certain sequence of DNA on the gene that codes this protein, whose basic transcription factors are activated by transcriptional control factors, and then RNA polymerase activity is enhanced by the above described mechanism. As a result, transcription from DNA to mRNA is promoted. Amino acids are recruited based on the mRNA code, synthesizing specific proteins. Since both transcriptional control factors and basic transcriptional factors are proteins, actions ascribed to hormone may well be elicited by transcriptional factors induced by respective hormones. For example, in the differentiation process of gonads, a series of transcriptional factors exert their actions in proper sequence, as exemplified by the effect of AD4BP. Inhibition or deletion of these transcriptional factors is known to have a result similar to that seen in lack of the effect of hormones.

When an organ is being formed, the orderly synthesis of new proteins and enzymes in proper timing and sequence is important. To accomplish this, many transcription-controlling factors manifest without mistaking the timing and sequence. It is probable that if an exogenous substance mimicking hormonal effects enters into body by escaping various controlling mechanism, which is immature at development stage, sequence of events that dominate normal organ development may become impaired or disrupted, resulting in various developmental abnormalities.

Summary

Cells synthesize and secrete various substances. For example, exocrine pancreatic cells synthesize digestive enzymes and secrete them into the duodenum through the excretory duct. This is called external secretion, or exocrine. However, the cell group called the Islet of Langerhans in the pancreas secretes substances such as insulin and glucagon synthesized in the cells, but does not have an excretory duct, rather, it secretes the substances into the circulation through the capillary vessels. This is called internal excretion, or endocrine. Hormones have been defined as substances secreted into the blood stream from specific endogenous glands and carried by circulation to act on target organs and express their specific effects. This mode of action is known as endocrine. Recently, other modes of action, in which the target cells are located neighboring the hormone-producing cells (paracrine); in which the target cells are hormone-producing cells themselves (autocrine); and in which the target cells incorporate the hormone precursors, and manufacture and secrete hormones (intracrine), have become known. In addition, it has been demonstrated that hormones are also secreted by tissues that are originally not endocrine glands, such as adipose tissues and blood vessels. Currently substances produced in cells that are involved in the controlling function the body are arbitrarily called hormones.

Hormones are secreted from endocrine glands [pituitary glands, thyroid glands, parathyroid glands, pancreas, adrenal cortex, medulla, gonads (testicles, ovaries), etc.], tissues of nervous system such as the hypothalamus; adipose tissues, heart and blood vessels; some hormones are carried into the blood stream by themselves or by binding with specific binding proteins, and exert their actions on organs. As a result of these actions, hormones dominate such biological functions as energy metabolism, development, growth, sex differentiation and reproduction, as well as regulation of metabolism required to maintain the bodily homeostasis, and also influence on the immune and nervous systems.

All hormones exert their actions by binding with receptors, some on cell membranes, while others migrate to the nucleus and exert their actions by binding with the receptors within the nucleus. Various endogenous and exogenous chemicals and antibodies are known to affect various processes, such as binding hormone with transport proteins in the blood, the binding with membrane receptors, and the signal transduction process is mainly consisting of a cascade of phosphorylation of proteins and enzymes. The disruption of this process causes abnormalities and disease. Other types of hormones are known to migrate into the nucleus and bind with nucleus receptors (which are in themselves proteins; in many cases, there are a number of different receptors for one hormone), which then bind with a specific sequence of the DNA of the gene and alter the transcription of the specific proteins through the actions of many transcription-controlling proteins. It is also known that endogenous and exogenous chemicals affect the binding of hormone with nucleus receptors, the binding of hormone-receptor complex with DNA, and the binding of cooperative proteins.