Hormones and Behavior: Basic Concepts

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Introduction

Behavioral endocrinology is the scientific study of the interaction between hormones and behavior. This interaction is bidirectional: hormones can affect behavior, and behavior can feedback to influence hormone concentrations. Hormones are chemical messengers released from endocrine glands that influence the nervous system to regulate the physiology and behavior of individuals. Over evolutionary time, hormones regulating physiological processes have been co-opted to influence behaviors linked to these processes. For example, hormones associated with gamete maturation such as estrogens are now broadly associated with the regulation of female sexual behaviors. Such dual hormonal actions ensure that mating behavior occurs when animals have mature gametes available for fertilization. Generally speaking, hormones change gene expression or cellular function, and affect behavior by increasing the likelihood that specific behaviors occur in the presence of precise stimuli. Hormones achieve this by affecting individuals' sensory systems, central integrators, and/or peripherial effectors. To gain a full understanding of hormone–behavior interactions, it is important to monitor hormone values, as well as receptor interactions in the brain. Because certain chemicals in the environment can mimic natural hormones, these chemicals can have profound effects on the behavior of humans and other animals.

Behavioral Endocrinology Techniques

A number of methods are used to gather the evidence needed to establish hormone–behavior relationships. Much of the recent progress in behavioral endocrinology has resulted from technical advances in the tools that allow us to detect, measure, and probe the functions of hormones and their receptors. These techniques, with a brief description, are listed in **Table 1**. Several of these techniques are the result of advanced research in behavioral endocrinology, including the time-honored ablation-replacement techniques, bioassays, as well as modern assays that utilize the concept of competitive binding of antibodies that include radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA; enzyme-linked immunoassay (EIA)), autoradiography, and immunocytochemistry. Other techniques commonly used in behavioral endocrinology include neural stimulation and single-unit recording, techniques that activate or block hormone receptors with drugs, and gene arrays and genetic manipulations including interfering with RNA and use of viral gene vectors to deliver novel genes directly into the brain. Because hormones must interact with specific receptors to evoke a response, many of these techniques are used to influence or measure hormone secretion, hormone binding, or the physiological and behavioral effects that ensue after hormones bind to their respective receptors.

Hormones

Hormones are organic chemical messengers produced and released by specialized glands called 'endocrine glands.' *Endocrine* is derived from the Greek root words *endon*, meaning 'within,' and *krinein*, meaning 'to release,' whereas the term *hormone* is based on the Greek word *hormon*, meaning 'to excite.' Hormones are released from these glands into the bloodstream (or the tissue fluid system in invertebrates), where they act on target organs (or tissues) generally at some distance from their origin. Hormones coordinate the physiology and behavior of an animal by regulating, integrating, and controlling its bodily function. Hormones are similar in function to other chemical mediators including neurotransmitters and cytokines. Indeed, the division of chemical mediators into categories mainly reflects the need by researchers to organize biological systems into endocrine, nervous, and immune systems, rather than real functional differences among these chemical signals. Hormones often function locally as neurotransmitters and also interact with neurotransmitters and cytokines to influence behavior.

Hormones can be grouped into four classes: (1) peptides or proteins, (2) steroids, (3) monoamines, and (4) lipid-based hormones. Generally, only one class of hormone is produced by a single endocrine gland, but there are some notable exceptions. It is important and useful to discriminate among the four types of hormones because they differ in several important characteristics, including their mode of release, how they move through the blood, the location of their target tissue receptors, and the manner by which the interaction of the hormone with its receptor results in a biological response. The major vertebrate hormones and their primary biological actions are listed in **Table 2**.

Although exceptions always exist, the endocrine system has several general features: (1) endocrine glands are ductless, (2) endocrine glands have a rich blood supply, (3) hormones, the products of endocrine glands, are

Table 1 Common techniques in behavioral endocrinology

Ablation (removal or extirpation) of the suspected source of a hormone to determine its function is a classic technique in endocrinology. Suspected brain regions that may regulate the behavior in question can also be ablated. Typically, four steps are required: (1) a gland that is suspected to be the source of a hormone affecting a behavior is surgically removed; (2) the effects of removal are observed; (3) the hormone is replaced, by reimplanting the removed gland, by injecting a homogenate or extract from the gland, or by injecting a purified hormone; and (4) a determination is made whether the observed consequences of ablation have been reversed by the replacement therapy.

Radioimmunoassay (RIA) is based on the principle of competitive binding of an antibody to its antigen. An antibody produced in response to any antigen, in this case a hormone, has a binding site that is specific for that antigen. Antigen molecules can be 'labeled' with radioactivity, and an antibody cannot discriminate between an antigen that has been radiolabeled (or 'hot') and a normal, nonradioactive ('cold') antigen. A standard curve is produced with several tubes, each containing the same measured amount of antibody, the same measured amount of radiolabeled hormone, and different amounts of cold purified hormone of known concentrations. The radiolabeled hormone and unlabeled hormone compete for binding sites on the antibody, so the more cold hormone that is present in the tube, the less hot hormone will bind to the antibody. The quantity of bound hormone can be determined by precipitating the antibody and measuring the associated radioactivity resulting from the radiolabeled hormone that remains bound. The unknown concentration of hormone in a sample can then be determined by subjecting it to the same procedure and comparing the results with the standard curve.

Enzymoimmunoassay (EIA), as RIA, works on the principle of competitive binding of an antibody to its antigen. EIAs do not require radioactive tags; instead, the antibody is tagged with a compound that changes optical density (color) in response to binding with antigen. Other than home pregnancy tests, most EIAs are developed to provide quantitative information. A standard curve is generated so that different known amounts of the hormone in question provide a gradient of color that can be read on a spectrometer. The unknown sample is then added, and the amount of hormone is interpolated by the standard curves. A similar technique is called 'enzyme-linked immunosorbent assay' (ELISA).

Immunocytochemistry (ICC) techniques use antibodies to determine the location of a hormone in cells. Antibodies linked to marker molecules, such as those of a fluorescent dye, are usually introduced into dissected tissue from an animal, where they bind with the hormone or neurotransmitter of interest. For example, if a thin slice of brain tissue is immersed in a solution of antibodies to a protein hormone linked to a fluorescent dye, and the tissue is then examined under a fluorescent microscope, concentrated spots of fluorescence will appear, indicating where the hormone is located.

Autoradiography is typically used to determine hormonal uptake and indicate receptor locations. Radiolabeled hormone is injected into an individual or into dissected tissue. Suspected target tissues are sliced into several very thin sections; adjacent sections are then subjected to different treatments. One section of the suspected target tissue is stained in the usual way to highlight various cellular structures. The next section is placed in contact with photographic film or emulsion for some period of time, and the emission of radiation from the radiolabeled hormone develops an image on the film. The areas of high radioactivity on the film can then be compared with the stained section to determine how the areas of highest hormone concentration correlate with cellular structures. This technique has been very useful in determining the sites of hormone action in nervous tissue, and consequently has increased our understanding of hormone–behavior interactions.

Blot tests use electrophoresis to determine in which cells specific DNA, RNA, or proteins are located. Homogenized tissue of interest is placed on a nitrocellulose filter, which is subjected to electrophoresis that involves application of an electric current through a matrix or gel that results in a gradient of molecules separating out along the current on the basis of size (smaller molecules move farther than larger molecules during a set time period). The filter is then incubated with a labeled substance that can act as a tracer for the protein or nucleic acid of interest: radiolabeled complementary deoxyribonucleic acid (cDNA) for a nucleic acid assay, or an antibody that has been radiolabeled or linked to an enzyme for a protein assay. If radiolabeling is used, the filter is then put over film to locate and measure radioactivity. In enzyme-linked protein assays, the filter is incubated with chromogenic chemicals, and standard curves reflecting different spectral densities are generated. Southern blots assay DNA; Northern blots assay RNA, whereas Western blots test for proteins.

In situ hybridization is used to identify cells or tissues in which mRNA molecules for a specific protein (e.g., a peptide hormone) are produced. The tissue is fixed, mounted on slides, and either dipped into emulsion or placed over film and developed with photographic chemicals. Typically, the tissue is also counterstained to identify specific cellular structures. A radiolabeled cDNA probe is introduced into the tissue. If the mRNA of interest is present in the tissue, the cDNA will form a tight association (i.e., hybridize) with it. The tightly bound cDNA, and hence the messenger RNA (mRNA), will appear as dark spots. This technique can be used to determine whether a particular substance is produced in a specific tissue.

Pharmacological techniques are used to identify hormones and neurotransmitters involved in specific behaviors. Some specific chemical agents can act to stimulate or inhibit endocrine function by affecting hormonal release; these agents are called 'general agonists' and 'antagonists,' respectively. Other drugs act directly on receptors, either enhancing or negating the effects of the hormone under study; these drugs are referred to as 'receptor agonists' and 'antagonists,' respectively.

Brain imaging techniques reveal brain activation during behaviors. Paired with endocrine manipulations or monitoring, imaging can provide important information about hormone–behavior interactions. Positron emission tomography (PET) scanning permits detailed measurements of real-time functioning of specific brain regions of people who are conscious and alert. PET gives a dynamic representation of the brain at work. Computer-assisted tomography (CT) scanner shoots fine beams of X-rays into the brain from several directions. The emitted information is fed into a computer that constructs a composite picture of the anatomical details within a 'slice' through the brain of the person. Magnetic resonance imaging (MRI) does much the same thing, but uses nonionizing radiation formed by the excitation of protons by radiofrequency energy in the presence of large magnetic fields. Functional MRI (fMRI) uses a very high spatial ($\sim$1 mm) and temporal resolution to detect changes in brain activity during specific tasks or conditions. When neurons become more active, they use more energy, and require additional blood flow to deliver glucose and oxygen. The fMRI scanner detects this change in cerebral blood flow by detecting changes in the ratio of oxyhemoglobin and deoxyhemoglobin.

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Table 1 Continued

Gene manipulations. In behavioral endocrinology research, common genetic manipulations include the insertion (transgenic or knockin) or removal (knockout) of the genetic instructions encoding a hormone or the receptor for a hormone. In knockout mice, behavioral performance can then be compared among wild-type (+/+), heterozygous (+/−), and homozygous (−/−) mice, in which the gene product is produced normally, produced at reduced levels, or completely missing, respectively. Inducible knockouts when specific genes are inactivated in adulthood promise to become important tools in behavioral endocrinology. An alternative approach involved gene silencing via RNA interference (RNAi), which is used to deplete protein products made in cells.

Gene arrays can be used to determine relative gene expression during the onset of a behavior, or a change in developmental state, or among individuals that vary in the frequency of a given behavior or hormonal state. Essentially, a miniscule spot of nucleic acid of known sequence is attached to a glass slide (or occasionally nylon matrix) in a precise location often by high speed robotics. This identified, attached nucleic acid is called 'the probe,' whereas the sample nucleic acid is the target. The identification of the target is revealed by hybridization, the process by which the nucleotides link to their base pair.

secreted into the bloodstream, (4) hormones can travel in the blood to virtually every cell in the body and can thus potentially interact with any cell that has appropriate receptors, and (5) hormone receptors are rather specific binding sites, embedded in the cell membrane or located elsewhere in the cell that interact with a particular hormone or class of hormones. As mentioned, the products of endocrine glands are secreted directly into the blood, whereas other glands, called 'exocrine glands,' have ducts into which their products are secreted (e.g., salivary, sweat, and mammary glands). Some glands have both endocrine and exocrine structures (e.g., the pancreas). Recently, the definition of an endocrine gland also had to be reconsidered. For example, adipose tissue produces the hormone, leptin, and the stomach produces a hormone called 'ghrelin.' Probably the most active endocrine organ, and the one that produces the most diverse types of hormones is the brain.

As single cells evolved into multicellular organisms, chemical communication within and between cells, as well as between individuals and populations, developed. The endocrine system evolved to become a key component of this complicated intra- and intercellular communication system, although other systems of chemical mediation exist. For example, chemical mediation of intracellular events is called 'intracrine mediation.' Some intracrine mediators may have changed their function over the course of evolution and now serve as hormones or pheromones. Autocrine cells secrete products that may feed back to affect processes in the cells that originally produced them. For example, many steroid–hormone-producing cells possess receptors for their own secreted products. Chemical mediators released by one cell that induce a biological response in nearby cells are called 'paracrine agents'; nerve cells are well-known paracrine cells. In several cases, a single hormone (especially peptides) can have autocrine, paracrine, or endocrine functions. For example, leptin stimulates expression of itself and its receptor. Generally, leptin is produced in adipose tissue and it functions as a hormone when released into the blood by regulating energy balance at the level of the hypothalamus. However, leptin is also produced in the anterior pituitary gland where it diffuses locally to influence thyroid-stimulating hormone (TSH) secretion (paracrine). Many chemical mediators display similar diversity in function.

Some hormones are water-soluble proteins or small peptides that are stored in the endocrine cell in secretory granules, or vesicles. In response to a specific stimulus for secretion, the secretory vesicle fuses its membrane with the cellular membrane, an opening develops, and the hormones diffuse into the extracellular space via a process called 'exocytosis.' The expelled hormones then enter the blood system from the extracellular space. Other hormones, such as steroid hormones, are lipid soluble (i.e., fat soluble), and because they can move easily through the cell's membrane, they are not stored in the endocrine cells. Instead, a signal to an endocrine gland to produce steroid hormones essentially serves as a signal to release them into the blood as soon as they are produced by the cellular machinery.

Hormone receptors, that are either embedded in the cell membrane or located elsewhere within the cell, interact with a particular hormone or class of hormones. Receptor proteins bind to hormones with high affinity and generally high specificity. As a result of the high affinity of hormone receptors, hormones can be very potent in their effects, despite their very dilute concentrations in the blood (e.g., $1\ \mathrm{ng\ ml^{-1}}$ of blood). However, when blood concentrations of a hormone are high, binding with receptors that are specific for other related hormones can occur in sufficient numbers to cause a biological response (i.e., crossreaction). Also, many hormones are structurally similar so that antibodies designed to attach to one hormone may cross react with other similarly shaped molecules (e.g., growth hormone and prolactin, the sex steroids, and the glycoproteins, viz., luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone).

But generally, hormones can directly influence only cells that have specific receptors for that particular hormone and served as target cells. The interaction of a hormone with its receptor begins a series of cellular events that either eventually lead to activation of enzymatic pathways

Table 2 Vertebrate hormones

Glands/hormone	*Abbreviation*	*Source*	*Major biological action*
Adrenal glands			
Mineralocorticoids			
Aldosterone		Zona glomerulosa of adrenal cortex	Sodium retention in kidney
11-Deoxycorticosterone	DOC	Zona glomerulosa of adrenal cortex	Sodium retention in kidney
Glucocorticoids			
Cortisol (hydrocortisone)	F	Zona fasciculata and z. reticularis of adrenal cortex	Increases carbohydrate metabolism; antistress hormone
Corticosterone	B	Zona fasiculata and z. reticularis of adrenal cortex	Increased carbohydrate metabolism; antistress hormone
Dehydroepiandro-sterone	DHEA	Zona reticularis of adrenal cortex	Weak androgen; primary secretory product of fetal adrenal cortex
Ovaries			
Estradiol		Follicles	Uterine and other female tissue development
Estriol		Follicles	Uterine and mammary tissue development
Estrone		Follicles	Uterine and mammary tissue development
Progesterone	P	Corpora lutea, placenta	Uterine development; mammary gland development; maintenance of pregnancy
Testes			
Androstenedione		Leydig cells	Male sex characters
Dihydrotestosterone	DHT	Seminiferous tubules and prostate	Male secondary sex characters
Testosterone	T	Leydig cells	Spermatogenesis; male secondary sex characters
Peptide and protein hormones			
Hormone	***Abbreviation***	***Source***	***Major biological action***
Adipose tissue			
Leptin (Ob protein)		Adipocytes	Regulation of energy balance
Adiponectin		Adipocytes	Modulates endothelial adhesion molecules
Plasminogen activator inhibitor-1	PAI-1	Adipocytes	Regulation of vascular hemostasis
Adrenal glands			
Met-enkephalin		Adrenal medulla	Analgesic actions in CNS
Leu-enkephalin		Adrenal medulla	Analgesic actions in CNS
Gut			
Bombesin		Neurons and endo-crine cells of gut	Hypothermic hormone; increases gastrin secretion
Cholecystokinin (pancreozymin)	CCK	Duodenum and CNS	Stimulates gallbladder contraction and bile flow; affects memory, eating behavior
Gastric inhibitory polypeptide	GIP	Duodenum	Inhibits gastric acid secretion
Gastrin		G-cells of midpyloric glands in stomach antrum	Increases secretion of gastric acid and pepsin
Gastrin-releasing peptide	GRP	GI tract	Stimulates gastrin secretion
Ghrelin		Stomach mucosa/GI tract	Regulation of energy balance
Glucogon-like peptide-1	GLP-1	L cells of intestine	Regulates insulin secretion
Motilin		Duodenum, pineal gland	Alters motility of GI tract
Secretin		Duodenum	Stimulates pancreatic acinar cells to release bicarbonate and water
Vasoactive intestinal polypeptide	VIP	GI tract, hypothalamus	Increases secretion of water and electrolytes from pancreas and gut; acts as neurotransmitter in autonomic nervous system
Peptide YY	PPY	GI tract	Regulation of energy balance/food intake
Heart			
Atrial naturetic factor	ANF	Atrial myocytes	Regulation of urinary sodium excretion
Hypothalamus			
Agouti-related protein	AGRP	Arcuate nucleus	Regulation of energy balance

Continued

Table 2 Continued

Glands/hormone	*Abbreviation*	*Source*	*Major biological action*
Arg-vasotocin	AVT	Hypothalamus and pineal gland	Regulates reproductive organs
Corticotropin-releasing hormone	CRH	Paraventricular nuclei, anterior periventricular nuclei	Stimulates release of ACTH and β-endorphin from anterior pituitary
Gonadotropin-releasing hormone	GnRH	Preoptic area; anterior hypothalamus; suprachiasmatic	Stimulates release of FSH and LH from anterior pituitary
Gonadotropin-inhibiting hormone	GnIH	Species-dependent loci	Inhibits release of LH (in birds)
Kisspeptin	KISS	Arcuate and anteroventral periventricular nuclei	Critical for normal puberty
Luteinizing hormone-releasing hormone	LHRH	Nuclei; medial basal hypothalamus (rodents and primates); arcuate nuclei (primates)	
Somatostatin (growth hormone-inhibiting hormone)		Anterior periventricular nuclei	Inhibits release of GH and TSH from anterior pituitary inhibits release of insulin and glucagon from pancreas
Somatocrinin (growth hormone-releasing hormone)	GHRH	Medial basal hypothala mus; arcuate nuclei	Stimulates release of GH from anterior pituitary
Melanotropin-release inhibitory factor (Dopamine)	MIF (DA)	Arcuate nuclei	Inhibits the release of MSH (no evidence of this peptide in humans)
Melanotropin-releasing factor	MRF	Paraventricular nuclei	Stimulates the release of MSH from anterior pituitary (no evidence of this peptide in humans)
Neuropeptide Y	NPY	Arcuate nuclei	Regulation of energy balance
Neurotensin		Hypothalamus; intestinal mucosa	May act as a neurohormone
Orexin A and B		Lateral hypothalamic area	Regulation of energy balance/food intake
Prolactin-inhibitory factor (Dopamine)	PIF (DA)	Arcuate nuclei	Inhibits PRL secretion
Prolactin-releasing hormone		Paraventricular nuclei	Stimulates release of PRL from anterior pituitary
Substance P	SP	Hypothalamus, CNS, intestine	Transmits pain; increases smooth muscle contractions of GI tract
Thyrotropin-releasing hormone	TRH	Paraventricular nuclei	Stimulates release of TSH and PRL from anterior pituitary
Urocortin		Lateral hypothalamus	CRH-related peptide
Liver			
Somatomedins		Liver, kidney	Cartilage sulfation, somatic cell growth
Angiotensinogen		Liver, blood	Precursor of angiotensins, which affect blood pressure
Ovaries			
Relaxin		Corpora lutea	Permits relaxation of various ligaments during parturition
Inhibin (folliculostatin)		Follicles	Inhibits FSH secretion
Gonadotropin surge-attenuating factor	GnSAF	Follicles	Control of LH secretion during menstruation
Activin		Sertoli cells	Stimulates FSH secretion
Pancreas			
Glucagon		α-cells	Glycogenolysis in liver
Insulin		β-cells	Glucose uptake from blood; glycogen storage in liver
Somatostatin		δ-cells	Inhibits insulin and glucagon secretion
Pancreatic polypeptide	PP	Peripheral cells of pancreatic islets	Effects on gut in pharmacological doses
Pituitary			
Adrenocorticotropic hormone	ACTH	Anterior pituitary	Stimulates synthesis and release of glucocorticoids
Vasopressin (antidiuretic hormone)	ADH or AVP	Posterior pituitary	Increases water reabsorption in kidney
β-endorphin		Intermediate lobe of pituitary	Analgesic actions

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Table 2 Continued

Glands/hormone	*Abbreviation*	*Source*	*Major biological action*
Follicle-stimulating hormone	FSH	Anterior pituitary	Stimulates development of ovarian follicles and secretion of estrogens; stimulates spermatogenesis
Growth hormone	GH	Anterior pituitary	Mediates somatic cell growth
Lipotropin	LPH	Anterior pituitary	Fat mobilization; precursor of opioids
Luteinizing hormone	LH	Anterior pituitary	Stimulates Leydig cell development and testosterone production in males; stimulates corpora lutea development and production of progesterone in females
Melanocyte-stimulating hormone	MSH	Anterior pituitary	Affects memory; affects skin color in amphibians
Oxytocin		Posterior pituitary	Stimulates milk letdown and uterine contractions during birth
Prolactin	PRL	Anterior pituitary	Many actions relating to reproduction, water balance, etc.
Thyroid-stimulating hormone (thyrotropin)	TSH	Anterior pituitary	Stimulates thyroid hormone secretion
Placenta			
Chorionic gonadotropin	CG	Placenta	LH-like functions; maintains progesterone production during pregnancy
Chorionic somatomammotropin (placental lactogen)	CS (PL)	Placenta	Acts like PRL and GH
Testes			
Müllerian inhibitory hormone	MIH	Fetal Sertoli cells of testes	Mediates regression of Müllerian duct system
Inhibin (folliculostatin)		Seminiferous tubules (and ovaries)	Inhibits FSH secretion
Activin		Sertoli cells	Stimulates FSH secretion
Thyroid/parathyroid			
Calcitonin	CT	C-cells of thyroid	Lowers serum Ca^{2+} levels
Parathyroid hormone	PTH	Parathyroid gland	Stimulates bone resorption; increases serum Ca^{2+} levels
Thyroxine (tetraiodothyronine)	T_4	Follicular cells	Increases oxidation rates in tissue
Triiodothyronine	T_3	Follicular cells	Increases oxidation rates in tissue
Parathyroid-related peptide	PTHrP	Parathyroid gland (and other tissues)	Regulation of bone/skin development
Thymus			
Thymosin		Thymocytes	Proliferation/differentiation of lymphocytes
Thymostatin		Thymocytes	Proliferation/differentiation of lymphocytes
Monoamine hormones			
Adrenal glands			
Hormone	***Abbreviation***	***Source***	***Major biological action***
Epinephrine (adrenaline)	EP	Adrenal medulla (and CNS)	Glycogenolysis in liver; increases blood pressure
Norepinephrine (noradrenaline)	NE	Adrenal medulla (and CNS)	Increases blood pressure
Central nervous system			
Dopamine	DA	Arcuate nuclei of hypothalamus	Inhibits prolactin release (and other actions)
Serotonin	5-HT	CNS (also pineal)	Stimulates release of GH, TSH, ACTH; inhibits release of LH
Pineal gland			
Melatonin		Pineal gland	Affects reproductive functions
Lipid-based hormones (eicosanoids)			
Hormone	***Abbreviation***	***Source***	***Major biological action***
Leukotrienes	LT	Lung	Long-acting bronchoconstrictors
Prostaglandins E_1 and E_2	PGE_1 and PGE_2	Variety of cells	Stimulates cAMP

Continued

Table 2 Continued

Glands/hormone	*Abbreviation*	*Source*	*Major biological action*
Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$	$PGF_{1\alpha}$ and $PGF_{2\alpha}$	Variety of cells	Active in dissolution of corpus luteum and in ovulation
Prostaglandin A_2	PGA_2	Kidney	Hypotensive effects
Prostacyclin I	PGI_2	Variety of cells	Increased second messenger formation
Thromboxane A_2	TX_2	Variety of cells	Increased second messenger formation

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or to a genomic response wherein the hormone acts directly or indirectly to activate genes that regulate protein synthesis. The newly synthesized proteins may activate or deactivate other genes, causing yet another cascade of cellular events see section 'Steroid Hormone' below.

When sufficient receptors are unavailable because of a clinical condition, or because previous high concentrations of a hormone have occupied all the available receptors and new ones have yet to be made, a biological response may not be sustained (see later). Such a reduction in the numbers of receptors may lead to a so-called endocrine deficiency despite normal or even supernormal levels of circulating hormones. For example, a deficiency of androgen receptors can prevent the development of male traits despite normal circulating testosterone concentrations. Conversely, elevated receptor numbers may produce clinical manifestations of endocrine excess despite a normal blood concentration of the hormone. Thus, in order to understand hormone–behavior interactions, it is sometimes necessary to characterize target tissue sensitivity (i.e., the number and type of receptors possessed by the tissue in question) in addition to measuring hormone concentrations.

Protein Hormones

Most vertebrate hormones are proteins. Protein hormones that comprise only a few amino acids in length are called 'peptide hormones,' whereas larger ones are called 'protein' or 'polypeptide hormones.' Protein and peptide hormones include insulin, the glucagons, the neurohormones of the hypothalamus, the tropic hormones of the anterior pituitary, inhibin, calcitonin, parathyroid hormone, the gastrointestinal (gut) hormones, ghrelin, leptin, adiponectin, and the posterior pituitary hormones. Protein and peptide hormones can be stored in endocrine cells and are released into the circulatory system by means of exocytosis. Protein and peptide hormones are soluble in blood, and therefore, do not require a carrier protein to travel to their target cells, as do steroid hormones. However, protein and peptide hormones may bind with other plasma proteins, which slow their metabolism by peptidases in the blood. Hormones are removed from the blood via degradation or excretion. The metabolism of a hormone is reported in terms of its biological half-life, which is the amount of time required to remove half of the hormone (radioactively tagged) from the blood. Generally, larger protein hormones have longer half-lives than smaller peptide hormones (e.g., growth hormone has 200 amino acids and a biological half-life of 20–30 min; thyroid-releasing hormone has three amino acids and a biological half-life of <5 min in humans). Again, a gut hormone such as cholecystokine (CCK) may function in a paracrine manner when released locally in the brain to affect behavior.

Steroid Hormones

The adrenal glands, the gonads, and the brain are the most common sources of steroid hormones in vertebrates. Vertebrate steroid hormones have a characteristic chemical structure that includes three six-carbon rings plus one conjugated five-carbon ring. In the nomenclature of steroid biochemistry, substances are identified by the number of carbon atoms in their chemical structure. The precursor to all vertebrate steroid hormones is cholesterol. The cholesterol molecule contains 27 carbon atoms (a C_{27} substance), although cholesterol itself is not a true steroid and can be stored within lipid droplets inside cells.

Because steroid hormones are fat soluble and move easily through cell membranes, they are never stored, but leave the cells in which they were produced almost immediately. A signal to produce steroid hormones is also a signal to release them. The range of responses can be a rather slow one: the delay between stimulus and response in biologically significant steroid production may be hours, although ACTH stimulates corticoid secretion within a few minutes and LH acts quickly to affect progesterone production during the periovulatory surge. In most cases, however, the signal to produce steroids is relatively slow; steroid hormones are not very water soluble. In the circulatory system, steroid hormones must generally bind to water-soluble carrier proteins that increase the solubility of the steroids and transport them through the blood to their target tissues. These carrier proteins also protect the steroid hormones from being

degraded prematurely. The target tissues have receptors for steroid hormones and accumulate steroids against a concentration gradient.

Upon arrival at the target tissues, steroid hormones dissociate from their carrier proteins and either interact with receptors embedded in the membrane or diffuse through the cell membrane into the cytoplasm or nucleus of the target cell, where they bind to cytoplasmic receptors. The amino acid sequence of steroid hormone receptors is highly conserved among vertebrates. Each steroid hormone receptor comprises three major domains: the steroid hormone binds to the C-terminal domain, the central domain is involved in DNA binding, and the N-terminal domain interacts with other DNA-binding proteins to affect transcriptional activation. Steroid receptors are kept inactive by the presence of corepressors (consisting mainly of heat-shock proteins (HSP)), which bind to the internal receptors and keep them inactive. It is the release of these HSP after formation of the hormone–receptor complex that activates the steroid receptor, and if not there already, the activated steroid–receptor complex is transported into the cell nucleus, where it binds to DNA sequences called 'hormone response elements' and stimulates or inhibits the transcription of specific mRNA. The effects of environmental, social, or other extrinsic or intrinsic factors on the regulation of specific coactivators have been understudied and represent yet another process by which individual variation in hormone–behavior interactions may be mediated. Environmental factors such as day length can determine whether photoperiod regulates whether steroids affect physiological and behavioral processes via slow (hours to days) genomic or fast (seconds to minutes) nongenomic pathways. For instance, in beach mice, estrogen rapidly ($<$15 min) increases aggression in short-but not long-day mice. This suggests that estrogen increases aggression via nongenomic actions on short days, but not on long days. Moreover, gene chip analyses indicated that estrogen-dependent expression of genes containing estrogen response elements in their promoters was decreased in the brains of short-day mice compared with that of long-day mice suggesting that the environment regulates the effects of steroid hormones on aggression in by determining the molecular pathways that are activated by steroid receptors. Transcribed mRNA migrates to the cytoplasmic rough endoplasmic reticulum, where it is translated into specific structural proteins or enzymes that produce the physiological response.

Thus, the actions of steroids on target tissues are based on three factors: (1) the steroid hormone concentrations in the blood, (2) the number of available receptors in the target tissue, and (3) the availability of appropriate coactivators. Blood concentrations of steroid hormones are also dependent on three factors: (1) the rate of steroid biosynthesis; (2) the rate of steroid inactivation by catabolism, which occurs mainly in the liver; and (3) the 'tenacity' (affinity) with which the steroid hormone is bound to its plasma carrier protein. Recently, it has been determined that different 'types' of steroid receptors exist. For example, three versions of the estrogen receptor (α, β, and γ) are currently recognized. Multiple versions of steroid receptors represent another mechanism by which responsiveness to steroid hormones can be regulated. Also, it appears that the brain can produce steroid hormones de novo and that the local effects of these steroids can have dramatic behavioral effects without altering blood concentrations of these hormones. These paracrine effects of steroids in the brain present special challenges to assessing hormone–behavior interactions.

How Might Hormones Affect Behavior?

All behavioral systems, including animals, comprise three interacting components: (1) input systems (sensory systems), (2) integrators (the central nervous system), and (3) output systems, or effectors (e.g., muscles). Again, hormones do not cause behavioral changes. Rather, hormones influence these three systems so that specific stimuli are more likely to elicit certain responses in the appropriate behavioral or social context. In other words, hormones change the probability that a particular behavior will be emitted in the appropriate situation. This is a critical distinction that affects conceptualization of hormone–behavior relationships. For example, female rodents must adopt a rigid mating posture (called 'lordosis') for successful copulation to occur. Females only show this posture when blood estrogen concentrations are high coincident with the maturing ova. Females adopt the lordosis posture in repsonse to tactile stimuli provided by a mounting male. Estrogens affect sensory input by increasing the receptive field size in sensory cells in the flanks. Estrogen affects protein synthesis, the electrophysiological responses of neurons, and the appearance of growth-like processes on neurons in the central nervous system, thus altering the speed of processing and connectivity of neurons. Finally, estrogen affects the muscular output that results in lordosis, as well as chemosensory stimuli important in attracting a mating partner.

How Might Behavior Affect Hormones?

The female rodent mating posture example demonstrates how hormones can affect behavior, but, as noted previously, the reciprocal relation also occurs, that is, behavior can affect hormone concentrations. For example, chemosensory cues from males may elevate blood estradiol concentrations in females, and thereby stimulate proceptive or male-seeking behaviors. Similarly, male mammals that lose an aggressive encounter decrease circulating

testosterone concentrations for several days or even weeks afterward. Similar results have also been reported in humans. Human testosterone concentrations are affected not only in those involved in physical combat, but also in those involved in simulated battles. For example, testosterone concentrations are elevated in winners and reduced in losers of regional chess tournaments.

Types of Evidence for Establishing Hormone–Behavior Interactions

What sort of evidence would be sufficient to establish that a particular hormone affected a specific behavior or that a specific behavior changed hormone concentrations? Experiments to test hypotheses about the effects of hormones on behavior must be carefully designed, and, generally, three conditions must be satisfied by the experimental results to establish a causal link between hormones and behavior: (1) a hormonally dependent behavior should disappear when the source of the hormone is removed or the actions of the hormone are blocked, (2) after the behavior stops, restoration of the missing hormonal source or its hormone should reinstate the absent behavior, and (3) finally, hormone concentrations and the behavior in question should be covariant, that is, the behavior should be observed only when hormone concentrations are relatively high and never or rarely observed when hormone concentrations are low.

The third class of evidence has proved difficult to obtain because hormones may have a long latency of action, and because many hormones are released in a pulsatile manner. Also, some pharmaceutical grades of steroids (e.g., esterfied steroids) have been altered to remain in circulation longer than endogenous steroids. Pulsatile secretion of hormones presents difficulties with making hormone–behavior inferences. For example, if a pulse of hormone is released into the blood, and then is not released for an hour or so, a single blood sample will not provide an accurate picture of the endocrine status of the animal under study. Completely different conclusions about the effect of a hormone on behavior could be obtained if hormone concentrations were assessed at their peak rather than at their nadir. This problem can be overcome by obtaining measures in several animals or by taking several sequential blood samples from the same animal and averaging across peaks and valleys. Another problem is that biologically effective amounts of hormones are vanishingly small and difficult to measure accurately. Effective concentrations of hormones are usually measured in micrograms (μg, 10^{-6} g), nanograms (ng, 10^{-9} g), or picograms (pg, 10^{-12} g). The development of techniques, such as the radioimmunoassay, has increased the precision with which hormone concentrations can be measured, but because of the multiple difficulties associated with obtaining reliable covariant hormone–behavior measures, obtaining the first two classes of evidence usually has been considered sufficient to establish a causal link in hormone–behavior relations.

The unique conditions of the laboratory environment may themselves cause changes in an animal's hormone concentrations and behavior that may confound the results of experiments; thus, it has become apparent that hormone–behavior relationships established in the laboratory should be verified in natural environments. The verification of hormone–behavior relationships in natural environments is challenging, but useful for differentiating laboratory artifacts from true biological phenomena. Establishing hormone–behavior interactions in the field presents other challenges including difficulties in reliability, treatment with exogenous hormones, and recaptures for hormone determinations. These difficulties can be overcome by noninvasive hormone determinations (e.g., fecal steroid assays), but again, coordination between lab and field studies is needed for a full appreciation of hormone–behavior interactions.

See also: Field Techniques in Hormones and Behavior.

Further Reading

Balthazart J and Ball GF (2006) Is brain estradiol a hormone or a neurotransmitter? *Trends in Neurosciences* 29: 241–249.

Brosens JJ, Tullet J, Varshochi R, and Lam EW (2004) Steroid receptor action. *Best Practices in Research and Clinical Obstetrics and Gynaecology* 18: 265–283.

Chen C, Chang YC, Liu CL, Chang KJ, and Guo IC (2006b) Leptin-induced growth of human ZR-75-1 breast cancer cells is associated with up-regulation of cyclin D1 and c-Myc and down-regulation of tumor suppressor p53 and p21WAF1/CIP1. *Breast Cancer Research and Treatment* 98: 121–132.

Hadley M and Levine JE (2007) *Endocrinology.* New York, NY: Benjamin Cummings.

Kiran SK, Scotti M-AL, Newman AEM, Charlier TD, and Demas GE (2008) Novel mechanisms for neuroendocrine regulation of aggression. *Frontiers in Neuroendocrinology* 29: 476–489.

Nelson RJ (2005) *An Introduction to Behavioral Endocrinology.* Sunderland, MA: Sinauer Associates.

Nelson RJ and Trainor BC (2007) Neural mechanisms of aggression. *Nature Reviews Neuroscience* 8: 536–546.

Norris DO (2007) *Vertebrate Endocrinology.* San Diego, CA: Elsevier Academic Press.

Trainor BC, Lin S, Finy MS, Rowland MR, and Nelson RJ (2007) Photoperiod reverses the effects of estrogen on male aggression via genomic and non-genomic pathways. *Proceedings of the National Academy of Sciences of the United States of America* 104: 9840–9845.