Non-genomic Effects of Steroid Hormones on Membrane Channels

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Abstract: Steroid hormones may possess two distinct actions, a delayed genomic influence and the rapid nongenomic effects, which may act in concert. Nongenomic effect may be mediated by putative membrane receptors or due to allosteric interactions of steroids with membrane proteins (*e.g*. ionic channels), inducing rapid changes in protein/receptor/channel activation or inhibition.

INTRODUCTION

Steroids are a class of natural or synthetic organic chemical compounds characterised by a molecular structure of 17 carbon atoms arranged in four rings conventionally denoted by the letters A, B, C, and D, as shown in Fig. (**1**). In this parent structure (referred to as the steroid nucleus), the carbon atoms are bonded to 28 hydrogen atoms. This class includes the sterols (steroid alcohols), bile acids, all the sex hormones, adrenal cortical hormones, cardiac

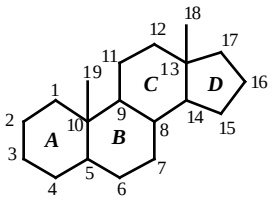

Fig (1). Chemical structure and numbering of the steroid skeleton.

glycosides, vitamin D3, sapogenins and some alkaloids. In the common theory of steroid hormone action, steroids bind to receptors present in the nucleus or in the cytosol, followed by translocation of the receptor-ligand complex to the nucleus, with subsequent modulation of transcription and protein synthesis. These genomic steroid effects, being characterised by their delayed onset of action and their dependence on transcription and protein synthesis, have been known for several decades. In contrast, very rapid actions of many natural and synthetic steroids, which are considered to be of nongenomic origin, have now been recognised more widely and characterised in detail. They may for example operate through transmembrane signal transduction involving increased intracellular calcium ions, modulation of protein kinase activities, phospholipid metabolism or modifications of the characteristics of membrane channels. This paper reviews the evidence that steroids can interact with plasma membrane and, among other responses, influence the activity of membrane channels on a time scale of seconds or a few minutes ; dramatic effects on membranes of excitable as well as other tissues have been demonstrated, suggesting a possibly modulatory role for certain steroid compounds.

The physiological and clinical relevance of these rapid effects is still largely unclear, but their existence *in vivo* has been clearly shown in various settings including human studies. Adrenocortical and gonadal steroid hormones can pass the blood-brain barrier ; the neurone excitability in the CNS can be modulated (nongenomically usually) by these compounds or their metabolites ("neuroactive" steroids), via alterations of the electrical properties of neuronal membranes. Drugs that specifically affect nongenomic steroid action may find applications in various clinical areas such as cardiovascular and central nervous system disorders, electrolyte homeostasis, infertility, etc.

CRITERIA FOR THE CLASSIFICATION OF NON-GENOMIC STEROID EFFECTS

Non-genomic steroid effects are at first characterised by their rapid onset of action, which occurs almost instantaneously (between a few seconds and 1-2 min after steroid application), whereas at least 30 min are required for the genomic response to steroids to occur. Such delay is explained by the time required for newly initiated transcription, translation and processing of proteins. Secondly, non-genomic effects are not prevented by inhibitors of DNA transcription and protein synthesis (*e.g*. cycloheximide or actinomycin D). Thirdly, a tool to distinguish genomic and non-genomic origins is to use steroids together with a macromolecule as bovine serum albumin, which prevents the steroid from entering the cells. If the compound still remains active, its site of action is likely to be the plasma membrane. However, endocytosis of the macromolecule, resulting in uptake of the active steroid into the cell, might obscure this conclusion. In addition, non-genomic effects of steroids are often observed at relatively high concentrations, sometimes two to three orders of magnitude higher than the normal plasma levels. In summary, the rapid time-course and the insensitivity of the

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signals to inhibitors of transcription or protein synthesis are the main types of evidence for the classification of a response as a non-genomic event [for review, see for example 1, 2, 3].

GONADAL STEROIDS

Gonadal steroid hormones can exert acute, nongenomic effects on several tissues, particularly on neural and vascular tissues.

ESTROGENS (17Β-ESTRADIOL)

The incidence of cardiovascular disease is lower in women before the menopause compared to men, while menopausal women have an incidence of coronary disease similar to that of men of the same age. Some of the increased risk for coronary heart disease in men has been attributed to the differences in lipoprotein levels; in particular, high-density lipoprotein cholesterol (HDL-C), a protective factor, is higher in women, but 17β-estradiol is known to have antiarrhythmic effects, possibly by acting on voltage-dependent membrane channels, attenuating myocardial and vascular contractility.

It was for example shown to slightly depolarise the membrane and attenuate the generation of action potentials in the isolated longitudinal smooth muscle cells of the pregnant rat by inhibiting both inward and outward currents [4]. In many tissues, it was found to reversibly inhibit voltage-dependent L-type calcium current (I_{CaL}), as at concentration 1 to 100 µM in rabbit arterial cells [5]. At 10 µM, it significantly and rapidly reduced I_{CaL} peak in A7r5 vascular smooth muscle cell line [6], in rat cardiomyocytes [7], in atrial myocytes of the human, and ventricular myocytes of guinea-pig and rat [8], as well as in guinea-pig atrial myocytes [9]. At 20 µM, it strongly inhibited Ca channel currents in smooth muscle cells isolated from rat myometrium [10] whereas at 30 µM, it decreased L-type Ca^{2+} current by 33% [11] and 80% [12] respectively in guinea pig ventricular myocytes. At 10 µM, it also significantly rapidly reduced peak T-type Ca^{2+} current in A7r5 vascular smooth muscle cell line [6] but only by about 10% in guinea-pig atrial myocytes [9].

The fact that the QT interval of the ECG is longer in females than in males has been known for more than 80 years now. Application of 10 µM 17β-estradiol prolonged the action potential duration in guinea pig ventricular myocytes [12], and this effect would result from the inhibition of three important currents which condition the depolarisation of the ventricular action potential : a calcium current ($I_{CaL)}$) and two potassium channels (the rapidly activating delayed outward K^+ current, or I_{Kr} and the slowly activating component, or I_{Ks}). At 10 µM, estradiol did not affect these currents but at 30 µM both of them were inhibited in guinea pig atrial myocytes [9, 12].

Extracellularly application of 10 µM 17β-estradiol for 10 min reduced inward rectifier K^+ current (I_{Kir}) in freshly isolated rat osteoclasts by about 30% [13]. The inwardly rectifying potassium current (i_{K1}) was reduced partly with 30 µM 17β-estradiol in male rat ventricular myocytes [14] and, in the range10 to 100 µM, in guinea pig ventricular myocytes [15].

Cystic fibrosis (CF) is an autosomal genetic disease associated with impaired epithelial ion transport. Mutations in the CF gene alter the primary sequence of the CF transmembrane conductance regulator (CFTR). 17β-estradiol as well as 17α-estradiol, its stereoisomer, caused a rapid and reversible inhibition of forskolin-stimulated chloride secretion across T84 epithelial cell monolayers [16]. 30 µM 17β-estradiol reduced the transient outward currents to approximately 50% of their maximal amplitudes in male rat ventricular myocytes [14]. Estradiol rapidly attenuates the response of $GABA_B$ receptors (cell hyperpolarization due to the activation of a K^+ conductance) in hypothalamic neurones [17]. Estradiol esters (2 to 25 µM in the external bath) possessed blocking effects on gap junctional communication in rat Sertoli cells and ventricular myocytes [18, 19]. The introduction into the cells of 250 nM unesterified estradiol was sufficient to reduce the intercellular coupling of rat cardiac myocytes (to approximately 50% of its level within 25 min; [20]).

ANDROGENS

Some studies suggest that testosterone levels are favourably associated with cardiovascular risk but, conversely, testosterone has also been shown to demonstrate beneficial effects on anginal symptoms and on parameters of myocardial ischemia in patients treated with this hormone (see [21]). Testosterone is the preferred ligand of the human androgen receptor in the myocardium and directly modulates transcription, translation, and enzyme function. Androgenic anabolic steroids are synthetic derivatives of testosterone that were developed as adjunct therapy for a variety of medical conditions. Today they are most commonly used to enhance athletic performance and muscular development. Both illicit and medically indicated anabolic steroid uses have been temporally and causally associated with many subsequent defects within each of the body systems, such as hypertension, ventricular remodelling, ischaemic cardiomyopathy, acute myocardial infarction and sudden cardiac death in humans. These effects persist long after their use has been discontinued and have significant impact on subsequent morbidity and mortality (for review, see [22].

Testosterone (1-100 µM) induced relaxation in isolated rabbit coronary artery and aorta, ascribed to an interaction with the potassium channels [21]. Long-term exposures to testosterone (96h, 17 to 69 µM) reversibly abolished gap junctional communication in two kinds of human transitional cell carcinoma cell lines (JTC-30 and JTC-32; [23], whereas in rat Sertoli cells or cardiac myocytes, 25 µM testosterone esters quickly induced a complete closure of junctional channels [24]. When directly introduced into the cells, testosterone rapidly reduced the cell-to-cell communication via gap junctional channels [18]. In contrast, testosterone had no effect on I_{Kir} in freshly isolated rat osteoclasts [13].

PROGESTERONE

Progesterone is known to modulate sperm functions by interacting with specific binding sites that, unlike the classic nuclear receptors, are located on the plasma membrane of the spermatozoon. Binding studies have revealed the presence of two classes of progesterone receptors in the human spermatozoon; one class has an elevated affinity constant (nanomolar) and is specific for progesterone, whereas the other class has an affinity constant in the micromolar range and binds equally well with other hydroxylated progesterone derivatives. Following exposure to progesterone, the main event is a rapid (within seconds) increase of the intracellular free calcium concentration, followed by a sustained rise lasting for several minutes (plateau phase). Both these calcium transients are dependent upon entry of extracellular calcium, but the nature of the calcium channel that mediates the effects of progesterone is, currently, unknown. It has been postulated that it may be: (*i*) part of the progesterone receptor; (*ii*) voltage-dependent; or (*iii*) operated by second messengers following activation of the progesterone receptor [for review, see 25]. Recent studies have shown that chloride ion efflux is vital for progesterone to promote the acrosome reaction. This effect is achieved by interaction with membrane receptor which resembles the neuronal $GABA_A$ receptors, responsible for hyperpolarization of the cell by opening a Cl^- channel [for review, see 26].

Progesterone and its metabolites, such as pregnanolone and pregnanediol, promote a rapid rise in intracellular free calcium ($[Ca^{2+}]_i$) in various cells (human sperm, platelets, ..) via a unique Ca^{2+} channel possibly similar to a store-operated channel (SOC) or a receptor-operated channel (ROC) [27].

Progesterone (10 to 100 µM) effectively blocked a broad spectrum of K^+ channels, but not Ca^{2+} or Cl^- channels in T lymphocytes, and this effect was specific since other steroid hormones had little or no effect [28] ; it also decreased (at up to 10 µM) the potassium conductances in MDCK cells [29] and hepatocytes [30]. Progesterone, in the micromolar range, can mediate a rapid transient decrease of human sperm cytosolic Cl^- by activating a steroid receptor/Cl^- channel resembling a $GABA_A$ channel [31] ; at 10-100 µM, this hormone enhanced GABA-induced chloride currents but, surprisingly, antagonises those induced by glycine [32]. At least at short-term, progesterone had no effect on some other channels, as for example I_{Kir} in freshly isolated rat osteoclasts [13], I_{CaL} in single guinea-pig atrial myocytes [9] or junctional channels in rat Sertoli cells [18].

NEUROSTEROIDS

Although the brain is a target site of steroid hormones supplied by peripheral steroidogenic glands, it is now established that the brain itself also synthesises steroids (called neurosteroids) either *de novo* from cholesterol or from steroid hormone precursors in a variety of vertebrates. In addition to endogenous steroids (such as pregnenolone sulfate, dehydroepiandrosterone sulfate (DHEAS), estradiol or progesterone), a number of synthetic steroids share their properties. Indeed, steroid compounds, highly lipophilic, easily cross the blood-brain barrier. Neuroactive steroids are capable (nongenomically usually) of modulating neurone excitability in the CNS, acting on a variety of G-protein-coupled receptors and membrane channels, with consequence on sleep patterns, reactions to stress, memory function, brain plasticity, vigilance, mood, etc… (for review, see [33, 34]. The anaesthetic properties of steroid hormones have been known for nearly 60 years now but the importance of their side effects (*e.g*. the toxic potential of the metabolites) limits their clinical use. Perhaps the most potent neurosteroid, 3-hydroxy-5-pregnane-20-one (also termed 3,5-tetrahydroprogesterone or allopregnanolone) has been shown, in the micromolar range, to dramatically alter the membrane currents caused by $GABA_A$ receptor activation [35]. The most frequently used among synthetic compounds is alphaxalone (5-pregnane-3-ol-11,20-dione), whose structure is related closely to some naturally occurring pregnane steroids, modulates and directly activates $GABA_A$ receptors in a number of neuronal preparations [see 36, 37].

A number of neurosteroids also interact with other receptors, such as NMDA, 5-HydroxyTrypamine type 3 (5HT-3) and glycine receptors and influence a variety of central nervous processes. Serotonin (5-HT) is a major neurotransmitter within the central nervous system, acting through multiple receptor subtypes, and one of them, 5-HT3, is a ligand-gated ion channel that shares structural features with $GABA_A$, glycine, and nicotinic acetylcholine receptors. Several steroids, including 17β-estradiol, 17α-estradiol, progesterone, testosterone or allopregnanolone but not pregnenolone or cholesterol, may act as functional antagonists at the 5-HT3 receptor [38]. The magnitude of NMDA-activated currents of cultured hippocampal neurones was approximately doubled in the presence of 100 µM of the neurosteroid pregnenolone sulfate [39]. This compound and some derivatives steroid failed to alter basal (unstimulated) $[Ca^{2+}]_i$ but, by enhancing NMDA-induced inward currents, induced elevations in intracellular Ca^{2+} concentration in cultured rat hippocampal neurones (at 50 µM, an approximately 300% potentiation of 5 µM NMDA responses was obtained). In contrast, 3-ol-5-pregnan-20-one sulfate (35S) is a negative modulator of NMDA-induced currents and inhibits NMDA-stimulated increases in intracellular calcium [40]. Pregnenolone sulfate rapidly and reversibly inhibits (EC_{50} : 3.7 µM) the strychnine-sensitive glycine receptor (a chloride channel protein belonging to the same superfamily of transmitter-gated ion channels as the $GABA_A$ receptor) in cultured chick spinal cord neurones [41].

In embryonic human DRG neurones, micromolar concentrations of alphaxalone directly activated Cl^- channels whose electrophysiological and pharmacological properties are distinct from those of Cl^- channels associated with GABAA receptors [37]. Several steroids, as allotetrahydrocorticosterone (THCC), dehydroepiandrosterone sulfate (DHEAS) and pregnanolone can rapidly and reversibly, in the range 0.1-100 µM, depress voltage-gated calcium currents in freshly isolated adult hippocampal CA1 pyramidal neurones [42].

MINERALOCORTICOIDS

Classically, aldosterone is a steroid hormone secreted from the adrenal cortex (however, in addition, there is an emerging evidence for aldosterone synthesis in the heart), which acts on kidney, colon and sweat/salivary glands to promote unidirectional sodium transport. Currently, there is an excellent experimental evidence for existence of rapid, non-genomic effects of aldosterone, acting for example directly on the central nervous system to raise blood pressure, and on the heart to cause cardiac hypertrophy and fibrosis [for recent reviews, see 34, 43]. In the nanomolar range, aldosterone may rapidly modulate ion fluxes of different cell types (for recent review, see [34] but, up to now, investigations targetted on the possible rapid, non-genomic effects of aldosterone, in the micromolar range, on membrane channels (for example calcium [44] or junctional [18] channels) gave negative results.

GLUCOCORTICOIDS

Glucocorticoids have profound anti-inflammatory and immunosuppressive actions when used therapeutically. The therapeutic dose is quite variable and depends on the disease, but ranges from very low to extremely high. Several glucocorticoids affect the membrane potential and membrane channels within minutes or seconds. Hydrocortisone (1 to 10 µM) increased the amplitude of the L-type calcium current of isolated guinea pig ventricular cells, inducing a positive inotropic action [45]. Hydrocortisone and corticosterone (10 µM) were the most efficient steroids to inhibit Cl^- channels in cultured epithelial cells [46]. Hydrocortisone (100-1000 µM) inhibited AChR channels of human embryonic kidney (HEK-293) -transfected cells by interacting with a specific site on the protein, probably located at the lipid-contacting surface of the protein [47]. Hydrocortisone and corticosterone esters (up to 25 µM) had no effect (at least at short-term) on the functional state of gap junctional channels in rat Sertoli cells [18].

VITAMIN D_3

The hormonally active form of vitamin D is 1,25(OH)2-vitamin D3 [1,25(OH)2D3]. This seco-steroid is the key mediator of the vitamin D endocrine system which, besides its biological effects via a signal transduction mechanism involving a nuclear receptor and modulation of gene transcription, exerts membrane nongenomic actions. In osteoblasts for example, they include effects on membrane voltage-gated calcium channels, phospholipase C activity, and the sodium/hydrogen antiport [48]. This hormone facilitates the opening of L-type Ca^{2+} channels currents in rat osteosarcoma cells [49], and the resulting increase in transmembrane Ca^{2+} influx augmented $[Ca^{2+}]_i$ within 2 min [50]. An activation of the L-type Ca^{2+} channels was found, in skeletal and cardiac muscle cells, to occur via a guanine nucleotide binding (G) protein-medicated stimulation of the adenylate cyclase/cAMP/protein kinase A messenger system [51]. $1\alpha,25(OH)_2D_3$ also promoted the rapid enhancement of outwardly rectifying Cl^- currents in osteoblasts in a concentration-dependent manner [52].

DISCUSSION

Steroid hormones may possess two distinct actions (which may act in concert), a delayed genomic action together with rapid non-genomic effects, for example quick changes in protein/receptor/channel activation or inhibition, inducing alterations in intracellular $[Ca^{2+}]$, modifications of activities of protein kinases and phosphatases, stimulation of NO production, participation of guanine nucleotide binding proteins (G proteins). These effects, now well documented in many tissues, are observed at high concentrations, acute, transient and non-specific.

POSSIBLE MECHANISMS OF ACTION

Non-genomic effects occur at high steroid concentrations and might be partly unique for certain compounds. This "specificity" might result from an interaction of the steroid with a membranous binding site with an affinity constant in the micromolar range or might mainly reflect properties such as lipophilicity, and thus should be rather termed "pseudospecificity".

Membrane Receptors and Binding Sites

Steroid binding sites localised in the cell membrane can allosterically modulate other functional proteins, as membrane channels. For example, several steroids are able to modulate ligand-gated ion channels, as the $GABA_A$ receptor, where allopregnanolone is a positive allosteric modulator, pregnenolone sulfate acts as a functional antagonist whereas progesterone and 17ß-estradiol are devoid of modulatory properties [see 38]. Generally, the steroids with a reduced A ring and an hydroxyl group at the 3α position are active on $GABA_A$ receptor complex [36]. At the N-methyl-D-aspartate (NMDA) receptor, 17ß-estradiol displays antagonistic properties whereas pregnenolone sulfate is a positive allosteric modulator. The structure of the steroids also plays an important role for the modulation of the 5-HT3 receptor : 17ß-estradiol, progesterone, and allopregnanolone are functional antagonists at this ligand-gated ion channel, whereas pregnenolone sulfate is inactive. Thus, the structure-activity requirements for modulation of ligand-gated ion channels by steroids also appear to be quite different between various members of this neurotransmitter receptor family.

Besides these effects as allosteric modulators, some steroids may interact with plasma membrane receptors; it is for example the case of testosterone, which increased $[Ca^{2+}]_i$ in male rat osteoblasts by activating nongenomic cell-surface receptors for testosterone that belong to the class of the membrane receptors coupled to a phospholipase C via a pertussis toxin-sensitive G-protein [53]. Several discrete actions of 17β-estradiol are considered mediated through membrane receptors, not isolated up to now, and relatively specific. Several non-genomic effects of estradiol are frequently counteracted by androgens [for review, see for example 54, 55], and intracellular application of 17β-estradiol are, in some cases, ineffective (*e.g.* on I_{Kir} channels; [13]). It is also the case for the nongenomic receptors of progesterone, thought to be a cell-surface

receptor having the properties of a calcium channel, chloride channel, bicarbonate/chloride exchanger, and $GABA_A$ [see 56]. These steroid effects were also observed when the compounds were conjugated to albumin (e.g. the rapid increase in $[Ca^{2+}]_i$, elicited by progesterone or estradiol in human platelets [27].

Membrane receptors for gonadal steroid would be particularly abundant in CNS [57]. The secosteroid hormone 1,25(OH)2-vitamin D3 rapidly activates voltage-dependent Ca^{2+} channels of the L-type in skeletal and cardiac muscle cells by a non-genomic mechanism which involves G-protein-medicated stimulation of the adenylate cyclase/cAMP/protein kinase A messenger system. The protein kinase C pathway also plays a role modulating 1,25(OH)2D3 signal transduction in muscle by cross-talk with the PKA system. The hormone sequentially activates phospholipases C and D providing diacylglycerol for PKC activation and inositol triphosphate for intracellular Ca2+ mobilisation. In addition, 1,25(OH)2D3 rapidly stimulates phospholipase A2 generating arachidonic acid for the eicosanoid pathway [51].

Possible Modifications in the Microenvironment of Membrane Proteins

High concentrations of steroids could have a non-specific effect on plasma membranes because of their lipophilicity, allowing their insertion into the lipid bilayer membrane, with resulting modification in the micro-environment of the membrane proteins, including membrane channels. Indeed, they are embedded in a core of lipids which remain in a gel state and maintain the tertiary structure of the protein in a particular conformation. The fluidity of the acyl chains probably provides the required freedom of motion, allowing proteins within the membrane to undergo conformational changes, rotational and/or translocational movements associated with their activity, which frequently depends on the physical state of the membrane lipids. Intercalation of the lipophilic steroids into the membrane bilayer could cause perturbations of lipid-lipid interactions that may, in turn, alter the function of membrane proteins. Steroids may displace lipids surrounding the proteins, allowing steroid-protein interactions to occur or, alternatively, steroids may also modify protein-protein interactions [57]. Steroid-induced changes in membrane biophysical properties, including fluidity and bilayer width have been reported. However, when the effects of testosterone, progesterone and 17β-estradiol on membrane fluidity were compared in a variety of membranes, testosterone had no effect on lipid movement whereas progesterone decreased it, in contrast with 17β-estradiol, which increased it [58]. Non genomic effects addressed here are observed at high steroid concentrations but the functional state of several membrane channels was found altered by some compounds and insensitive to other compounds, showing that these effects cannot be regarded as unspecific. Such structure/activity diversity was for example found for gap junctional, I_{CaL} or I_{Kir} channels. In the first case, 17β-estradiol and testosterone derivatives induced comparable current inhibition whereas progesterone was without effect [18] ; in contrast, 17β-estradiol reduced the intensity of I_{CaL} in single guinea-pig atrial myocytes [9] and I_{Kir} in freshly isolated rat osteoclasts [13] whereas progesterone and testosterone had no influence. Conversely, progesterone reduced I_{Ca} in human intestinal smooth muscle cells whereas estradiol was ineffective [59]. The different efficiencies of the steroid compounds to alter the activities of the channels might then be consistent with a mechanism involving a direct incorporation of steroid compounds of appropriate shape and size into the lipidic domains that maintain the proper orientation of membrane channels in the bilayer, modifying their properties.

PHYSIOLOGICAL AND CLINICAL SIGNIFICANCE OF NON-GENOMIC RAPID ACTIONS

While steroid concentrations in the nanomolar range are sufficient to activate intracellular steroid receptors, the effects on various membrane channels require micromolar concentrations. The plasma concentrations of gonadal steroids are usually in the nanomolar range, but considerably higher concentrations may occur locally around tissues where they are synthesised, and that there may be an enrichment of steroids at the receptor-membrane interface due to their lipophilic properties.

The direct introduction of unesterified 17β-estradiol into the cytosol impaired the opening state of junctional channels at a concentration of 250 nM (sufficient to reduce the intercellular coupling of rat cardiac myocytes to approximately 50% of its level within 25 min [20]), not far above the range observed during pregnancy in women, where the serum level can reach up to 105 nM [60]. Circulating progesterone concentrations reach 0.5 µM in the rat [see 34]. Concentrations >1 µM are considered as realistic concentrations for the anaesthetic steroids (e.g. alphaxalone ; [36]). Biochemical measurements have estimated progesterone concentrations to be as high as 20 µM within the placenta, and concentrations in the vicinity of trophoblasts producing progesterone might be even higher [see 28]. General plasma concentrations might also reach high levels for example during estrogen replacement therapy and could still be considerably higher in steroid drug abuses (*e.g.* of anabolic steroids).

Steroid synthesis and metabolism have been shown to exist in the CNS, and the effects have been observed in both the central and peripheral nervous systems. The major groups of neuroactive steroids, and their metabolites, have been progesterone, deoxycorticosterone, and some androgens, notably DHEA. These compounds show increased concentrations both in blood and in the brain following stress and they have also been associated with anxiolytic effects and antiepileptic activity. In the periphery, some of these compounds show remarkable inhibitory effects on the secretion of catecholamines and other neurotransmitters. Due to their chemical properties, steroids cross the blood-brain barrier where they have profound effects on a variety of G-protein-coupled receptors and membrane channels, with consequence on sleep patterns, the reaction to stress, memory function, brain plasticity, vigilance, mood, etc. [see for example 33].

In general, fast effects by corticosteroids induce inhibitory effects on cellular firing (although regional differences seem to exist), suppressing transmission carried by amino acids, particularly when the activity is elevated in comparison to resting level; modulatory inputs are enhanced. Prolonged activation of glucocorticoid receptors can implicate the integrity of neuronal circuits by allowing considerable influx of calcium ions during depolarisation. Of the gonadal hormones, estradiol mainly exerts excitatory actions, in both the rapid and the delayed mode. Progesterone is predominantly inhibitory, usually with a short delay in onset. The effect of androgens on neuronal excitability has not yet been studied in detail. Finally, neurosteroids and A-ring reduced steroids in general induce rapid effects on firing patterns, probably by acting on ligand-gated ion channels. The diverse actions of steroid hormones on single cell activity have consequences for the excitability in local circuits in which these cells participate. In this way steroid hormones add an essentially new aspect to the regulation of functional processes in the brain, during physiological conditions but also when networks are implicated during diseases and disorders. Fluctuations in gonadal steroid hormone concentrations, for example, may be involved in the development and course of the respective underlying disorders. Nausea is frequently observed during the first trimester of pregnancy, whereas psychiatric disturbances are more common during the postpartum period. The mechanism for many of the effects of these steroids is via their action on membrane ion channels. Activation of the GABAA receptor complex, resulting in the opening of its central chloride channel, is the major target of the neuroactive steroids, resulting in repolarisation of the plasma membrane and inhibition of further neuronal firing. The properties of these neuroactive steroids have resulted in their use as therapeutic agents in the treatment of anxiety, insomnia, convulsions, epilepsy, and possibly for the alteration of pain thresholds.

A number of rapid, non-genomic effects of progesterone have also been found in oocytes and sperm ; in the latter, the possibility to ameliorate the human sperm acrosome reaction gives hope to improve assisted reproductive techniques in sub-fertile patients [for review, see 34].

Many steroid hormones exert direct actions on the cardiovascular system at its different levels of organisation, thus enabling adjustment to the changing demands during reproduction (gonadal steroids), stress (adrenal steroids), and solar seasons (vitamin D, soltriol). Besides their positive effects on plasma lipids, estrogens have antagonistic influence on many membrane channels which may account for their anti-arrhythmic effects and contributing to their cardioprotective actions and to the gender- and age- based differences in cardiac diseases.

In conclusion, many steroid compounds affect the functional state of membrane channels, sometimes by interacting with putative membrane receptors, yet unidentified, frequently by allosteric interactions of steroids with specific channel-forming structures in the plasma membrane, for example by entering the lipid bilayer, modifying the biophysical properties of the lipid belt surrounding the channel, and/or the lipid-protein relations at the channel-membrane interface, thereby allosterically modulating the function of ion channels in a structure-specific manner. The effects of the steroid-BSA complexes are not contradictory to this assumption because parts of the steroid molecules may enter into the extracellular lipid component of the cell membrane.

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