—Review—

Steroid Hormones, their Receptors and Neuroendocrine System

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Abstract

The brain is an important target organ for circulating steroid hormones secreted from peripheral organs such as the adrenal cortex, testis and/or ovary. In other words, these peripheral organs control the central nervous system. Steroid hormones substantially influence brain development, reproduction, sexual differentiation, cognition, memory, behavior, and so on. These effects are mediated by steroid hormone receptors, which directly regulate gene expression. The steroid hormone receptor superfamily is an intracellular ligand-regulated transcription factor. All members, including the glucocorticoid receptors (GR), mineralocoroticoid receptors (MR), estrogen receptors (ER), progesterone receptors (PR) and androgen receptor (AR), mediate the expression of a gene by binding to hormone responsive elements (HREs) as dimmers in a ligand-dependent manner. In particular, steroid hormones have an important role for the regulating neurons and cells, which are associated with the neuroendocrine and endocrine regulation system, because many neuroendocrine neurons and cells express the steroid hormone receptors, such as estrogen receptor (ER), androgen receptor (AR) and corticosteroid receptors.

In this review, first the localization of GR and MR immunoreactivities in the brain is introduced, and secondly, the effects of change of GR expression in neurons are examined by several morphological approaches. Third, the interaction of GR expression and pituitary cell function is introduced. Finally, the recent topics on the control system of feeding regulation in the central nervous system, which also closely involves steroid hormone action, are discussed.
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Key words: steroid hormone, steroid hormone receptors, hippocampus, hypothalamus, pituitary

Introduction

Steroid hormones, when mediated through their receptors, play crucial roles in neuronal development and plasticity in vertebrates[1]. Their sites of action are primarily on the genes which regulate gene expression followed by protein synthesis. Although steroid hormones, as well as thyroid hormones, vitamin D and retinoid, are small hydrophobic molecules that differ in chemical structure and function, they all diffuse directly across cell plasma membranes and bind to intracellular receptors. These receptors are structurally related, constituting the nuclear receptor superfamily, and they are activated after the binding of their specific receptors[2]. The regulation of gene expression by nuclear receptors is mediated through the subcellular distribution of inactive receptors, redistribution of activated receptors to nuclear

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domains, and direct interaction between nuclear receptors and co-factors.

In the brain, the hippocampus and hypothalamus are the main targets of steroid hormones; through their receptors, steroid hormones affect these areas[3]. There are many physiological function neurons in the hippocampus and hypothalamus. Neuroendocrine regulation is an important function which occurs in these areas. Many neurons, which have neuroendocrine function, show the expression of steroid receptors, indicating that numerous neuroendocrine neurons are strongly regulated by steroid hormones. Steroid hormones may closely regulate not only function, but also the morphological construction.

In this review, the functional and morphological dynamics of neuroendocrine neurons and cells under different steroid conditions are discussed.

Distribution of Glucocorticoid Receptor (GR) and Mineralocorticoid Receptor (MR) Immunoreactivities in the Rat Brain

GR immunoreactive cells are widely distributed from the olfactory bulb of the forebrain to the gracile-cuneate nuclei of the medulla oblongata. The highest densities of GR immunoreactive cells are observed in the subfields of cerebral cortex, olfactory cortex, hippocampal formation, amygdala, septal region, dorsal thalamus, hypothalamus, cerebellar cortex, locus coeruleus and dorsal nucleus of raphe. On the other hand, MR immunoreactive cells are also well distributed in the hippocampus, hypothalamus, and cerebral cortex[4]. In particular, hippocampal formation is the main target region of corticosteroids, and both GR and MR are well expressed[5,6]. Colocalization of GR and MR has been observed by duel immunofluorescent method using a confocal laser scanning microscopy. GR and MR-immunoreactivities are colocalized in CA1 and CA2 pyramidal neurons and in the granule cells of the dentate gyrus, while MR immunoreactivity is seen in the CA3 and CA4 pyramidal neurons (**Fig. 1**). Colocalization of MR and GR immunoreactivities is also observed in the parvocellular, but not in the magnocellular region of the paraventricular nucleus (PVN). Subcellular distribution of GR is predominantly observed in the cell nucleus, and some endoplasmic reticulum and Golgi apparatus show a weak immunoreaction. No immunoreactivity is observed in the mitochondria, lysosome, matrix or cell membrane by immunoelectron microscopy. On the other hand, the subcellular distribution of MR is more cytoplasmic in comparison with that of GR-immunoreactivity, while the ratio of cytoplasmic to nuclear distribution of these receptors differs among regions. The effect of corticosteroids on the distribution of GR and MR immunoreactivities was examined by bilateral adrenarectomy (ADX) and corticosterone replacement after ADX. One week after ADX the nuclear GR immunoreactivity was completely diminished, whereas the MR immunoreactivity remained intact, just as that observed in the sham-operated rats (**Fig. 1**). ADX and corticosterone replacement did not induce marked changes in the localization pattern or profile in the MR immunoreactivity in the cells of the CA3, CA4 pyramidal neurons[4-6].

Change of Morphology of Pyramidal Neurons under Different Corticosteroid Conditions

Intracellular labeling with lucifer yellow (LY) was performed to analyze the morphological changes of hippocampal neurons at 2 weeks after ADX and with replacement of corticosterone after ADX; special attention was given to the subpopulation of the hippocampus, CA1, CA3, and the dentate gyrus. The quality of the LY intracellular injection was evaluated on the basis of the filled fine structures such as the dendritic spines and terminal tips[7]. Confocal laser scanning microscopic examination of LY-filled cells revealed that the CA1 pyramidal cells and dentate granule cells of ADX rats appeared to have smaller dendritic trees with few dendritic branches and segments than those of sham-operated (control) rats, however the morphology of CA3 pyramidal cells was similar between the sham and ADX rats. In contrast, the ADX+CORT2w rats showed no decrease in dendritic trees of CA1 pyramidal cells and dentate granule cells. However CA3 pyramidal cells of ADX+CORT2w rats

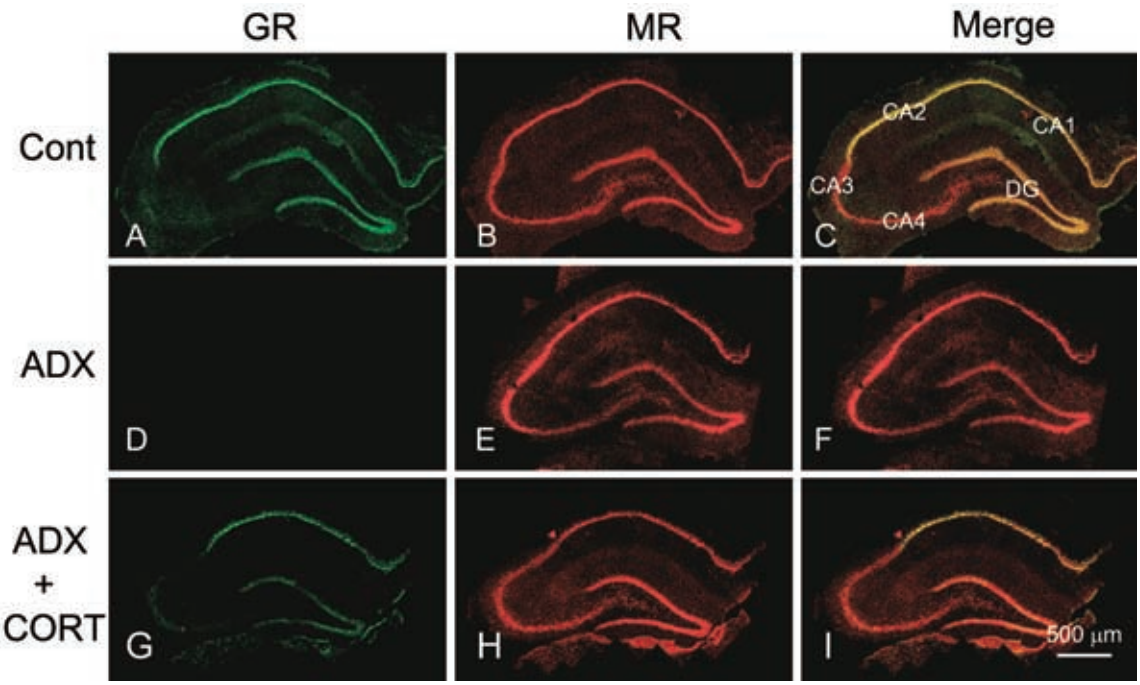

Fig. 1 Dual-immunohistochemical images for the GR (green) and MR (red) immunoreactivity in the hippocampus. The merged image (yellow) showed the colocalization of GR and MR in the CA1 and CA2 subregion of pyramidal cells and granular cells of the dentate gyrus in the sham-operated control (A, B, C), ADX (D, E, F) and ADX and corticosterone replacement (G, H, I) experimental groups. Only GR immunoreactivity disappeared under the ADX condition (D), while MR immunoreactivity did not change (E). Bar = 500 μm.

appeared to have larger dendritic trees with more dendritic branches and segments compared to the sham and ADX rats; these qualitative differences were particularly significant in the basal dendritic trees of CA3 pyramidal cells (**Fig. 2**).

High voltage electron microscopy (HVEM) is a useful tool for analysis of three-dimensional ultrastructure, and in particular for the stereographic observation of the spines of dendrites[8,9]. Typically, a high density (number) and various types of spines with an enlarged head and thin tail are observed in the CA1 and CA2 of pyramidal neurons under normal (control) conditions. After ADX, the density (number) decreases and the shape of spines is clearly changed. However, these morphological changes are recovered by corticosterone replacement (**Fig. 3**). These morphological changes are not observed in the CA3 and CA4 pyramidal neurons, which do not express GR.

Interestingly, similar morphological changes after ADX are observed in CA1 and CA2 pyramidal neurons with aging. HVEM stereo-observation was used to evaluate the dendritic spine density of CA1 neurons in young (2 months) and aged (24 months) rats. HVEM revealed a significant reduction in the number and the density of dendritic spines both in the apical and basal dendrites of CA1 pyramidal neurons with some transformations in the aged rats (**Fig. 4**), suggesting that aging causes several structural changes in neurons, particularly in dendrites and their spines in the hippocampal pyramidal neurons. These morphological alterations may be involved in behavioral and cognitional conditions with aging.

Glucocorticoids and Pituitary Cells

It is well known that the hypothalamo-pituitary-adrenal axis forms a feedback system and this axis shows sensitive responses to changes of circulating corticosterone content. In the hypothalamo-pituitary axis, GR immunoreactivity has been observed in hypothalamic neurons containing corticotropin

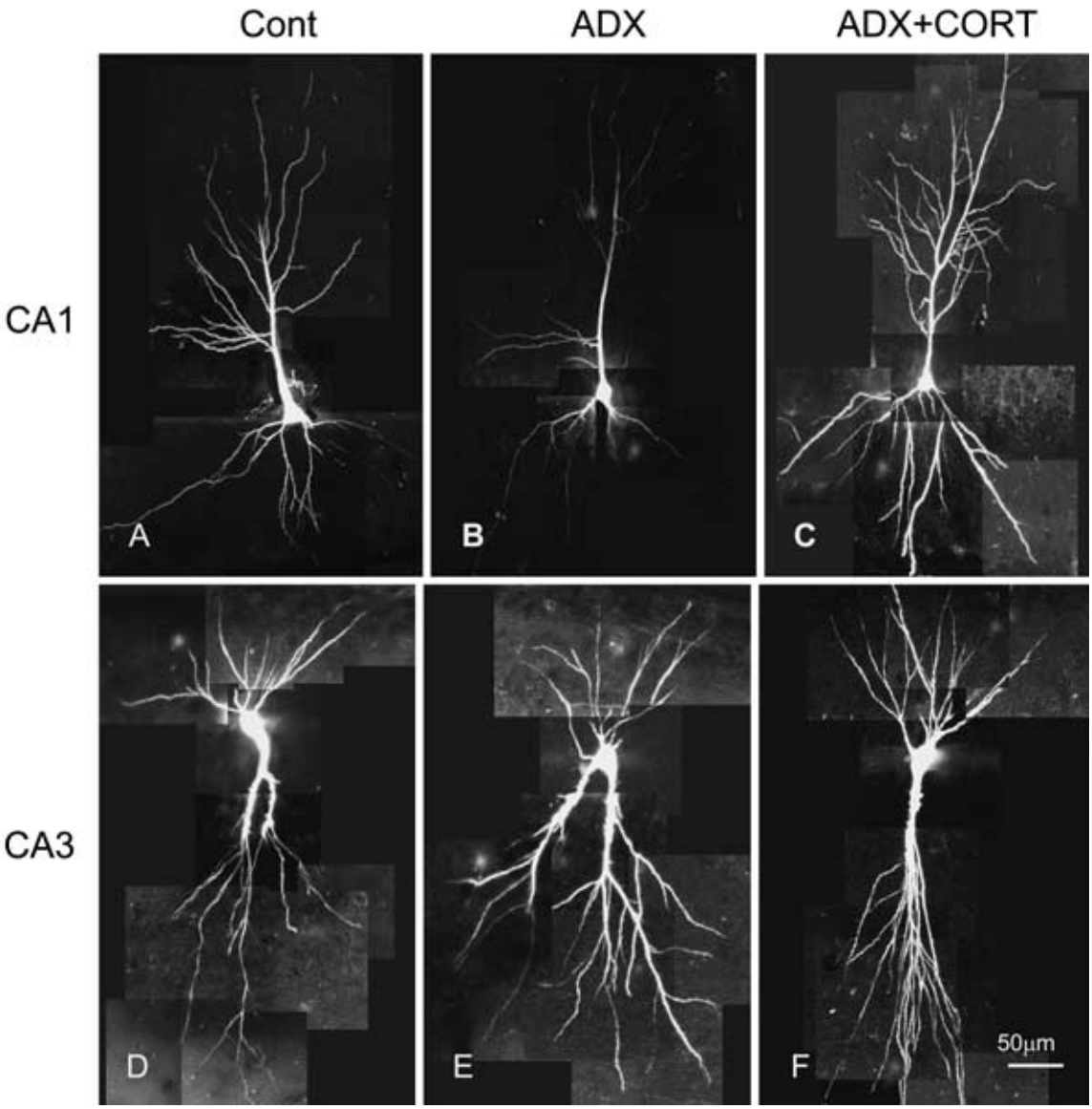

Fig. 2 Photomicrographs of hippocampal neurons intracellularly labeled with Lucifer yellow in CA1 pyramidal cells (A, B, C) and CA3 pyramidal cells (D, E, F). A decrease in the number of dendritic branches observed in CA1 pyramidal neurons after ADX (B) and recovery was observed in the ADX + corticosterone replacement group (C), while these obvious morphological changes were not observed in the CA3 pyramidal cells under different corticosteroid conditions. Bar = 50 μm.

releasing hormone (CRH), thyrotropin-releasing hormone (TRH), growth hormone-releasing hormone (GRH) and somatostatin. On the other hand, in the pituitary gland, GR immunoreactivity is detected in the growth hormone (GH)-, adrenocorticotropic hormone (ACTH)- and thyroid stimulating hormone (TSH)-producing cells. Folliculo-stellate (FS) cells, which are non-hormone producing cells, also express GR. In the posterior pituitary gland, pituicytes immunostained for S100 protein express GR[10]. This means that glucocorticoids directly influence certain pituitary cells in order to regulate cell function, including the synthesis and/or secretion of hormones.

Annexin-1 (also called lipocortin-1), a 37-kD member of the annexin superfamily of Ca^{++} and phospholipid-binding protein, is expressed in FS cells and annexin-1 is thought to be a candidate for paracrine agents to modulate the release of pituitary hormones. It was first described as a glucocorticoid inducible protein in macrophages and has been shown to play a key role as the mediator of

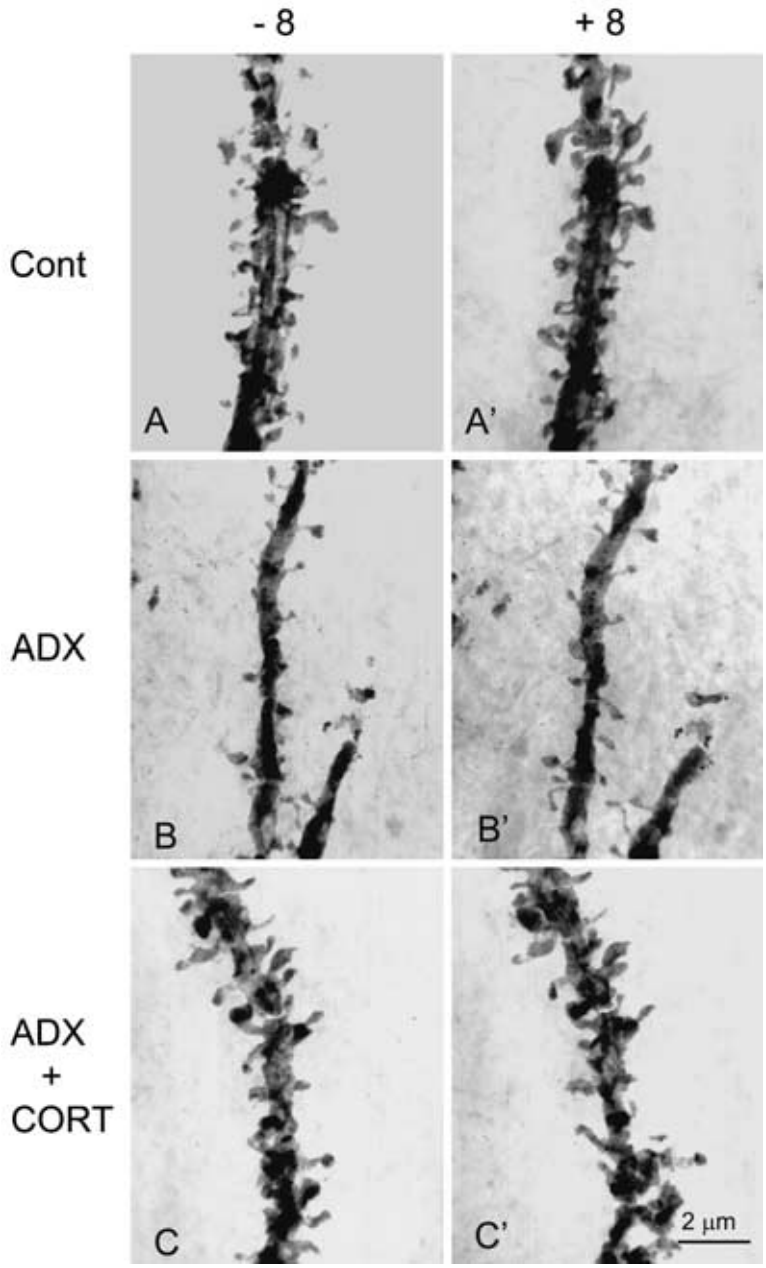

Fig. 3 Stereopairs (tilted ± 8) observed by high voltage electron microscopy of dendrites of CA1 pyramidal neurons in the sham-control (A, A'), ADX (B, B') and ADX + corticosterone replacement (C, C') groups. A decrease in the number of dendritic spines was observed in the ADX conditions (B, B'), whereas corticosterone treatment to ADX rats showed recovery in the number (C, C'). Bar = 2 μm.

glucocorticoid action in immune/inflammatory cells and in the neuroendocrine system[11,12]. The localization of annexin-1 binding sites on rat anterior pituitary cells has been demonstrated by fluorescence-activated cell (FAC) analysis and electron microscopy in somatotropes, lactotropes, corticotropes, thyrotropes and gonadotropes[13], suggesting that annexin-1 is directly involved in the function of hormone-producing cells in the anterior pituitary gland. Further, annexin-1 located in FS cells has been advocated as one of the candidates for paracrine agents produced by FS cells that modulate the release of pituitary hormones. Annexin-1 immunoreactivity is usually observed in

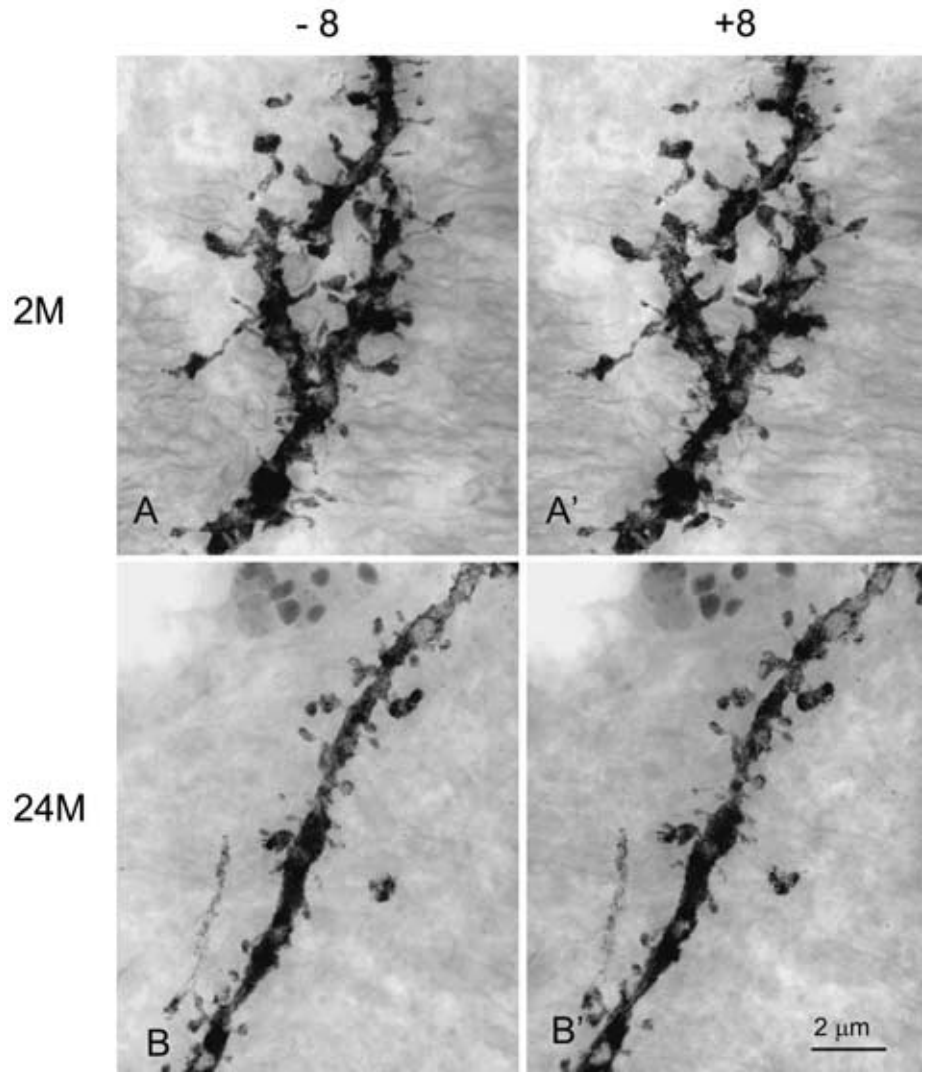

Fig. 4 Stereopairs (tilted ± 8) observed by high voltage electron microscopy of dendrites of CA1 pyramidal neurons in the 2-month young rat (A, A') and 24 month aged rat (B, B'). An obvious decrease in the number of spines and the morphological changes of dendritic spines were observed in the aged rat. Bar = 2 μm.

the cytoplasm, especially intense immunoreactivity was detected in the follicle surface of FS cells under control condition. After adrenalectomy, annexin-1 immunoreactivity almost disappeared, but the immunoreactivity recovered with corticosterone replacement (**Fig. 5**). The expression of GR immunoreactivity in the nucleus of FS cells also showed a pattern similar to annexin-1 related to the changes in the corticosteroid conditions[14]. This has indicated that glucocorticoids regulate the annexin-1 expression, and demonstrated the translocation of annexin-1 from intracellular to pericellular sites in FS cells[14,15].

On the other hand, it has been studied that the exocytosis sensitivity of GH cells to growth hormone-releasing hormone under different corticosterone environments is different among the subsets of GH cells[16]. GH cells in the rat anterior pituitary have been morphologically classified into three subtypes: type I (mature type) containing large secretory granules about 350 nm in diameter, type II (intermediate) containing a mixture of large and small granules, and type III (immature) containing small granules[17]. The number of exocytotic figures was low in all subtypes of GH cells in sham-operated control rats. Growth hormone-releasing hormone (GRH) treatment induced a significant increase in exocytosis in each subtype of GH cell, particularly in

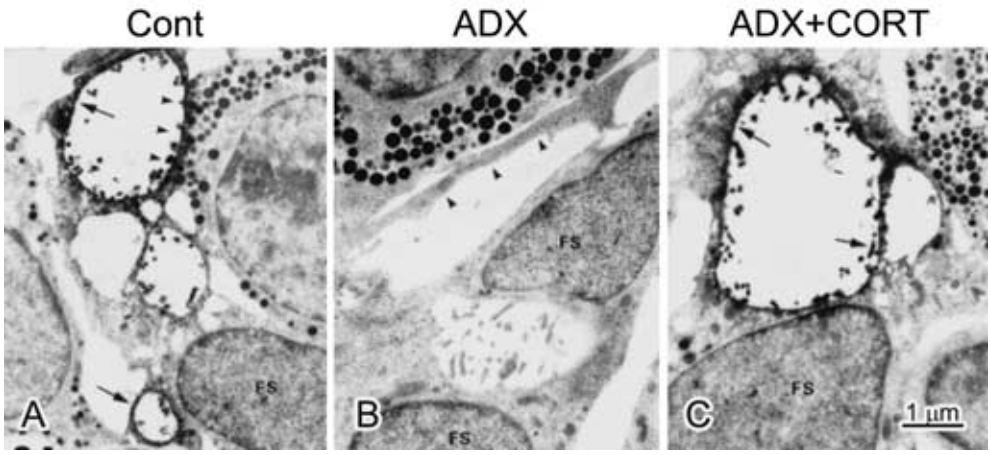

Fig. 5 Immunoelectron micrographs for annexin-1 in folliculo-stellate (FS) cells in sham-operated control (A), ADX (B) and corticosterone treatment to ADX rats (C). An intense immunoreaction was observed along the follicle lumen (**arrows**) and the processes of FS cells showed intense immunoreactivity surrounding a granulated endocrine cell (**arrowheads**) (A). No immunoreaction was observed in ADX rats (B), and recovery was observed after replacement with corticosterone in ADX rats (C). Bar = 1 μm.

type I (mature) and type II (intermediate) GH cells in the control rats. GRH treatment for 4 days after ADX showed a slight increase in exocytosis. Corticosterone replacement given to ADX rats induced a clear recovery of the excytotic response to GRH to the control level (**Fig. 6**). These results indicate that the secretion of GH stimulated by GRH is closely related to corticosteroids, and that the sensitivity of GRH differs among the three subtypes of GH cells[16].

Steroid Hormones and the Control System of Food Intake in the Central Nervous System

It is well known that the hypothalamus is the major center controlling food intake and body weight. Some studies have identified the ventromedial hypothamlamic nucleus (VMN) as the "satiety center", while lateral hypothalamic area (LHA) is termed the "hunger center". Recently, it has been demonstrated that the profound hyperphagia and obesity of *ob/ob* mice results from an autosomal recessive mutation of the gene encoding leptin[18], a hormone secreted by adipocytes; this sutudy provided compelling evidence of a second adiposity signal. Leptin receptors are expressed by neurons involved in energy intake such as NPY neurons and POMC neurons in the arcuate nucleus of the hypothalamus[19,20]. Several subsequent observations have indicated that leptin has an important role in the central nervous system control of energy homeostasis. **Fig. 7** shows a model for the second-order neuronal signaling pathway for food intake and energy homeostasis. The hypothalamus, including the paraventricular nucleus (PVN), zona incerta, perifornical area (PFA) and the lateral hypothalamic area (LHA) are abundantly supplied by axons from the arcuate nucleus (ARC) NPY and POMC neurons, which express the leptin receptor. These complex neuronal networks are involved with food intake and it is suggested that they play an important role in regulating the neuronal function of each other[21]. It is also well known that food intake disorders are preferentially expressed in women, suggesting that estrogen affects the neuronal network involved in food intake. Our recent study has indicated that there is an obvious sex difference in the orexin neurons, which is a key neuron in the LHA for the control of food intake (**Fig. 8**). Orexin, discovered in 1998[22,23], has been reported to be affected by the nutritional state of the animal. Orexin activates two closely related G-protein-coupled receptors, termed orexin-1 and orexin-2 receptors, which are also highly conserved

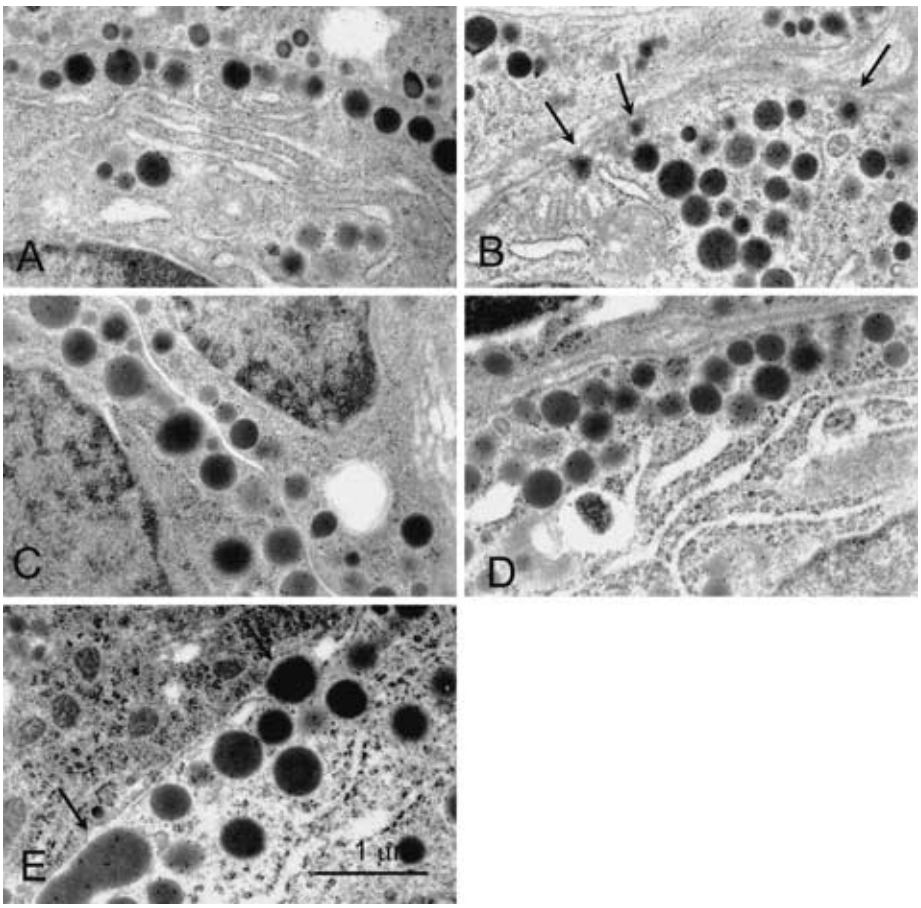

Fig. 6 Immunoelectron micrographs of GH cells under control (A), GRH injection to control (B), ADX (C), GRH injection to ADX (D) and GRH injection to corticosterone-treated ADX (E) rats. In the case of GRH injection to control (B), and to ocotritosterone-treated ADX rats, a clear increase in exocytosis (**arrows**) was observed. Bar = 1 μm.

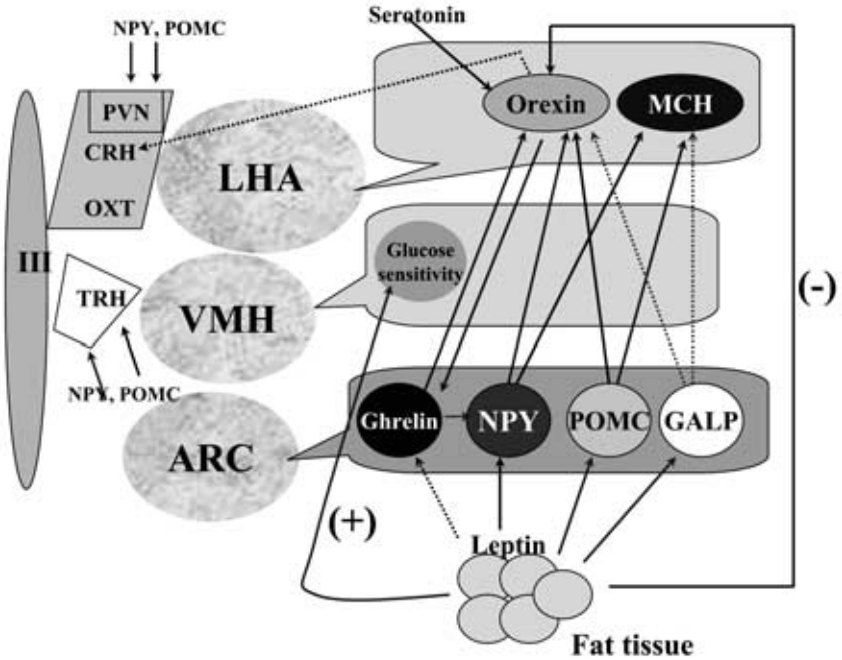

Fig. 7 Schematic drawing of the neuronal network involved with the appetite regulation in the hypothalamic area.

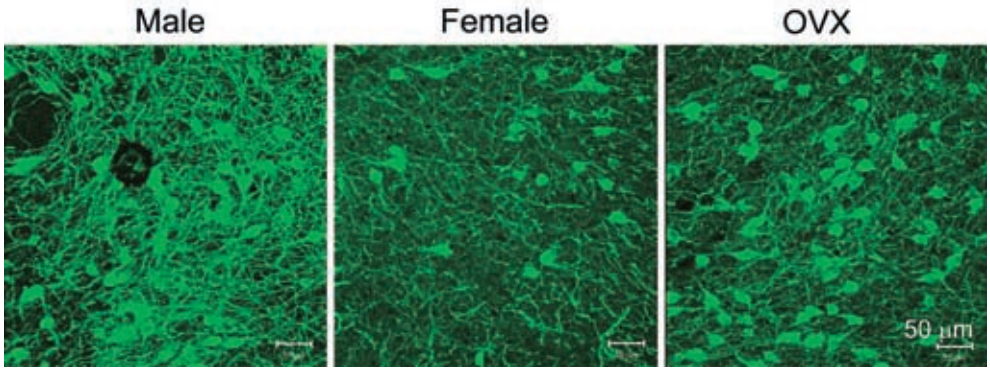

Fig. 8 Sections (25 μm thickness) immunofluorescent stained with orexin-A in the lateral hypothalamic area (LHA) in the male, female, and OVX-rats were imaged in the Z-series focal plane, by capturing each 1 μm with a confocal scanning microscopy and merged. The density of the immunoreaction was preferentially higher in the male as well as in OVX rats than in female rats. Bar = 50 μm.

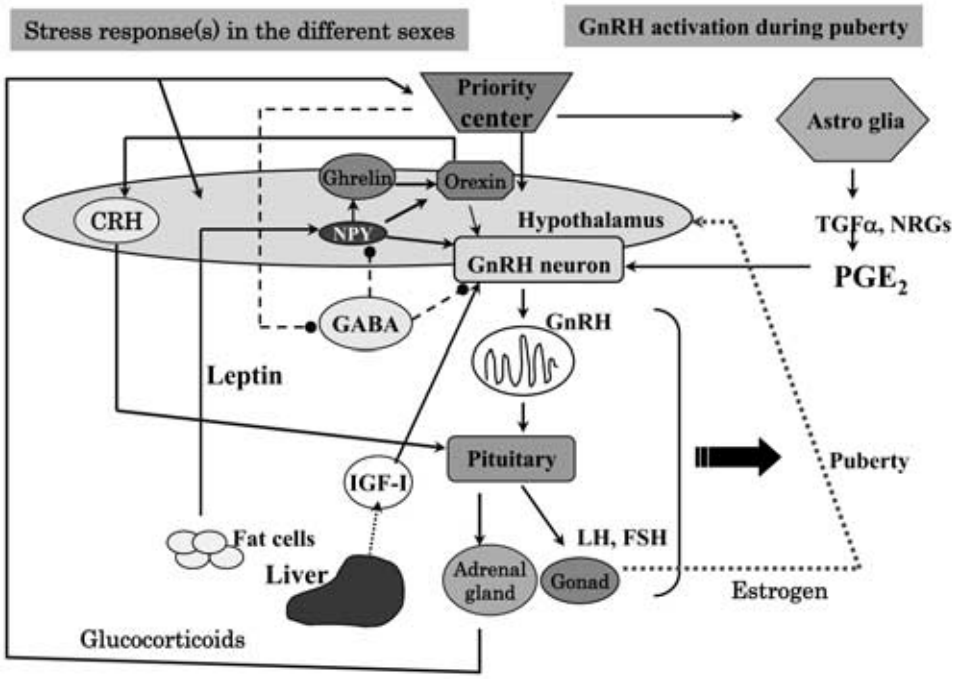

Fig. 9 Schematic drawing of the interaction between the feeding control neuronal network and stress response neuronal system or the neuronal system for sex behavior control such as puberty expression. These complex networks communicate with each other. Steroid hormones such as glucocorticoid and estrogen are suggested to closely interact in the systems.

across mammalian species. These receptors have been indicated to distribute within the hypothalamus including in the suprachiasmatic nucleus (SCN), PVN, ARC, VMN and the LHA. The extensive anatomical distribution of orexin fibers and orexin receptors suggests a diversity of autonomic, neuroendocrine and behavioral functioning. Additionally, these feeding regulation systems seem to be closely related to the stress response system and the sexual behavior response system in the brain, because the orexin system also is contacted to these via different neuronal circuits. Thus, it is hypothesized that the neuronal system is involved with food intake and the regulation of energy homeostasis interacts with another neuronal system which communicates and informs the status of body

energy metabolism (**Fig. 9**). In particular, the expression of steroid hormone receptors such as glucocorticoid receptor (GR), estrogen receptor (ER) and androgen receptor (AR) in certain neurons within these neuronal complexes seems to be important. The evolution of the analysis of these neuronal interactions should be moved forward.

References

1. Kawata M: Roles of steroid hormones and their receptor in structural organization in the nervous system. Neurosci Res 1995; 21: 1–46.
2. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schultz G, Umesono K, Blumberg B, Kastner P, Mark L, Chambon P, Evans RM: The nuclear receptor superfamily: the second decade. Cell 1995; 83: 835–839.
3. Kawata M, Yuri K, Ozawa H, Nishi M, Ito T, Hu Z, Lu H, Yoshida M: Steroid hormones and their receptors in the brain. J Steroid Biochem Molec Biol 1998; 65: 1–6.
4. Morimoto M, Morita N, Ozawa H, Yokoyama K, Kawata M: Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brains: an immunohistochemical and in situ hybridization study. Neurosci Res 1996; 26: 235–269.
5. Ito T, Morita N, Nishi M, Kawata M: In vivo and in vitro immunocytochemistry for the distribution of mineralocorticoid receptor with the use of specific antibody. Neurosci Res 2000; 37: 173–182.
6. Han F, Ozawa H, Matsuda K, Nishi M, Kawata M: Colocalization of mineralocorticoid receptor and glucocorticoid receptor in the hippocampus and hypothalamus. Neurosci Res 2005; 51: 371–381.
7. Xie CX: Morphological changes on the pyramidal and granule neurons and glial cells in the hippocampus of the rat brains under different corticosteroid conditions. J Kyoto Pref Univ Med 2002; 111: 189–202.
8. Hama K, Arii T, Kosaka T: Three-dimensional morphometrical study of dendritic spines of the granule cell in the rat dentate gyrus with HVEM stereo images. J Electron Microsc Tech 1989; 12: 80–87.
9. Hama K, Arii T, Ito Y: High-voltage electron microscopy in neurocytology. J Electron Microsc 2000; 49: 1–4.
10. Ozawa H, Ito T, Ochiai I, Kawata M: Cellular localization and distribution of glucocorticoid receptor immunoreactivity and the expression of glucocorticoid receptor messenger RNA in rat pituitary gland: a combined double immunohistochemistry study and in situ hybridization histochemical analysis. Cell Tissue Res 1999; 295: 207–214.
11. Gerbicke-Haerter PJ, Schobert A, Deiter P, Honeer P, Hertting G: Regulation and glucocorticoid-independent induction of lipocortin 1 in cultured astrocytes. J Neurochem 1991; 57: 175–183.
12. Strijbos PJ, Tilders FJ, Carey F, Forder R, Rithwell NJ: Localization of immunoreactive lipocortin-1 in the brain and pituitary gland of the rat. Effect of adrenalectomy, dexamethasone and colchicine treatment. Brain Res 1991; 553: 249–260.
13. Christian HC, Taylor AD, Flower RJ, Morris JF, Buckingham JC: Characterization and localization of lipocortion 1-binding sites on rat anterior pituitary cells by fluorescence-activated cell analysis/sorting and electron microscopy. Endocrinology 1997; 138: 5341–5351.
14. Ozawa H, Miyachi M, Ochiai I, Tsuchiya S, Morris JF, Kawata M: Annexin-1 (lipocortion-1)-immunoreactivity in the folliculo-stellate cells of rat anterior pituitary: the effect of adrenalectomy and corticosterone treatment on its subcellular distribution. J Neuroendocrinol 2002; 14: 621–628.
15. Morris JF, Christian HC, Chapman LP, Epton MJ, Buckingham JC, Ozawa H, Nishi M, Kawata M: Steroid effects on secretion from subsets of lactotrophs: role of folliculo-stellate cells and annexin-1. Arch Physiol Biochem 2002; 110: 54–61.
16. Ozawa H, Han F, Kawata M: Exocytosis sensitivity to growth hormone-releasing hormone in subsets of GH cells in rats under different corticosterone conditions. Ultrastructural study using microwave irradiation for fixation and immunocytochemistry. J Endocrinol 2004; 183: 507–515.
17. Kurosumi K, Koyama T, Tosaka H: Three types of growth hormone-producing cells in the rat anterior pituitary as studied by immunogold electron microscopy. Arch Histol Japon 1986; 49: 227–247.
18. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425–432. (Erratum in: Nature 1995; 374: 479)
19. Cheung CC, Clifton DK, Steiner RA: Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. Endocrinology 1997; 138: 4489–4492.
20. Baskin DG, Breininger JF, Schwartz MW: Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. Diabetes 1999; 48: 828–833.
21. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG: Central nervous system control of food intake. Nature 2000; 404: 661–671.
22. De Lecea L, Kilduff TS, Peyron C, Gao XB, Foye PE, Danielson PE, Fukuhara C, Battenberg ELF, Gautvik VT, Bartlett FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG: The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA 1998; 95: 322–327.
23. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JRS, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbargy NA, Bergsma DJ, Yanagisawa M: Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding. Cell 1998; 92: 573–585.

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