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Rapid signaling of steroid hormones in the vertebrate nervous system

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1. ABSTRACT

Steroid hormones easily cross the blood–brain barrier because of their physicochemical lipid solubility. The hormones act through nuclear receptor-mediated mechanisms and modulate gene transcription. In contrast to their genomic actions, the non-genomic rapid action of steroid hormones, acting *via* various types of membrane-associated receptors, reveals pharmacological properties that are distinct from the actions of the intracellular nuclear receptors. As a result, non-genomic rapid actions have gained increased scientific interest. However, insight into the phylogenic and/or comparative actions of steroids in the brain is still poorly understood. In this review, we summarize recent findings concerning the rapid, non-genomic signaling of steroid hormones in the vertebrate central nervous system, and we discuss (using a comparative view from fish to mammals) recently published data regarding the mechanism underlying physiology and behavior.

2. INTRODUCTION

Steroids are a large family of chemical substances comprising many hormones and vitamins built on cyclopentanoperhydrophenanthrene, a tetracyclic nucleus (*i.e.*, steroid skeleton) (see Figure 1) (1). Cholesterol is the precursor of the five major classes of steroid hormones: estrogens, progestins, androgens, glucocorticoids and mineralocorticoids *in vivo* (Figure 1) (1, 2). In vertebrates, steroid hormones supplied by the peripheral endocrine glands regulate numerous important functions not only in the peripheral organs but also in the central nervous system (CNS) during development and this regulation persists into adulthood (2-7). Peripheral steroid hormones have a small molecular weight (less than 400) and easily cross the blood-brain barrier because of their physicochemical lipid solubility. They act directly on neuronal tissues through nuclear receptor-mediated mechanisms and modulate nuclear transcriptions. Thus, the steroid hormones trigger genomic actions that result in

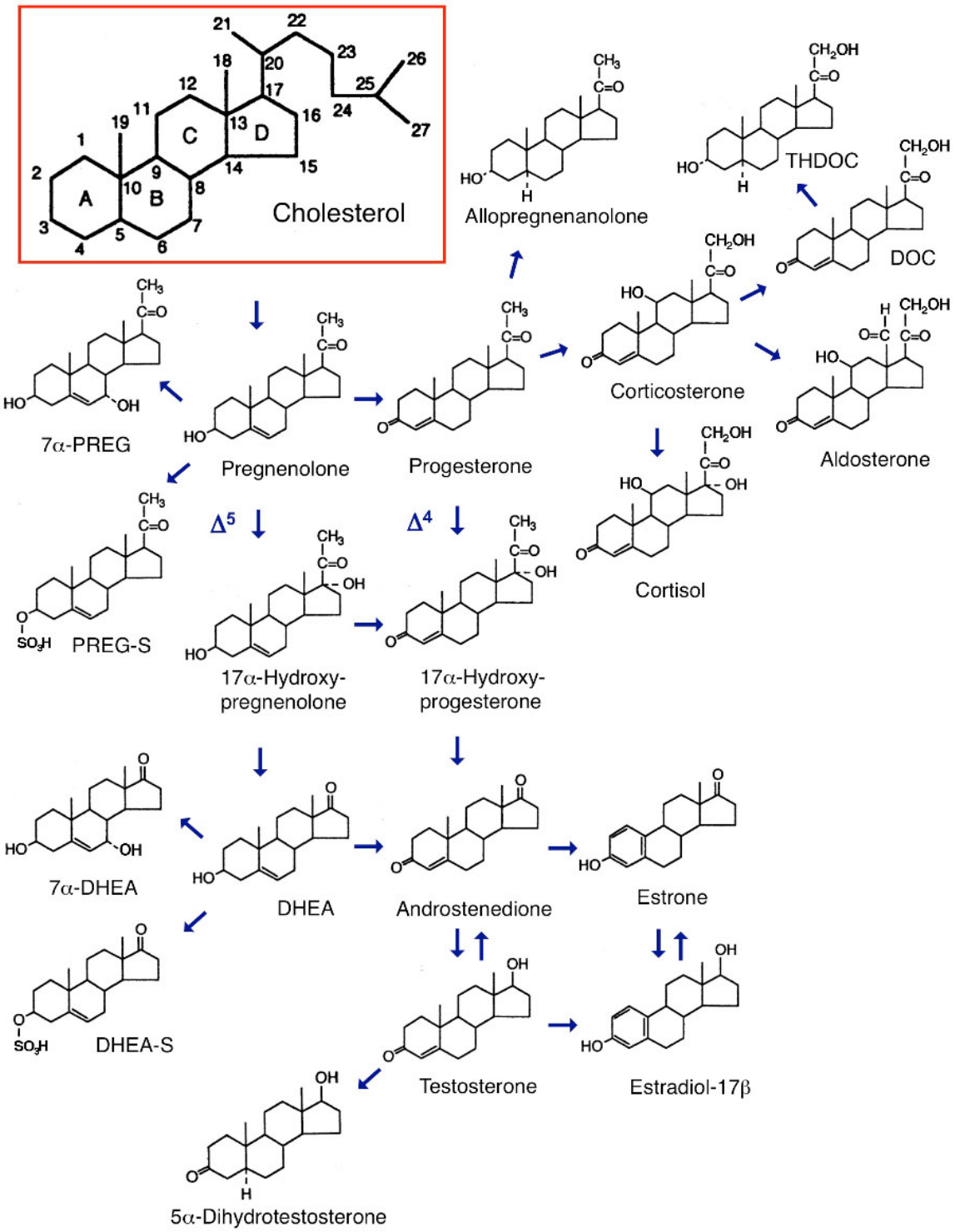

Figure 1. The major pathway of steroid hormone synthesis from cholesterol. The core of steroids is composed of seventeen carbon atoms bonded together that take the form of four fused rings: three cyclohexane rings (designated as rings A, B, and C in cholesterol) and one cyclopentane ring (the D ring). The basic skeleton of a steroid with numbering of carbon atoms in gonane is also indicated in cholesterol (marked in red). PREG-S, pregnenolone sulfate; 7α-PREG, 7α-hydroxypregnenolone; DHEA, dehydroepiandrosterone; 7α-DHEA, 7α-hydroxydehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; THDOC, 3α,5α-tetrahydrodeoxycorticosterone.

physiological effects (Figure 2) (2, 3, 7-9). The specific binding of a ligand to nuclear receptors translocates the receptor/ligand complex into the nucleus, where it binds to deoxyribonucleic acid (DNA) and modulates gene transcription (Figure 2) (2, 3, 5, 7, 9-15). These actions of steroid hormones are called genomic actions.

The effects of many steroid hormones develop slowly; however, some effects occur very rapidly and last for only a few minutes before inducing behavioral responses (16-19). Using only traditional models (*e.g.*, organizing actions during development), it is difficult to explain the rapid responses of steroid hormones since it takes time for the steroid hormones to bind to nuclear receptors and induce the transcriptional changes that underlie protein synthesis. Hence, it has been proposed that the activating (*i.e.*, non-genomic) effects of steroid hormones occur *via* a membrane-associated receptor (2, 3, 16-24). In the mammalian CNS, data concerning the differences between "organizing actions" and "activating actions" have now been accumulated. The non-genomic rapid actions of steroid hormones that occur *via* various types of membrane-associated receptors consequently reveal pharmacological properties that are distinct from the actions of the intracellular nuclear receptors. They have gained increased scientific interest (16, 17). To date, target molecules and/or sites for the non-genomic effects of steroid hormones on the CNS have been suggested for most classes of steroid hormones – including the estrogens (section 3.1), progestins (section 3.2), androgens (section 3.3), glucocorticoids (section 4.1), mineralocorticoids (section 4.2), and several neurosteroids (section 5) that are synthesized *de novo* within the CNS (21-23, 25). However, there is little phylogenic and/or comparative insight concerning the actions of steroid hormones in the CNS. In this review, we summarize recent findings on rapid, non-genomic signaling of steroid hormones in the vertebrate CNS and discuss recently published data regarding the mechanisms (from a comparative view from fish to mammals) underlying physiology and behavior (Table 1).

3. SEX STEROIDS

Sex steroids are biologically important hormones that are mainly derived from the gonads in vertebrates. The ovaries secrete major amounts of estrogens (estrone, estriol, and estradiol-17β [the most important in mammals)) and progestins (pregnenolone, 17,20β-dihydroxy-4-pregnan-3-one (an important progestin in teleosts), and progesterone (the most important in mammals)). The principal function of the estrogens and progesterone in the peripheral reproductive system is to maintain the cellular proliferation and growth of the tissues in the sexual organs during the estrus cycle and to develop the secondary sexual characteristics in females. The androgens (5α-dihydrotestosterone, androstenedione, 11-ketotestosterone (an important androgen in teleosts), dehydroepiandrosterone (DHEA), and testosterone (the most important in mammals)) have a broad spectrum of physiological and behavioral functions. They play a role in the development and maintenance of the male reproductive system and play an important role in the expression of secondary sexual characteristics in males. In the classic concept, sex steroids exert these effects by binding to and activating their specific nuclear receptors in target cells: the estrogen receptor (ER), the progesterone receptor (PR), and the androgen receptor (AR). In addition to these genomic actions, there is increasing evidence that sex steroids are capable of inducing rapid signaling processes in the CNS (independent of transcriptional changes) *via* membrane-associated receptors that have recently been identified (Figure 2).

3.1. Estrogens

Estrogens secreted by the ovary have various regulatory functions in the nervous system such as reproduction, cognition, mood, neuroprotection, neurotrophism, and pain perception (2, 4, 5, 18, 26-28). The classic nuclear ERs – ERα and ERβ – are expressed in neurons and are mainly localized in the nucleus (2). It is widely accepted that these two nuclear receptors exert their regulatory effects through genomic actions (2, 5). However, nuclear receptor-mediated genomic actions do not explain all aspects of estrogenic effects on the nervous system and accumulating evidence suggests that rapid non-genomic actions are crucially involved in their effects (19, 29-31). The non-genomic actions of estrogen occur within a period of a millisecond to few minutes. This is distinct from a genomic action, which needs gene transcription and protein synthesis. Rapid cellular response to estrogens could be initiated at the plasma membrane, which would require the presence of a membrane-associated ER. Observations by immunoelectron microscopy reveal that ERα and ERβ are also localized in extranuclear areas, which include the dendritic spine and axon terminal of hypothalamic and hippocampal neurons (26, 32-34). In addition, it has been reported that caveolin proteins are important for estrogen-mediated signaling in hippocampal neurons, suggesting that ERα and ERβ are localized to the plasma membrane (30, 35, 36).

There are other potential membrane ERs, in addition to the classic ERs. G protein-coupled ER (GPER) 1, formerly known as GPR30, is a member of the seven-transmembrane G protein-coupled receptor (GPCR); GPER1 activates rapid signal cascades, following estradiol-17β binding. ER-X and Gq-coupled membrane ER (Gq-mER) are also putative membrane ERs. Their encoding genes have not been cloned, but they are definitely different from ERα, ERβ, and GPER1 (37-41).

Possible mechanisms that bring about rapid cellular responses to estrogens that have been proposed are activation of signal transduction pathways and modulation of neurotransmitter receptors. ERα, ERβ, and ER-X are functionally coupled with mitogen-activated protein kinase (MAPK) signaling, whereas GPER1 and Gq-mER activate G proteins, followed by activation of their signal transduction cascades in response to the estrogens (19, 29, 37, 42-45). Ionotropic and metabotropic neurotransmitter receptors are both modulated by estrogen stimulation. Estradiol-17β treatment promptly changes *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) binding to each

Table 1. Summary of the major origins and physiological functions of steroid hormones

Steroids	Major origin	Physiological function[1]
Estrone	Gonads	Estrogen
Estriol	Gonads	Estrogen
Estradiol-17β	Gonads/CNS	Estrogen/Neurosteroid
Pregnenolone	Gonads/CNS	Precursor/Neurosteroid
Pregnenolone sulfate	CNS	Neurosteroid
17α-Hydroxypregnenolone	Gonads	Progestin
7α-Hydroxypregnenolone	CNS	Neurosteroid
Progesterone	Gonads/CNS	Progestin/Neurosteroid
17α-Hydroxyprogesterone	Gonads	Progestin
17,20β-Dihydroxy-4-pregnan-3-one	Gonads (fish)	Progestin
Testosterone	Gonads/CNS	Androgen/Neurosteroid
5α-Dihydrotestosterone	Gonads	Androgen
Androstenedione	Gonads	Androgen
Dehydroepiandrosterone	Adrenal/CNS	Androgen/Neurosteroid
7α-Dehydroepiandrosterone	CNS	Neurosteroid
Dehydroepiandrosterone sulfate	CNS	Neurosteroid
11-Ketotestosterone	Gonads (fish)	Androgen
Corticosterone	Adrenal/CNS	Glucocorticoid/Neurosteroid
Cortisol	Adrenal/CNS	Glucocorticoid/Neurosteroid
	Interrenal (fish)	Glucocorticoid/Mineralocorticoid
Aldosterone	Adrenal/CNS	Mineralocorticoid/Neurosteroid
11-Deoxycorticosterone	Interrenal (fish)	Neurosteroid/Mineralocorticoid
3α,5α-Tetrahydroprogesterone (Allopregnanolone)	CNS	Neurosteroid
3α,5α-Tetrahydrodeoxycorticosterone	CNS	Neurosteroid

[1]Refer details for the text

ionotropic receptor in several brain regions (46). As for metabotropic receptors, activation of membrane-associated ERα and ERβ promotes the transactivation of metabotropic glutamate receptor (mGluR) signaling, without requiring glutamate (30, 47), while Gq-mER-Gq activation induces desensitization of the γ-aminobutyric acid (GABA) subtype B receptors, a metabotropic receptor (31, 40). These mechanisms modulate ion channels, protein synthesis, and spine plasticity, and ultimately alter brain functions that influences behavioral changes (19, 26, 29-31).

The rapid actions of estrogens may also be functional in glial cells. ERα and ERβ localized in plasma membrane have been observed in astrocytes and oligodendrocytes. In hypothalamic astrocyte cultures, estradiol-17β stimulates calcium flux and subsequently induces neuroprogesterone synthesis; this process can be blocked by an mGluR1a antagonist (48, 49). In oligodendrocyte cultures, estradiol-17β elicits the rapid activation of MAPK signaling pathways, suggesting that membrane-associated ERα and ERβ contribute to the differentiation, survival, and function of oligodendrocytes (50).

In 2005, two groups independently reported localizing GPER1 as a membrane-bound ER in several cancer cell lines (42, 43). Estradiol-17β stimulation of GPER1-expressing cells results in the activation of G protein and subsequently the activation of adenylyl cyclase, intracellular calcium mobilization, and the accumulation of phosphatidylinositol-3,4,5-trisphosphate in the nucleus. GPER1 is widely expressed in mammalian tissues (*e.g.*, reproductive, digestive, and cardiovascular systems) (51, 52). In addition to mammalian species (*e.g.,* mouse, rat, primate, and human) (42, 43, 45, 53-55), a homolog of GPER1 is found in teleosts (56-58). This suggests that the function of this gene is evolutionarily conserved. In the teleost zebrafish (*Danio rerio*), endogenous estrogens produced by the follicle cells inhibit or delay the spontaneous maturation of oocytes; GPER1 mediates this action of estrogen (59). GPER1 is widely expressed throughout rodent brain tissues such as the cerebral cortex, hippocampus, striatum, basal forebrain cholinergic nuclei, and nuclei of hypothalamus and brain stem (53, 60-62). The cellular localization of the GPER1 is a matter of debate. Some groups, including the present authors, have demonstrated that the GPER1 is localized on the endoplasmic reticulum and Golgi apparatus (43, 60, 61), while other groups have demonstrated that the GPER1 is localized on the plasma membrane (42, 63). The endoplasmic reticulum and Golgi apparatus localizations of this GPCR may seem strange, but estradiol-17β is a membrane-permeable molecule that can diffuse across the plasma membrane and easily bind to GPER1 – even if the receptor is localized in intracellular organelles.

The hypothalamus and the preoptic area are closely related to the action of the estrogens (2, 5). In the magnocellular neurons of the paraventricular and supraoptic nuclei, oxytocin neurons express GPER1 at the messenger ribonucleic acid (mRNA) and protein levels; by contrast, vasopressin neurons do not express GPER1 (60). The expression of ERβ by oxytocin neurons has previously been described (64). However, the presence of a nuclear receptor does not fully explain estrogen functions in these neurons (65). The selective expression of GPER1 suggests that GPER1 may primarily serve to non-genomically transduce estrogen actions in the hypothalamo-neurohypophyseal system. In the paraventricular nucleus, GPER1 is co-localized with corticotrophin-releasing hormone (CRH) (66). It has been demonstrated that exposing the paraventricular nucleus to an agonist specific

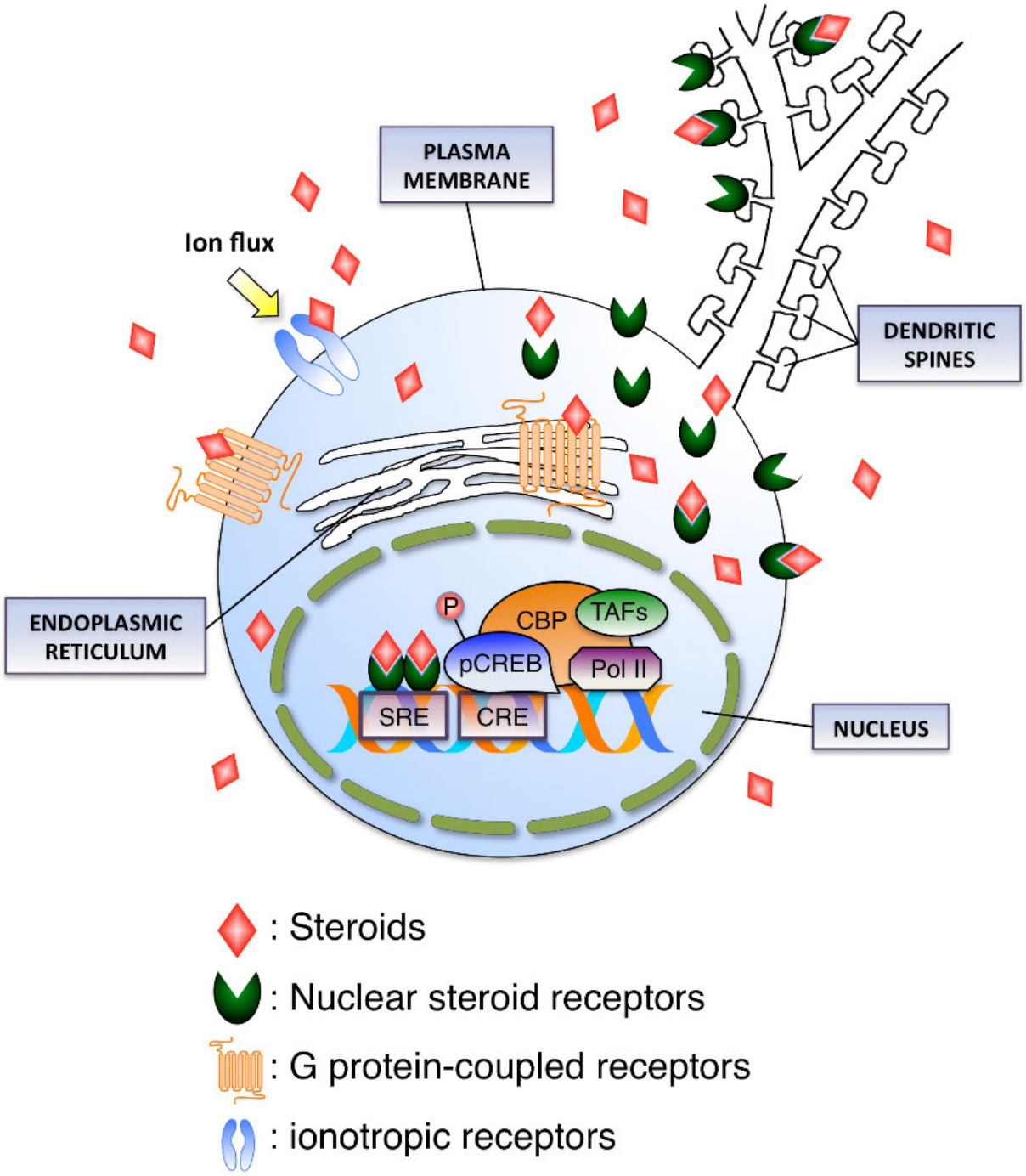

Figure 2. Schematic diagram of genomic and non-genomic actions of steroid hormones in neurons.Steroid hormones easily cross the plasma membrane because of their lipid solubility. The hormones act through nuclear receptor-mediated mechanisms and modulate gene transcription. In addition to the genomic actions in the nucleus, the nuclear steroid receptors may be localized in extranuclear areas, which include the dendritic spine and axon terminal of neurons to induce the spinogenesis, or to modulate the synaptic transmissions locally. Caveolin proteins may be important for steroid-mediated signaling in neurons, suggesting the plasma membrane localization of nuclear steroid receptors. G protein-coupled receptors for steroid hormones may be localized in the plasma membrane or membrane structures of the endoplasmic reticulum to induce the second messenger systems. Ionotropic receptors for the conventional neurotransmitters may also be targets for the non-genomic actions of steroid hormones. SRE, steroid response element; CRE, cyclic AMP response element; pCREB, phosphorylated CRE-binding protein; CBP, CREB-binding protein; TAFs, TATA element-binding protein-associated factors; Pol II, RNA polymerase II.

for the GPER1 (67) desensitizes the 5-HT_{1A} receptor, thereby suppressing the 5-HT-mediated secretion of oxytocin and adrenocorticotropic hormone (ACTH) from the anterior pituitary (66).

Gonadotropin-releasing hormone (GnRH) neurons, which are located in the preoptic area, are critical in controlling fertility. Depending on the concentration of estradiol-17β during the estrous cycle, the hormone inhibits or stimulates GnRH release. In a brain slice culture, estradiol-17β induces increased firing activity of the GnRH neurons, but knockdown of GPER1 by using a small interfering RNA molecule completely abrogates this estrogenic effect. By contrast, the specific GPER1 agonist G1 induces a similar increase in firing activity that is observed with estradiol-17β (54, 55).

The hippocampus is also an important target for the actions of estrogen (18). GPER1 is expressed at both the mRNA and protein levels in the pyramidal cells in the cornus Ammonis (CA) 1 to CA3 region of the hippocampus and in the granule cells of the dentate gyrus; however, the protein expression level in the dentate gyrus cells is lower than its level in the hippocampal neurons in the CA1 to CA3 region (53, 61, 62). The GPER1 expression level is not different between the sexes in adults, but is higher in newborn male infants (61). These observations suggest that GPER1 plays an important role in hippocampal functions by transducing estrogen signals. Based on results from a model using a hippocampal-derived cell line, the GPER1 agonist G1 has been reported to have a neuroprotective effect (68).

Estrogen signaling is also functional in the spinal cord and peripheral nervous system (e.g., in the dorsal root ganglia (DRG) and pelvic autonomic ganglia) (2, 69, 70). GPER1 is expressed in the spinal cord from the cervical level to the sacral level in male and female rats (71, 72). GPER1-immunoreactivity is localized in the neuronal somata and in the dendritic and axonal processes and/or their terminals in the spinal dorsal horn and ventral horn of gray matter. GPER1-immunoreactive cells are also localized in the glial cells in white matter. Selective spinal dorsal rhizotomy indicates that GPER1 protein is synthesized in the DRG neurons and then transported *via* dorsal root to the terminals of nociceptive afferents in the superficial layer of spinal dorsal horn (laminae I and II) (72).

The estrogens reportedly influence pain sensitivity, pain threshold, and pain tolerance in females during the menstrual cycle in humans and during the estrus cycle in rodents (73-75). ERα and ERβ may play a role in these somatosensory system effects.

The cell size of DRG neurons is classified into three groups, and generally based on the different physiology of DRG neurons. The localization of ERα and ERβ primarily in the nucleus of small and medium-sized DRG neurons suggests that the estrogens may modulate nociceptive signaling at the primary afferent level (69, 76). An *in vitro* study using rat DRG neuron culture demonstrated that estradiol-17β attenuates adenosine triphosphate (ATP)-induced intracellular calcium ($(Ca^{2+})_i$) concentration transients, which is a putative nociceptive signal (77, 78). Estradiol-17β conjugated to bovine serum albumin, which does not penetrate the plasma membrane, has the same effect as estradiol-17β alone, suggesting that a membrane-associated ER mediates the nociceptive response (77). Furthermore, estrogens are ineffective in modulating ATP-induced $(Ca^{2+})_i$ flux in a DRG neuron culture from ERα knockout (ERαKO) mice but not from ERβ knockout (ERβKO) mice, indicating that the rapid action of estradiol-17β is mediated through membrane-associated ERα (78). However, little is known about the acute effects of nociception that occur *via* nuclear ERα and ERβ *in vivo*. On the other hand, the GPER1 is localized in the somata of small, medium, and large-sized DRG neurons (72). Using the selective GPER1 agonist G1 to mimic estrogen function, a recent pharmacological approach showed that estrogens act acutely on the GPER1 to produce protein kinase Cε (PKCε)-dependent mechanical hyperalgesia *in vitro* and *in vivo* in rats; this effect is similar to that of estrogen (79). Taken together, these results suggest that the GPER1 may play a role in estrogen-mediated non-genomic signal transduction mechanisms in the somatosensory system.

3.2. Progestins

The most important progestin is progesterone. In the normal non-pregnant female, progesterone is secreted from the corpus luteum cells only during the latter half of the ovarian cycle. As do most steroids, progesterone exerts its effects by binding and activating specific nuclear receptors (10, 11). According to a common theory of steroidal actions, progesterone's effects are mediated by binding to its cognate PR (classically defined as ligand-activated transcription factors) (10, 11). PRs occur as two isoforms – PR-B (a 115 kDa full length form) and PR-A (an 80 kDa N-terminally-truncated form) – that are derived from a single gene that is generated either from an alternative transcriptional site or from a translational site (10, 80). These isoforms are functionally distinct in terms of their ability to activate target genes (81). PR-B is known to function as a transcriptional activator of genes responsible for progesterone; PR-A seems to function as a transcriptional inhibitor of steroid hormone receptors, including ERs and PRs (81, 82). As with the estrogens, nuclear receptor-mediated genomic actions do not explain all aspects of progesterone's actions.

A putative membrane-associated progesterone-binding protein has recently been identified, characterized, and purified from porcine liver membranes (83). A full-length complementary DNA (cDNA) molecule encoding this putative progesterone-binding protein was subsequently isolated from the porcine vascular smooth muscle cell cDNA library (84). On the other hand, the same protein was found to be identical to an approximately 25 kDa molecule, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced protein (called 25-Dx). In independent studies, an adrenocortical inner zone-specific antigen (IZAg) was isolated from the rat adrenal gland by using affinity

chromatography (85-88) and was shown to be identical to 25-Dx (89). To date, cDNA molecules encoding 25-Dx has been cloned from human (90), pig (84), rat (89, 91), and mouse (92) models. Two isoforms of 25-Dx have been identified in humans (90).

25-Dx protein has a hydrophobic-membrane-anchoring region at the primary structure of the C-terminus (89) and is believed to form a dimeric complex *in vivo* (83). The UV-visible absorption and electron paramagnetic resonance spectra of the purified protein shows that 25-Dx is a heme-binding protein present in the endoplasmic reticulum membrane of the adrenal cortex (93). Peripheral distributions of 25-Dx has been reported in organs such as the liver, lung, kidney, adrenal cortex, smooth muscle, and ovary (89).

An important non-genomic action of progesterone, acting *via* 25-Dx, is the rapid stimulation of $(Ca^{2+})_i$ influx during the acrosome reaction in human sperm (94). Another prominent examples for 25-Dx actions have been reported that 25-Dx may play a role in steroid hydroxylation in the adrenal cortex (85, 88, 91, 93), and may mediate cell death from oxidative damage in MCF-7 human breast cancer cells (95). In the rat ovary, the protein is now referred to as progesterone membrane receptor component (PGRMC) 1. There is now strong evidence that PGRMC1 mediates the anti-apoptotic actions of progesterone in rat granulosa and luteal cells and that it interacts with other membrane proteins such as plasminogen activator inhibitor RNA-binding protein-1 (96-98).

25-Dx is expressed in the sexually dimorphic regions of the hypothalamus in rats, suggesting that 25-Dx may play a role in the female reproductive behaviors such as lordosis (99). 25-Dx is reportedly a novel ventral midline-associated protein, called ventral midline antigen (VEMA), that may play a role in axonal path finding or patterning during development (100, 101). On the other hand, evidence for acute effects of progesterone on the cerebellum has been reported from over the past two decades. Smith and colleagues demonstrated that progesterone infusion into the cerebellum rapidly enhances an inhibitory response of the Purkinje cell (24, 102, 103). According to Ke and Ramirez, high-affinity binding sites for progesterone have been found in cell membranes of the rat cerebellum (104). Photoaffinity labeling with progesterone also identified progesterone-binding proteins in mouse cerebellar membranes (105). Taken together, these results suggest that progesterone acts on Purkinje cells *via* membrane-associated progesterone-binding proteins. Sakamoto *et al.* reported that 25-Dx-immunoreactivity is found in the somata of cerebellar Purkinje cells and in proximal primary dendrites (106). In the developing cerebellum of neonates, the cerebellar Purkinje cells and external granule cells express 25-Dx (106, 107). A series of studies by Tsutsui and colleagues demonstrated that the rat Purkinje cell, a cerebellar cortical neuron, actively produces progesterone *de novo* from cholesterol – but only during neonatal life – and progesterone, acting as a neurosteroid, promotes dendritic growth, spinogenesis, and synaptogenesis (23, 108-114). Therefore, 25-Dx may also play a role in regulating cerebellar cortical formation and/or neurosteroidogenesis in the developing cerebellum (106, 107) (as for neurosteroids, please refer the following section 5). 25-Dx may likewise guide the regeneration of axons after spinal cord injury in rats (115). The expression of 25-Dx in structures involved in the production of cerebrospinal fluid and in osmoregulation, and the up-regulation of 25-Dx after brain damage point to its novel and potentially important role in maintaining water homeostasis after a traumatic brain injury (116). Taking these findings into consideration, 25-Dx may act as a putative membrane PR to mediate the non-genomic actions of progesterone in the CNS and in peripheral organs (83, 99, 116, 117).

Membrane PRs in a fish model, the spotted seatrout (*Cynoscion nebulosus*), have been identified (independent of the discovery of 25-Dx); these PRs are completely different from 25-Dx (118-120). In this fish, progesterone induces oocyte maturation, activates MAPK activity, and inhibits adenylyl cyclase activity; these actions are inhibited in the presence of pertussis toxin, suggesting that the receptor mediating oocyte maturation activates an inhibitory G protein (118, 119). Computer analyses of the deduced amino acid sequence has consistently predicted that the protein would be located in the plasma membrane and would have seven transmembrane domains (118). Thus, the identified membrane PR is believed to be a seven-transmembrane GPCR (118) and may induce oocyte maturation *via* the membrane PR in this fish. The homologues have been identified – at least 3 isoforms (*e.g.,* PRα, PRβ, and PRγ) – in several vertebrate species, including human, mouse, pig, *Xenopus*, zebrafish, and *Fugu*; the homologues have a highly conserved nucleotide sequence, deduced amino acid sequences, and similar structures as those found in the spotted seatrout membrane PR (119, 120). PRα is the most characterized membrane PR subtype. It has been cloned from fish and mammals (120).

In the spinal cord, progesterone has long been shown to exert non-genomic pleiotropic actions on neurons and glial cells and on other cells that can synthesize progesterone *de novo* (121-123). Although PRα is expressed in most neurons, astrocytes, and oligodendrocytes, Labombarda *et al.* have recently reported wide distribution of membrane PRα in the mouse spinal cord (124). The membrane PRα isoform is thus likely to play a significant role in the neuroprotective and promyelinating effects of progesterone in the spinal cord (124). The actions of the other membrane PR subtypes in the vertebrate CNS nevertheless remain poorly understood.

3.3. Androgens

Androgens also play a key role in the sexual differentiation of the nervous system, regulation of reproductive behavior, neuroendocrine responses, and aggressive behavior (6, 7, 125, 126). In most vertebrates from fish to mammals, androgens strongly facilitate the expression of aggressive behavior, and regulate mood and aggressiveness for this purpose (2, 7, 12, 13). In humans, the androgens (which include testosterone, 5α-

dihydrotestosterone, androstenedione, and the adrenal androgen DHEA) could also instantaneously induce libido and improve the sexual activity in both males and females (7, 127-129). In older women, testosterone replacement seems to decrease dysphoria (128). In men, however, the mechanisms of testosterone on mood are currently unclear (128). Based on findings obtained by locally manipulating the testosterone level in the hypothalamic preoptic area of fish, this area is known to be involved in controlling reproductive behaviors such as sexual and aggressive behaviors in fish and other higher vertebrates (130, 131).

Under natural conditions, plasma androgen levels increase in eels during their reproductive migration from the sea to the river (132). This coincides with a rapid change in the eel's habitat and behavior. The eel stops eating and starts migrating, which suggests an important shift in brain dopaminergic systems and a narrowing of olfactory focus that results in reduced feeding, reduced detection of alarm cues, and improved detection of pheromones (132, 133). Since these responses are very rapid, the androgens may not simply mediate the nuclear AR mechanisms that modulate gene transcription. However, there are no reports in vertebrates concerning membrane-associated AR-like molecules as the direct targets of the non-genomic actions of androgens.

Testosterone (or the non-aromatizable 5α-dihydrotestosterone) is active in the brain and testis in mammals. In fish, 11-ketotestosterone plays an important role in testicular maturation and spermatogenesis, although testosterone is a major androgen in the brain. On the other hand, the enzymatic activity of cytochrome P450 aromatase, a key enzyme that forms estrogen from testosterone, is extraordinarily higher (approximately 100 times or more) in the teleost fish brain than it is in the mammalian brain (134, 135). This suggests a lower concentration of androgens in the teleost brain. Moreover, only teleosts in vertebrates, at least two different subtypes of nuclear AR, such as ARα and ARβ, are identified, which are transcribed by the distinct genes (136, 137). However, the expression dominance of the AR subtypes at the protein level or activity between brain and gonad is still poorly understood. These results taken together suggest that different mechanisms could exist in androgen signaling in the brain and in the gonads in fish. Future attention should focus on a comparative study of androgen signaling in fish and in mammals to reveal the conserved property underlying the genomic and non-genomic actions of androgens in the vertebrate CNS.

4. CORTICOIDS

Adrenal corticosteroids (*e.g.,* glucocorticoid, cortisol (in humans) or corticosterone (in rodents), mineralocorticoid, and aldosterone) readily enter the brain in target neural cells and exert markedly diverse effects such as stress responses, mood regulation, learning, memory, and sodium appetite. These effects are regulated *via* two nuclear receptor systems – the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). GRs and MRs are classically regarded as ligand-inducible transcription factors, which are known as genomic receptors, both types of receptors predominantly reside in the cytoplasm in the absence of a ligand, but they are quickly translocated into the nucleus upon binding with a ligand (14). Classic GRs selectively bind to naturally occurring corticosteroids and synthetic glucocorticoids; MRs bind to corticosteroids and aldosterone with a very high affinity (*e.g.*, about a 10-fold higher affinity for corticosteroids), compared with that of GRs (15). In addition to genomic receptors, there is increasing evidence that corticosteroids are capable of rapid signaling processes, independent of transcriptional changes, *via* membrane-bound MRs and GRs (Figure 2) (20).

4.1. Glucocorticoids

Direct anatomical distribution of GRs on membranes and synapses in amphibians and mammals gives evidence for possible membrane-bound receptors (138, 139). By using green fluorescent protein (GFP)-GR knockin mice, Usuku *et al.* also demonstrated the localization of GR in the neurites of cultured hippocampal neurons (140). Joels and colleagues reported that the MR potentially regulates glutamate release from the presynapse (141). In this section, the rapid actions of corticosteroids that occur *via* putative membrane GRs in the CNS are summarized, and their direct actions on synaptic transmission are especially focused.

Stress is essential for health, but if there is a failure in coping with stress, the action of the stress hormones, (*i.e.,* corticosteroids) becomes dysregulated, thereby precipitating a higher susceptibility to psychopathology. Corticosteroid secretion can be increased at any time in response to a stressor. A rapid stress-induced secretion of corticosteroids is a sign of health and resilience as long as the secretion is turned off effectively. The corticosteroid surge after stress actually promotes behavioral adaptation. However, if the hormone response is inappropriate, excessive, or prolonged, cognition becomes impaired and emotion become imbalanced (14, 15). GRs activate many life-sustaining mechanisms, in addition to their role in the stress response. For example, GRs are located in almost every cell of the body and stimulate glycogen deposition and regulate immune function, which mediate physiological homeostasis (14). Accumulating evidence obtained from the CNS demonstrates that GRs also affect neuronal plasticity by acting directly and indirectly through second messengers, such as the G proteins, to modulate different signal transduction mechanisms. Furthermore, GRs may exert their effects at the membrane, where they can rapidly modulate synaptic transmission and modulate the conductance of specific ion channels, which in turn modulates neuronal excitability (20).

Evidence for functional membrane GRs has been accumulated over the past two decades. Orchinik and Moore in the early 1990s clearly showed the presence of GRs in the neuronal membranes of the *Taricha granulosa* (a rough-skinned newt); the GRs were associated with G proteins and modulated intracellular signaling (138). Borski and colleagues, who used the tilapia teleost (*Oreochromis mossambicus*), found that the acute inhibitory effects of

cortisol on prolactin release occur through a non-genomic mechanism involving interactions with the plasma membrane and inhibition of the Ca^{2+} and cyclic adenosine monophosphate (cAMP) signal transduction pathways (142-145). The search for mammalian membrane GRs occurred more than ten years later. Tasker and colleagues demonstrated membrane GRs in the rat hypothalamus by using *in vitro* electrophysiological recordings (146). Kawato and colleagues reported the possible existence of GR proteins in synaptosomal fractions (enriched in post-synaptic membranes) from rat hippocampal slices (147). Johnson and colleagues moreover showed a direct anatomical localization of membrane GRs in the post-synaptic density (139). Johnson and colleagues used three criteria to categorize a receptor as a membrane-bound receptor that directly regulates associated cell surface ion channels: i) the receptor response latency must be less than 30 minutes (148, 149); ii) the response must be replicated using a corticosteroid conjugated with a membrane-impermeable protein such as bovine serum albumin (150); and iii) the application of protein synthesis inhibitors must not affect the corticosteroid-induced responses. In the absence of new protein synthesis, signaling responses are likely to be membrane-mediated, with or without involving a local second messenger system.

In contrast to presynaptically located membrane MRs exhibiting an increase in neuronal excitability (141), membrane GRs are predominantly distributed in postsynaptic sites and may exert various effects in a region-specific manner. Ffrench-Mullen demonstrated that membrane GRs may decrease neuronal excitability by reducing calcium entry *via* voltage-gated calcium channels (151). Kawato and colleagues indicated that corticosteroids exert acute effect on NMDA receptor-mediated Ca^{2+} elevation in hippocampal slices through putative membrane GRs (152). Tasker and colleagues showed that in hypothalamic parvocellualr neurons the corticosteroids rapidly activate membrane GRs associated with G-protein; this induces cAMP/protein kinase A (PKA)-dependent endocannabinoid (eCB) synthesis and release (153). eCBs then act in a retrograde manner to suppress glutamate release from excitatory synaptic terminals *via* cannabinoid receptor 1 (CB1) activation (146, 153). They recently found that corticosteroids activate divergent intracellular G protein signaling pathways. Activated Gαs leads to eCB synthesis (as mentioned above) and activation of the Gβγ dimer leads to neuronal nitric oxide synthase (nNOS) stimulation and nitric oxide (NO) synthesis in hypothalamic magnocellular neurons. The released NO feeds back onto inhibitory synaptic terminals to facilitate GABA release (154). Thus, post-synaptic membrane GR activation can decrease post-synaptic excitation in the paraventricular region of hypothalamus in mammals.

Coming full circle, Moore and colleagues used the model proposed by Tasker for their newt experiments and reported that glucocorticoids inhibit sensory-evoked stimulation of medullary neurons and that courtship clasping is modulated by eCB signaling (155). In contrast to the findings from the paraventricular region of hypothalamus, Campolong *et al.* recently demonstrated that membrane GRs activate the Gs-cAMP/PKA pathway, thereby inducing eCB synthesis; this inhibits GABA release from GABAergic terminals *via* retrograde eCB signaling in the basolateral amygdala (BLA). These actions disinhibit norepinephrine release and activate post-synaptic β-adrenoreceptors, thereby increasing the consolidation of emotionally aversive memories. These results provide the first *in vivo* evidence of the existence of this pathway in mammals (156).

Given the evidence that membrane GRs regulate synaptic transmission in the CNS, it is crucial to define the structure of the receptors and how they differ from or are similar to their genomic counterparts. The initial evidence has been obtained from an MR study that used a mouse line with forebrain-specific ablation of the MR (141). This did not induce in these mice the rapid corticosterone-mediated increase in miniature excitatory postsynaptic current (mEPSC) frequency and glutamate release that is observed in wild-type mice. These findings suggest that membrane MRs and genomic MR could be encoded by the same genes and, at least initially, could have the same structure. Mostly the same stories for membrane MR are accepted in the case of membrane GR, although the molecular identity of membrane GRs remains to be clarified.

How can a single receptor act as both transcription factor and a membrane receptor? An answer comes from research findings on the ERs, as described in the previous section and in other studies (157). Post-translational palmitoylation of genomic receptors is a necessary process in associating the receptors with a cell membrane. Furthermore, caveolar localization of the receptors is another important component of their membrane association (157). In non-neuronal cells, caveolar are required for localization of the membrane GRs and for membrane GR regulation of protein kinases (158). However, there is still no evidence that the GRs are associated with caveolae in the CNS (139). On the other hand, there are some reports indicating that antagonists for the classic genomic GRs do not interfere with the suppression of mEPSC frequency, although corticosteroids are only active when administered on the outside of the plasma membrane (153). These findings indicate the possibility that an unknown GPCR is responsible for these actions. Future study is required to clarify the molecular identity of the membrane GRs.

How can these rapid cellular effects of corticosteroids contribute to the stress response in the CNS? More than half a century ago in 1941, Selye reported rapid physiological effects of steroid compounds (159). Since then, potential signal transduction mechanisms and response properties of membrane GRs and membrane MRs have been surveyed. This has revealed aspects of the neurobiology of stress – especially the rapid effects of stress at the synapse. With respect to hypothalamo-adrenal-pituitary axis regulation, it has been known for a long time that high levels of corticosteroids can exert a fast negative control through CRH and ACTH. The inhibition of the hypothalamo-adrenal-pituitary axis through corticosteroid feedback could be regulated by hormonal actions at

different central targets – either indirectly by their actions in the hippocampus or directly by their actions in the hypothalamus and pituitary. Tasker and colleagues first presented evidence for the rapid direct inhibition of CRH neurons by corticosteroids, and provided a mechanism for the fast direct feedback inhibition of CRH release by corticosteroids in hypothalamic CRH neurons *via* putative membrane GRs (153). They have more recently proposed a model of rapid corticosteroid regulation of the magnocellular neurons in the supraoptic nucleus (SON) (154). The rapid corticosteroid inhibition of the glutamatergic system *via* eCB system and the facilitation of GABAergic synaptic inputs to magnocellular neurons *via* NO system are likely to have robust functional consequences for the release of vasopressin and oxytocin (160). This overall corticosteroid inhibitory effect likely underlies the negative feedback in the release of neurohypophysial hormones. These rapid inhibitory feedback actions during the stress response may play an incorporative role in regulating neuroendocrine function and play a neuroprotective role in the hypothalamus since eCBs and NO have anti-inflammatory actions in the brain (161).

Furthermore, glucocorticoids – acting *via* non-genomic interactions with norepinephrine and intracellular signaling systems in the BLA – enhance the long-term consolidation of emotionally arousing memory (162). Glucocorticoids also interact with the eCB system in the BLA. Roozendal and colleagues demonstrated that eCBs in the BLA modulate memory consolidation of emotionally arousing experiences, and they suggest that CB1 activity in the BLA is critically involved in mediating non-genomic glucocorticoid effects on memory consolidation (156, 163).

4.2. Mineralocorticoids

4.2.1. Mammals

The principal role of the aldosterone-MR is to control Na^+ homeostasis. Na^+ reabsorption in the kidney by the epithelial Na^+ channel (ENaC) is regulated by mineralocorticoid signaling (164). MR knockout mice ($MR^{-/-}$) cannot reabsorb Na^+ at the distal tubules and therefore die 8–12 days after birth because of massive salt and volume loss (165). Hypovolemia, hyponatremia, and hypotension in mammals stimulate aldosterone secretion, which evokes sodium appetite. Deoxycorticosterone acetate (DOCA), an aldosterone analog, dramatically increases sodium appetite, which suggests that a significant part of the mechanism of sodium homeostasis depends on aldosterone. Aldosterone's effects occur rapidly in the CNS, therefore it is believed that aldosterone acts by a direct non-genomic membrane receptor and not by an intracellular genomic MR only (166).

In terrestrial animals, water and Na^+ are constantly lost at the same time. When water balance is disrupted (*e.g.,* hypovolemia), animals need to ingest Na^+, as well as water. Therefore, thirst and sodium appetite are closely related, and the dipsogenic hormone angiotensin II (ANGII) principally induces sodium appetite (167). Intracerebroventricular treatment with aldosterone enhances ANGII-induced drinking and sodium uptake (168). It is believed that the mineralocorticoids modify the functional ANGII receptor subtype and reduce the tonic inhibitory signal against sodium appetite in the CNS; this enables ANGII to stimulate sodium intake (169-171). On the other hand, ANGII robustly stimulates aldosterone secretion. There is also evidence that aldosterone itself directly induces sodium appetite.

MR expression has been reported in many brain areas (172), but aldosterone-sensitive cells need to express 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) to prevent glucocorticoid binding since the circulating concentration of glucocorticoid is several hundredfold higher than that of aldosterone. The nucleus tractus solitarius (NTS) matches this criterion (173) and it lacks the problem involved with the blood-brain barrier (174); this enables circulating aldosterone direct access to the nucleus. Dietary sodium deprivation activates the 11β-HSD2 neurons; they are rapidly inactivated by voluntary sodium ingestion (175). Since sodium deprivation-induced activation of 11β-HSD2 neurons also occurs in the adrenalectomized rat, sodium appetite may not only be induced by the mineralocorticoids. It remains unknown whether 11β-HSD2 neurons are sensitive to ANGII and other hormones.

Psychological stress also causes sodium appetite in several mammalian species (176-178). Stress activates the hypothalamo-pituitary-adrenal axis and stimulates the release of glucocorticoids. Stress-induced sodium appetite seems to be mediated by glucocorticoid-activated MRs. However, Shelat *et al.* reported that glucocorticoids induce sodium appetite in rats but only when administered in combination with mineralocorticoids (179). Despite these results in animal models, studies on humans or primates have indicated that stress has no effect on sodium intake (180, 181).

MR also binds to corticosteroids. High circulating levels of corticosteroids, resulting from stress, quickly and reversibly stimulate hippocampal glutamatergic transmission *via* the non-genomic actions of MR (182). A recent study demonstrated the localization of MRs in mammalian synapses. Prager *et al.* reported that the MR has been anatomically localized to extra nuclear sites, including presynaptic terminals, neuronal dendrites, and dendritic spines (183). Furthermore, they found MRs in the post-synaptic membrane densities of excitatory synapses (183).

In addition to the glucocorticoids' actions on the MR, aldosterone also activates the MR and GR in mammals. This complicated corticosteroid signaling makes it difficult to identify the aldosterone-MR pathway specifically (*e.g.*, MR structure, second messengers) in sodium appetite or in the CNS of mammals.

4.2.2. Fish

It is generally acknowledged that the ability to synthesize aldosterone evolved in the *Sarcopterygii* (*e.g.,* lungfish, coelacanth) and that the *Actinopterygians* (*e.g.,* teleost fish) do not possess this mineralocorticoid due to their lack of aldosterone synthase (184-186). Therefore, the

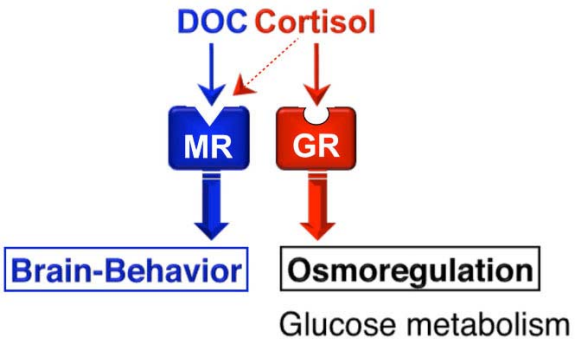

Figure 3. Schematic diagram of corticosteroid signaling in the teleost fish. Cortisol, acting through the glucocorticoid receptor (GR), plays a central role in osmoregulation in teleosts. The role of the mineralocorticoid receptor (MR) in osmoregulatory organs seems to be minor, although MR is also sensitive to cortisol. On the other hand, 11-deoxycorticosterone (DOC; a teleostean mineralocorticoid) activates only the MR (but not the GR). Mineralocorticoid signaling appears to be important for regulating brain function and/or behaviors in teleosts.

circulating aldosterone level is lower than what is likely to affect the fish's GR or MR (185, 187). Cortisol is the major corticosteroid released from the teleost interrenal (which is analogous to the adrenal cortex in the tetrapod) and is believed to carry out both glucocorticoid and mineralocorticoid activity (188). Cortisol induces the transactivation of the GR and MR in the fish, which is similar to its actions in mammals since the mammalian GR and MR are sensitive to cortisol (185, 187, 189). However, 11-deoxycorticosterone (DOC), a precursor of aldosterone, is a circulating corticosteroid that is present in significant concentrations in teleosts and can interact physiologically with MRs in the fish (187, 190-192). Expression studies of fish MR and GR indicate that transactivation of the MR is more sensitive to DOC than to cortisol, whereas the GR is specific to cortisol (185, 187, 190, 192). These effects differ from those in mammals in which MR and GR can both be activated by aldosterone and glucocorticoids (189, 193).

In addition to the presence of a specific MR ligand in the teleost, MR mRNA is found in a wide variety of tissues. Studies interestingly have shown that MR mRNA levels are relatively modest in tissues involved in ion regulation, but considerably higher in the brains of most teleosts that have been examined (190, 194, 195). Furthermore, recent studies from our laboratory and other researchers have shown that glucocorticoid signaling (*i.e.*, cortisol-GR) may play a more important role than mineralocorticoid signaling (DOC-MR) in differentiation of the osmoregulatory organs in teleosts (196, 197). Based on these findings, teleost fish seem to be a unique and useful model for studying the biological actions of the mineralocorticoid system in the brain and in behavior (Figure 3) (192, 198).

Recent studies suggest that the MR in the brain is involved in the teleost behavior, which agrees with research findings of highly expressed MR in the brain. The mudskipper (*Periophthalmus modestus*), an amphibious goby in estuaries, prefers an aquatic habitat several hours after undergoing intracerebroventricular injection with DOC (our unpublished results). The MR in the brain seems to be involved in a preference for an aquatic habitat, which may be related to the induction of sodium appetite by mineralocorticoid signaling that has been reported in mammals (see above). In the carp (*Cyprinus carpio*), the MR and the GR are expressed in key components of the stress axis such as the forebrain pallial areas (which are homologous to the mammalian hippocampus) (130); the CRH cells in the preoptic nucleus; and the ACTH cells in the pituitary pars distalis. Following exposure to stressors, the mRNA levels of the MRs and GRs are regulated similarly. Since water-deprived mudskippers are stressed (199), the MR in the fish brain appears to play a role in stress responses (192). MR expression is high in the forebrain of the plainfin midshipman fish (*Porichthys notatus*), especially in the "sneaking" males (194). On the other hand, feeding rainbow trout (*Oncorhynchus mykiss*) with corticosteroid receptor (CR) antagonists inhibits aggression, which suggests that the MR (and the GR) are involved in this behavior (198). Thus, the MRs of the teleost fish seem to be involved in regulating various brain functions and/or behaviors.

There are at present several gene manipulation methods available, including gene knock-out and knock-down technologies, to utilize in the medaka (*Oryzias latipes*) and zebrafish (200-202). In medaka, the high expression of MR mRNA has been observed in the brain, especially in the cerebellum (our unpublished result). To acquire a clearer understanding of the biological and physiological roles of the MR in the teleost brain, future investigations should focus on these model fishes. By using the Targeting Induced Local Lesions IN Genomes (TILLING) method (203), we have recently identified three missense mutations in the ligand binding domain of the medaka MR, and we are currently establishing the fish with functionally deleterious MR.

In the sea lamprey (*Petromyzon marinus*), a member of the oldest vertebrate lineage, a single CR has been identified (204). Stolte *et al.* grouped the lamprey CR with other vertebrate MRs, suggesting that the MR gene is ancestral to the GR gene (205). Close *et al.* very recently found that 11-deoxycortisol (a potent endogenous ligand of the lamprey CR) is regulated by the hypothalamo–pituitary axis and by acute stress, which implies that the CR is involved in the stress response; however, the tissue distribution of this receptor is unclear (206). Further study on ancestral corticosteroid signaling may explain the action (and perhaps the original function) of corticosteroids in the brain and in behavior and explain the intriguing evolution of the role of corticosteroids.

5. NEUROSTEROIDS

The view concerning the sources and actions of steroid hormones has recently broadened. A number of studies have reported that *de novo* steroidogenesis from

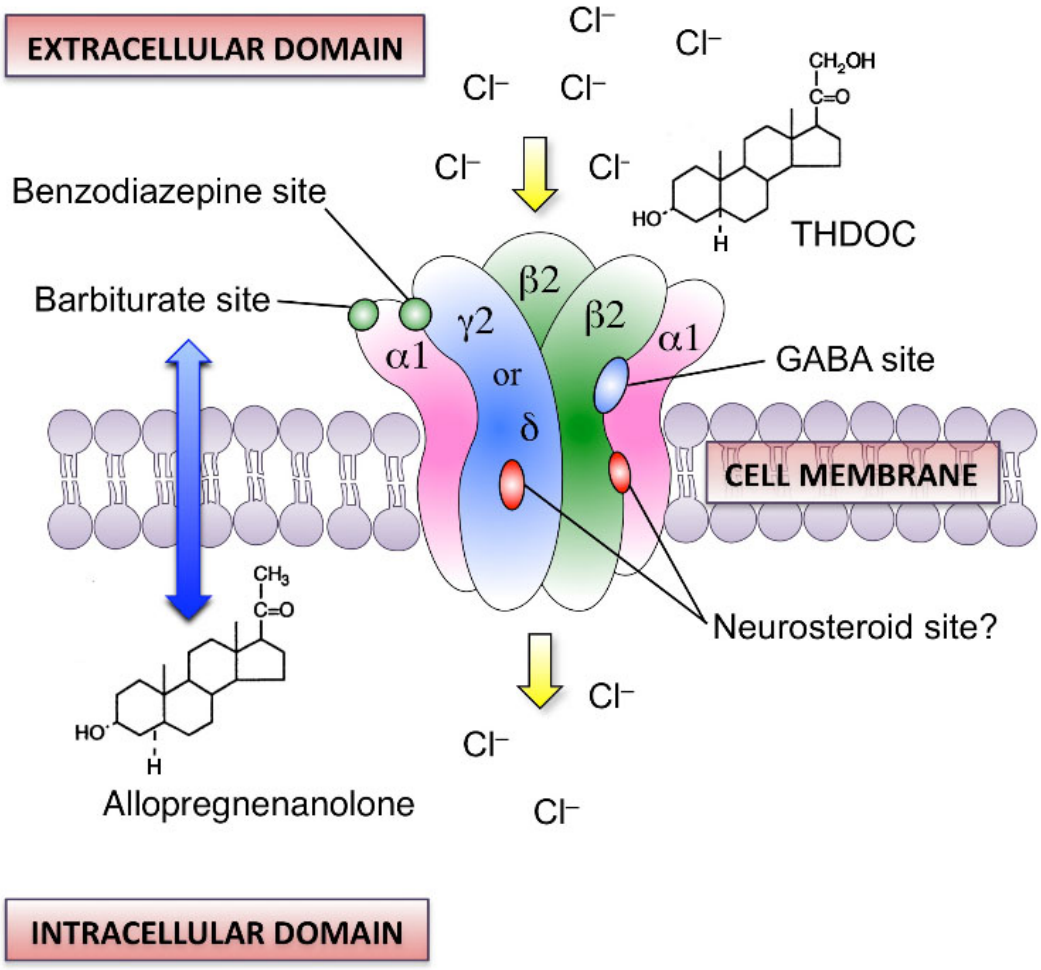

Figure 4. Schematic diagram of neurosteroid signaling in the brain through a γ-aminobutyric acid (GABA) system. The pentameric structure of a $GABA_A$ receptor, which constitutes GABA-gated Cl^- channels that mediate the fastest inhibitory neurotransmission in the brain. The image shows allopregnanolone acting through non-genomic means, rather than through intra-nuclear steroid receptors that promote classic genomic actions. Currently, seven different classes of $GABA_A$ receptor subunits with multiple variants (α1–6, β1–3, γ1–3, σ1–3, δ, ε and θ) are known. Most $GABA_A$ receptors *in vivo* comprise α-, β- and γ-subunits, in a probable stoichiometry of 2α:2β:1γ, and may contain binding sites for modulators such as benzodiazepines, barbiturates and neurosteroids. The δ-subunit can substitute for the γ-subunit. Although no absolute subunit specificity for neurosteroid modulation of $GABA_A$ receptors has been found, the presence of α- and γ- or δ- subunits could affect allopregnanolone or 3α,5α-tetrahydrodeoxycorticosterone (THDOC) modulation. In addition, the hydrophobic steroids may be able to interact at the interphase of the membrane phospholipids and receptor proteins.

cholesterol is a conserved property of the vertebrate brain (where it is synthesized by neurons and glial cells). Steroids synthesized *de novo* in the CNS are called *neurosteroids (*for reviews, see references (21-23, 25)). The basic observation is that certain biologically active steroids are present in higher concentrations in the CNS than in the blood (21-23, 25). Recent findings indicate that some neurosteroids may mediate their actions by rapid non-genomic mechanisms, rather than by genomic actions. It has been proposed that several CNS-specific neurosteroids (*e.g.,* such as 3α,5α-tetrahydroprogesterone (allopregnanolone), 3α,5α-tetrahydrodeoxycorticosterone (THDOC) and 7α-hydroxypregnenolone (7α-PREG)) are synthesized *de novo* in the vertebrate CNS; these neurosteroids may mediate their actions through a panel of ion-gated channel receptors or metabotropic GPCRs for the classic neurotransmitters (21-23, 25, 207, 208).

The patch-clamp method indicates that allopregnanolone (a progesterone metabolite) may mediate its action through ion-gated channel receptors (*e.g.,* $GABA_A$ receptor) through allosteric modulation, rather than through intra-nuclear steroid receptors that promote classic genomic actions (Figure 4) (21, 208-212). The genetic knockout of the δ subunit of the $GABA_A$ receptors resulted in a significant decrease in the sensitivity of mice

to the sedative/hypnotic, anxiolytic, and pro-absence effects of neurosteroids, including the synthetic analog alphaxalone (3α-hydroxy-5α-pregnan-11,20-dione) and pregnanolone, suggesting a central involvement of δ-containing $GABA_A$ receptors in the non-genomic actions of neurosteroids (Figure 4) (213-215). $GABA_A$ receptors are well known molecular targets for a variety of sedative-hypnotic drugs, including the benzodiazepines and barbiturates (Figure 4) (210, 216, 217). The affinity of allopregnanolone for the $GABA_A$ receptor is equal to or greater than that of the benzodiazepines or barbiturates (210, 211, 217). The exogenous administration of allopregnanolone shows robust antidepressant properties in rodent models of depression, and high endogenous allopregnanolone levels in the animal brain are associated with less depressive behavior (218-222). Furthermore, allopregnanolone may regulate rapid nerve growth in cultured rat hippocampal neurons (223), and may also promote neurogenesis in the rat hippocampal subgranular zone (224). The administration of allopregnanolone significantly increases the proliferation of rodent and human neural progenitor cells *in vitro* through the $GABA_A$ receptor and through L-type Ca^{2+} channel-dependent mechanisms (225, 226).

On the other hand, allopregnanolone may play a role in epilepsy. Progestogen therapy clinically reduces seizures in some menopausal women (227); therefore, allopregnanolone may similarly protect against seizures through its actions at the $GABA_A$ receptor (227).

Progesterone and allopregnanolone may enhance (through membrane receptor-mediated mechanisms) sexual motivation, receptivity, and proceptivity in female rats and mice (228-230). On the other hand, THDOC is a metabolite of the mineralocorticoid deoxycorticosterone and it is responsible for the sedative and antiseizure activity of deoxycorticosterone in animal models (231). Like allopregnanolone, THDOC is an extremely potent positive allosteric modulator of $GABA_A$ receptor function and allows $GABA_A$ receptors to remain open for a longer period of time and permits increased chloride ion flux (Figure 4) (231, 232). Moreover, the neurosteroid pregnenolone sulfate (PREG-S) infusion into the rat cerebellar slice rapidly enhances, in a dose-dependent manner, the frequency of inhibitory postsynaptic currents (IPSCs) within 1 minute of perfusion. The IPSCs recorded in the Purkinje cells were completely blocked by bicuculline, a $GABA_A$ receptor antagonist, suggesting that they are also mediated by $GABA_A$ receptors (23, 108, 233, 234). On the contrary, a direct effect of GABA on the regulation of neurosteroid biosynthesis also reported in the brain of frog (*Rana ridibunda*) (235). GABA, acting on $GABA_A$ receptors at the hypothalamic level in frogs, inhibits the activity of several key steroidogenic enzymes, including 3β-hydroxysteroid dehydrogenase (3β-HSD) and cytochrome $P450_{C17}$ (17α-hydroxylase) (235).

In a lower vertebrate model, the brain of the Japanese red-bellied newt (*Cynops pyrrhogaster*) actively produces 7α-PREG from pregnenolone through the actions of the key enzyme, cytochrome P450 7α-hydroxylase ($P450_{7\alpha}$) (207, 236). 7α-PREG activates the dopaminergic system, thereby stimulating locomotor activity and changes during the annual breeding cycle; its maximum level occurs in the spring breeding period, when locomotor activity increases (207, 236). The adenohypophyseal hormone prolactin may act on neurons in the magnocellular preoptic nucleus, which expresses cytochrome $P450_{7\alpha}$, to stimulate 7α-PREG synthesis in breeding male newts (237). In addition, the brain of a poultry bird, the Japanese quail (*Coturnix japonica*), produces 7α- and 7β-PREG, which act as a key factor for inducing locomotor activity and mediating the action of melatonin that underlie diurnal locomotor rhythms (238, 239).

In the mammalian brain (*e.g.,* rodents and humans), the formation of 7α-hydroxylated neurosteroids such as 7α-PREG and 7α-hydroxydehydroepiandrosterone (7α-DHEA) has been reported (240-243). In mammals, 7α-hydroxylation of DHEA may be part of a metabolic pathway to more potent derivatives (244). 7α-DHEA is more active than DHEA in preventing the hypoxic death of neurons *in vitro (*245). However, the function of 7α-PREG is still unclear in the mammalian CNS. On the other hand, DHEA and its sulfate ester (DHEA-S) are abundant neurosteroids in the mammalian CNS (246, 247). Compagnone and Mellon (248) reported that DHEA selectively increases the length of axons and the incidence of varicosities and basket-like process formations in cultured cerebral neurons, whereas DHEA-S selectively promoted branching and dendritic outgrowth *in vitro*. These steroids may play crucial and different roles in organizing the cerebral cortex (248). Furthermore, DHEA rapidly increases free intracellular calcium by activating NMDA receptors, which suggests it has non-genomic actions in the CNS (248). The neurosteroids PREG-S and DHEA-S have also been implicated as powerful modulators of memory-enhancing processes and sleep states in young and aged rodents, depending on the integrity of cholinergic systems (249-251).

6. CONCLUSION

In the classic concept, steroid hormones act just as a transcriptional factor *via* their specific nuclear receptors located broadly in the vertebrate CNS. These actions are slow to develop because it takes time to form a steroid/nuclear receptor complex, bind it to the DNA molecule, and induce transcriptional changes that underlie protein synthesis. Most steroid hormones have now gained increased scientific interest for the rapid steroid actions that occur through non-nuclear receptors, as mentioned above (Table 1). Steroid hormones have a long history of study, of course, but more recently developed analyses and a better understanding of the rapid non-genomic actions between steroid hormones and their membrane-associated receptors (or target sites) may have opened the door to new research for behavioral neuroendocrinology and clinical investigations.

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Abbreviations: ACTH: adrenocorticotropic hormone, AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, ANGII: angiotensin II, AR: androgen receptor, ATP: adenosine triphosphate, BLA: basolateral amygdala, CA: cornus Ammonis, cAMP: cyclic adenosine monophosphate, CB1: cannabinoid receptor 1, CBP: cyclic adenosine monophosphate response element-binding protein-binding protein, cDNA: complementary deoxyribonucleic acid, CNS: central nervous system, CR: corticosteroid receptor, CRE: cyclic adenosine monophosphate response element, CRH: corticotrophin-releasing hormone, DHEA: dehydroepiandrosterone, 7α-DHEA: 7α-hydroxydehydroepiandrosterone, DHEA-S: dehydroepiandrosterone sulfate, DNA: deoxyribonucleic acid, DOC: 11-deoxycorticosterone, DOCA: deoxycorticosterone acetate, DRG: dorsal root ganglion, eCB: endocannabinoid, ENaC: epithelial Na^+ channel, ER: estrogen receptor, GABA: γ-aminobutyric acid, GFP: green fluorescent protein, GnRH: gonadotropin-releasing hormone, GPCR: G protein-coupled receptor, GPER1: G protein-coupled estrogen receptor 1, Gq-mER: Gq-coupled membrane estrogen receptor, GR: glucocorticoid receptor, 3β-HSD: 3β-hydroxysteroid dehydrogenase, 11β-HSD2: 11β-hydroxysteroid dehydrogenase type 2, IPSC: inhibitory postsynaptic current, IZAg: adrenocortical inner zone-specific antigen, MAPK: mitogen-activated protein kinase, mEPSC: miniature excitatory postsynaptic currents, mGluR: metabotropic glutamate receptor, MR: mineralocorticoid receptor, mRNA: messenger ribonucleic acid, NMDA: *N*-methyl-D-aspartate, nNOS: neuronal nitric oxide synthase, NO: nitric oxide, NTS: nucleus tractus solitarius, $P450_{C17}$: cytochrome

P450 17α-hydroxylase, $P450_{7\alpha}$: cytochrome P450 7α-hydroxylase, pCREB: phosphorylated cyclic adenosine monophosphate response element-binding protein, PGRMC: progesterone membrane receptor component, PKA: protein kinase A, PKCε: protein kinase Cε, Pol II: ribonucleic acid polymerase II, PR: progesterone receptor, 7α-PREG: 7α-hydroxypregnenolone, PREG-S: pregnenolone sulfate, SON: supraoptic nucleus, SRE: steroid response element, TAFs: TATA element-binding protein-associated factors, THDOC: 3α,5α-tetrahydrodeoxycorticosterone, TILLING: Targeting Induced Local Lesions IN Genomes, VEMA: ventral midline antigen

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