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Steroid Hormone Receptors in Target Cell Membranes

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Numerous reports of rapid steroid hormone effects in diverse cell types cannot be explained by the generally prevailing theory that centers on the activity of hormone receptors located exclusively in the nucleus. Cell membrane forms of steroid hormone receptors coupled to intracellular signaling pathways may also play an important role in hormone action. Membrane-initiated signals appear to be the primary response of the target cell to steroid hormones and may be prerequisite to subsequent genomic activation. Recent dramatic advances in this area have intensified efforts to delineate the nature and biologic roles of all receptor molecules that function in steroid hormone–signaling pathways. This work has profound implications for our understanding of the physiology and pathophysiology of hormone actions in responsive cells and may lead to development of novel approaches for the treatment of many cell proliferative, metabolic, inflammatory, reproductive, cardiovascular, and neurologic defects.

Key Words: Steroid hormone action; plasma membrane; receptor.

Introduction

The broad physiologic effects of steroid hormones in the regulation of growth, development, and homeostasis have been known for decades. Often, these hormone actions culminate in altered gene expression *(1)*, which is preceded by nutrient uptake and other preparatory changes in the synthetic machinery of the cell *(2)*. Owing to certain homologies of molecular structure, specific receptors for steroid hormones, vitamin D, retinoids, and thyroid hormone are often considered a receptor superfamily. The actions of ligands in this steroid receptor superfamily are commonly postulated to be mediated by receptors in the cell nucleus. On binding ligand, nuclear receptors associate with target genes and permit selective transcription. This genomic mechanism is generally slow, often requiring hours or days before the consequences of hormone exposure are evident. However, steroids also elicit rapid cell responses, often within seconds. The time course of these acute events parallels that evoked by peptide agonists, lending support to the conclusion that they do not require precedent gene activation *(2–5)*. Rather, many rapid effects of steroids, which have been termed *nongenomic*, appear to be owing to specific recognition of hormone at the cell membrane. Although the molecular identity of binding sites remains elusive and the signal transduction pathways require fuller delineation, there is mounting evidence that steroid action is initiated by plasma membrane receptors.

A current challenge is to determine the relation of rapid responses to steroid hormones to intermediate and long-term effects. Some questions that arise in this context include the following: Is specific membrane binding responsible merely for cellular entry of the hormone? Do plasmalemmal receptors escort ligand to the nucleus? Are the membrane binding sites coupled to rapid signal transduction systems that also act in concert with nuclear transcription factors? Are the membrane receptors identical to nuclear receptors, modified forms, or entirely different entities? This review explores these important issues. In preparing this work, more than 1200 references providing significant evidence for rapid steroid actions and for membrane forms of steroid receptors were identified. Only a fraction of these citations can be presented here, and the reader is referred to several recent reviews in this area *(3–7)*.

Estrogens

As with other steroid hormones, biologic activities of estrogen in breast, uterus, and other tissues are considered to be fully mediated by a specific high-affinity receptor in cell nuclei. Estrogens are accumulated and retained in responsive cells, and it has been commonly assumed that the steroid diffuses passively to intracellular receptors. However, estradiol is a lipophilic molecule that partitions deep within the hydrocarbon core of lipid bilayer membranes, even those devoid of relevant receptors *(3)*. Several investigations now demonstrate that steroid hormones enter target cells by a membrane-mediated process that is saturable

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Table 1
Brief Chronology of Selected Reports Documenting Occurrence and Activity of Membrane Steroid Hormone Receptors[a]

Steroid	Year	Observation	Reference
Estradiol	1967	Elevation of uterine cAMP by estrogen within seconds	*11*
	1975	Rapid endometrial cell calcium mobilization by estrogen	*9*
Corticosterone		Binding to plasma membranes of rat liver	*108*
Estradiol	1976	Effects on electrical activity of neurons	*20*
	1977	Specific plasma membrane binding sites for estrogen	*16*
Cortisol		Electrophysiologic effects on neurons	*21*
Progesterone	1978	Induction of oocyte maturation by steroid linked to a polymer	*29*
Estradiol	1979	Increased proliferation of cells with membrane ER	*17*
	1980	Molecular properties of ERs in liver plasma membrane	*13*
Vitamin D	1981	Rapid intestinal cell calcium uptake	*109*
Progestin	1982	Specific binding to oocyte surface and role in meiotic maturation	*30*
		Steroid receptor of 110 kDa on oocyte surface by photoaffinity labeling	*31*
Corticosterone	1983	Binding to synaptic plasma membranes	*50*
Estradiol	1983	Increase in density of microvilli at endometrial cell surface within seconds	*112*
	1984	Primary internalization of ER in endometrial plasma membrane vesicles	*104*
Thyroid hormone	1985	Characterization of plasma membrane binding sites	*47*
Estradiol	1986	High-affinity binding sites in breast cancer cell plasma membranes	*26*
		Altered breast cell membrane potential, density of microvilli within seconds	*110*
Glucocorticoid	1987	Correlation between membrane receptor and apoptosis in lymphoma cells	*53*
Vitamin D	1989	Rapid activation of phospholipase C (PLC) in rat intestine	*5,14*
		Activation of calcium channels in osteoblasts	*63*
Thyroid hormone		Rapid induction of glucose uptake	*42*
Progesterone	1990	Stimulation of calcium influx in human sperm	*33*
	1991	Calcium uptake mediated by sperm cell surface-binding sites	
		Action at plasma membrane of human sperm	*34*
Corticosterone		Correlation of neuron membrane receptors with behavior in newts	*51*
Aldosterone		Rapid effects on Na^+/H^+ exchange	*111*
Glucocorticoid	1993	Antigenic similarity between membrane and intracellular receptors	*54*
Estradiol		Binding and stimulation of HER-2 membrane receptor	*90*
	1994	Activation of adenylate cyclase signaling pathways	*12*
Vitamin D		Isolation of a plasma membrane receptor from chick intestine	*88*
Aldosterone		Identification of membrane receptor in human lymphocytes	*86*
Estradiol	1995	Membrane receptor with antigenic identity to nuclear receptor	*7,78*
		Greater nongenomic responses of membrane receptor–enriched neural cells	
Androgen		Rapid increase in cytosolic Ca^{++} in Sertoli cells	*36*
Estradiol	1997	Membrane action and PLC regulation	*14*
		Isolation of membrane binding-proteins from rat brain	*81*
Vitamin D	1998	Blocking of hormone activation of PKC by antibody to membrane receptor	*65*
Estradiol	1999	Rapid Ca^{++} mobilization required for activation of MAPK	*10*
		Rapid actions in neurons from ERα knockout mice	*94*
		Reduction of membrane ER expression by antisense to nuclear ER	*80*
		Membrane and nuclear ERα, and ERβ, each expressed from single transcript	*25*
		Activation of G-proteins, IP_3, adenylate cyclase, and MAPK by membrane ER	
Androgen		Rapid activation of MAPK pathway in prostate	*37*
Progesterone		Cloning and expression of binding protein from liver microsomal membrane	*85*
Vitamin D	2000	Ligand-induced nuclear translocation of plasma membrane receptor	*89*
Estradiol		Surface receptor in endothelial cells recognized by monoclonal ERα antibody	*79*
		Interaction of ERα with regulatory subunit of phosphatidylinositol-3-OH kinase	*113*
		Rapid tyrosine phosphorylation of Raf-1 and activation of MAPK resulting in prolactin gene expression in pituitary cells	*114*

[a]More than 1200 publications on membrane steroid receptors have appeared in the past 30 yr. Of these, only representative examples are listed here. The potential roles of alternate *(25)* or variant *(56)* forms of steroid hormone receptors and other membrane-signaling molecules *(90,94)* remain to be clarified.

and temperature dependent *(3,8)*. Moreover, it is well established that estrogen can trigger in target cells rapid surges in levels of intracellular messengers, including calcium *(9, 10)* and cyclic adenosine monophosphate (cAMP) *(11,12)*, as well as activation of mitogen-activated protein kinase (MAPK) *(13)* and phospholipase (*14)* (Table 1). These data have led to a growing consensus that the traditional genomic model of estrogen action does not explain the rapid effects of estrogens and must be expanded to include membrane receptors as a component of cell signaling *(2–7,15)*.

The first unequivocal evidence for specific membrane-binding sites for estradiol-17β (E_2) was reported in 1977 *(16)*. Intact uterine endometrial cells equipped with estrogen receptor (ER), but not ER-deficient control cells, bound to an inert support with covalently linked E_2. In addition, target cells that bound could be eluted selectively with free hormone, and cells so selected exhibited a greater proliferative response to estrogens than cells that did not bind *(17,18)*. Further investigations have continued to provide compelling evidence for the occurrence of a plasma membrane form of ER and support for its role in mediating hormone actions (*3)* (Table 1).

Selye *(19)* first demonstrated that steroids at pharmacologic concentrations elicit acute sedative and anesthetic actions in the brain. However, electrical responses to *physiologic* levels of E_2 with rapid onset have since been reported in nerve cells from different brain regions *(4,20,21)*. Similarly, certain vasoprotective effects of estrogen appear attributable to membrane receptors *(15,22)*. Estrogen-induced release of uterine histamine *in situ* has long been associated with rapid enhancement of the microcirculation by a process that excludes gene activation *(2)*. Reinforcing these observations are new data detailing the role of nitric oxide (NO) in vascular regulation by estrogen. Normal endothelium secretes nitric oxide, which relaxes vascular smooth muscle and inhibits platelet aggregation. Estrogens elicit abrupt liberation of NO by acute activation of endothelial NO synthase without altering gene expression, a response that is fully inhibited by concomitant treatment with specific ER antagonists *(23)*. This estrogenic effect may be mediated by a receptor localized in caveolae of endothelial cell membranes *(24)*. Such observations require extension, because several independent cell-signaling complexes that appear to participate in signal transduction to the nucleus also associate with caveolar structures *(2,3,22)*.

Estrogen deficiency is associated with significant bone loss, and research on the potential role of membrane ERs in regulating bone mass has increased. Evidence for membrane-binding sites and acute effects of estrogen with an onset within 5 s has been reported in both osteoblasts and osteoclasts *(5,13)*. The effects of estrogens on bone homeostasis also appear to involve rapid activation of MAPK *(13)*, as has also been demonstrated in certain other target cells *(10,15,25)*.

When exposed to E_2 conjugated to fluorescein-labeled bovine serum albumin (BSA), human breast cancer cells exhibit specific surface staining *(7,26)*. Since E_2-BSA is considered membrane impermeant, these conjugates, developed primarily for use as immunogens and for affinity purification of nuclear ERs, have also been used to assess the membrane effects of estrogen. However, in light of the fact that E_2-BSA is unstable in solution, especially in the presence of cells and their enzymic products, and releases measurable amounts of free steroid *(27)*, data relying only on the use of estradiol conjugates to test for membrane effects of steroids need especially careful scrutiny. It is clear that more stable, cell-impermeant derivatives of estradiol should be developed for evaluating membrane receptors.

Progestogens and Androgens

As documented for estrogens, several physiologic effects of progestogens and androgens appear to be regulated, in part, by membrane receptors. Progesterone controls components of reproductive function and behavior. Some of these activities are mediated by interaction with neurons in specific brain regions, and membrane effects appear to be important in this process *(4,28)*. Meiosis in amphibian oocytes is initiated by gonadotropins, which stimulate follicle cells to secrete progesterone. The progesterone-induced G_2/M transition in oocytes was among the first convincing examples of a steroid effect at plasma membrane, since it could be shown that exogenous, but not intracellularly injected, progesterone elicited meiosis and that many progesterone-stimulated changes occurred even in enucleated oocytes *(29–32)*. Moreover, this process may be related to progesterone-induced increments in intracellular Ca^{++} and release of diacylglycerol species that elicit a cascade of further lipid messengers *(32)*.

Progesterone elicits rapid effects on membrane receptors, second messengers, and the acrosome reaction in human sperm *(33–35)*. Assay of acute sperm responses to progesterone in subfertile patients is highly predictive of fertilizing capacity *(35)*. Effects of the steroid, present in the cumulus matrix surrounding the oocyte, appear to be mediated by elevated intracellular Ca^{++}, tyrosine phosphorylation, chloride efflux, and stimulation of phospholipases, effects attributed to activation of a membrane-initiated pathway. Indeed, two different receptors for progesterone, apparently distinct from genomic ones, have been identified at the surface of human spermatozoa *(35)*; nevertheless, a monoclonal antibody (MAb) against the steroid-binding domain of human *intra*cellular progesterone receptor (PR) inhibits progesterone-induced calcium influx and the acrosome reaction in sperm *(35)*.

As with estrogens and progestogens, androgens promote a rapid increase in cytosolic Ca^{++} in their cellular targets *(36)*. Other effects of androgens that are not attributable to genomic activation include acute stimulation of MAPK in prostate cancer cells *(37)*. The androgen, 5β-dihydrotestosterone, induces vasodilation of aorta, which may be owing to direct action of the steroid on membranes of smooth muscle cells leading to modulation of calcium channels *(38)*. In osteoblasts, membrane receptors for androgen appear to be coupled to phospholipase C (PLC) via a pertussis toxin–sensitive G-protein that, after binding testosterone, mediates rapid increments in intracellular calcium and inositol triphosphate (IP_3) *(39)*. Of note, Benten et al. *(40)* report that testosterone elicits Ca^{++} mobilization in macrophages that lack intracellular androgen receptor (AR). These cells

express an apparent G-protein-coupled AR at the cell surface that undergoes agonist-induced internalization.

Thyroid Hormones

Thyroid hormones are well known to regulate energy expenditure and development, and membrane-initiated effects may contribute to these responses. Triiodothyronine (T_3) rapidly stimulates oxygen consumption and gluconeogenesis in liver *(41)*. T_3 also promotes an abrupt increase in uptake of the glucose analog, 2-deoxyglucose, in responsive tissues by augmenting activity of the plasma membrane transport system for glucose *(42)*. In rat heart, T_3 elicits a positive inotropic effect, increasing left ventricular peak systolic pressure, as early as 15 s after hormone *(43)*. In each tissue investigated, alterations in intracellular Ca^{++} induced by thyroid hormone appear to modulate signal transduction to the cell interior *(41–44)*.

Membrane-initiated effects of T_3 have been documented in bone cells by means of inositol phosphate signaling *(45)*, and in brain through calcium channel activation *(46)*. T_3 can also influence other cell processes, including the exocytosis of hormones and neurotransmitters *(46)*, rapid effects that may be attributable to mediation by membrane receptors *(44)*. Although uptake of T_3 can occur concomitantly with receptor-mediated endocytosis of low-density lipoprotein, and likely accompanied by carrier proteins, uptake of T_3 itself has also been reported to occur in numerous tissues by means of a high-affinity, stereospecific, and saturable process *(45,47,48)*, as found for steroid hormones *(3,8)*.

Glucocorticoids

In addition to their long-established effects on mobilization of energy sources by promoting catabolism and the induction of enzymes involved in gluconeogenesis, glucocorticoids have profound effects on neuron signaling and on induction of apoptosis in lymphocytes, phenomena that appear to be membrane-initiated events. Kelly et al. *(21)* found that glucocorticoids rapidly altered neuron-firing patterns, and many studies have verified these effects *(4,6,28)*. These molecular events lead to glucocorticoid modulation of specific brain functions, such as the rapid response of hypothalamic somatostatin neurons to stress *(49)*. Such abrupt changes in neuron polarization are reinforced by findings of specific, saturable binding of corticosterone to neuron membranes *(50,51)*. Specific, high-affinity corticosterone binding to calf adrenal cortex plasma membrane is also identified by use of the biologically active radioligand [^{3}H]corticosterone *(52)*.

Glucocorticoids also play an important role in the regulation of immune function and inflammation. In lymphoproliferative diseases, glucocorticoids are in wide use as therapeutic agents, but the cellular mechanism leading to the therapeutic effect remains unclear. In several studies using both cell lines and freshly prepared leukemia or lymphoma cells, the presence of a membrane receptor for glucocorticoids has been implicated in modulating apoptosis and cell lysis *(7,53–55)*. Moreover, in lymphocytes, the membrane-binding site is antigenically related to the intracellular glucocorticoid receptor (iGR) and may be a natural splice variant form of the intracellular receptor *(7,55,56)*. A potential parallel to the ER transfected in Chinese hamster ovary (CHO) cells *(25)* is evident.

Aldosterone and Digitalis-Like Steroids

Beyond its classic functions of promoting renal reabsorption of sodium and excretion of excess potassium, aldosterone enhances sodium absorption from colon and urinary bladder. In each tissue, the mineralocorticoid effect is owing to enhanced activity of amiloride-sensitive sodium channels. Aldosterone rapidly augments Na^+/H^+ exchange *(6,57)*. This function is Ca^{++}- and protein kinase C (PKC)-dependent but independent of nuclear receptor activation, transcription, and protein synthesis *(6,58)*. Similarly, "nongenomic" action of aldosterone has also been reported to underlie its acute effects on cardiac function and on sodium transport in vascular smooth muscle cells *(6,58)*.

Digitalis-like compounds are often forgotten members of the steroid superfamily. These plant-derived agents elicit inotropic and chronotropic effects on the heart but also affect many other tissues. Endogenous steroidal ligands, termed *digitalis-like* or *ouabain-like* factors, have been found in sera of humans and other animals with blood volume expansion and hypertension *(59,60)* and may be released from adrenal cortex *(60)*. These ligands elicit inhibition of membrane-associated Na^+,K^+-ATPase, likely the principal receptor for these agonists. It is notable that the steroid-binding domain of Na^+,K^+-ATPase and that of nuclear hormone receptors share significant amino acid sequence homology *(61)*. In addition to membrane actions of these compounds on Na^+,K^+-ATPase, ouabain-induced hypertrophy in myocytes is accompanied by promotion of Ca^{++} flux and initiation of protein kinase–dependent pathways leading, in turn, to specific changes in transcription and altered expression of early response- and late-response genes *(62)*. Thus, the biologic effects of digitalis-like compounds, long considered the exception to the concept of exclusive genomic influence, may render them more closely integrated with the steroid hormone superfamily than was previously recognized.

Vitamin D Metabolites

Membrane-initiated effects of the seco-steroid hormone, 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$), are well documented in bone and cartilage. In osteoblasts, Caffrey and Farach-Carson *(63)* elucidated possible connections between rapid effects of $1,25(OH)_2D_3$, requiring milliseconds to minutes, and longer-term effects owing to gene expression. Their laboratory was the first to show activa-

tion of calcium channels by $1,25(OH)_2D_3$ *(63)*. Calcium, which can signal gene expression through multiple pathways, promotes key phosphorylation events in certain bone proteins *(5)*. Osteoblasts exhibit rapid changes in IP_3 and diacylglycerol in response to vitamin D metabolites via activation of PLC *(5,14)*. Other bone cells with rapid responses to vitamin D metabolites include osteosarcoma cells and chondrocytes *(5,64)*. The latter system is particularly intriguing because chondrocytes elaborate matrix vesicles that appear critical in bone mineralization. The matrix vesicles, which lack nuclei, exhibit specific, saturable binding of $1,25(OH)_2D_3$, especially when derived from growth zone chondrocytes *(65)*.

Other rapid effects of vitamin D occur in a variety of cell types. Muscle cells respond within seconds to $1,25(OH)_2D_3$ via several mediators that alter cardiac output in some instances, while acute activation of calcium channels in skeletal muscle promotes contraction *(5,66)*. Of note, in lymphoproliferative disease, $1,25(OH)_2D_3$ appears to prime monocytic leukemia cells for differentiation through acute activation or redistribution of PKC, Ca^{++}, and MAPK *(5, 67)*. In pancreas and intestine, activation of membrane-associated signaling pathways results in vesicular exocytosis. Pancreatic β-cells respond to $1,25(OH)_2D_3$ with enhanced intracellular Ca^{++} coupled to increased insulin release *(68)*. In intestine, $1,25(OH)_2D_3$ stimulates exocytosis of transported vesicular calcium and phosphate. These cellular events may be related to vitamin D–promoted alterations in the levels of α-tubulin *(5)*, thereby influencing assembly of microtubules and possibly providing a means for vectorial transport of absorbed ions. Several signal transduction pathways have been found to respond rapidly to exogenous $1,25(OH)_2D_3$, including activation of protein kinases and promotion of abrupt increments in Ca^{++}, but integration of these signaling cascades with the physiologic response of enhanced ion absorption remains to be established *(5,68,69)*.

Investigations with vitamin D congeners have recently indicated the potential hormonal nature of $24,25(OH)_2D_3$, once thought to represent merely the inactivation product of precursor $25(OH)D_3$. Acute effects of $24,25(OH)_2D_3$ have been observed in bone cells and in intestine; $24,25(OH)_2D_3$ also inhibits rapid actions of $1,25(OH)_2D_3$ *(5)*. This may explain why abrupt effects of $1,25(OH)_2D_3$ often fail to be observed in vivo *(70)*: normal, vitamin D–replete subjects have endogenous levels of $24,25(OH)_2D_3$ sufficient to inhibit acute stimulation of calcium transport by $1,25(OH)_2D_3$, thus providing a feedback regulation system *(69)*.

Retinoids

Retinoic acid exerts diverse effects in the control of cell growth during embryonic development and in oncogenesis. It is widely considered that effects of retinoids are mediated through nuclear receptors, including those for retinoic acid as well as retinoid X receptors *(1)*. However, other retinoid response pathways appear to exist, independent of nuclear receptors *(71)*. Cellular uptake of retinol (vitamin A) may involve interaction of serum retinol-binding protein with specific surface membrane receptors followed by ligand transfer to cytoplasmic retinol-binding protein *(72)*. In this regard, targeted disruption of the gene for the major endocytotic receptor of renal proximal tubules, megalin, appears to block transepithelial transport of retinol *(73)*. It is noteworthy that megalin may also be implicated in receptor-mediated endocytosis of $25(OH)D_3$ in complex with its plasma carrier *(74)*. In addition, retinoic acid binds mannose-6-phosphate (M6P)/insulin-like growth factor-2 (IGF-2) receptor with moderate affinity and appears to enhance its receptor activity *(75)*. M6P/IGF-2 receptor is a membrane glycoprotein that functions in binding and trafficking of lysosomal enzymes, in activation of transforming growth factor-β, and in degradation of IGF-2, leading to suppression of cell proliferation. The concept of multiple ligands binding to and regulating the function of a single receptor is relatively novel but has important implications for modulating and integrating the activity of seemingly independent biologic pathways.

Properties of Membrane Receptors for the Steroid Superfamily

Despite renewed interest in membrane steroid receptors, the physical identity of receptors with high binding affinity for ligand remains elusive. Isolation and structural characterization of these molecules remains to be accomplished. They may be known membrane components (e.g., enzymes, ion channel subunits, receptors for nonsteroid ligands), with previously unrecognized binding sites for steroids, new forms of steroid hormone receptors, "classic" receptors complexed with other membrane-associated proteins, or truly novel membrane proteins.

Estrogens and Progestogens

Efforts to isolate and purify membrane receptors that mediate rapid effects of steroids are under way in several laboratories (Table 2). Early work on purification of ER from uterus and liver plasma membranes suggested that it was a protein species with high-affinity, saturable binding specific for estradiol-17β *(16,18)*. The molecular size of solubilized receptor was in the range of intracellular ER *(18,76)*. Other work to isolate plasma membrane estrogen-binding proteins identified the 67-kDa species characteristic of nuclear receptor, but additional proteins of variant size ranging from 28 to 200 kDa were also revealed *(77)*. To determine whether membrane ER had antigenic homology with nuclear ER, Pappas et al. *(78)* used antibodies prepared to different functional epitopes of intracellular receptor and demonstrated surface labeling in nonpermeabilized rat pituitary cells by confocal scanning laser microscopy. Recent work by Russell et al. *(79)* has demonstrated, by means

Table 2
Representative Examples of Physical Properties of Membrane-Associated Receptors for Ligands of Steroid Hormone Superfamily[a]

Ligand	MW (kDa)	K_d (*M*)	Binding capacity (fmol/mg protein)	Homology with nR	Tissue	Reference
Estradiol	51–78[b]	2.8×10^{-10}	526	ND	Rat hepatocytes	*18*
	105–148[c]					
	11–67	3.6×10^{-10}	370	ND	Rabbit uterus	*77*
	67			Yes	CHO cell (ER transfected)	*25*
Progestin	110	5×10^{-7}		ND	Amphibian oocyte	*30*
	110	1×10^{-6}		ND		*31*
	28,56	6.9×10^{-8}	Variable	ND	Porcine liver	*84*
Vitamin D	65	7×10^{-10}	240	No	Chick intestine	*88*
		1.7×10^{-11}	124	No	Rat growth chondrocytes	*65*
		2.8×10^{-11}	100	No	Rat resting chondrocytes	
	36	1×10^{-8}		ND	Rat osteoblast-like cells	*87*
Aldosterone	50	1.1×10^{-8}	350	No	Pig liver	*86*
Glucocorticoids		1×10^{-7}		ND	Rat synapses	*50*
	97–150	2.4×10^{-7}	384	Yes	S-49 lymphoma cells	*55*
		5.1×10^{-10}		ND	Amphibian synapses	*51*
Thyroid hormone	145	2×10^{-9}	320	No	Human placenta	*47*
		6×10^{-10}		ND	Rat myoblasts	*48*

[a]Only representative examples of steroid-binding membrane macromolecules are presented here. Please refer to text for additional references. Homology of membrane macromolecules to nuclear receptor forms (nR) is noted; MW, apparent molecular weight; ND, not determined.
[b]High salt (0.4 *M* KCl).
[c]Low salt (0.01 *M* KCl).

of monoclonal anti-ERα, that human endothelial cells possess surface-binding sites for estrogen (see Table 1). In evaluating the source and distribution of membrane ER, target cells with expression of ERα were treated with antisense oligonucleotide to nuclear ERα to suppress expression of receptor protein *(80)*. This approach significantly reduced expression of membrane as well as nuclear forms of ER. Using an alternate method to assess receptor origin, Razandi et al. *(25)* transfected cDNA for ERα and ERβ into CHO cells, which do not normally express ER. The transfections resulted in ER expression in both nuclear and membrane fractions, suggesting that membrane and nuclear ER are derived from a single transcript. In addition, both ERα and ERβ were expressed in membranes, and both receptors were capable of activating G-proteins, MAPK, as well as DNA synthesis *(25)*. In related studies, the acute stimulation of endothelial nitric oxide synthase (eNOS) by estrogen was reconstituted in COS-7 monkey kidney cells cotransfected with ERα and eNOS, but not by transfection with eNOS alone *(23)*.

Binding molecules for estrogen and progesterone, comprising several molecular species, were isolated from brain synaptosomes by affinity chromatography and characterized by electrophoresis and Western blot *(15,81)*. Microsequencing of one E_2-binding protein indicated that the high-affinity site corresponds to the subunit of an ATPase/ATP synthase. In addition, some studies suggest that estrogen bound to sex hormone–binding globulin, a plasma protein, also binds with specificity to membrane sites recognizing the liganded transport protein *(82)*. These transport-protein interactions promote cAMP generation via the intermediacy of G-proteins. However, further characterization of receptors for such steroid:protein complexes is not available, and it must be recalled that estrogen is in noncovalent association with its plasma protein carrier and dissociates readily therefrom *(83)*.

Binding of progesterone to plasma membrane of amphibian oocytes is specific, saturable, and temperature dependent *(31,32)*. Photoaffinity labeling with the synthetic progestin [^{3}H]-R5020, followed by gel electrophoresis, revealed progestin binding to both 80- and 110-kDa proteins in oocyte cytosol, whereas only the 110-kDa R5020-binding protein was present in oocyte plasma membrane. A progesterone-binding protein (msPR) was identified in crude microsomal, rather than purified plasmalemmal, membranes from porcine liver *(84,85)*. On solubilization, a moderate-affinity site with a dissociation constant (K_d) of 69 n*M* was found, but, after further purification, affinity decreased to K_d of 228 n*M*. The final fraction contained two novel peptides of 28 and 56 kDa. Expression of msPR-cDNA in CHO cells led to slightly increased progesterone binding in microsomes, and administration of an antibody against msPR reduced rapid progesterone-initiated Ca^{++} increases in sperm *(85)*. Whether this work represents the first successful cloning

and expression of a steroid receptor associated with cell mem-branes will have to await confirmation. However, Falkenstein et al. *(85)* suggest that the native *plasma* membrane PR may actually be an oligomeric protein complex of about 200 kDa, composed only in part by 28- and 56-kDa peptides.

Glucocorticoids, Aldosterone, and Vitamin D

Progress has been made in the isolation and characterization of plasma membrane receptors for glucocorticoids, aldosterone, and $1,25(OH)_2D_3$, although at this writing, evidence of cloning of the cDNA for any of these proteins is lacking. The membrane glucocorticoid receptor (mGR) was purified from lymphoma cells by immunoaffinity binding with an MAb coupled to Sepharose-4B; the protein displayed properties similar to iGR *(55)*. Scatchard analysis of mGR yielded a K_d of 239 n*M* and B_{max} of 384 fmol/mg of protein, representing a somewhat higher number of binding sites but a lower affinity than that of the iGR. Peptide maps revealed some sequences that were unique to the membrane form *(55,56)*. Further data suggest that the mGR in lymphoma cells is a transcript variant of the iGR *(56)* (Table 2). Properties of the aldosterone membrane receptor have been analyzed by means of [^{125}I]-aldosterone photoaffinity labeling. The protein has an apparent molecular mass of 50 kDa and appears to be distinct from intracellular receptor *(86)*.

The pursuit of membrane receptor for $1,25(OH)_2D_3$ (pmVDR) by affinity isolation has been hampered by the fact that most ligand derivatives lack sufficient binding activity. Nevertheless, work by Baran et al. *(87)* indicates that the vitamin D analog, [^{14}C]-1α,25-dihydroxyvitamin D_3 bromoacetate, does exhibit a moderate degree of specific binding to a 36-kDa protein in plasma membranes of rat osteoblast-like cells. Using sequence determination and Western blot, the labeled membrane protein was identified as annexin II, part of a family of membrane-binding proteins previously implicated in the regulation of Ca^{++} signaling, tyrosine phosphorylation, and apoptosis. Partially purified plasma membrane proteins and purified annexin II exhibited specific and saturable binding for [^{3}H]-1α,$25(OH)_2D_3$, and antibodies to annexin II inhibited [^{14}C]-1α,$25(OH)_2D_3$ bromoacetate binding to plasma membranes and also inhibited hormone-induced increases in intracellular calcium in osteoblast-like cells. Hence, these initial results *(87)* suggest that annexin II may serve as a receptor for rapid actions of $1,25(OH)_2D_3$ in rat osteoblast-like cells, but it is not known if this receptor system functions in other cell types. In independent studies, classic biochemical strategies, coupled with analyses of specific binding, were used to isolate the vitamin D membrane receptor (pmVDR) from intestinal epithelium of chicks *(88)*. Basal-lateral membranes were solubilized with detergent and subjected to ion-exchange and gel filtration chromatography. Binding activity eluted with a protein of 65 kDa, with a K_d of 0.7 n*M* *(88)*. A highly specific antibody toward plasma membrane VDR failed to recognize the nuclear receptor in Western analyses. On the other hand, a commercially available MAb generated against the "classic" nuclear receptor reacted with many proteins in nuclear fractions of chick intestine, including a band that comigrated with authentic recombinant protein, but did not detect VDR in basolateral membranes *(89)*. Antibody to the plasma membrane receptor, but not to the nuclear receptor, blocked hormonal activation of PKC. The 65-kDa protein was also observed to bind the affinity ligand, [^{14}C]-1α,25-dihydroxyvitamin D_3 bromoacetate, and labeling was diminished in the presence of excess nonradioactive ligand *(89)*. Electron microscopic studies of duodena vascularly perfused with control media, $1,25(OH)_2D_3$, or $24,25(OH)_2D_3$ followed by immunochemical staining revealed that $1,25(OH)_2D_3$, but not control media or $24,25(OH)_2D_3$, resulted in dramatically enhanced nuclear localization of the putative membrane receptor *(89)*.

Varied Forms of Steroid Hormone Receptors in Plasma Membranes

Collectively, current findings suggest that membrane receptors for steroid hormones are, in certain instances, transcriptional copies (estrogen) or variants (glucocorticoids) of nuclear receptors and, in other instances, products apparently unrelated to intracellular receptors (aldosterone and vitamin D). There is evidence for alternatively spliced transcripts of several steroid receptors, and these variant receptors give rise to proteins of different molecular size and, possibly, modified properties *(56)*. Membrane insertion of receptors in primary transcript form would likely require one or more hydrophobic regions, and post-translational modification of receptor protein leading to cell membrane targeting may also occur, including phosphorylation, glycosylation, and addition of lipid anchors or other modifications, such as palmitoylation or myristoylation. Surface steroid hormone receptors may also be part of a mul timeric complex including a "classic" nuclear receptor but bound to as-yet-unidentified transmembrane proteins and coupled to membrane-associated signaling molecules *(3,7, 15,79)*. Alternatively, plasma membrane receptors for steroids may have several common structural features with, but may be distinct from, the intracellular steroid hormone receptors *(88,89)*. In the case of retinoic acid and estradiol, binding to known membrane proteins, such as M6P/IGF-2 receptor *(75)* or HER-2 receptor *(90)*, respectively, may modulate some ligand effects. Progesterone appears to interact directly with oxytocin receptor, a G-linked protein at the cell surface, and inhibits some functional effects of oxytocin signaling, thus suppressing uterotonic activity of oxytocin *(91)*. Progesterone congeners also bind with moderate affinity to γ-aminobutyrate type A ($GABA_A$) receptors that comprise ligand-gated ion channel complexes *(4, 28)*. Absence of the γ-subunit of $GABA_A$ receptor in appropriate knockout mice results in a significant decrease in

sensitivity to neuroactive steroids such as pregnanolone *(92)*. Similarly, acute vascular relaxation induced by pharmacologic levels of E_2 may be mediated by its binding to the regulatory subunit of Maxi-K channels in membranes *(93)*, thus supporting the view that some effects of steroids, at least at high micromolar concentration, may be mediated by known membrane receptors with previously unrecognized steroid-binding sites.

Using ERα gene knockout (ERKO) mice, Gu et al. *(94)* showed that rapid actions of estradiol at 50 nM on kainate-induced currents in hippocampal neurons still occur, and the effect is not inhibited by ICI 182,780, a pure antagonist of hormone binding to both ERα and ERβ. These investigators suggest that a distinct estrogen-binding site exists in neurons and appears to be coupled to kainate receptors by a cAMP-dependent process. However, it is important to note that alternatively spliced forms of ERα *(95)*, as well as ERβ *(96)*, can occur in ERKO mice, thus complicating the interpretation of these results. Moreover, uterine tissues of ovariectomized ERKO mice exhibit 5–10% of the estradiol binding present in wild-type uteri *(95,97)*, and the significance of these residual estrogen-binding sites in ERKO target cells is unclear. Nonetheless, further development of double ERα and ERβ gene knockouts and perfection of this new technology should prove important in deciphering the contribution of "classic" and novel receptor forms in hormone action.

In future work, it will be important to pursue isolation and characterization of constituent proteins from homogeneous plasma membranes prepared in the presence of proteinase inhibitors *(18,76,98)*. Verification of their purity should be confirmed by use of a balance sheet for enzyme or other membrane markers *(18,76)*. Screening for activity of receptor would benefit from the use of independent approaches, such as ligand binding with radio- or photoaffinity-labeled steroids and immunoassay directed toward known intracellular receptors *(15,31,55,78,86)*. These several approaches may detect membrane receptors originating from a transcript other than that of intracellular receptor. As with the mixed steroid hormone–binding protein systems known to occur within cells and in their extracellular fluids, it may well be that multiple forms of receptor proteins for steroids coexist in plasma membranes, thus complicating efforts to isolate and characterize the individual binding species in this cell compartment. Our efforts to understand ligand-receptor interactions are often limited by simplistic "lock-and-key" models that may not accurately reflect the true state of complex molecular signaling cascades. Study of the molecular organization of several neurotransmitter receptor families has already shown that extraordinary biologic variability occurs, with multiple "keys" and multiple "locks" sometimes involved in ligand-receptor recognition *(99)*. We must consider the existence of similar high-affinity, but possibly multivalent and multifunctional, receptors in the steroid hormone superfamily *(75,91–93)*.

Perspectives

Ever since the discovery of chromosomal puff induction by ecdysone, cell regulation by steroid hormones has focused primarily on a nuclear mechanism of action. However, even the venerable steroid hormone ecdysone elicits rapid membrane effects that may facilitate later nuclear alterations *(100)*. Indeed, membrane-initiated responses appear to be the cell's earliest response to steroids and may be prerequisite to subsequent genomic responses (*2,3,7, 10*; see also Fig. 1). Coupling of surface membrane, cytoplasmic, and nuclear responses may offer a progressive, ordered expansion of initial signal. Accordingly, the terms *genomic* and *nongenomic* may not accurately define such a response continuum *(101)*. Future investigations should focus on potential interactions of membrane and nuclear steroid receptors that may promote activation of transcription and other specific hormonal responses. Molecular details of cross-communication between steroid and peptide receptors are also beginning to emerge *(3,98)*, and membrane steroid receptors may be in a pivotal location to promote convergence among diverse signaling pathways (Fig. 1). Indeed, the consequences of steroid hormone recognition at the outer cell membrane of target, but not nontarget, cells are shared by numerous other classes of regulatory molecules (cf. ref. *102*), including peptide hormones, neurotransmitters, drugs, plant lectins, mitogens, and antibodies *(3)*. Although the agonists are manifold, the signaling mechanisms are few. Primary signal recognition at the surface would be fleeting, but the mutual specificities and affinities are high, and thus sufficient for setting the appropriate signal transduction chain in motion. However, until the current surge of renewed focus on this problem, identification of these instantaneous triggering interactions for steroid hormones has accumulated relatively slowly, having been limited by technical and microanalytic barriers that are now being surmounted.

Ligand-receptor interactions depend on an extensive array of extracellular and intracellular partners to localize to membrane microdomains, recruit signaling molecules, and trigger intracellular signaling pathways. As the consequences of surface interactions are analyzed in greater depth, it will be important to evaluate further the biologic role of rapid internalization of steroid-binding sites from plasma membranes via endocytotic-lysosomal pathways *(2,3,88,101,103–105)*. These membrane-initiated events may involve cytostructural elements or scaffold proteins that contribute to signal propagation to the nucleus and the nuclear-protein matrix (*2,101,104–107*; Fig. 1). Thus, antibodies specific to intestinal membrane VDR reveal a vitamin D–induced redistribution of membrane receptor, a protein that appears distinct from intracellular receptor, to the nucleus within 5 min of binding ligand *(89)*. It is unknown whether the membrane receptor has inherent DNA- or coregulator-binding capacity to alter transcription; alter-

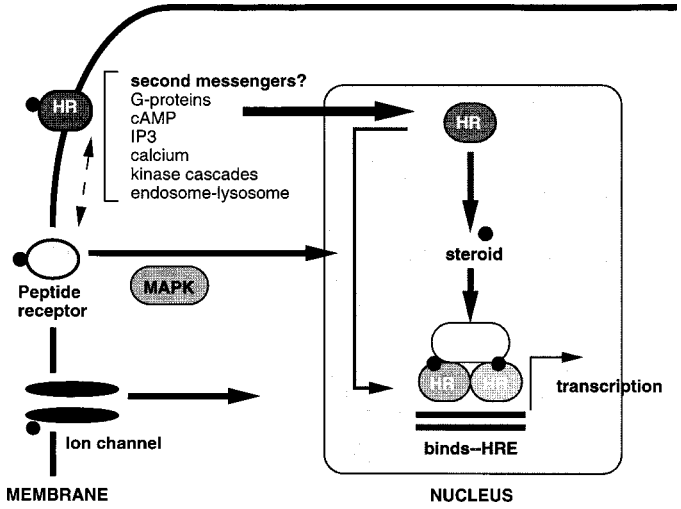

Fig. 1. Postulated mechanism of action of steroid hormones (black circles) in target cells with steroid hormone receptor (HR). In most current models, steroid binding to HR is believed to promote alterations in receptor conformation favoring enhanced association with coactivator proteins and with specific hormone-responsive elements (HRE) in the nucleus, leading, in turn, to initiation of selective gene transcription. However, the latter model fails to account for numerous, rapid cell responses to steroid treatment (see Table 1 and text). These deficiencies in the genomic model of hormone action require integration with the latter observations. In the model shown here, steroids may also bind to a membrane HR, with potential for promotion of hormonal responses via a complementary pathway that may cross-communicate or interact directly with the genomic mechanism. As noted in the text, membrane HR may be known molecules (kinases, ion channels, other receptors) with previously unrecognized binding sites for steroid, new isoforms of HR in membranes, "classic" forms of HR complexed with other membrane-associated proteins, truly novel membrane proteins, or a combination of these. Available evidence indicates that liganded membrane HR may affect one or more of several pathways, including modulation of ion channels, leading to enhanced flux of ions, notably Ca^{++}; interaction with peptide membrane receptors; and activation of G-proteins, nucleotide cyclases, and MAPK, with resultant increases in their catalytic products (see Table 1). These membrane interactions may promote phosphorylation of HR itself via steroid-induced or ligand-independent pathways. The intricate array of physiologic responses of cells to steroid hormones may occur as a consequence of a synergistic feed-forward circuit in which steroids activate cell membrane signaling pathways that act, in turn, to enhance the transcriptional activity of HR (Table 1). Active reconsideration of the unqualified genomic model of nuclear receptor action is ongoing, and the probable importance of alternate signaling pathways elicited by surface recognition is now increasingly evident.

natively, it could serve to shuttle ligand to the nuclear-localized fraction of receptor. As has frequently been noted from these laboratories (cf. ref. *105*), the cellular mechanisms governing the further transport and targeting of signaling molecules are powerful avenues of current investigation.

Many issues remain to be resolved for fuller understanding of the biologic actions of steroid hormones. Foremost among these is the structural characterization of membrane steroid hormone receptors. It is now abundantly clear that the nuclear receptor–mediated mechanism as the sole means by which steroid hormones act is incomplete *(2,3,5,7,15, 107)*. It is likewise unmistakable that membrane effects of steroid hormones represent an established phenomenon that is by no means to be construed as alternative to the genomic pathway, and that demands continued investigation. Indeed, the chain of membrane-initiated events is helping to account for the relatively prolonged, apparent silence between the capture of the hormone at the surface of its preferential target and the eventual outcome in augmented genomic activities. In challenging the dogma that steroid hormones act exclusively via intracellular receptors, the membrane receptor experiments reviewed here provide a persuasive paradigm for a potentially new class of drugs for human therapy. The clinical use of steroid hormone agonists and antagonists has substantially changed the course of many hormone-related diseases, but side effects of many agents currently in use are also significant. In-depth analysis of the relative contributions of nuclear and membrane-initiated activities in steroid receptor biology may lead to the development of pharmaceutical agents that exert differential activities in the two pathways, thus favoring more selective drug delivery and promoting the emergence of novel approaches for treatment of many cell metabolic and proliferative defects.

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