MINIREVIEW

Membrane-Initiated Steroid Actions and the Proteins that Mediate Them (44338)

CHERYL S. WATSON*,[1] AND BAHIRU GAMETCHU†

Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77555-0645, and Department of Pediatrics,† Medical College of Wisconsin, Milwaukee, Wisconsin 53226*

Abstract. **Membrane-initiated or nongenomic activities of steroids contribute to the effects of steroids on a wide variety of target organs, including those with low receptor numbers, recently discovered due to the increased sensitivity of new measurement techniques. Membrane-initiated responses are the cell's rapid and first response to steroids. The responses that emanate from the membrane can have direct functional consequences, such as secretion of other peptide hormones or rapid behavioral changes. Other rapid responses are prerequisites for subsequent genomic responses. The wide variety of signal transduction schemes employed by various tissues and hormones are summarized and discussed in terms of the identity of proteins that mediate these responses. Probable mixed binding systems for steroids in plasma membranes are compared to similar multiple hormone–binding protein systems in extracellular fluids and inside cells. These issues are related to steroid-dependent tumor growth, developmental and therapeutic apoptosis, and the actions of endocrine disrupters. The integration of membrane-initiated effects with genomic mechanisms results in the complete cellular response to steroids.** [P.S.E.B.M. 1999, Vol 220]

Steroid hormones and related compounds act as ligands for nuclear receptors that alter transcription of genes and thus eventually result in alteration of a variety of functions (1–3). However, a major deviation from widely accepted dogma on the exclusivity of genomic steroid action has developed from the observation that at physiological levels, these hormones sometimes elicit very rapid effects (beginning seconds to minutes after hormone exposure) that can be manifested without RNA or protein synthesis. Although this idea was proposed by Szego (4–6) in early biochemical investigations of estrogenic mechanisms, the major advances in our understanding of steroidal actions from the 1960s to 1990s focused primarily on genomic actions, with very few investigators addressing rapid or nongenomic actions.

The time dependence of different actions of steroids presents a useful way of mechanistically categorizing different types of steroid actions. Very rapid effects like ion fluxes, triggering of action potentials, or discharging of secretory vesicles happen within a time frame of seconds to minutes. Somewhat slower responses might arise from the activation of enzymes or pumps, whose products would be generated immediately, but would build up to detectable and functional levels only over time. Long-term responses, such as those that we associate with gene expression mediated by steroid receptors acting as transcription factors, require synthetic events eventually altering functional or structural proteins or their levels. These responses can reversibly regulate tissues for temporary, hormonally dictated changes in service. Finally, long-term events may develop over the maturational time course of the organism to produce permanent developmental tissue remodeling. Steroid receptors participate in all of these activities, but through different mechanisms.

[1] To whom requests for reprints should be addressed at Department of Human Biological Chemistry and Genetics, Route 0645, University of Texas Medical Branch, Galveston, TX 77555-0645. E-mail: cswatson@utmb.edu http://cellbio.utmb.edu/watson/watson.htm

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Szego suggested that the cellular synthesis of macromolecules *via* the genomic mechanism of estrogen action must be preceded by preparative changes that support such synthetic activity (reviewed in Ref. 7). One of the primary prerequisites for making new macromolecules should be the import into the cell of the raw materials, thus coordinating signals and energy sources needed to effect such large tasks. The most likely initial cellular response to prepare for further investments in a hormonally altered outcome is rapid action at the point of entry to the cell, the cell membrane. Signals initiated at the membrane, and then associated with speedy signal cascades, can affect import of sugars, ions, amino acids, and other substrates to support subsequent cell changes. Thus, rapid membrane actions of steroids might coordinate uptake of such substrates with the genomically regulated events they precede and support.

Another related concept that has been modified dramatically recently is the broadened designation of target tissues for steroid hormones with the discovery of ever-expanding arrays of receptor subtypes to diversify further the possible actions of steroids. It is well established that thyroid hormone receptor (TR), retinoic acid receptor (RAR), retinoid X receptor (RXR), and peroxisome proliferator receptors (PPARs) have multiple subtypes contributed by both multiple genes and multiple splicing patterns of those genes (3) expressed in a tissue- and development-specific way. Only relatively recently have the concepts of multiple genes and multiple splice variants of ER, GR, and AR been recognized, now allowing these receptors to be considered as arrays of multiple forms of steroid receptors mediating different kinds of functions. For example, ERα and ERβ, products of different genes, are expressed in different but intersecting subsets of tissues (8), though the functional consequences of the multiple splice variants of ERα are currently unknown (9). The roles of multiple forms of the glucocorticoid receptor (α and β) are not understood (10), though multiple splice variants of GRα are very tissue-specific (11–13). One splice variant has been associated with the expression of a membrane form of the receptor [(14, 15) and unpublished data]. Development of more sensitive methods to detect steroid receptors has now allowed tissues with low receptor levels to be included as targets for steroid actions. Recently introduced experimental tools with increased sensitivity include hormone binding to receptor [although multiple receptor forms are often detected together, when their affinities for a class of steroids are similar (16)], recognition of specific receptor epitopes along with immunodetection amplification methods, and hybridization to specific transcripts that encode receptors. Thus a new category of tissues with lower levels of receptors has been defined (17–19) and offers new opportunities to decipher the functional meaning of variant receptor messages and their encoded proteins, perhaps including the membrane steroid receptors.

Cellular Functions Associated with Nongenomic Actions

The types of cellular functions that have been assigned to members of the steroid receptor superfamily are vast and diverse, and will not be reiterated here (3, 20). However, we will consider the types of functions that have been attributed to the nongenomic actions of steroids. This will perhaps enlighten our consideration of what mechanisms serve these functions and what protein(s) mediate the initial signal. Some steroids have been implicated in functions that require relatively rapid membrane fusions. These include estrogen-induced exocytosis, resulting in secretion of other active peptide hormones (21, 22), and progesterone-induced membrane fusions that activate sperm for fertilization (23–26). Hormonal signals often force cells to make a decision about whether they will divide, differentiate, or apoptose. Such signals are often first perceived at the membrane, where the cell must quickly begin to import substrates to support two of these activities or alter cellular ion concentrations that activate enzymes to support the latter consequence for tissue remodeling. Cell cycle events are often triggered by phosphorylation of key cell cycle proteins (27). This switch is set, and the cell passes through its cycle restriction point long before synthetic events in the cell begin. Steroids have been shown to elicit phosphorylations of proteins that control both meiotic (28) and mitotic (29, 30) decisions. Behavioral responses often must be elicited quickly and be coordinated with other physiological events. Steroids evoke rapid responses in the brain (31–47), especially in connection with procreative behaviors that must quickly succeed hormone elevation to facilitate or inhibit reproduction (48–51). Smooth muscle responses to steroids are rapidly developed in a variety of tissues, including the very clinically important vasodilation and vasorestriction responses in cardiovascular tissues (19, 52–60). Cell migration (61), cell motility (24), movement and redistribution of proteins within the plasma membrane (62), and cell shape changes (7, 63) have been observed as membrane-initiated or rapid effects of steroids and steroid-like compounds. Other rapid responses are on trans-cellular transport of structural calcium (64), uptake of energy sources (65, 66) necessary for a variety of functions, and sometimes assistance in the import of steroids themselves into cells (67). The activity of other membrane receptors for neurotransmitters are said to be modified rapidly by steroids through mechanisms that are still unclear (36, 68–75) and could possibly involve interactions with membrane steroid receptors.

Cellular Mechanisms Associated with Membrane-Initiated Steroid Actions

Despite the fact that membrane-initiated steroid effects were understudied for many years, new attention has recently been focused on this area. Therefore, it is now timely to survey examples of signaling mechanisms that presum-

ably emanate from the initial interaction of steroid with a membrane receptor or binding protein. Here we will address the type of signals that have been described and classify them with respect to the relevant mechanism and their signaling connections. Although this review of the material will be mechanism-centered and not tissue- or function-centered, we will also comment on the tissues and hormones involved in particular mechanisms. In many cases only a single mechanism has been assessed for a given tissue. Therefore, we cannot yet understand the generality of these concepts for given tissues, organs, or disease processes in those locations until each type of mechanism has been assessed more systematically. It should be noted that genomic effects on the synthesis of the cellular machinery used in generating these signals have been well established in most cases, and are not reviewed here.

Ion fluxes are common consequences of the rapid workings of steroidal compounds. The channel opening most commonly affected (or more frequently measured) is Ca^{++} (24, 26, 52, 55, 57, 59, 60, 76–95). These examples include the actions of glucocorticoids (95), progestins (24, 26, 76–80), vitamin D (52, 81, 91), thyroid hormone (94), androgens (81, 82, 90), mineralocorticoids (55), and estrogens (59, 60, 83–89, 93). The types of tissues (or cell lines representing them) using this mechanism are also very diverse. They include ovarian cells (76, 82) and oocytes (77–79, 96), sperm (24, 26, 80, 92), prostate (93), cardiac and vascular smooth muscle cells (52, 55, 57, 59, 60), anterior pituitary cells (83–85), endometrium (86), bone (81, 87–89, 91), splenic or thymic lymphocytes (90, 95), and brain (35). K^+ ions also move across membranes in response to steroids, and sometimes the activation of a K^+ channel is thought subsequently to activate or inhibit neighboring Ca^{++} channels (35, 58, 97). Na^+ (53, 98), often in combination with other ions such as K^+ (97), H^+ (54, 99–101), or Cl^- (92, 102–104), has also been reported to be affected. These ion fluxes sometimes result in cellular pH changes (53, 89), or the raising or lowering of membrane potentials sufficient to trigger, inhibit, or change the firing rate of action potentials in permissive tissues (31–34, 68, 70). Practically the same spectrum of hormones and tissues is involved in these further examples. These additional ion fluxes in response to steroids have been noted in the following tissues: brain (31–34), kidney (98), oocyte (97), sperm (92), bone (89, 103), liver (94), vascular system cells (53, 54, 58), and lymphocytes (99, 100, 102). Hormones that elicit such responses include glucocorticoids (31–34, 98), progestins (92, 97), vitamin D (103), thyroid hormone (94), mineralocorticoids (53, 54, 99, 100, 102), and estrogens (58, 89). No pattern has yet emerged from this collection of examples, and the principle illustrated seems to be that of diversity. The ions generated can act upon their own receptors, channel proteins, enzymes, or other forms of calcium-binding regulatory proteins to generate a variety of subsequent messages and actions.

Other well-known second messengers generated by membrane receptor activation are also seen in steroid-perturbed systems. Cyclic nucleotide level changes, for both cAMP (4, 43, 52, 89, 105–108) and cGMP (51, 79, 89, 109–111), and G-protein involvement (35, 76, 112) can be seen as a result of steroid actions. Turnover of various lipid signaling molecules such as inositol phosphates (56, 76, 78, 87, 91, 99, 102, 113–115) and diacylglycerol (DAG) (54, 87, 113, 116, 117) are induced, along with the activity levels of phospholipases C (54–56, 76, 82, 87, 118, 119), D (116, 117) and A2 (117). Rapid generation of the short-lived nitric oxide signal has also been measured as a result of steroid stimulation (120). Again a spectrum of these ligands generates such signals including glucocorticoids (35, 51, 107), progestins (76, 79, 112, 113, 116), vitamin D (52, 91, 109, 110, 114), thyroid hormone (118), androgens (82, 111), mineralocorticoids (54–56, 99, 102), and estrogens (4, 87, 89, 106, 108, 115). A variety of tissues and cell types have so far been shown to be involved in these mechanisms. These include pituitary (107), brain (35, 51), ovary (76, 82), oocytes (79, 112, 113, 116), cardiac and vascular muscle (52, 54–56), bone (87, 89, 91, 117), colon (114), uterus (4, 115, 118), lymphocytes (99, 102), mammary cells (106), and prostate (108).

A major consequence of signals generated in these systems is the activation of various kinases and the consequent alteration of the phosphorylation state of important regulatory proteins. Multiple phosphorylations could present different pathways of action, or be at different points in the same cascade. Kinases are also frequently controlled by their own phosphorylation status. Examples of rapid steroid-induced changes in kinase activities include many different categories of protein kinases including both tyrosine and serine/threonine kinases (29, 121), cyclic nucleotide-, Ca^{++}-, inositol phosphate-, or DAG-dependent kinases (24, 35, 41, 54, 76, 94, 101, 113, 118, 122–124), and kinases associated with cell proliferation such as MAP kinase (19, 30, 123, 125–128) and other ERKs (19, 29, 30, 62, 128, 129). One wonders if further analysis will also reveal participation of phosphatase activities in these schemes, although no examples of this part of the general mechanism have yet appeared.

Cell proliferation–associated kinases and phosphatases can change the phosphorylation status of key cell cycle regulatory proteins (27). Many different phosphate addition or removal enzymes could affect the phosphorylation state of the cyclins. The status of cyclins (and their binding partner inhibitors and activators) are critical to a cell's decision to divide. So far, these molecules have been more frequently studied from the biased viewpoint that steroids will induce their synthesis and not affect their activity (130).

Finally, steroids have been seen to cause the rapid phosphorylation of transcription factors (29, 44, 128, 131, 132). Relevant mechanisms include the phosphorylation of the steroid receptors themselves (133), since it is known that

these transcription factors are exquisitely responsive to both ligand-dependent and ligand-independent phosphorylations. We are just beginning to learn how many of these gene regulatory proteins are controlled by their phosphorylation state. The discovery that transcription factors can be modulated by steroid-induced changes in phosphorylation status provides a satisfying mechanistic link to the genomic actions of steroids and supports the idea that the two mechanistic arms of steroid action are effectively linked and interleaved. This category also illustrates why "nongenomic" effects of steroids can easily have "genomic" consequences, and suggests that a more appropriate label for these actions of steroids might be "membrane-initiated."

Steroid and neurosteroid effects on 5HT-1A (134), GABA-$_A$ (71–75, 104) acetylcholine (68, 69), dopamine (135), and kainate (70) neurotransmitter receptors have been noted, and are usually attributed (speculatively) to binding of the steroid at an alternative (allosteric) site on the neurotransmitter receptor. Although this may be the case, it may also be that these receptors interact with steroid receptors in the plane of the plasma membrane. The experimental data that are used to support the first conclusion could also be used to support the second one. That is, cell types in which the cloned neurotransmitter receptor cDNAs were introduced and expressed, either have [in the case of the Xenopus oocyte (136, 137)] or could have (as yet undiscovered) membrane steroid receptors. Studies suggesting that the steroid binds to the neurotransmitter receptor itself could also be explained by binding of steroid to a steroid receptor that, in turn, is a binding partner for the neurotransmitter receptor in the membrane.

It is apparent that there is tremendous variation in the signal transduction mechanisms used by steroids in different tissues, and sometimes within tissues under different circumstances. So certain tissues and cell types appear in many of the above categories of signal transduction molecule associations. Some of these examples can be parts of the same sequential cascade. Alternatively some might be part of separate initial signals that converge where cascades intersect or diverge when multiple different signals are generated. Whereas some of these effects may remain separate by virtue of different subcellular locations or different dose-response sensitivities, other signals may sum to provide a final signal outcome that integrates the magnitude of signals from multiple inputs. Careful dissection of the temporal order, inhibitor sensitivity, and subcellular localization of each of these signals (24, 62, 121) may eventually make sense of these redundancies and their final relationship to individual or coordinated functions. Redundancies in signal generation in some tissues are perhaps related to the number of different functions linked to these signals. Like peptide hormone and growth factor receptors, a steroid receptor residing in the plasma membrane could be connected to alternative or multiple signaling mechanisms by multiple interactions with binding partners in or near the membrane. Thus, our current knowledge of membrane-initiated actions of steroids is still very sketchy and incomplete. Now the task will be to collect enough examples of these membrane-initiated actions to begin to make sense of the organization of the responses and any principles that can be gleaned from these examples. This will be the basis for organizing our thinking to formulate hypotheses about the design of the steroid-induced signal transduction schemes and their pathway interactions.

Proteins that may Mediate Membrane-Initiated Steroid Effects

The few systems in which rapid actions of steroids have been linked to a specific protein mediator have so far failed to fit into a single theme or suggest a functional grouping. Whereas some membrane steroid receptors have been identified as being antigenically related to their intracellular counterparts, others have not. Many investigators have assumed that membrane-initiated actions of steroids will be accomplished by a protein different from the classical intracellular receptor that mediates nuclear actions of the hormone. These assumptions have been based on several arguments. One is that membrane responses, while usually just as sensitive (in the picomolar to nanomolar range) as nuclear responses, exhibit some differences in pharmacological specificity or sensitivity hierarchies (25, 53, 138–142). However, receptor residence in lipid membranes, as opposed to the aqueous interior of the cell, may impose different conformational restrictions (143) on the receptor, and consequently after the geometry of the steroid binding pocket. Likewise, the binding of known specific antagonists of the nuclear receptors such as antiestrogens (e.g., tamoxifen, ICI 187,780) and anti-glucocorticoid/progestins (e.g., RU486) could be affected by binding-pocket conformations. A survey of the available data demonstrates an equal likelihood that well-known nuclear receptor antagonists will block these responses, or not, for many different classes of steroids on many different rapid effects (19, 29, 30, 43, 56, 59, 60, 72, 76, 81–83, 85, 87, 90, 93, 101, 103, 113, 126, 128, 131, 132, 144–147), probably suggesting that multiple different steroid-binding proteins are involved in these responses. Another argument for membrane-initiated functions being accomplished by unique proteins is that when membrane-isolated receptors have been identified, their size is different from that of the corresponding intracellular receptor (100, 148). While this argument could be one reason to consider unique proteins, it does not fully consider the possibilities that proteolytic cleavage into fragments may occur during the course of protein isolation (a common occurrence with steroid receptors) or that membrane targeting may involve the addition or deletion of peptide sequence or other chemical moieties to a known protein. Rapidly evolving information concerning the structure and functions of members of the cytokine/growth hormone/prolactin receptor superfamily provide an interesting and potentially relevant analogy. In this gene family, a single gene can give rise to multiple membrane, cytoplasmic, and transcriptional

activities (149, 150). This is an issue that may also be resolved by allowing ourselves to consider the possibility that the plasma membrane contains mixed binding systems for steroids.

A favored method of demonstrating the presence of membrane steroid receptors is with so-called impeded ligands (18, 19, 35, 36, 49, 76, 87, 89, 90, 113, 145, 151–154). These conjugates usually contain multiple molecules of a given steroid covalently attached per single molecule of bovine serum albumin (BSA), rendering the steroid impermeable to the cell membrane (if endocytosis is minimized by use of low temperatures or inhibitors). Such compounds have also been shown to mediate very rapid effects of steroids. The activity of steroids on the outside of cells should caution those who claim that ligands not sufficiently lipophilic to gain access to the inside of the cell by diffusion through the membrane, will be inactive. Since there are several different types of steroid-binding proteins, binding of these impeded ligands could jointly involve several different proteins that may all be involved in steroid-induced, membrane-initiated functions. It is possible that different laboratories arguing over the "true identity" of the membrane steroid receptors could be studying different proteins that are functional components of the same or different steroid-induced pathways.

Our own laboratories have addressed the issue of membrane steroid receptor identity by using well-known antibodies raised against the intracellular versions of the receptor for estrogens (using eight antibodies) and glucocorticoids (using three antibodies). These antibodies specifically recognize different epitopes spanning the length of the proteins (13, 21, 22, 151, 152, 155). In both cases we found that the cell surface–attached version of the protein was larger than the intracellular version, but that it also included smaller fragments (22, 156, 157). Our antibody-labeled membrane steroid receptors were present in a punctate pattern (illustrated by the examples in Figure 1) that patched and capped when live Ab-stained cells were incubated at temperatures above 4°C (21, 156). The uneven distribution and aggregated appearance of these membrane steroid receptors were similar to those visualized by other methods involving derivatized steroids (19, 151, 154, 158, 159). While the multivalent nature of both the antibodies and some derivatized ligands may be the reason for this aggregation, we have also found a clustered appearance for receptors visualized on cells fixed before the antibodies were applied (Norfleet and Watson, unpublished data).

The cell types on which we have immuno-identified membrane steroid receptors come from the list of those previously cited to exhibit rapid signals and functions in response to steroids (83, 95). We identified membrane estrogen receptors on the GH_3/B6 cell line, derived from an anterior pituitary tumor of the rat. The membrane version of the glucocorticoid receptor was characterized in lymphoid cancer cell lines from mice (S-49 cells) and humans (CCRF-CEM cells) and in leukemic cells from human patients. This

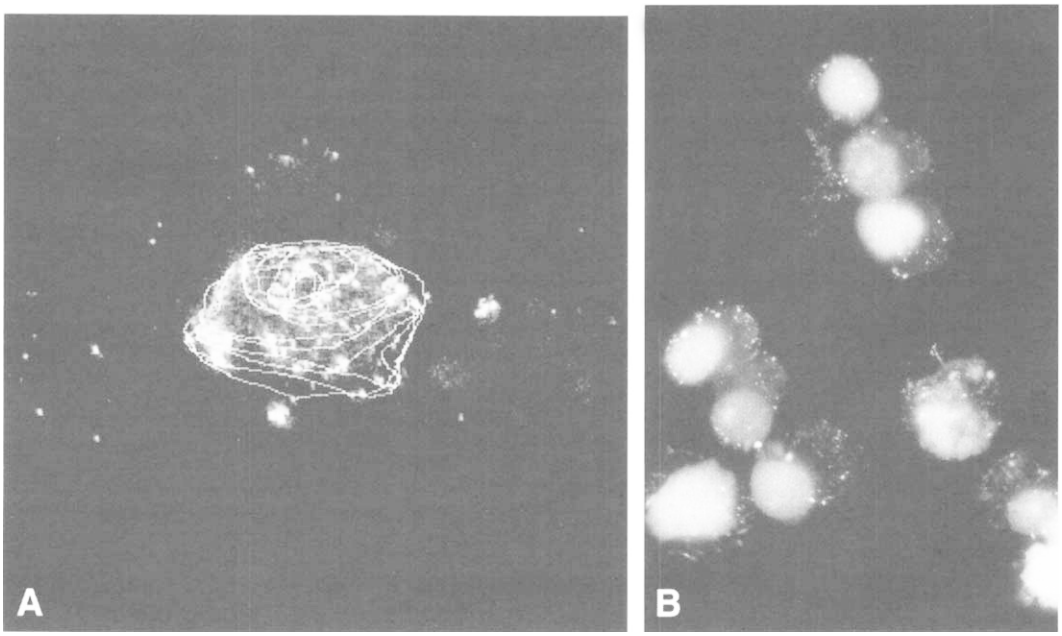

Figure 1. Identification of two steroid receptors in plasma membranes by cross-reactivity with intracellular steroid receptor–specific antibodies. This suggests that steroid binding proteins present in plasma membranes are immunologically similar to their nuclear transcription factor counterparts. (A) Confocal microscopic analysis (3-dimensional reconstruction) to demonstrate an uneven plasma membrane localization for the estrogen receptor-α on GH_3/B6 pituitary tumor cells. Epitope-purified R3 antibody to the intracellular estrogen receptor (antipeptide polyclonal antibody) was used to stain live cells. The antigen was visualized using a cy3-tagged anti-rabbit secondary antibody (light irregular spots and clumps). The continuous horizontal lines depict the contour lines of the cell. (Reproduced from FASEB J **9:**404–410, 1995). (B) Punctate staining of the membrane glucocorticoid receptor on a human leukemic cell line (CCRF-CEM) with an antipeptide polyclonal antibody to the hinge region of the human intracellular glucocorticoid receptor. The secondary Ab is visualized by a fluorescein tag. The nuclei of these cells are counterstained with propidium iodide. (Reproduced with permission from Am J Physiol **273:**E571–E583, 1997).

receptor was correlated with the hormone-induced lymphocytolytic effects in these cells (13, 155–157, 160–163). The progesterone receptors on sperm acrosomal membranes (73, 92, 145) and granulosa cells (72) have also been related to their intracellular protein counterparts *via* epitope recognition. In addition, some antibody staining for progesterone (164) and estrogen (165) receptors on dendrites appear punctate (unlike cytoplasmic staining) and could also be evidence for membrane forms of the intracellular receptors. Nevertheless, some sequences for membrane receptors for other steroids have recently been reported not to match that of their nuclear receptor counterparts (148, 166, 167). It may be helpful to consider that steroid binding to membrane components may not only reflect binding to receptor, leading to signal transduction, but could also represent binding of steroid to other important functional molecules. These other functions may include hormone transport into the cells or transfer to other recipient molecules, protection from metabolism, and even enzymatic conversions. Therefore, steroid binding to whole cells or membrane preparations probably reflects the hormone's affinity for a variety of different proteins that are present in the plasma membrane for different purposes; these proteins may constitute a mixed binding system necessary for the comprehensive business of steroids. Such mixed binding systems are known to exist both intracellulary and in the circulation, and besides receptors, include transporters or importers, enzymes, and other binding proteins of unknown or disputed function. What evidence is there that some of these other nonreceptor proteins play a role in nongenomic actions of steroids and reside in plasma membranes? Proteins that bind hormones in the circulation have, in some cases, been shown to be necessary for control of cell proliferation, a response possibly initiated at the cell membrane (108, 168). It is possible that blood-borne steroid-binding proteins dock at plasma membrane sites (108, 169–171) to deliver steroids to membrane steroid receptors or steroid importers (67).

Possible Membrane Steroid Receptor Roles in Hormonally-Induced Pathologies or Therapeutic Interventions

Many of the signaling mechanisms leading to cell proliferation, death, or differentiation originate from the membrane-derived second messenger systems and result in phosphorylation events that alter cell cycle proteins responsible for sending the cell along the proliferation/death pathways. There are multiple examples of steroids causing any or even all of these outcomes in a given tissue (172), and the membrane form of the receptor may be responsible for the onset of this cascade, resulting in eventual replication or elimination of cells. There is direct evidence that the membrane glucocorticoid receptor is involved in apoptosis and is cell cycle-regulated (163). The involvement of membrane glucocorticoid receptors in the natural process of developmental culling of immature mouse thymic cells was shown recently in experiments where expression of the mGR correlated with surface antigens marking developmentally immature cells; these are the cells that are also susceptible to glucocorticoid-induced apoptosis (173). Interestingly, cancer cells, which are sometimes thought of as developmentally-arrested, also express this receptor, which has become the basis for therapeutic, glucocorticoid-induced apoptosis for leukemias (155). Thus, we can speculate that alterations in the expression (either upregulation or downregulation) of the membrane-resident forms of steroid receptors might play an important role in the development, growth, or demise of steroid-regulated cancers, as well as providing potential sites for pharmacological intervention. Increasing the scope of our knowledge to include membrane actions of steroids may provide many new insights into the mechanisms of tumor growth and other pathologies and therapeutic approaches to address them.

The public has recently become very concerned about a large number of compounds termed "endocrine disrupters" that can contaminate our environment and food and water supplies. These dietary or environmental contaminants often are steroid hormone mimetics for estrogens, androgens, and progestins (174–176). It is currently unclear how these compounds carry out their hormonal effects (for example on cell proliferation, behavior, or production of sex-specific proteins), when all tests to date have shown them to be extremely weak in assays measuring "nuclear transcription effects" *via* actions on gene expression or on artificial reporter gene constructs. Only one test of these mimetic compounds has thus far been conducted on the membrane-initiated actions of steroids (60) in which they were found to be active. Therefore, we may be missing their most potent effects. Since cells respond to steroids and related compounds both at the membrane and later in the nucleus, combinations of these compounds that act at either site may "team up" to be potent hormonal cocktails, with mechanistically complementary activities. It is certain that these compounds exist in our environment in highly complex mixtures, and may also act in concert with endogenous steroidal compounds.

The Whole Cell Response to Steroids: Integration of Nongenomic and Genomic Effects

Finally, although this review has emphasized the role of steroids acting at the level of the plasma membrane, we must think of these actions as components of the entire enterprise of steroid action on the whole cell, as illustrated in the cartoon in Figure 2. In this capacity, the membrane portion of the signaling mechanism would simply be the "first alert" to the cell to prepare for further synthetic action or to perform functions whose actions must occur quickly, such as mediating a behavioral response, secreting other hormones that will mediate such a response, or switching the activity of a crucial regulator by phosphorylation. The importance of the classic and elegant work on the actions of steroid receptors as nuclear transcription factors has been largely understood and accepted. However, these tran-

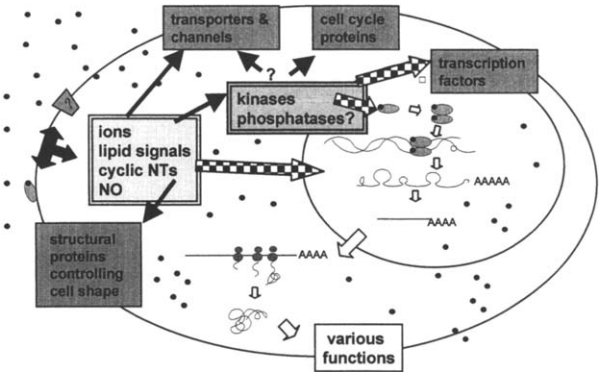

Figure 2. The two branches of the cellular mechanism of steroid action and their interactions are shown by the different arrow styles. The small black circles represent steroids that can act both intracellularly and extracellularly with the shaded ovals that represent steroid receptors. The classic mechanism of steroid action is depicted by the sequence using hollow arrows. This involves the binding of liganded, dimerized nuclear receptors to specific gene response elements and the consequent synthesis and processing of messenger RNAs that code for specific proteins leading to a variety of well-defined functions. The sequence depicted by the solid black arrows outlines actions initiated by the membrane receptor for steroids; the tripartite arrow leading to the shaded trapezoid in the membrane indicates that this could be accomplished by interaction with a variety of other membrane-resident signal transduction proteins (protein partners), depending on which ones were available in a given cell type. This interaction then leads to the release of cell type–specific second messengers (box with double outlines), which in turn may affect enzymatic modifiers (lightly shaded box with double outlines) or directly affect their target molecules. Darker shaded boxes list final functions in this pathway (such as transport of molecules into and out of the cell, change in cell shape, and/or post-translational modifications of cell cycle or transcription factor proteins in ways that affect their activities). Some of these membrane-initiated effects can eventually lead to downstream genomic actions *via* transcription factor modification or second messenger–responsive transcription factor pathways. The convergence of the genomic and nongenomic systems is shown by the black and white checked arrows. Interaction and integration lead to a complete and complex cellular response.

scriptional activities do not explain all the functional consequences of the actions of steroid hormones. The challenge in the near future is to begin to unravel the many details and themes of steroid action at the membrane, and to begin to understand the integration of membrane-initiated events in the complex overall actions of steroids on cell function. It seems highly likely that an expanded understanding of the range of cellular mechanisms used by steroids will facilitate studies of steroid actions both in nonclassical target tissues and *via* mimetic compounds that may have either injurious or therapeutic effects.

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