Regulatory Patterns

11

Contents

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In this chapter we will present examples of endocrine regulation. A single hormone in the endocrine system is something like an individual in a family tree. Different sources exist which determine the creation and development of a single person, and his or her progeny are dependent not on him or her alone, but also on many other people.

That is like the release of a hormone. From everywhere, messengers touch the receptor of the hormone-producing cell, some force release, others retention, some new synthesis, others arrest. An endocrine cell has to integrate all these different influences and decide what to do. This cannot be achieved by intelligence, but by an interplay of control elements in the related signal transduction pathways of the different receptors or by complex interaction while activating genes, or both.

If a cell actually releases an hormone, the stimulating forces prevailed. These are, however, often blocked by high concentrations of the very same hormone, or by other, downstream hormones. For example, gonadotropin-releasing hormone (GnRH) triggers in the pituitary release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH); these trigger in the gonads estradiol and testosterone synthesis. The latter are, in turn, strong inhibitors of GnRH release and LH/FSH secretion.

11.1 Feedback Control

Figure 11.1 demonstrates a simple feedback control pathway. In the thyroid, on stimulation by thyroid-stimulating hormone (TSH), thyroxine (T_4) is synthesized and released (1 in Fig. 11.1). By T_4 and by triiodothyronine (T_3) derived therefrom through the action of deiodinase, however, TSH release in the pituitary is blocked (2 in Fig. 11.1). TSH release is stimulated by thyrotropin-releasing hormone (TRH; 3 in Fig. 11.1). This TRH release (4 in Fig. 11.1) is equally blocked by T_4 (5 in Fig. 11.1), whereby T_4 has to cross the blood–brain barrier.

T_4 synthesis is additionally controlled, for example, by circadian rhythms not shown here. TRH release in the brain is multiply influenced by other neurons and hormones: by serotonin (6 in Fig. 11.1) and by noradrenaline (7 in Fig. 11.1). TSH release is negatively influenced by (hypothalamic) dopamine (8 in Fig. 11.1) and somatostatin (9 in Fig. 11.1). Estrogens in the circulation enhance TSH release

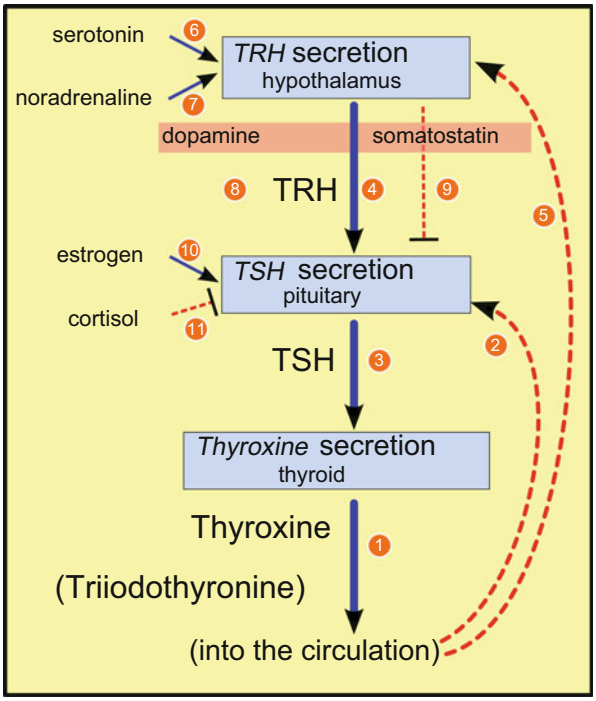

Fig. 11.1 Synthesis of thyroxine and its control. *Blue, continuous lines* indicate stimulation, *red, broken lines* indicate inhibition; *numbers* are explained in the text. *TRH* thyrotropin-releasing hormone, *TSH* thyroid-stimulating hormone

(10 in Fig. 11.1), and the glucocorticoid cortisol suppresses TSH formation (11 in Fig. 11.1).

In very similar ways, other hormones are regulated by different additional hormones, neuronal interactions, and other soluble messengers. In the following pages, we will provide several examples.

11.2 Regulator Circuits

11.2.1 Pressure and Stress

Stress was defined by Selye (1936, 1950) as "the nonspecific response of the body to any demand upon it." Hunger, injury, coldness, fear, cardiac arrest, exhaustion, capture, and arrest as well as social stress result, according to Selye, in the same unspecific reactions of the organism manifesting themselves in a strong elevation of the levels of glucocorticoids and adrenaline in the circulation. There might be specific reactions to any to these threats, called stressors; however, according to Selye, the unspecific aspect of the reaction should be called stress.

An easily comprehensible experiment with accountants was reported by Lennart Levi and colleagues (for an overview, see Levi 1989). The mode of payoff for the pay slip was changed in a daily manner. On odd days the payoff was due to the number of entries accomplished on that day; on even days there was no such check. Whereas on even days the workload remained as before, the quota increased by 14 % on odd days compared with even days. In parallel, adrenaline and noradrenaline were analyzed in urine. The amount of secreted catecholamines on odd days was 40 % above that on even days. Additionally 11 of 12 women reported fatigue, backache, and pain in the shoulders and arms—only on odd days. They felt hurried.

These women stressed themselves. They did not want to lose income, were especially motivated, but were overshooting and their organisms reacted to this psychic pressure. By psychic strain, obviously, stress can be induced. Stress can thus be regarded as a reaction of the central nervous system manifested in other organs.

Modern endocrinology at the dusk of the twentieth century took the physiology of stress as the interaction of two pillars: the hypothalamic–pituitary–adrenal axis, on the one hand, and the sympathetic nervous system, on the other. Both are coupled by mutual communications between centers in the paraventricular nucleus and the locus coeruleus.

In the paraventricular nucleus, parvocellular neurons synthesize corticotropin-releasing hormone (CRH), arginine vasopressin (AVP), or CRH and AVP. After CRH release into the median eminence portal system, ACTH and endorphins are released in the pituitary. This ACTH stimulates in the adrenal glands adrenaline production and release (see Sect. 7.1) and steroid hormone synthesis, above all synthesis of glucocorticoids.

In the locus coeruleus, noradrenergic neurons particularly stimulate sympathetic nerve cells. The locus coeruleus nerves project, for example, to the parvocellular neurons of the paraventricular nucleus, and these latter project to the noradrenergic neurons of the locus coeruleus in a mutual way; in this interaction CRH and AVP act as neurotransmitters. The entire system is further controlled by unknown zeitgebers—in any case CRH, AVP, and ACTH are released in one to three pulses per hour and in a daily rhythm where evening/night pulses are greater than those during the day (Fig. 11.2). Furthermore, many other neurons from brain areas and peripheral organs act via sympathetic and other nerves on the paraventricular nucleus and locus coeruleus neurons. The limbic system is tightly coupled to the stress response. Other hormones such as neuropeptide Y (NPY) and substance P are also involved: NPY stimulates CRH release and inhibits locus coeruleus neurons, whereas substance P acts in the opposite way (Strakis and Chrousos 1997).

The central role of the mutual interaction of the paraventricular nucleus and locus coeruleus has been questioned in recent years. In the last 10 years, several authors have reported on specific reactions to stressors which cannot be reconciled with the definition of Selye.

One aspect of stress regulation is the action of glucocorticoids in the brain. The patterns is most complex. Corticosterone and cortisol were observed in brain; released from the adrenal gland, they are transported by corticosteroid-binding

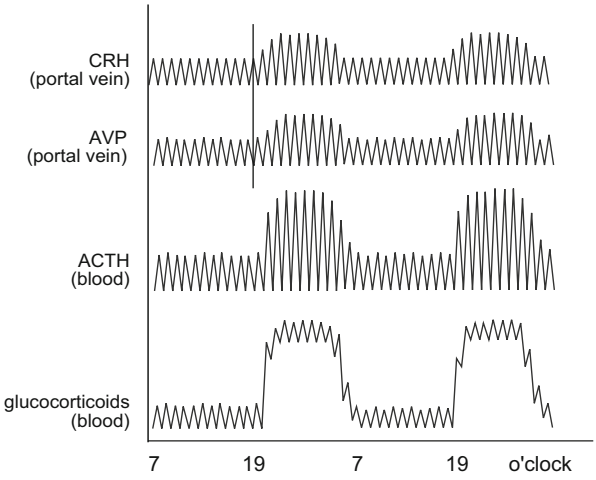

Fig. 11.2 Pulsatile and circadian activity of the hypothalamic–pituitary–adrenal axis. The pulse shift from corticotropin-releasing hormone (*CRH*) to arginine vasopressin (AVP) is indicated by the *vertical bar*. The amplitude changes in a circadian manner. Following the pulsatile CRH/AVP release, pituitary adrenocorticotropic hormone (*ACTH*) is released in pulses and stimulates adrenal synthesis and release of glucocorticoids. Elevated AVP/CRH pulses in the evening and during the night result in markedly enhanced glucocorticoid levels still oscillating with a frequency of 20 min (Redrawn from Chrousos 1998)

globulin or unbound (may be attached to albumin). Whether corticosteroid-binding globulin can cross the blood–brain barrier has not been analyzed; steroids, as far as we know, are able to do so. In the periventricular tissue around the blood capillaries, 11-β-hydroxysteroid dehydrogenase (11β-HSD) type 2 is strongly expressed, converting cortisol into the inactive 11-deoxycortisol (see Fig. 6.21).

In many animal species, corticosterone (Fig. 6.21) instead of cortisol is the active glucocorticoid. This is not converted by 11β-HSD type 2. Corticosterone, too, binds to corticosteroid-binding globulin, but with a somewhat lower affinity. Furthermore, cortisol is removed from cells of the blood–brain barrier by the multidrug resistance P-glycoprotein; this multidrug resistance protein serves, in general, to move toxic substances. Obviously, cortisol is regarded as something foreign, whereas corticosterone is not. Thus, there are two mechanisms which favor the use of corticosterone in the brain.

Corticosterone binds preferentially to mineralocorticoid receptor (MR) and with reduced activity to glucocorticoid receptor (GR); both nuclear receptors are transcription factors. To make the situation even more complex, hippocampus cells express another 11β-HSD, of type 1, which converts corticosteroid to cortisol, which, again, prefers to bind to GR (Kloet 2003). Whether 11β-HSD type 1 is widely distributed and whether its expression is required for glucocorticoid activity in the central nervous system (CNS) has not been reported.

These findings still leave open the question whether cortisol is active in the brain. Corticosterone might bind to MR and GR. Maintaining homeostasis is achieved via MR, whereas GR-induced activities seem to facilitate recovery after disturbances of homeostasis (Kloet 2003).

11.2.2 Calcium Metabolism

Since there are closed blood circuits, the free calcium concentration in blood is a fairly exact 1 mmol/l. This is exactly the calcium concentration in saltwater, from which it is inferred that all organisms are derived from saltwater organisms.

In humans calcium is taken up with the food (1 in Fig. 11.3), brought into the blood, and stored there complexed with albumin (2 in Fig. 11.3); albumin–calcium serves as a buffer for the blood calcium concentration. Calcium from blood is incorporated into bone (3 in Fig. 11.3), or filtered from blood by the kidney (4 in Fig. 11.3) and if required resorbed from the primary filtrate (5 in Fig. 11.3).

The calcium level is controlled by calcium concentration sensing receptors in the surface of parathyroid cells (6 in Fig. 11.3) or kidney cells (7 in Fig. 11.3). The sensor is a heptahelical G-protein-coupled membrane receptor. When the sensor is stimulated by elevated calcium concentrations, it activates phospholipase to enhance the level of inositol trisphosphate (IP_3); by this messenger, resorption in the kidney is inhibited and filtered calcium is secreted in urine (8 in Fig. 11.3). In the parathyroid gland, IP_3 inhibits the synthesis of parathormone (9 in Fig. 11.3).

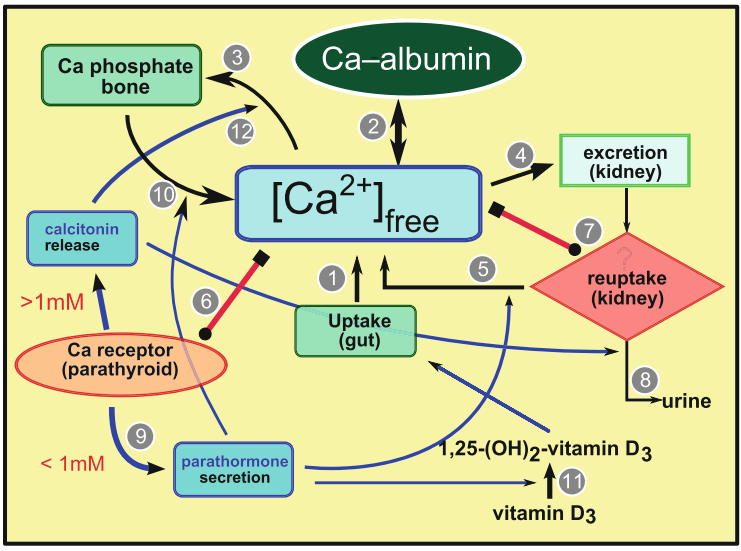

Fig. 11.3 Major components of calcium metabolism

If the calcium secretion leads to a deprivation of calcium, IP_3 release is blocked and resorption in the kidney and parathormone synthesis in the parathyroid gland are induced.

By parathormone, the level of available calcium is increased by desorption from bone (10 in Fig. 11.3). Simultaneously, the conversion of 25-hydroxyvitaminD_3 to 1,25-dihydroxyvitaminD_3 (calcitriol) (11 in Fig. 11.3) is triggered, which then allows uptake of calcium in the gut and its transport through the gut wall.

The antagonist of parathormone is calcitonin; its formation is triggered by elevated (greater than 1 mM) calcium levels. This hormone blocks resorption in the kidney and facilitates calcium incorporation into bone (12 in Fig. 11.3).

11.3 Regulation of Reproduction

Progeny and reproduction are among the most important characteristics of life. Since the debut of science, the study of these phenomena has occupied investigators. The differences between women and men have been obvious since primeval times, and children learn the functions of sexual organs at the latest in their first sex education at school.

The influence of hormones on reproduction is common knowledge to any woman taking or having taken the pill. The role of hormonal regulation during the menstrual cycle is less well known. The hormonal control of male reproductive capacities is almost unknown. The male sex hormone testosterone receives more attention when its abnormal levels are used to help defend a person accused of rape in court.

11.3.1 Regulation of GnRH

Reproductive rhythms are controlled via GnRH. Sexual activity in animals is allowed when sufficient food is available and the social fabric allows it; in many animals, a seasonal rhythm maintains control of sexual activity. Additionally, only pulsatile GnRH release induces pulsatile release of gonadotropins, which in turn regulate gonadal activity. These observations are reflected in the regulation of GnRH; neurotransmitters such as NPY, endorphins, CRH, and galanin are messengers of these environmental conditions which by controlling GnRH formation and release allow reproduction.

The perikarya of the GnRH neurons are present in the hypothalamus, mainly in the preoptic area and in the median eminence, and in addition in the lamina terminalis and septal area (Fig. 11.4). The former have been shown to be innervated strongly by other neurons, while the latter are only sparsely connected with other neurons.

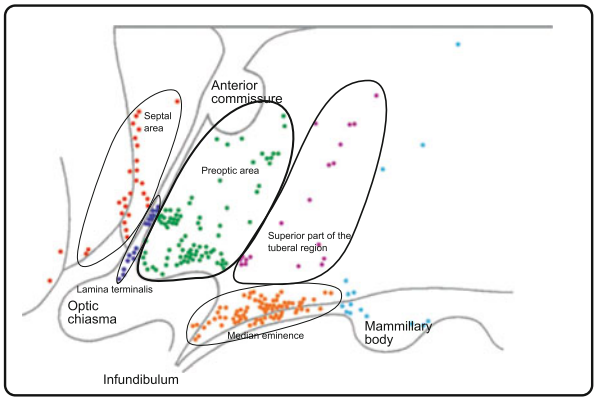

Fig. 11.4
Gonadotropin-releasing hormone (GnRH)-secreting neurons in the human hypothalamus. The perikarya of GnRH neurons are in the *labeled areas*. GnRH neurons are strongly innervated by other neurons, with the exception of those in the septal area (From Dudas and Merchenthaler 2006)

The following neurotransmitters influence GnRH neurons (Fig. 11.5): γ-aminobutyric acid (GABA), NPY, substance P, endogenous opiates such as endorphins and leu-enkephalin, CRH, galanin, catecholamines (dopamine and noradrenaline), and neurotensins (Dudas and Merchenthaler 2006) (Fig. 11.5).

Tuberoinfundibular dopaminergic (TIDA) neurons, which release dopamine, are important control elements for GnRH release. In those areas where TIDA neurons are found, there are also many NPY, galanin, endorphin, and substance P neurons, whereas CRH neurons are mainly located in the infundibulum and the preoptic area (Dudas and Merchenthaler 2006). TIDA neurons, furthermore, express estrogen receptor, allowing gonadal feedback inhibition of GnRH release (Mitchell et al. 2003). The latest addition to the list of GnRH regulators is kisspeptin (Messager et al. 2005).

Suppression of reproductive activity as a consequence of stress has been established in mammals. The central and peripheral stress systems are thought to play the prominent role: CRH directly suppresses GnRH release via synaptic contact of CRH axons to dendrites of GnRH neurons in the medial preoptic nucleus. There are differences between rodents and primates. Endogenous opiates (from proopiomelanocortin) can mediate several CRH effects, in part with respect to the menstrual cycle, to the species, or to the sex. Cytokines from the CNS are equally involved in GnRH regulation: IL-1 inhibits GnRH neuron activity and reduces GnRH synthesis and release. These IL-1 effects are mediated in part by endogenous opiates (endorphins and enkephalins) and by central prostaglandins.

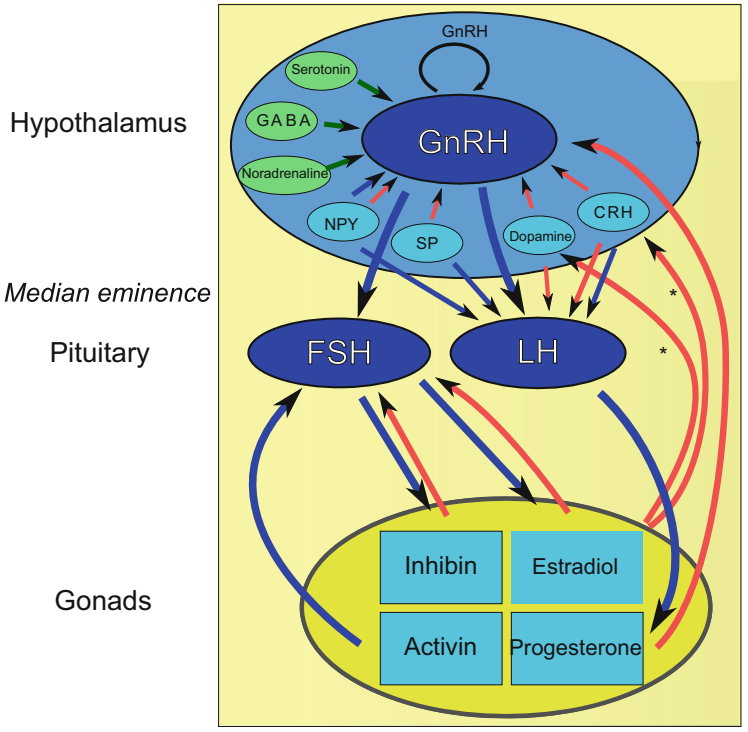

Fig. 11.5 Hormonal interplay for successful reproduction. *Blue arrows* amplifying endocrine secretion, *red arrows* mitigation of endocrine secretion, *green arrows* neurotransmitter activity [tuberoinfundibular dopaminergic neurons have been found to express estrogen receptors and may thus exert feedback control on gonadotropin-releasing hormone (*GnRH*) release], *asterisks* indicate that other neurons are potentially estrogen receptor positive and may participate in this feedback control, *CRH* corticotropin-releasing hormone, *FSH* follicle-stimulating hormone, *GABA* γ-aminobutyric acid, *LH* luteinizing hormone, *NPY* neuropeptide Y, *SP* substance P (From Dudas and Merchenthaler 2006)

11.3.2 Regulation of the Menstrual Cycle

Regulation of the female menstrual cycle occurs in four organs: in the hypothalamus, in the pituitary, in the ovaries, and in the uterus. We are talking about the hypothalamic–pituitary–gonadal axis.

The first essential variable indispensable to reproduction is *pulsatile release of GnRH* in the hypothalamus. There are only some thousand GnRH-secreting neurons in the mediobasal hypothalamus and in the preoptic nucleus. Triggered by γ-aminobutyric acid (GABA) and owing to mutual axonal connections of GnRH neurons, a coordinated secretion in the median eminence occurs. Whether galanin coexpressed in many GnRH neurons plays a role in GnRH secretion is an open question. GnRH secretion is reduced or entirely blocked by stress and its mediator CRH.

Without pulsatile release—that is, with continuously elevated or missing GnRH levels—LH/FSH release is efficiently blocked. The molecular mechanisms for this effect are unknown. Failure of GnRH receptor recycling and thus lack of cell surface expression of the GnRH receptors might explain short-term unresponsiveness of gonadotropic cells to GnRH. A possible block of synthesis has not yet been proven, but may be the simplest explanation for long-lasting LH/FSH suppression.

Because of this LH/FSH deficiency, continuous dosing of GnRH or GnRH analogs has been used for temporary contraception.[1]

Following pulsatile release of GnRH into the portal system of the infundibulum, LH and FSH release is induced in the pituitary gonadotropic cells.

FSH initiates maturation of cohorts of primordial ovarian follicles into primary and secondary follicles (meiosis). LH stimulates in theca cells around the follicles synthesis of testosterone, which after diffusion into the follicles is converted into estradiol. The largest follicle synthesizes amounts of estradiol which inhibit pituitary FSH release. In cows, and possibly in general, this follicle's growth is no longer dependent on FSH, but the growth of the other follicles still is, and thus they degenerate on lack of FSH. In addition to estradiol, the follicular fluid contains a protein heterodimer: inhibin. In concert with estradiol, inhibin blocks specifically pituitary FSH release, but not LH release, although both LH and FSH have been found together in the secretory granules. Inhibition is obviously due to blocking of FSH synthesis. Follistatin is also involved in this process.

Although only the dominant follicle grows further, androstenedione, testosterone, 17-hydroxyprogesterone, and estradiol concentrations in blood increase steadily. Shortly before ovulation there is a LH surge. Finally, the follicle bursts and liberates together with the oocyte its hormonal content. The estradiol stimulates growth of the uterine endometrium. This allows nidation of the fertilized egg within a restricted time window and which may grow into a new individual.

The corpus luteum develops from the remnants of the burst follicle. The corpus luteum provides progesterone, which inhibits degeneration and rejection of the uterine endometrium. Progesterone together with estradiol from the former follicle decreases hypothalamic GnRH release. This progesterone synthesis is maintained for only a few days and finishes if fertilization does not occur (see the next paragraph) after 7–8 days. As a consequence, blood supply to the endometrium is blocked, and the latter degenerates until it is rejected during the following menstruation. Without inhibition by estradiol and progesterone, hypothalamic GnRH release is reinitiated, FSH is made in the pituitary, and the next wave of follicles start to grow. The cycle restarts.

In the case of fertilization, the fertilized egg starts to divide while still in the ovarian duct. After the 32-cell stage, cells fuse to the morula, which gives rise to the blastocyst, with an outer layer of trophoblastic cells and the inner cell mass. The fetus develops from the inner cell mass, and the placenta develops from

[1] Permanent contraception with these analogs is not desirable owing to permanently repressed estradiol levels and the risk of osteoporosis.

the trophoblastic layer. The trophoblastic cells perform two roles: they mediate nidation into the uterine endometrium and they synthesize the pregnancy hormone choriogonadotropin. With this choriogonadotropin, progesterone synthesis in the corpus luteum is maintained, progesterone levels remain elevated, and degeneration of the endometrium is inhibited. As long as trophoblastic cells of the blastocyst and later in the placenta secrete choriogonadotropin, progesterone synthesis in the corpus luteum is ensured. In addition, progesterone blocks pulsatile GnRH release from the median eminence and LH/FSH release from the pituitary.

11.3.3 Puberty

Men and women are not fertile immediately postpartum. The reproductional competence is acquired later in life, in most individuals after the 11th to 13th birthday. Pituitary hormonal release and gonadal hormonal release in the newborn drop off within a few weeks. Menarche, the time of first ejaculation or first menstruation, is in the middle of a long period of sexual maturation starting with enhanced adrenal activity at the age of about 8 years, called adrenarche. First DHEA and DHEA sulfate secretion is enhanced, then 1–2 years later androstenedione secretion is enhanced, but the reasons for this change are still unknown. The formation of these androgens starts the growth of primary and secondary sexual organs and the development of pubic hair.

Up to puberty no pulsatile LH or FSH is measurable. The earliest measurable parameters are nightly FSH pulses strongly dependent on GnRH pulses. These GnRH pulses are induced centrally without any gonadal costimulation since puberty occurs in individuals without functional gonads. About 1 year after the first occurrence of FSH pulses, LH pulses can be measured. These FSH or LH pulses are not yet regularly timed and of constant amplitude as in adults, but are relatively rare and with largely varying frequency. As a reaction to these gonadotropin pulses and because of the development of Leydig cells in boys and the first growing ovarian follicles in girls, testosterone and estrogen levels in blood increase and they become measurable. Mood changes are explained by these irregular gonadotropin pulses and thus changing steroid levels. The first FSH pulses induce only incomplete follicular development, and ovulation does not yet occur. In the course of puberty, pulses are more regular, follicle development reaches later stages, and finally the first ovulation occurs. Menarche is reached.

In boys, the first ejaculation is not at the end of puberty, since regular GnRH pulses and coupled LH and FSH pulses are not as frequent and regular as in men, where about 18 pulses per day is normal. Enhanced FSH release forces the formation of seminiferous tubules. Under the influence of LH, precursor cells develop into Leydig cells, which mainly release testosterone.

The enhanced testosterone formation coinciding with enhanced growth hormone (GH) levels induces strong mood changes: the teenager shows—compared with childhood—an enhanced aggressive behavior although the environment, obviously, did not change: the “only” change is the maturation of the endocrine system.

Apart from the hormones of reproduction, other hormones, especially GH, insulin, and insulin-like growth factor (IGF) 1, are produced in greater amounts. With GH levels reduced, puberty is retarded.

11.3.4 Menopause

After the arrival of sexual competence, men and women are fertile for quite some time, until in women at the age of about 50 years menstruation fails to occurs. When pregnancy can be excluded—for example, in the absence of choriogonadotropin—then menopause has been reached. Since many follicles mature during the follicular stage of the menstrual cycle, with one or perhaps two arriving at ovulation, one assumes that the storage of egg cells/follicles capable of growing has been emptied. In 35 years there are about 13 cycles per year; in total 455 cycles. With 20 growing follicles per cycle, about 9,000 follicles have matured. Why the rest of the about 100,000 egg cells counted at birth do not mature is not known. About 220 follicles should mature per cycle in order to expend 100,000 primordial follicles within 455 cycles.

In men, sperms are not formed antepartum, but are first made during puberty. As long as the hormonal endowment in a man of 70 years—an age at which women are normally no longer fertile–allows there to be sufficient LH, FSH, and testosterone production, then the man is still fertile. For men andropause is presumed to be equivalent to menopause in women, but a fixed time point cannot be estimated.

Gamete development differs remarkably in men and women:

- By two meiotic divisions, four sperms arise, but a single haploid egg arises. Those chromosomes not utilized in women, which are called polar bodies, are removed from the egg.
- The first meiotic division happens in women already in utero; the second one happens after ovulation.
- In men both meiotic divisions occur during sperm development in the adult testis.

The frequency of genetically arising malformation in newborns rises with the age of both parents. There is no evidence that there is a sex bias in gamete errors. Obviously, several primary factors are balanced:

- Advantages in men: a single sperm of many fertilizes the egg, errors are diluted.
- Disadvantages in men: errors might be acquired in sperm stem cells during the long fertilization period.
- Advantages in women: after the first division an enrichment of reduplication mismatches does not occur; three quarters of the chromosomes will be removed.
- Disadvantages in women: a single egg is not selected from many other egg cells; once an error is there, it will be propagated.

In the past only the mother's age was reflected on when arguing for genetic counseling, but today the father's age is also taken into account.

11.4 Glucose Metabolism

11.4.1 The Origin of Blood Glucose

The organism derives glucose from three different sources: from food, from glucose stores, and from glucose synthesis:

1. *Glucose from food.* Glucose is taken up from the gut by a transporter protein which simultaneously takes up two sodium ions per glucose molecule (sodium–glucose cotransporter 1; Lee et al. 1994). The same transporter takes up other sugars as well.
2. *Glucose storage.* When in excess, glucose is stored as glycogen mainly in the liver. When glucose is needed, these stores can be emptied and glycogen can be converted into free glucose.
3. *Gluconeogenesis.* In an inverted process of glucose oxidation to CO_2, glucose can be newly generated from intermediates using energy. Gluconeogenesis mostly occurs in the liver.

11.4.2 Regulators and Control Variables

Insulin is the characteristic hormone of glucose regulation (see Sect. 4.7). Its antagonist is glucagon. Both hormones are released from specialized cells of the pancreas (see Sect. 10.6).

The control variable of glucose metabolism (Fig. 11.6) is the glucose concentration in blood. Dependent on the glucose concentration, there are two stages: *fasting* and *sated*. The threshold is 5 mM glucose. Below 5 mM there is fasting, above 5 mM, there is satiation.

11.4.3 Glucose-Dependent Gene Expression in the Liver

The liver switches between the two states depending on the glucose concentration: in the sated state, glucose is removed—by catabolization or by storage as glycogen; in the "fasting" state, glucose is synthesized or is provided by emptying glycogen stores. The regulating enzyme is the intracellular glucokinase, which converts glucose to glucose 6-phosphate (Glc6P), which is then used further. Intracellular regulation is possible owing to the high availability of glucose transporter 2 on the liver cell membrane (1 in Fig. 11.6).

On other cells there are glucose sensors from the family of glucose transporters: sodium–glucose cotransporter 3 (Diez-Sampedro et al. 2003). Owing to the activity of the sensors, there is a gene expression switch from glucose utilization to glucose production and vice versa (2 in Fig. 11.6). In the liver, at high glucose concentration, glucokinase expression is enhanced and thus Glc6P production is triggered, which leads either to catabolism or to glucose storage (3 in Fig. 11.6). When the glucose

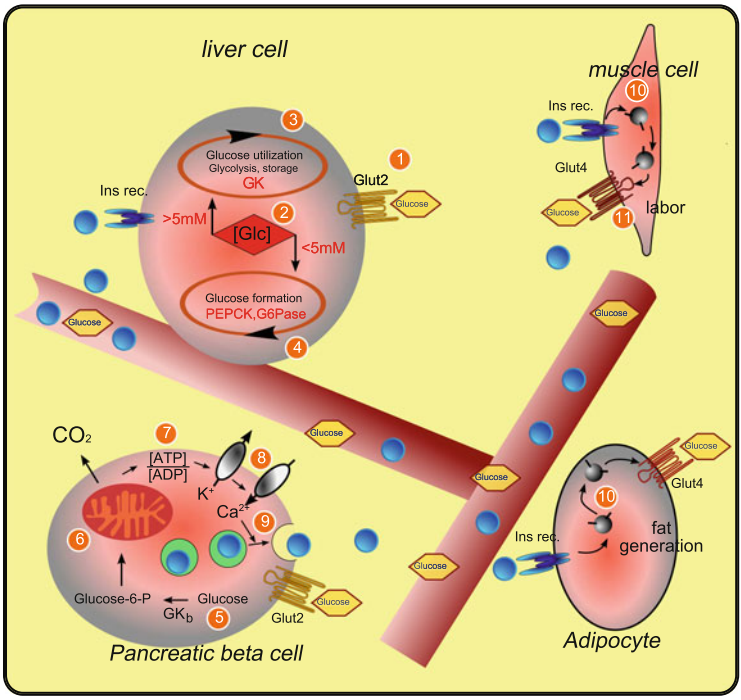

Fig. 11.6 Elements of glucose metabolism. *Blue spheres* insulin, *GK* glucokinase, GK_b glucokinase content of β cell, *Glc* glucose, *glucose-6-P* glucose 6-phosphate, *Glut* glucose transporter, *G6Pase* glucose 6-phosphatase, *Ins rec.* insulin receptor, *PEPCK* phosphoenolpyruvate carboxykinase

concentration is low, Glc6P is made available from stores or by gluconeogenesis, and the glucose produced following its cleavage is secreted into the circulation (4 in Fig. 11.6).

The rate-determining enzyme of glucose anabolism is phosphoenolpyruvate carboxykinase, whose expression is stimulated at low glucose concentration. For the conversion of Glc6P into glucose, glucose 6-phosphatase is required, and this also has a glucose-concentration-dependent expression.

Insulin has a role in glucose storage in the form of glycogen.

11.4.4 Glucose-Dependent Insulin Secretion in the Pancreas

Insulin is released in the pancreas at high glucose concentration:

1. Glucose is gated into pancreatic β cells by glucose transporter 2.
2. After phosphorylation of glucose to Glc6P (5 in Fig. 11.6), this Glc6P is catabolized in the mitochondria into CO_2 (6 in Fig. 11.6).

3. Since ATP is gained in the catabolism, the cytosolic ATP-to-ADP ratio is increased (7 in Fig. 11.6).
4. At an elevated ATP-to-ADP ratio, potassium ions are transported from the cell via a potassium channel (8 in Fig. 11.6).
5. This generates a voltage change at the membrane and induces the opening of calcium channels and an influx of calcium ions.
6. These in turn induce fusion of secretory insulin vesicles with the cell membrane (9 in Fig. 11.6), and the vesicle content is released. The released insulin acts as a hormone in the regulation of blood glucose.

11.4.5 Insulin-Dependent Procedures

In the liver, insulin stimulates Glc6P storage as glycogen. Additionally, insulin induces release of amino acids from liver cells to be used in muscle cells (insulin triggered) for protein synthesis.

In muscle cells, insulin triggers, mediated by insulin receptor (10 in Fig. 11.6), gating of preformed glucose transporter 4 from intracellular vesicles to the membrane, thus stimulating glucose uptake (11 in Fig. 11.6). The glucose taken up is used to support muscle activity.

Similarly, insulin triggers uptake of glucose into adipose cells mediated by glucose transporter 4, where glucose is used for fatty acid synthesis.

11.4.6 Glucagon and Blood Glucose Level Increase

Whereas at elevated glucose concentration insulin is secreted from β cells, at low glucose concentration pancreatic α cells secrete glucagon (see Sects. 4.7 and 10.6). In liver cells, glucagon stimulates protein kinase A, the key enzyme in gluconeogenesis and glycogen catabolism, both reactions enhancing the level of available glucose.

11.5 Appetite and Hunger

Human ingestion is controlled consciously and unconsciously (Fig. 11.7). We are able to control our eating behavior only in part: it is common experience that bursts of ravenous appetite send our good intentions packing. An autonomous regulation separated from conscious control (again in part) is active. The purpose of this control is a long-lasting constancy of body weight and therefore safeguarding of the individual's survival. To achieve this, food and energy uptake and energy consumption have to be balanced.

In this control system comprising the brain, adipose tissues, and gastrointestinal (GI) tract, hormones from the GI tract, from adipocytes, and from neurons are involved. The presentation of these circuits is limited to an overview owing to space restrictions; in addition, many details have not been fully and sufficiently analyzed.

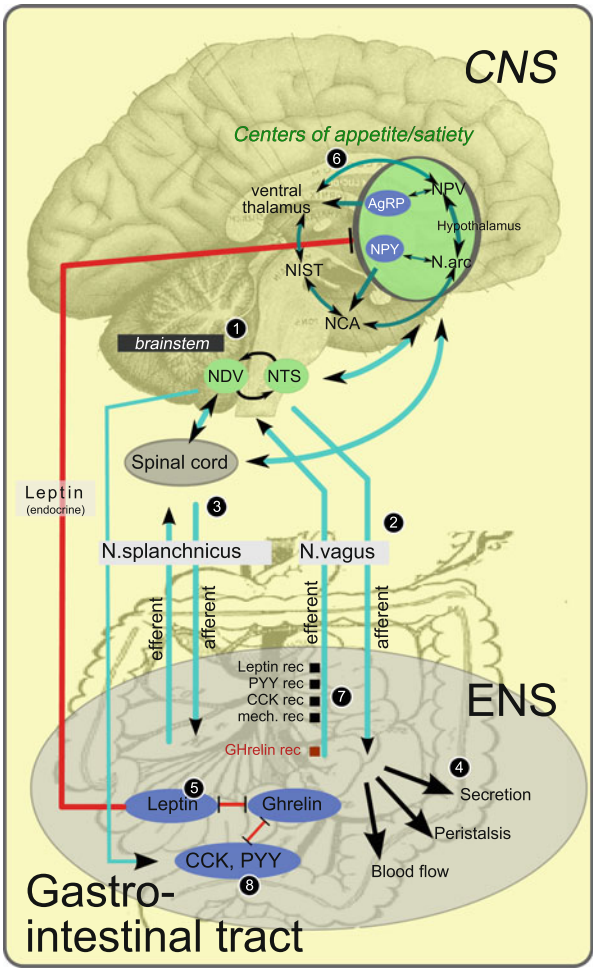

Fig. 11.7 Brain–belly interplay: regulation of appetite and hunger. *Red lines* inhibitory endocrine actions, *blue-green lines* control by nerves and synapses, *AgRP* agouti-related peptide, *CCK* cholecystokinin, *CNS* central nervous system, *ENS* enteric nervous system, *mech. rec* mechanoreceptor, *N.arc* arcuate nucleus, *NDV* dorsal motor nucleus of the vagus nerve *NCA* central nucleus of the amygdala, *NIST* bed nucleus of the stria terminalis, *NPV* paraventricular nucleus, *NPY* neuropeptide Y, *NTS* nucleus of the solitary tract, *PYY* peptide tyrosine tyrosine, *rec* receptor (From Konturek et al. 2004; Gray 1918, Fig. 715)

Konturek et al. (2004) reviewed the regulation of appetite and body weight.

11.5.1 Central Nervous System

In the CNS (Fig. 11.7) the nucleus of the solitary tract regulates gut secretion, gut movement, and blood supply. Satiety and appetite are controlled in different centers,

mainly in the arcuate nucleus and the paraventricular nucleus, as well as in the ventromedial and lateral hypothalamus. Also involved are the central nucleus of the amygdala, the interstitial nucleus, and the ventral thalamus.

11.5.2 Parasympathetic Fibers of the Vagus Nerve

Starting from the brainstem (1 in Fig. 11.7), the vagus nerve interconnects multiple organs of the GI tract. About 20,000 nerves connect to different cells of the GI tract.

11.5.3 Sympathetic Fibers of the Splanchnic Nerve

Synapses of the splanchnic nerve connect the GI tract with the brain (3 in Fig. 11.7).

11.5.4 Enteric Nervous System

A specialized nervous system is generated by about 100 million nerve cells of the enteric nervous system. These nerves stimulate and inhibit the entire GI events in an intrinsic way, whereas the vagus nerve and the splanchnic nerve interconnect the GI system with the brain. Secretion of digestion enzymes, salts, or hydrochloric acid, peristalsis, and the circulation are controlled by these nerves (4 in Fig. 11.7).

11.5.5 Endocrine Mediators and Neuropeptides

The following hormones are among those involved in the balance of hunger and satiety:

- *Leptin*. Leptin (see Sect. 4.8.1) is made in adipose cells, and the amount released is dependent on their number. Using leptin, adipose cells signal the nutritional status and contribute to the regulation of the body weight in the long run. Simultaneously, leptin is involved in short-term food intake since its release is elevated postprandially. Leptin acts not only in the brain (6 in Fig. 11.7), but also via a paracrine loop in adipose tissue (Jequier and Tappy 1999) as well as in the GI tract (Meier and Gressner 2004) (5 in Fig. 11.7). In the GI tract leptin antagonizes the actions of ghrelin, whereas in the hypothalamus, especially in the arcuate nucleus, the synthesis and release of NPY and agouti-related peptide (AgRP) are blocked. This leptin action is mediated by the long splice variant (OB-Rb) of the leptin receptor.
- *Cholecystokinin* (CCK). CCK (see Sect. 4.10) is made in the I cells of the duodenum. By binding of CCK to its receptors on the ends of the vagus nerve (7 in Fig. 11.7), satiety is signaled to the brain. Antagonistic receptor blockade results in prolonged feeding.

- *Peptide tyrosine tyrosine* (PYY). PYY (see Sect. 4.10) is most probably released under central control from endocrine gut cells. PYY acts antagonistic to ghrelin (8 in Fig. 11.7).
- *NPY*. NPY is one of the most intensively studied endocrine molecules of the third millennium: more than 5,600 articles published between 2000 and 2014 had a major focus on NPY. Central NPY is a major player in food intake, together with AgRP (see Sect. 4.3.4). NPY/AgRP knockout mice are fully viable and fertile, and do not exhibit anorexia; however, when NPY is administered intracerebrally, these knockout mice demonstrate all the symptoms related to foraging.

  NPY belongs to a family of proteins characterized by the pancreatic polypeptide fold (see Sects. 4.3 and 4.10 and Fig. 4.41). It is synthesized in hypothalamic neurons, mainly in the arcuate nucleus, but also in brainstem neurons projecting into the hypothalamus and in endocrine cells of the GI tract. In the brain it acts mostly as a neurotransmitter via synapses between neurons; furthermore, NPY is released by neurosecretory cells in the median eminence and acts in the pituitary.

  Apart from its role in food intake, NPY is involved in the hypothalamic–pituitary–adrenal axis by the control of CRH, it coregulates heart frequency via NPY-immunopositive neurons, and its acts in angiogenesis and wound healing.

  A signal sequence mutation of human NPY (Leu←Pro; see the framed **L** on the dark background in Fig. 4.40) results not in adiposity, but in elevated cholesterol levels and an enhanced risk of cardiovascular diseases. In Finland, about 14 % of the population is affected. In another study, not only NPY levels were decreased, but so were noradrenaline and insulin levels, and the blood glucose level was elevated. The heart frequency was enhanced.
- *AgRP*. AgRP binds, for example, to melanocortin receptors; in appetite regulation, melanocortin 4 receptor (MC4-R) has a special role. Formed in neurosecretory cells of the basal hypothalamus (in the arcuate nucleus), AgRP binds to MC4-R mainly in centers of satiety and appetite in the paraventricular nucleus and other hypothalamic nuclei as well as in the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the ventral thalamus. By these ligand–receptor interactions, food intake is blocked.

  MC4-R has different ligands: α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH) inhibit appetite, and AgRP and agouti protein enhance appetite. Melanocortin 3 receptors might also be involved in appetite regulation.
- *Ghrelin*. Ghrelin is a recently identified ligand for the long-known GH secretagogue receptor (GHS-R1), which when bound by small synthetic peptides and nonpeptide molecules triggers release of GH. GHS-R1a is a heptahelical membrane receptor; however, GHS-R1b, which is generated after alternative RNA splicing with retention of an intron with an early stop codon, is a heptahelical membrane protein with unknown function.

11.5.6 Hormone Receptors on Nerve Cells

Hormone and neuropeptide receptors involved in the control of the balance between hunger and satiety are present on a number of neurons in the ventromedial and arcuate hypothalamus (NPY receptor, MC4-R, leptin receptor) as well on the vagus nerve, signaling from the GI tract to the brainstem and to additional centers: PYY receptor, leptin receptor, CCK receptor, and GH secretagogue receptor.

11.5.7 Mechanoreceptors

The same vagus nerve signals extension and contraction of the stomach and gut wall to the brain with the help of mechanoreceptors.

Figure 11.7 illustrates how the regulation between the CNS and the enteric nervous systems occurs: hormones act on receptors present on nerve cells or on other cells with effector functions (acid secretion, enzyme production, muscle contraction).

The scheme sketches only a complex cross talk of endocrine and neuronal elements: in the GI tract peripheral signals, such as hunger-triggering peptides ghrelin and PYY, and satiety-inducing CCK or mechanoreceptors measuring stomach wall extension elicit in concert hunger or satiety, which ends food intake. Additional signals—for example, insulin secretion from the pancreas triggered by a carbohydrate diet—also induce satiation.

Signaling of satiety or hunger happens in the CNS. When the above-mentioned hormones enter the brain (leptin or insulin) or by neuronal connections act in the hypothalamus (PYY, CCK, ghrelin), neurotransmitter signaling is either stimulated or blocked. One example is AgRP, which by acting on MC4-R reduces appetite. Increased release of NPY, however, owing to elevated ghrelin levels, generates hunger. Since in order to maintain a constant body weight not only food intake but also energy consumption is relevant, the latter's parallel regulation of ingestion is expedient and necessary: sating neuropeptides with an action on energy consumption increase this consumption, whereas hunger-generating neuropeptides diminish it.

Apart from the interactions outlined, many other neuropeptides are involved in central regulation of appetite: for example, α-MSH, CRH, and orexins; monoamines such as serotonin also have a role.

The hormone leptin, which is released from white adipose tissue, is, in contrast to the other GI tract and pancreatic hormones/neuropeptides, an element of a long-term regulatory circuit. Its synthesis and release are dependent on the amount of fat, and thus provide a feedback between food intake and energy stores. To act in the hypothalamus (mainly in the arcuate nucleus), leptin has to enter the brain. Because it is a peptide of 16 kDa, passive diffusion across the blood–brain barrier is not possible. It has to be specifically gated across the blood–brain barrier. It could well be that the short splice variant of the leptin receptor (OB-Ra) fulfills this transport function.

During evolution organisms have been confronted with lack of food rather than with plenty of it. Adaptations have therefore evolved which react sensitively to lack of food and only insensitively to a surplus of food—that is, exceeding the required amount for homeostasis. The transport capacity for leptin is exhausted in humans and rodents of normal weight, so enhanced leptin levels in obese situations cannot be transmitted and do not find an adequate reaction in the hypothalamus. This effect is aggravated owing to a decrease of leptin transport capacity in obesity and consequently a further reduced signal transduction via OB-Rb. The result is the so-called leptin resistance.

Hunger is triggered by ghrelin in the GI tract and by ghrelin, NPY, and AgRP in the CNS. Ghrelin from the stomach acts via the vagus nerve on the CNS, where these ghrelin signals lead to NPY and AgRP release in the ventrobasal hypothalamus (arcuate nucleus). Ghrelin secretion from neurosecretory hypothalamic cells also contributes to NPY and AgRP release.

The functional antagonist of ghrelin is leptin: leptin blocks efficiently synthesis and release of ghrelin in the brain and in the GI tract and thus generates satiety. Ghrelin, in turn, blocks leptin. Leptin might penetrate the blood–brain barrier in order to reach in neurohemal organs, for example, the median eminence axons of ghrelin neurons and block its release there. In rats, it was shown that intranasally administered leptin reaches the CNS and can act there (Fliedner et al. 2006; Schulz et al. 2004).

In contrast to leptin, the other ghrelin antagonists act via receptors on the vagus nerve and not by direct action in the CNS. PYY is an effective blocker of hunger.

11.5.8 Feeding Circuits in the Brain

For a couple of years it has been well known that the lateral hypothalamic area (LHA) is crucial for the regulation of appetite and hunger:

- Numerous neurons in the LHA express melanin-concentrating hormone (MCH) and orexins (Jobst et al. 2004). These MCH/orexin neurons are blocked by synaptic contacts of proopiomelanocortin/cocaine- and amphetamine-regulated transcript (CART) neurons from the arcuate nucleus; the latter are stimulated by leptin. Leptin as a product of adipocytes thus signals into the LHA via the arcuate nucleus to the brain excess of food and blocks feeding by inhibiting the release of MCH and orexins from the LHA.
- The proopiomelanocortin/CART neurons, on the other hand, trigger the release of TRH and CRH from the paraventricular nucleus.
- In the arcuate nucleus there are some NPY/AgRP neurons expressing the leptin receptor which react to leptin. The fact that only the transcription factor SOCS-3, but not Fos, is stimulated by leptin in these cells is interpreted to mean that these neurons are inhibited by leptin. In the proopiomelanocortin/CART neurons, however, both SOCS-3 and Fos are found; therefore these cells are thought to be stimulated.

- AgRP and α-MSH compete for the same MC-R4, α-MSH being the agonist, AgRP the antagonist. In concert with NPY, AgRP stimulates hunger, whereas α-MSH blocks feeding, as leptin does. Ghrelin acts differently: it stimulates the AgRP/NPY neurons in the arcuate nucleus. NPY antagonists block the impact of ghrelin on the hypothalamus.

The role of the nucleus of the solitary tract on the regulation of food has long been underestimated:

- Being close to the area postrema where the blood–brain barrier is permissive, access of hormones to the receptors in the nucleus of the solitary trac is feasible.
- Gastrointestinal peptides such as CCK and pancreatic polypeptide as well as leptin bind to nucleus of the solitary tract receptors.
- In addition, pancreatic polypeptide, glucagon-like peptide 1, and leptin act via their receptors on the vagus nerve into the nucleus of the solitary tract.
- The melanocortin receptor is equally found in the nucleus of the solitary tract. When α-MSH or an agonists is injected intracebrally into the fourth ventricle, which is close to the nucleus of the solitary tract, feeding is blocked, whereas the injection of an antagonist induces feeding.

The nucleus of the solitary tract signals into the nuclear region of the nucleus accumbens, which is an important reward center. This action is mediated mainly by dopamine signals. When in the nucleus accumbens tyroxine hydrolase is missing, mice stop feeding. When dopamine is injected into the nucleus accumbens in these mice, they feed and favor tasty over unsavory food.

Endogenous opoids when injected in the nucleus accumbens stimulate the preference for sugar and lipid-rich food. Mutual GABAergic connections between the LHA and the nucleus accumbens possibly a have role in the choice of food. Since the nucleus accumbens bears MCH receptors, it might be influenced by synaptic contacts to MCH neurons of the LHA or by released MCH in an endocrine way.

Important players of the central food regulation are thus the arcuate nucleus, the paraventricular nucleus, the LHA, the nucleus of the solitary tract in the brainstem and finally the two regions of the nucleus accumbens in the basal forebrain. The hormones stimulating feeding are NPY, AgRP, ghrelin, MCH, and orexins; those blocking food uptake are mainly α-MSH and leptin.

11.5.9 Hunger and Food Intake in *Drosophila melanogaster*

NPY's role in food intake in vertebrates has an invertebrate analog: Neuropeptide F (NPF). Directly after molt, *Drosophila melanogaster* larvae are fixed on food intake, and some hours later they stop feeding and start wandering. Both stages, feeding and wandering, differ considerably in NPF expression: only feeding larvae express NPF in the four brain neurons and the pairwise neurons of the ventral cord; in wandering larvae these neurons are devoid of NPF. Using molecular techniques, Wu et al.

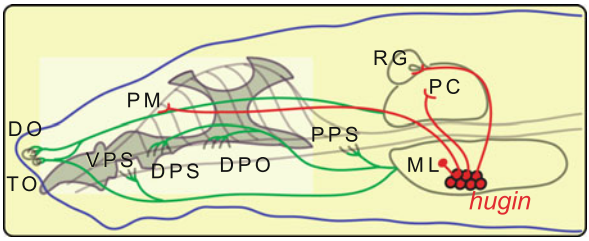

Fig. 11.8 Network of hugin neurons in the subesophageal ganglion. Hugin neurons, their expression restricted to the subesophageal ganglion, are in contact with taste nerves (*gray lines*): dorsal organ (*DO*), terminal organ (*TO*), dorsal pharyngeal sense organ (*DPS*), ventral pharyngeal sense organ (*VPS*), posterior pharyngeal sense organ (*PPS*), dorsal pharyngeal organ (*DPO*). Hugin neurons project to the maxillary lobe (*ML*), to the pharyngeal muscle (*PM*), to the ring gland (*RG*), and into the protocerebrum (*PC*) (*red lines*) (From Melcher and Pankratz 2005)

(2003b) demonstrated that overexpression of NPF in larvae prolongs the feeding phase, whereas elimination of NPF leads to avoidance of food. Absence of NPF induced aversion to a glucose-containing diet. In the laboratory, wandering larvae try to dig into agar. This behavior has also been observed in NPF-deficient larvae.

In their search for genes involved in the food intake of fly larva, Melcher et al. (2007) found two genes: *klumpfuss* (*klu*) and *pumpless* (*ppl*). Flies with defects in these genes are unable to bring food from the pharynx into the esophagus (Melcher et al. 2007; Melcher and Pankratz 2005). These flies die early from malnutrition. The gene product of *ppl* belongs to the glycine cleavage system; *klu* encodes a zinc finger protein, mainly expressed in developing nerves. In *klu* mutants, it was shown that the expression of several neuropeptides was increased: corazonin, NPF, hugin, adipokinetic hormone, pigment-dispersing factor, and cardioacceleratory peptide (CAP).

Hugin (the *D. melanogaster*[2] pyrokinin) is exclusively expressed in perikarya of the subesophageal ganglion. These neurons project into the central brain, to the ring gland (corpus cardiacum, corpus allatum), and to the pharyngeal muscle. The hugin neurons in fly larvae receive signals from several sensory organs (Fig. 11.8).

Sex peptide also stimulates food intake, at least in inseminated female flies. Sex peptide is generated in the testes, and during copulation is transferred with the sperms into the female. Activation of food intake facilitates vitellogenesis and egg deposition. When sex peptide is mutated in males (by molecular techniques), the females after copulation with these males do not lay eggs. And they are sought for copulation, as described before (see Sect. 5.4.3), by other males (Carvalho et al. 2006).

It is not yet understood how the different signals contribute to the complete situation. The relation among food intake, growth, and molting is discussed in Sect. 11.7.

[2]Drosophila melanogaster.

11.6 Growth

Embryonic growth requires an exact time and space control of gene expression, activator and inhibitor release, and secretion of hormones and other proteins, and a sufficient supply of maternal nutrients. During embryonic growth, the entire organism is to be built—bones and all other organs and body parts. Postpartum human growth, in the sense we discuss below, is mostly restricted to bone growth. Three stages are distinguished: the first one with rapid growth until the third year of life, then until puberty a stage with decelerated but steady growth, and during puberty again a stage with very rapid growth until the final body height is achieved.

11.6.1 Epiphyseal Cartilages

Bone growth occurs in the epiphyseal cartilages (Fig. 11.9). Here chondrocytes proliferate and thus enhance cartilage mass. Since this chondrocyte growth happens perpendicular to the bone in one bone slice, a new volume element is created as a

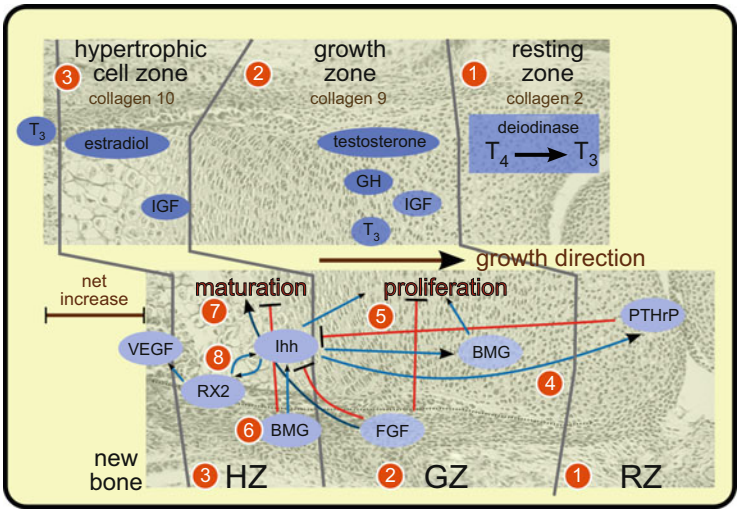

Fig. 11.9 Endocrine and paracrine control in the epiphysis. *In the upper part* the epiphyseal anatomy is depicted with the hormones active in the separate zones; *in the lower part* paracrine control by locally formed factors is depicted. For linear growth, the epiphysis has to move away from the bone's center. By the addition of new bone at the epiphyseal boundary (*left*), bone length increases. Thus, the epiphysis migrates. The epiphysis is marked by three zones: the rest zone (*RZ*), the growth zone (*GZ*), and the hypertrophic zone (*HZ*). Endocrine growth regulation is achieved by growth hormone (*GH*), insulin-like growth factor (*IGF*), thyroid hormone, corticoids, and sex hormone. A paracrine control is exerted by Indian hedgehog (*Ihh*), parathormone-related peptide (*PTHrP*), bone morphogenetic protein (*BMG*), and fibroblast growth factors (*FGF*). For the generation of new bone, vascular endothelial growth factor (*VEGF*) is required. *RX2* runx2, T_3 triiodothyronine, T_4 thyroxine (Adapted from Eerden et al. 2003; background image from Darl R. Swartz, Lafayette Center For Medical Education, Indianapolis, IN, USA)

disk in the bone and the bone's ends migrate away from each other. When you look at the X-rays of a child's hand, you will notice the epiphyses as dark cross sections: there appear more phalanges than are obvious, since in epiphyseal cartilages only cartilage is created, and this is permeable with regard to X-rays. X-ray-impermeable calcium phosphate is incorporated only at later stages.

11.6.2 Zonal Organization of the Epiphysis

The first zone, the *rest zone* (1 in Fig. 11.9), with the chondrocyte stem cells recognizable in small assemblies in sections is characterized by collagen type 2.

Close to the rest zone there is the *growth zone* (2 in Fig. 11.9). Therein chondrocytes divide and create the new volume. Since the newly formed cells cannot move to one side—owing to the rapid creation of extracellular matrix—the cells form columns. The growth zone cell are characterized by collagen type 9.

When chondrocytes have been dividing for some time, their volume strongly increases in the *hypertrophic zone* (3 in Fig. 11.9) before they die. Stimulated by vascular endothelial growth factor, blood capillaries extend into the space generated and osteoblasts migrate into the space. The latter form the new bone. The hypertrophic zone is characterized by collagen type 10.

11.6.3 Regulation by Hormones

GH, IGF1, the thyroid hormones thyroxine and triiodothyronine, glucocorticoids, and the sex hormones testosterone and estradiol are major regulators of bone growth. These act in an endocrine fashion on the hormone receptors on epiphyseal cells.

Within the epiphyses, proliferation and maturation of chondrocytes is additionally controlled by two paracrine factors—Indian hedgehog (Ihh) peptide and parathormone-related peptide (PTHrP), with its receptor—and furthermore by fibroblast growth factors (FGFs) from the transforming growth factor β (TGF-β) family, by heparan sulfate biosynthesis, by bone morphogenetic proteins (BMPs), and by a member of runt transcription factor family, runx2 (RX2).

GH release is triggered by GH-releasing hormone (GHRH) and ghrelin from somatotropic cells of the anterior pituitary and is inhibited by somatostatin. Its actions are partially direct, and in part are mediated by IGF1. Both the GH receptor and the IGF receptor need to be expressed in chondrocytes for regular bone growth. Both receptors enhance cellular division of stem cells and chondrocytes by facilitating mitosis and blocking arrest and maturation into hypertrophic chondrocytes.

There are two IGFs—IGF1 and IGF2, the latter mainly expressed before birth. They exert their functions in several control circuits:

- *Proliferation.* In fibroblasts, muscle, skin, epithelial, and bone cells, cells of male and female gonads, and several tumor cells, cell division is triggered by IGF1.
- *Cell death.* In hematopoietic cells and in some tumor cells, IGF1 blocks apoptosis.
- *Cellular differentiation.* Myoblasts, osteoclasts, osteoblasts, chondrocytes, neuronal cells, and adipocytes start to differentiate on being triggered by IGF1.
- *Cellular functions.* IGF1 actions have been described in endocrine and immune cells: IGFs stimulate hormone synthesis and release in theca and granulosa cells (see Sect. 10.8). They stimulate thymulin release from thymus epithelium. In the adrenal cortex, IGF1 enhances the number of membrane ACTH receptors and thus increases ACTH actions.

The actions of IGF1 are controlled by IGF-binding proteins (IGFBPs). IGFBPs lower the availability of IGF. In the epiphysis—in concert with IGF—they mediate mitosis and proteoglucan synthesis. These IGFBPs are in turn controlled by IGF, insulin, and TGF-β. The IGF receptor has been found on chondrocytes of the growth zone and the hypertrophic zone, but not the rest zone.

Thyroxine (T_4) is an important growth regulator. It is converted by deiodinase into the active triiodothyronine (T_3) (4 in Fig. 11.9). Deiodinase is expressed in the epiphysis and enhances by local conversion availability of T_3. The intracellular T_3 receptor is expressed in chondrocytes of the rest zone and growth zone and in osteoblasts of newly formed bone. T_3 stimulates chondrocyte maturation.

Glucocorticoids such as cortisol induce bone resorption and block bone-forming osteoblasts, which delays growth in children treated with glucocorticoids. Under normal conditions, glucocorticoids are required to block chondrocyte mitosis for T_3-induced chondrocyte conversion into hypertrophic cells. In the epiphysis, cortisol can be inactivated by a 11β-HSD.

The role of steroids appears obvious given that male sex hormones induce larger body sizes than do female ones.

The different control elements influencing the body form are far from well understood, whereas some major steps of sex-specific regulation have become apparent (Gatford et al. 1998).

The pulse frequency of GH release is effected by GHRH and somatostatin. There is evidence that in women only GHRH secretion and in men release of both GHRH and somatostatin are sex specifically controlled. In growing men compared with young women, this leads to more frequent pulses with higher amplitudes and lower nadirs. Likewise, the number of IGF and GH receptors differs with the sex as do the IGF1 levels and the concentrations of IGFBP. The synthesis of soluble GH-binding proteins (by cleavage of the membrane receptor) appears equally to differ with the sex.

In the epiphyses, estrogens and androgens have opposite functions: androgens stimulate chondrocyte growth (androgen receptor is expressed in the epiphysis), whereas estrogens stimulate chondrocyte maturation (Eerden et al. 2003) A young man with an estrogen receptor defect showed extreme growth owing to a failure to close the epiphyses. Two other individuals with aromatase defects showed similar growth. Whereas the latter two profited from estradiol substitution, the former was estradiol resistant. Sex-specific differences in the androgen–estrogen balance appear to be at the origin of different body sizes in men and women by directly influencing epiphyseal activity.

In addition to these systemic regulators, the paracrine roles of the transcription factor Ihh and PTHrP have been analyzed by Vortkamp et al. (1996) and others. The role of BMPs from the TGF-β family in heparan sulfate synthesis was also found recently.

Hedgehog proteins are so-called morphogens with indispensable function during embryonic morphogenesis. By binding to the Patched receptor, they release the protein Smoothened from Patched–Smoothened complexes, which then induces cellular reactions. Without hedgehog, Smoothened cannot be released.

The Ihh variant is expressed in chondrocytes becoming hypertrophic. Ihh diffuses in the direction of the bordering perichondrium at the epiphyseal margin and stimulates there via TGF-β PTHrP release from the rest zone (4 in Fig. 11.9). PTHrP in turn diffuses into the space in between the growth zone and the hypertrophic zone and inhibits Ihh expression (5 in Fig. 11.9). BMPs stimulate and FGFs inhibit this Ihh expression. Thus, BMPs block maturation of and stimulate chondrocyte proliferation by an Ihh-mediated BMP release in the growth zone (6 in Fig. 11.9). FGFs in turn inhibit proliferation and induce chondrocyte maturation (7 in Fig. 11.9). Furthermore, in an enhancer circuit, Ihh induces RX2 release and RX2 stimulates Ihh release (8 in Fig. 11.9).

Without control of Ihh release, dwarfism occurs. Ihh apparently controls the rate of chondrocyte maturation.

PTHrP had been related to abnormal calcification in tumors (Suva et al. 1987). Vortkamp et al. (1996) identified its role in the epiphysis.

The balance between Ihh and PTHrP has a decisive role in the control of growth, Ihh expression, and chondrocyte maturation. Mutants of Ihh, Patched, PTHrP, and their receptors impair normal growth.

11.7 Growth and Molt in Ecdysozoans

The body form of insects and crustaceans is determined not by an internal skeleton, but by an exoskeleton. This does not allow steady growth, and growth in these animals means rejection of the exoskeleton (molt) and formation of a new one. During this process, the new skeleton is formed first; thereafter, the animal leaves the old skin, and the new skeleton needs to harden, which is a lengthy process and may last several days in lobsters or several hours in dragonflies, during which time the animals are immobile and rather defenseless.

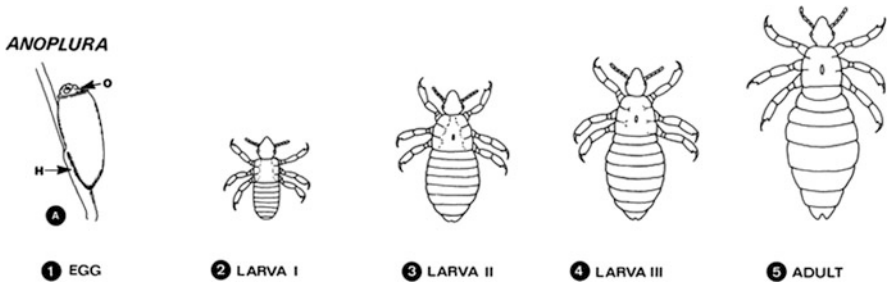

Fig. 11.10 Hemimetabolous development in lice (Anoplura). The egg (*O*) fixed on some hair (*H*) develops into a nymph, which molts twice into another, larger nymph. The third molt leads to the adult animal (From Mehlhorn 2001)

Crustaceans may molt without a limit to the number of molts. In insects several patterns can be distinguished:

- *Ametabolous insects.* The forms of the freshly molted insects resemble those of the adults. During molt it is only the size which increases, no additional organs develop, and gonads mature gradually.
- *Hemimetabolous insects.* These insects perform a partial metamorphosis. After hatching, the larvae, called nymphs, increase in size after each molt. The adult insect acquires by the last molting sexual maturation and, for example, wings. Hemimetabolous insects do not undergo pupation (Fig. 11.10).
- *Holometabolous insects.* Holometabolous insects undergo the characteristic pupation during their development. Larvae molt several times. The different stages are called instar; the fifth instar is the larva after the fifth molt. At the end of development, the larva pupates. The adult insect, the imago, ecloses from the pupa

In addition to insects and crustaceans, there are other invertebrates which molt. According to molecular genetics, Kinorhyncha, Loricifera, Priapulida, Nematoda (e.g., *Caenorhabditis elegans*), Nematomorpha, Lobopodia, Onychophora, Tardigrada, and Arthropoda share a common ancestor. All these have in common shedding of the exoskeleton. The superphylum is called Ecdysozoa.

The molting processes, and those processes during pupation, are controlled by hormones. Neuropeptides from the brain, juvenile hormone from the corpora allata, cholesterol-derived ecdysteroid from the prothoracic gland, and additional peptide hormones from the periphery are woven into a network of complex interactions.

Whether, how often, and when a larva/nymph undergoes molt depends on several environmental and intrinsic factors: Environmental factors include the temperature, the length of days or nights, and the richness of food supply and its composition. The intrinsic factors are, for example, synthesis of different hormones, such as insulin-like peptide (ILP) 2 and other ILPs in *D. melanogaster*, and synthesis of prothoracicotropic hormone (PTTH), the hormone that triggers ecdysone formation in the prothoracic gland or conversion of ecdysone into 20-hydroxyecdysone in the periphery. The process of molting is also controlled in an endocrine manner:

Generation of the new exoskeleton depends on ecdysteroids. Stripping of the old skin is also initiated by the decline in the amount of ecdysteroids. The movement whereby the animal removes its old skin is a specific behavior that is determined by hormones. In nematodes, for example, is has been shown that loss of one molt hormone blocks stripping of the exoskeleton, which ultimately leads to the animal's death.

Finally, the outcome of a molt is determined by hormones: whether the molt of a nymph again leads to the next nymph stage or whether it will be the final molt (eclosion) and lead to a sexually mature adult which acquires, for example, wings is dependent on hormones. In holometabolous species, pupation occurs before eclosion. There are, however, some hemimetabolous species with a final molt resembling pupation: the two last nymph stages of thrips (Thysanoptera) do not take up food and form a kind of cocoon. Some coccid nymph stages were also observed not to feed any further (Chapman 1998, p. 368).

11.7.1 Regulation of Growth in Insects

The life cycle of insect larvae usually has three phases: food uptake, wandering, and molting. The larvae cycle through these severalfold. The transitions are most interesting.

With use of molecular techniques it has been observed in *D. melanogaster* that the body length which a individual will reach is directly related to the expression of hormones, Especially, ILP, its receptors, and the insulin receptor substrate Chico are required for lipid utilization in flies. Without ILP, ILP receptor, or insulin receptor substrate there are developmental disturbances where larval molts are delayed and the final size is diminished. Overexpression of ILP, in contrast, results in an enhanced length. It is interesting to note that flies where ILP expression was reduced—for example, by destruction of certain ILP neurons in the brain—live longer than comparable wild-type flies. It is also noteworthy that such a phenotype of animals with defects of development and enhanced life span can equally be generated by feeding of yeast-deficient nutrition.

Short NPF controls ILP expression (see Sect. 11.5.9). Defect mutants with low or no short NPF expression are similarly development deficient and longer living than ILP- or ILP-receptor-defective animals. Overexpression of the transcription factor Foxo, which is downregulated by ILP in adipose cells, likewise enhances the life span of flies.

11.7.2 Hormones and Postembryonic Development

The hormone for development of ecdysozoans is ecdysone (Fig. 6.23, **55**), together with its derivatives. In insects, it is synthesized in the prothoracic gland, and in crayfish it is synthesized in the Y organ. Insects cannot generate cholesterol, the precursor of ecdysone; they have to acquire it together with their nutrition. Transition from one larval stage to the next is preceded and triggered by

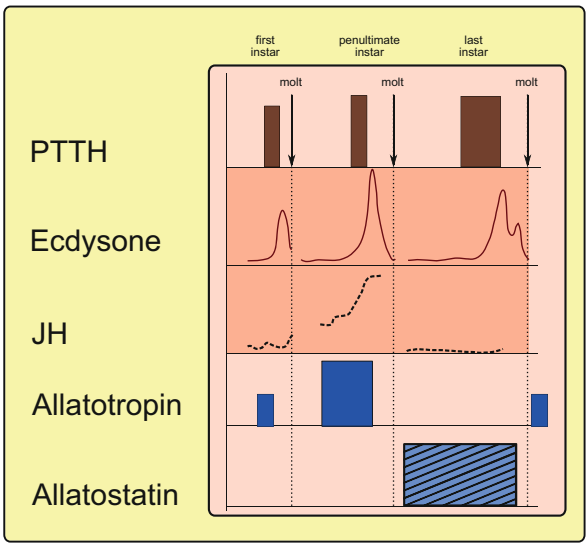

Fig. 11.11 Interactions of juvenile hormone (*JH*) and ecdysone: prothoracicotropic hormone (*PTTH*)

ecdysone bursts. Ecdysone-like compounds such as 3-dehydroecdysone, which is made in *Manduca* in parallel to ecdysone, are converted in the hemolymph into ecdysone. In peripheral tissue, mainly in the midgut, ecdysone is oxidized to 20-hydroxyecdysone (Fig. 6.23, **56**). This is the active hormone which binds to the ecdysone receptor, a nuclear receptor active as a transcription factor. Ecdysone synthesis is stimulated by PTTH.

Juvenile hormone (JH) is measurable during postembryonic development until the last larval or nymphal stage has been reached, then JH synthesis is downregulated. JH synthesis in the corpora allata is stimulated by allatotropin and is blocked by allatostatins (Fig. 11.11).

The synthesis of both terpenes—JH and ecdysone—is stimulated and repressed by neuropeptides. Allatotropin and PTTH facilitate and allatostatins and prothoracicostatic hormone suppress JH or ecdysone synthesis. PTTH induces prothoracic ecdysone synthesis by acting through its receptor Torso possibly via the extracellular-signal-regulated kinase pathway and also stimulates Ca^{2+}/calmodulin and cyclic AMP, whereas allatotropins act by hydrolysis of phosphatidylinositol. Both neuropeptides are released from a few brain neurons. By which stimuli allatotropin release is triggered is largely unknown. In adult flies (*Phornia regina*) it could be reproducibly observed that allatotropin is released 8 h after a protein meal. In larvae, however, such studies have not been reported.

The release of PTTH is coupled to the activity of the circadian clock since the zeitgeber neurons in *Rhodnius prolixus* (vector of trypanosomiasis; Chagas disease) were found in the direct neighborhood of PTTH neurons. That way, there is a circadian rhythm for PTTH release in these bugs, and for ecdysone synthesis and release. This rhythm is, in addition, dependent on daylight (Steel and Vafopoulou 2006; Vafopoulou et al. 2007). Whether the ecdysone levels in the hemolymph and peripheral tissues depend on this rhythm and whether other stimuli control the amount of ecdysone released is an open question.

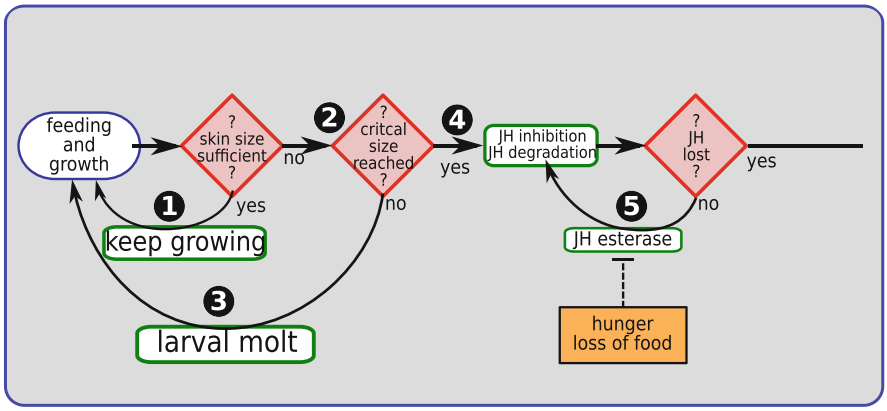

Fig. 11.12 Development of metamorphosis. *JH* juvenile hormone, *PTTH* prothoracicotropic hormone

All authors who have analyzed JH synthesis have stated that in the last larval stage of hemimetabolous insects, no JH could be found in the hemolymph. The corpora allata are still present, since in the adult animal JH is again synthesized and important for gonad maturation. There is thus a temporary JH release blockage possibly effected by allatostatins. The detailed analysis of these finding remains to be done.

11.7.3 Linkage of Growth and Metamorphosis

The question of when a holometabolous larva will pupate and when it will only undergo a larval molt is an unsolved problem of entomological endocrinology. Some years ago, it was observed that there is a critical size after which the insect can begin metaphorphosis and undergo a pupal molt. No biochemical basis for the determination of the critical size has been identified, except in hemipterans (*Dipetalogaster maximus*). There, Nijhout (1984) identified stretch receptors—that is, nerve cells that react to stretching. Similar mechanoreceptors are supposed to exist in the human atrium and to be involved in blood volume regulation via release of atrial natriuretic peptide (ANP). Stretch receptor neurons in the *D. maximus* abdomen signal to the brain. This release triggers an ecdysone pulse. If saline is injected into the abdomen of *Oncopeltus fasciatus* larvae, the PTTH–ecdysone axis can be triggered and large milkweed bugs developing after this treatment are miniaturized. In bloodsucking *Rhodnius prolixus*, a single blood meal can trigger abdominal stretch receptors to induce molting (or metamorphosis).

In the dung beetle *Onthophagus taurus*, PTTH and ecdysone are induced when dung has been taken up; depriving the larvae of the nutrition and thus bringing forth an early end of food intake can induce PTTH and later metamorphosis.

In the tabacco hornworm (*Manduca sexta*), PTTH release and ecdysone synthesis are negatively controlled by JH. After removal of the JH-forming corpora allata,

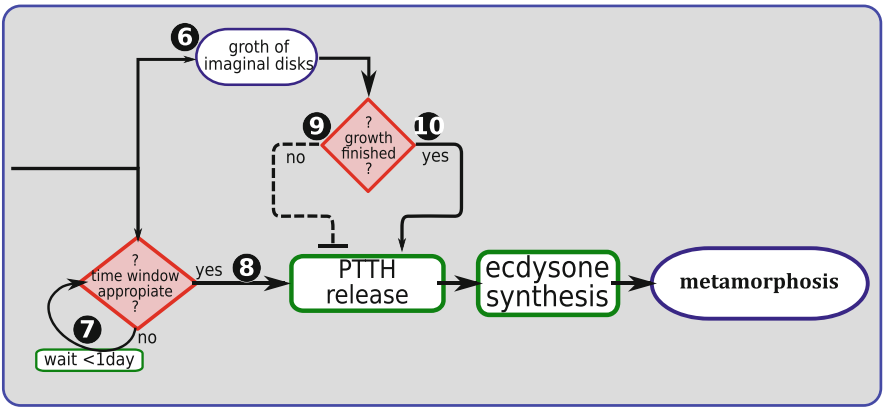

Fig. 11.12 (continued)

precocious metamorphosis occurs. On the other hand, JH administration delays metamorphosis. Once the critical size has been reached, JH generation is blocked by unknown mechanisms. In *M. sexta*, the cessation of JH synthesis occurs after the onset of feeding in the final larval instar and is thought to be due to both nervous inhibition and the loss of the methyltransferease such that the glands secrete only JH acid. Simultaneously, there is an increase in the activity of JH-degrading JH esterase, which converts JH I or JH II (Fig. 6.5, **24**) into the inactive acid (see Fig. 6.8). Expression of this enzyme is directly coupled to sufficient nutrition: when the larva fasts, JH esterase expression is blocked; with nutrients in surplus, JH is degraded and metamorphosis is initiated.

PTTH release in *M. sexta*[3] is permitted in a narrow time window; once this has passed, PTTH can be released only during the same period of the next day. Because PTTH neurons are in close contact with neurons of the circadian clock, a direct synaptic linkage is assumed.

During metamorphosis new organs develop from the imaginal disc. These do not develop as long as JH is present. After reduction of the JH levels with the help of JH esterase, the imaginal discs may mature. During their own development they signal with an unknown messenger that PTTH release should be suppressed. After reaching their final size, the imaginal discs stop this inhibitory signal for PTTH, and its release can occur.

Figure 11.12 summarizes the different control elements and developmental stages:

1. During the larval/nymph stage permanent control tests whether growth—that is, cellular division—is still possible. The "how" of this control is unknown; if growth is still allowed, feeding occurs.

[3] Manduca sexta.

2. If the skin has become to narrow, but the critical size has not yet been reached, a molt to the next larval stage is initiated.
3. Once the critical size is reached, JH synthesis and release are blocked (howsoever) and JH esterase inactivates remaining JH.
4. As long as JH is still present, the organism waits and lets JH be further reduced.
5. Once JH has gone, the inhibition of imaginal disc development is lifted.
6. Simultaneously, it is tested whether the time window is open for PTTH synthesis and release.
7. If it is not, it is necessary to wait until the next day.
8. If the time window is open, the signal for PTTH release is given.
9. If, however, the imaginal discs are not yet ready, this signal is postponed.
10. If all imaginal discs are ready, the block of PTTH release is finally abolished and PTTH can be released.

Stimulated by PTTH, ecdysone is synthesized and metamorphosis is initiated.

11.7.4 Regulation of Ecdysis

Initiated by ecdysone, a new epidermis is generated below the old skin. To trigger this, ecdysone is peripherally converted into 20-hydroxyecdysone. 20-Hydroxyecdysone interacts with a nuclear receptor dimer (ecdysone receptor/ultraspiracle[4]); the ligand–dimer complex migrates into the nucleus and acts as a transcription factor on ecdysone-responsive elements.

In the cases studied, PTTH release is brief at a particular time or in response to a particular stimulus such as stretch receptors in *R. prolixus* or *D. maximus*. It comes as a pulse and initiates ecdysone release from the prothoracic gland. Then it is thought, but it has not been proven, that ecdysone (or 20-hydroxyecdysone) acts on the prothroacic gland in a positive-feedback manner to increase the amount released. At high levels of ecdysone and 20-hydroxyecdysone, the feedback on the glands becomes negative and perhaps prothoracicostatic hormone and/or other peptides act to turn off the glands.

It has been observed that although the level of endogenous ecdysone decreases in the hemolymph, administration of exogenous ecdysone or 20-hydroxyecdysone delays ecdysis; this way, the process is regulated, but it has not been analyzed further.

When the ecdysone concentration in the hemolymph has dropped under a threshold, ecdysis is prepared. Corazonin is the first hormone known to be involved. It is expressed in lateral neurosecretory cells of the brain with axons into the corpora cardiaca and the corpora allata as well as onto neurons of the ventral nerve cord. There are no ecdysone-responsive elements in the corazonin gene. Therefore, a direct control of corazonin expression by ecdysone receptor is unlikely.

[4] An insect analog of retinoid X receptor

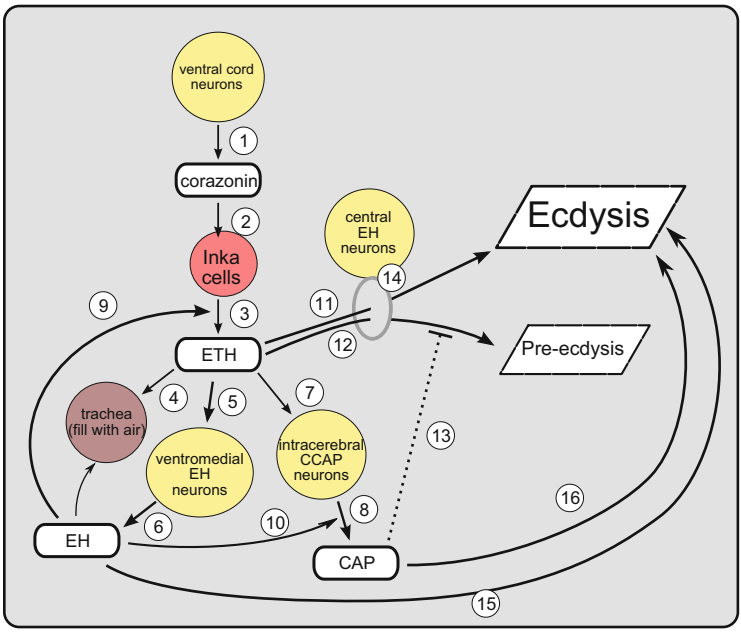

Fig. 11.13 Regulation of ecdysis. Neurons (*large circles*) or neuroendocrine cells (*small circles*) secrete hormones (*rounded rectangles*). These induce release of additional hormones (*solid lines*) or block their actions (*dotted lines*). *CAP* cardioacceleratory peptide, *CCAP* crustacean cardioacceleratory peptide, *EH* eclosion hormone, *ETH* ecdysis-triggering hormone (According to Clark et al. 2004 with additions)

To initiate ecdysis, a few neurons of the brain and the ventral cord secrete corazonin (1 in Fig. 11.13) into the hemolymph. In the Inka cells (2 in Fig. 11.13) in the vicinity of the trachea, corazonin binds to its receptors and stimulates release of ecdysis-triggering hormone (ETH).

ETH is formed in Inka cells stimulated by ecdysteroids, but is stored in vesicles. Release is triggered by corazonin (Zitnan et al. 2007; Zitnan and Adams 2005). This acts locally on the trachea (4 in Fig. 11.13), which—influenced by ETH and eclosion hormone (EH)—is inflated. Endocrine-active ETH acts on ventromedial EH-releasing neurons (5 in Fig. 11.13). This EH in turn empties the ETH stores of Inka cells to stop further ETH release before ecdysis occurs. Pre-ecdysis comprises the generation of the new exoskeleton and resorption of the old one, leaving only a marginal old skin. Further pre-ecdysis behavior is characterized in the tobacco hornworm (*M. sexta*), for example, by rhythmic contractions about 1 h before ecdysis by which the old skin is successively lost. For these contractions, pre-ecdysis-triggering hormone and ETH have to act directly on abdominal ganglia in the tobacco hornworm.

Once the level of ETH had been reduced, ecdysis can be initiated, which means the stripping of the old exuviae. ETH still acts on CAP neurons in the

pars intercerebralis (7 in Fig. 11.13), which—triggered by EH—release CAP (8 in Fig. 11.13). This CAP blocks pre-ecdysis (13 in Fig. 11.13) and stimulates ecdysis (14 in Fig. 11.13).

EH is an indispensable mediator of ecdysis: It enhances in a feedback loop ETH release from Inka cells (9 in Fig. 11.13). Additionally it controls the behavioral pattern of ecdysis (15 in Fig. 11.13), the typical and necessary contractions to strip the old exoskeleton. Characteristic of EH action is a strong increase of the level of cyclic GMP in neurons of the subesophageal, thoracic, and abdominal ganglia. Prothoracicostatic hormone/myoinhibitory peptide (allatostatin type B) acts together with CAP as a booster and initiator of the ecdysis program (16 in Fig. 11.13), which finally lets the larva or the adult leave the old cuticle.

The last step of molting, sclerotization of the new cuticle, is initiated by the hormone bursicon. This is mainly made in abdominal ganglia and released in neurohemal organs. Without bursicon, *D. melanogaster* fails to spread its wings (Dewey et al. 2004). Bursicon is coexpressed with CAP. Thus, EH-triggered CAP release might also induce bursicon release.

The photographs of the blue-eyed darner (Fig. 11.14) illustrate the process: the ecdyseal behavior leading to the shedding of the old cuticle and the sclerotization of the new cuticle.

11.7.5 Postembryonic Development in Holometabolous Species

In holometabolous insects, additional hormones act during metamorphosis.

As long as JH is synthesized and reaches its targets, development whereby new organs are formed from imaginal discs is inhibited. With the JH level too low to block gene expression, the molt program occurs. With the protein Methoprene-tolerant, a basic helix–loop–helix (bHLH) protein, identified as the JH receptor, the roles of Methoprene-tolerant, germ-cell-expressed bHLH-PAS, and ultraspiricle might become clearer.

Development from a larva to an imago includes destruction of old tissues and generation of new organs: whereas the butterfly larvae have stemmata (simple eyes), the imagines possess complex compound eyes. Compound eye development from an imaginal disc is inhibited by JH. This JH action is mediated by Methoprene-tolerant (Parthasarathy et al. 2008).

Wing formation also originates from imaginal discs. Gonad development is achieved at the transition from the larva to the imago. In some insects there are gills at larval stages which are lacking in the adult. Whereas larvae take in food by grating, the butterflies use a relatively long proboscis for taking up nectar. The development of these diverse organs has not been analyzed in such detail to exclude additional endocrine actions.

The nervous system also undergoes changes during metamorphosis. For example, corazonin neurons of *D. melanogaster* become apoptotic about 6 h after initiation of metamorphosis (Choi et al. 2006). Other neurons also undergo apoptosis, with the result of a visible shrinking of the ventral nerve cord.

Fig. 11.14 Blue-eyed darner (*Aeshna cyanea*) before, during, and after ecdysis

11.8 Regulation of Blood Pressure, Osmolarity and Blood Volume

Blood pressure and osmolarity are regulated by nerve cells as well as endocrine circuits. Osmolarity regulation (with individual thresholds of 280–295 mOsm) is mainly done by hormones. Cells close to the blood–brain barrier measure osmolarity via sodium channels.

11.8.1 Integration of Several Control Circuits

For hemostasis regulation, several circuits are entangled: pressure determination and pressure control, blood volume regulation, and control of osmolarity. Since pressure is a function of volume and osmotic pressure, such an interplay can be understood: with three circuits involved, it is not easy to provide an overview.

Blood pressure is determined in the aorta wall, in sinuses of the carotid arteries, and in the periphery. The receptors and the biochemical mechanism have not yet been identified. Many nerves transfer the information into the nucleus of the solitary tract, which in turn has nerve cells controlling contraction or dilatation of vessel wall muscles (the neuronal circuitry of baroreceptor and peripheral adjustment is called baroreflex); other neurons inform the locus coeruleus and the diagonal band of Broca, from where neurons project into the paraventricular nucleus and the supraoptic nucleus, inducing AVP release in the posterior pituitary.

Osmolarity is detected by osmoreceptors (sodium channels) at the blood–brain barrier. Neuronal interplay of the subfornical organ and the vascular organ of the lamina terminalis with the paraventricular nucleus and supraoptic nucleus increases or blunts AVP and oxytocin release. AVP stimulates renal expression of aquaporin 2 (Aq2) proteins, which resorb water from the primary urine. Furthermore, resorption of sodium from the urine is reduced, with an overall effect of reduced osmolarity.

Blood volume is measured in the atria. Stretch receptors have not been characterized. By the atria, ANP is released; ANP by reduction of water resorption and sodium resorption in the kidneys and by direct effects on blood vessels contributes to volume regulation.

Following the increased AVP effect on retention of water in urine, the urine salt concentration is enhanced. An increased sodium concentration in urine is measured in cells of the macula densa and leads to renin secretion from the nearby juxtaglomerular cells. From the angiotensinogen precursor in blood, renin cleaves angiotensin I, which is converted by angiotensin-converting enzyme into angiotensin II. Angiotensin II then stimulates adrenal aldosterone synthesis and release. Aldosterone, in turn, induces fusion of sodium channels to the apical membranes of renal tubuli with the effect of sodium back-resorption. Enhanced sodium levels in urine thus lead via renin, angiotensin II, and aldosterone to an enforced sodium resorption.

On the other hand, angiotensin II acts at the blood–brain barrier and triggers the neurons in the subfornical organ and the vascular organ of the lamina terminalis,

which then stimulate hypothalamic paraventricular nucleus and supraoptic nucleus magnocellular neurons to release AVP.

11.8.2 Osmoreceptors at the Blood–Brain Barrier

Nerve cells are separated from the circulation by myelin sheaths and by capillaries with thickened walls (mainly protoplasmic astrocytes surround the capillaries). There are only a few places in the brain where this blood–brain barrier is permeable. In Fig. 11.15 these areas are labeled by black circles with orange letters: the subfornical organ and the vascular organ of the lamina terminalis together with the area postrema and the choroid plexus are called circumventricular organs and are

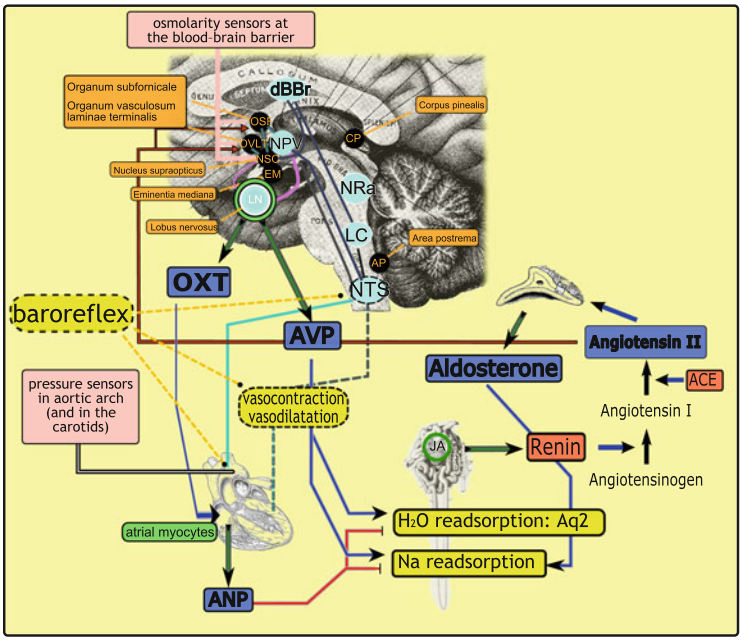

Fig. 11.15 Central and endocrine control of blood pressure and osmolarity. Hemostasis control occurs by action in concert of the brain, the brainstem, the heart plus the vessel system, the adrenal glands, and the kidneys. Several circuits are entangled: osmolarity, pressure (baro) reflex, and water balance. The major hormones are arginine vasopressin (*AVP*), aldosterone, angiotensin II, and atrial natriuretic peptide (*ANP*). Those areas where the blood–brain barrier is permeable and estimation of osmolarity via osmoreceptors or the transfer of molecules from blood to the brain is possible are labeled by *black circles* and *orange letters*. Major centers of hemostasis regulation are marked by *light blue circles*. Angiotensin II synthesis in the brain is not shown for reasons of clarity. Centers from where thirst originates are not yet known. *Blue arrows* endocrine stimulation, *red arrows* endocrine inhibition, *green arrows* regulation on axons and synapses. *ACE* angiotensin-converting enzyme, *Aq2* aquaporin 2, *dBBR* diagonal band of Broca, *JA* juxtaglomerular apparatus, *LC* locus coeruleus, *NPV* paraventricular nucleus, *NRa* raphe nucleus, *NTS* nucleus of the solitary tract, *OXT* oxytocin (Modified from Gray 1918; Krstić 1991; image by H.J. Heikenwälder)

Table 11.1 The atypical Na_x sodium channel

Name	Species	Tissue/cell type	Size (amino acids)	Gene	Chromosome locus	GenBank no.
Nav2.1	Human	Heart, uterus, muscle	1,682	*SCN7A*	2q21-23[a]	M91556
Na-G	Rat	Astrocytes	Partial sequence	*SCN7a*	3q21	M96578
SCL11	Rat	PNS (DRG)	1,702			Y09164
Nav2.3	Mouse	Heart, uterus, muscle	1,681			L36179

From Goldin (2001)
DRG dorsal root ganglion, *PNS* peripheral nervous system
[a]George et al. (1994)

located in the anterior ventricular part of the third ventricle (AV3V). Not labeled is the median nucleus of the preoptic region. In the supraoptic nucleus, median eminence and posterior pituitary as well as in the pineal gland and in the area postrema, the blood–brain barrier is opened.

Sodium channels (Table 11.1) detecting osmolarity are formed by a single protein: Na_x. This protein was known in humans and rodents for some time before its function was discovered. Owing to some structural and phylogenetic peculiarities, it was called voltage-dependent sodium channel type 2 (Goldin 2001). Watanabe et al. (2000) found Na_x expressed in the circumventricular organs and showed that Na_x knockout mice had a deficient liquid uptake. Other groups then confirmed these results (Grob et al. 2004; Hiyama et al. 2004, 2002). Hiyama et al. (2004) demonstrated by adenoviral transfer of an intact Na_x gene the repair of the defect in these knockout mice, thus proving that Na_x is necessary and sufficient for osmoreception in circumventricular organs.

Signals from the circumventricular organs also reach the paraventricular nucleus and the supraoptic nucleus, where magnocellular neurosecretory cells synthesize oxytocin and AVP, which are released in the posterior pituitary. The subfornical organ and the vascular organ of the lamina terminalis thus enhance oxytocin and AVP release.

At the same time the AV3V is connected to the raphe nuclei and the locus coeruleus by adrenergic nerves. From there the kidney is controlled by adrenergic nerves: circulation, resorption, and renin production, which, as described later, are also controlled in an endocrine manner.

11.8.3 Angiotensin II Receptors at the Blood–Brain Barrier

Angiotensin II is a key hormone of circulation control. It is derived by the endopeptidase actions of the renal enzyme renin on the angiotensinogen precursor and of angiotensin-converting enzyme on angiotensin I. Apart from stimulating adrenal aldosterone synthesis, angiotensin II plays an important role in control of AVP (and oxytocin) release, as demonstrated by the expression of angiotensin

receptor in neurons close to the blood–brain barrier and by the action of serum angiotensin II levels on the brain.

At the same time—but not depicted in Fig. 11.15—angiotensin II is expressed and posttranslationally modified in neurons of the AV3V. These angiotensin II neurons were identified in the subfornical organ, vascular organ of the lamina terminalis, and median nucleus of the preoptic region, and the axons reach the paraventricular nucleus and supraoptic nucleus. With angiotensin II administered intracerebrally, treated animals stop all other activities and search for water. Intracerebral angiotensin II is thus regarded as a thirst signal. On the other hand, AVP release and oxytocin release are initiated, which facilitates water resorption in the kidney, thereby reducing water loss.

11.8.4 AVP Release in the Posterior Pituitary

AVP release and oxytocin release from the pituitary are under neuronal control: Via synapses, membrane potentials are generated which are relayed to the axon ends in the posterior pituitary and which induce fusion of secretory vesicles with the plasma membrane, whereby the vesicle content is released into the pericapillary space and via sieve plates into the capillaries.

In addition from noradrenaline (sympathetic system), angiotensin II (from angiotensinergic nerves of the subfornical organ and vascular organ of the lamina terminalis) and GABA are active as neurotransmitters. GABAergic nerves from the diagonal band of Broca route signals from the locus coeruleus and the nucleus of the solitary tract. These signals have been generated by receptors estimating blood pressure in the aortic arch or in the carotid sinuses. The baroreflex includes AVP release mediated by the nucleus of the solitary tract and the locus coeruleus and neuronal feedback on the vessel musculature as well: by vascular constriction, blood pressure is promptly enhanced, or is decreased by vasodilatation as promptly.

11.8.5 The Role of Oxytocin

Oxytocin is released from the posterior pituitary in reaction to volume increases signaled by the nucleus of the solitary tract or locus coeruleus. Oxytocin can bind oxytocin receptors in the atrium. This leads to ANP release, which triggers renal secretion of sodium and potassium, leading to volume reduction. Oxytocin receptors have also been found in the kidney, which suggests a direct oxytocin effect on renal regulation of hemostasis. Not shown in Fig. 11.15 is the observation that ANP receptors are present on oxytocin neurons in the AV3V, which creates an enhancer loop against a volume increase.

11.8.6 Thirst and the Endocrine System of the Brain

After intracerebral administration of angiotensin II, treated animals stop all other activity and search for water (see Sect. 11.8.3). Since neurons in the subfornical

organ and the vascular organ of the lamina terminalis form angiotensin II themselves, we may assume that angiotensin II is the crucial signal in the CNS which signals the need for water. Those centers transforming the angiotensin II signal into conscious or unconscious activity have not yet been identified. In humans, enhanced activity has been observed in the cingulate cortex—that is, in the limbic system—by positron emission tomography with volunteers; in animals, the lateral parabrachial nucleus and the central nucleus of the amygdala are involved (McKinley and Johnson 2004) The lateral parabrachial nucleus in turn receives signals from the area postrema (where the blood–brain barrier is permeable) and from the nucleus of the solitary tract, where the signals of peripheral baroreceptors are integrated.

Since thirst originates prima facie from lack of water which cannot be compensated for by internal recruitment of water from interstitial spaces and by resorption, it is understandable that all mechanisms dealing with blood volume or osmolarity finally lead to the demand to add water from external sources.

11.8.7 Biochemistry of Water and Sodium Resorption

Blood is filtered in the renal glomeruli. The primary urine from the capsule of Bowman is transferred in the loop of Henle. Here and in the adjacent distal tubules, hormone-controlled resorption of water and ions occurs.

The expression and membrane positioning of the water transporter aquaporin 2 (Aq2) is positively controlled by AVP. This molecule is preformed in intracellular vesicles. On binding of AVP to its vasopressin receptor, vesicle fusion with the membrane occurs and Aq2 molecules allow water import into nephrocytes. On the basal side of nephrocytes, constitutively expressed aquaporin 3 and aquaporin 4 are present to transport the water back into the circulation. Without AVP, the Aq2 receptors are again internalized and may be transported back to the membrane if required.

Sodium resorption uses on the apical side the endothelial sodium channel (ENaC) consisting of three similar subunits (α, β, and γ). The usual sodium gradient between urine and cytosol is sufficient to transport sodium ions into the cell. On the basal side, sodium is pumped from the cell to the circulation by Na^+/K^+-ATPase, which pumps potassium into the cell. ENaC expression and Na^+/K^+-ATPase expression are stimulated by aldosterone. In addition to the amiloride-sensitive ENaC, there is a second sodium channel—the bumetanide-sensitive sodium cotransporter—which allows resorption of sodium ions, and also sugars, amino acids, phosphate, and sulfate. The expression of this molecule is also dependent on aldosterone.

ENaC receptors are further controlled by the binding of an ubiquitin ligase (Nedd4). Phosphorylation of ENaC blocks this binding and allows a longer presence of the channels in the apical membrane. The differential regulation of ENaC thus contains several hormone-controlled steps: transcription and translation triggered by aldosterone, membrane transport and complex formation controlled by AVP and ANP, and finally internalization and degradation by Nedd4.