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Review

Emerging Diversities in the Mechanism of Action of Steroid Hormones

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The classical genomic action of steroid hormones acting through intracelluar receptors is well recognized. Within this concept of action, questions regarding the ultimate fate of the hormone and lack of a tight correlation between tissue uptake and biological activity with receptor binding remain unanswered. Evidence has accumulated that steroid hormones can exert non-classical action that is characterized by rapid effect of short duration. In most of these cases, the hormone effect occurs at the membrane level and is not associated with entry into the cell. The possible mechanisms for these non-classical actions are: (a) changes in membrane fluidity; (b) steroid hormone acting on receptors on plasma membranes; (c) steroid hormones regulating $GABA_A$ receptors on plasma membranes; and (d) activation of steroid receptors by factors such as EGF, IGF-1 and dopamine. Data have also been obtained indicating that receptor-mediated insertion of steroid hormones into DNA may take place with the steroid acting as a transcription factor. These new proposed mechanisms of action of steroid hormones should not be viewed as a challenge to the classical mechanism. These diverse modes of action provide for an integrated action of hormones which may be rapid and of short duration or prolonged to address the physiological needs of the individual.

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INTRODUCTION

Extensive work in the late fifties and the early sixties laid the foundations on which the current classical concepts of the mechanism of action of steroid hormones is based. In the mid-fifties it was believed that the major action of steroid hormones, particularly estrogens was oxidation–reduction of hydroxyl groups mediating hydrogen transfer from NADH to NADP and NADPH to NAD [1]. However, in 1959 Bush and Mahesh [2] demonstrated that oxidation–reduction of the 11β-hydroxyl group in corticosteroids was not essential for biological activity because the 11β-hydroxyl-corticosteroids were active without further metabolism. This finding was followed by the work of Jensen and his collaborators who demonstrated that estradiol by itself in the absence of further metabolism was biologically active [3]. Furthermore, there existed in estrogen responsive tissues a system that was capable of concentrating and retaining the active hormone. The ability of the rat ventral prostrate to concentrate 4-androstene-3-17-dione and testosterone was also reported in the early sixties along with the statement that chloroform soluble radioactivity appeared in the RNA containing fractions [4, 5]. Mahesh and Greenblatt first demonstrated in 1963 that clomiphene citrate which contained weak agonist and strong antiestrogenic properties inhibited the specific uptake of [^{3}H]estradiol not only in the uterus but the pituitary as well [6]. The stereospecificity of the binding of estrogens in rat uterine subcellular fractions was demonstrated by Noteboom and Gorski in 1965 and based on the release of [^{3}H]estradiol by proteases, the investigators concluded that the [^{3}H]estradiol was bound to a protein [7]. The estrogen receptor containing fraction was isolated from the rat uterus using sucrose gradient techniques and further characterized by Gorski and collaborators [8, 9]. The two-step process of hormone binding and activation of the receptor to its biologically active form was then described by Jensen and his collaborators [10, 11]. These discoveries led to the classical concept of intracellular hormone receptors. The major mechanism of action of steroid hormones consists of specific steroid hormone binding to its receptor, transformation of the bound receptor to the active form,

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and binding of the steroid–receptor complex to DNA followed by gene activation [12–15]. The various steps involved have been the research focus of several outstanding laboratories.

In spite of the merits of the classically recognized mechanism of action of steroid hormones, extensive evidence has also accumulated in the literature suggesting other modes of action of steroid hormones in a variety of tissues and sites (Tables 1–5). For instance some of the actions of steroid hormones take place in time intervals of a few seconds and cannot be inhibited by protein synthesis inhibitors. Additionally, some actions of steroid hormones can be exerted even when the entry of the steroid into the cell is blocked by covalent binding to a large protein or in brain preparations lacking cell nuclei. Finally, there is growing evidence that a precise relationship between the binding of a given steroid hormone to its receptor and its biological activity is frequently not present. This review will be devoted to the emerging evidence and concepts of diverse modes of action of steroid hormones which may occur either in conjunction with the classical action or as an alternate mechanism in selected situations. The possible mechanisms underlying the non-classical effect of steroid hormones will also be discussed.

CLASSICAL ACTION OF STEROID HORMONES

Since the classical mechanisms of action of steroid hormones have been extensively investigated and are the subject of several excellent reviews [12–15], only the major steps will be briefly summarized. It is generally accepted that steroid hormones enter the cell by the process of diffusion although some evidence of an active transport has also been cited [16]. Steroid hormone receptors may be located in the cytoplasm (glucocorticoids) or within the cell nucleus (estrogens and progesterone). Steroid hormone action is initiated by the hormone binding to specific receptors in target cells. This ligand binding promotes dissociation of heat shock proteins from the nascent receptor. The heat shock protein dissociated receptor is then capable of allosteric changes and dimerization needed for binding to DNA. Several phosphorylation steps follow which convert the receptor to the transcriptionally active form. The transcriptionally active receptor–DNA complex activates recruitment and stabilization of transcriptional factors at the target gene promoter and activation of RNA polymerase II to initiate new mRNA synthesis which codes for protein required for hormone action. Usually the time taken for new protein synthesis is 30 min or longer.

While our knowledge of the genomic mechanism of action of steroid hormones is well advanced, the exact role of the ligand has not been fully elucidated. While it is true that the essential steps involving the receptor (such as removal of heat shock protein, dimerization and phosphorylation by either activating phosphorylation enzymes or by inhibiting their degradation) can be carried out *in vitro* in the absence of ligand [17], nevertheless, under physiological conditions the steroid hormone ligand usually activates the key steps needed for action. Thus, it is generally accepted that the binding of various ligands to their cognate receptors in the cell nucleus is the critical step in the transmission of the hormonal response. Given that the magnitude of hormonal responses has been shown to be governed by the stereospecificity and concentration of the ligand, this information must somehow be communicated to the gene via receptor–ligand–DNA interaction. One possibility would be that the relative strength of binding of the ligand to the receptor causes a specific and quantitative conformational change in the receptor protein which then interacts in a specific and quantitative fashion with DNA. However, it is well known that receptor binding of various ligands does not always correlate with hormonal activity. For instance, in the case of estrogens, many compounds which are known to be more active than the natural hormone estradiol bind very poorly to the estrogen receptor [18, 19]. In fact, one of the most active estrogens known, i.e. 11β-acetoxyestradiol, has less than 1% of the binding to the estrogen receptor relative to estradiol [20]. Conversely, certain compounds which bind strongly to the estrogen receptor compared to estradiol have poor estrogenic activity [21–22]. It is of further interest that uptake of various estrogens in uterine tissue correlates with hormonal activity but not with receptor binding [18]. In trying to explain these findings *in vivo*, one has to take into account the delivery of the steroid to the target tissue. For example, the action of compounds which have receptor binding but low activity and uptake could be due to rapid clearance upon hepatocellular passage. However, compounds with high hormonal activity and uptake but low receptor binding could not be rationalized on this basis. Taken as a whole, such findings led to the conclusion by Brooks *et al.* [23] that the character and extent of hormonal responses to estrogen analogs are not directly related to their affinity for the estrogen receptor. Murdoch *et al.* [24] have recently shown the binding of the estrogen receptor to DNA is the same whether or not the ligand is bound to the receptor. In the case of glucocorticoids, Spanjaard and Chin, using receptor constructs in which the ligand

Table 1. *Evidence supporting non-classical mechanisms of steroid hormones*

(1) Certain effects of steroid hormones are so rapid (seconds to minutes) that they preclude a classical genomic mechanism.
(2) These effects occur even if the steroid hormone is *not* allowed to enter the cell.
(3) The rapid effects are not blocked by protein synthesis inhibitors.
(4) The effects of steroid hormones are stereospecific suggesting a specific membrane receptor mediated event.
(5) Many steroid hormones exhibit potent biological activity with little to no affinity for the classical intracellular steroid hormone receptor.

binding domain has been removed, [25] have shown that the hormone enhances transcriptional activity. Taken as a whole, these data suggest that some additional step(s) involving recognition of the concentration and stereospecificity of the ligand must be involved in order for the hormone to regulate the gene. One emerging hypothesis is that one of these additional steps could be a receptor mediated interaction of the ligand with DNA. This possible addendum to the classical hypothesis of hormone action will be discussed in detail in the context of new experimental findings at the end of this review.

NON-CLASSICAL ACTIONS OF STEROID HORMONES

Extensive evidence has accumulated in the literature suggesting that steroid hormones and their metabolites can act in a variety of tissues via non-classical mechanisms of action. The lines of evidence supporting the non-classical actions of steroid hormones include rapid effects and action in which entry into the cell and nuclear interaction have been precluded. Table 1 summarizes the evidence supporting the non-classical modes of action of a variety of steroid hormones. While not all-inclusive, it displays the extensive amount of work performed in this area supporting non-classical mechanisms.

Progesterone and progesterone metabolites

Table 2 summarizes the actions of progesterone and its metabolites that occur within seconds or minutes while Table 3 summarizes actions that take an hour or longer but still show evidence of a non-classical action. However, the possibility of a combination of both classical and non-classical action cannot be ruled out.

Anesthetic and antiepileptic effects of progestins. The pioneering work of Selye in 1942 reported that progesterone and other steroids, when administered via injections to rats, could produce prolonged anesthesia lasting up to 2 h or longer [26]. This observation led to the development of a variety of steroidal anesthetics [27]. Several of these anesthetics were the 3α,5α- and 3α,5β-forms of progesterone metabolites which reportedly do not bind to the classical intracellular progesterone receptors [28–30]. Recent studies suggest that the anesthetic and antiepileptic properties of these progestins may be due to interactions with the $GABA_A$ receptor system. This subject will be discussed in detail under "Mechanisms of Non-Classical Actions of Steroid Hormones" in this review. Nevertheless, it is clear that progestins can exert rapid depressive effects within the CNS.

Affirmation of the potent CNS depressant effect of progesterone was further supported by the observations of Bäckström [31] who observed that the incidence of seizures in epileptic women during the menstrual cycle was lowest during the period when plasma progesterone levels were highest. Furthermore, Rosciszewska *et al.* [32] showed that epileptic seizure activity in women was associated with low levels of progesterone metabolites in the blood. Bäckström and coworkers [33] subsequently demonstrated that intravenous progesterone infusion significantly attenuated epileptic discharge frequency in women with partial epilepsy. The latency of the effect was 1 h. This led the investigators to suggest that the effect was actually due to the metabolism of progesterone to more potent metabolites [33]. Luntz-Leybman *et al.* recently confirmed that progesterone metabolites such as 3α-hydroxy-5α-pregnan-20-one (3α,5α-tetrahydroprogesterone; 3α,5α-THP) can potently inhibit nicotine-induced seizures in male mice [34]. This effect appears to be due to the ability of progesterone metabolites to potently modulate the $GABA_A$ receptor system. These findings could have physiological implications as Freeman *et al.* [35] have shown that significantly elevated plasma levels of 3α,5α-THP in females are correlated with fatigue, confusion and delayed immediate recall. This further demonstrates that progestins can potently suppress brain functions perhaps via modulation of $GABA_A$ receptor activity in the brain.

Lordosis behavior. Progestins have also been shown to induce female mating behavior (lordosis) in estrogen-primed rats within minutes [36]. Rodriguez-Manzo *et al.* [37], showed that implantation of 3β-hydroxy-5β-pregnan-20-one (3β,5β-tetrahydorprogesterone; 3β,5β-THP) into the medial preoptic optic area (MPOA), induced lordosis behavior within 30 min in estrogen-primed ovariectomized rats. Progesterone was less effective than its 3β,5β-metabolite. Gorski and coworkers [36] found that progesterone implanted in the MPOA was ineffective; however, when implanted in the caudal mesencephalic reticular formation, it induced lordosis behavior in estrogen-primed ovariectomized rats with a latency of 3–4 min. The authors considered this to be too rapid for a genomic action and suggested that a non-genomic mechanism of action was involved. Together, these studies suggest that progesterone acts within the CNS to influence LHRH and dopamine release by a membrane rather than a genomic mechanism. Further evidence supporting this conclusion was provided by the studies of Brann *et al.* [38] who demonstrated that the LH releasing activity of the 3α-hydroxy-5α-pregnan-20-one, a progesterone metabolite that does not bind to the intracellular progesterone receptor, was blocked by the $GABA_A$ antagonist picrotoxin but not the antiprogestin RU486.

Effects on single neuron excitability. The whole-body studies described in the preceding paragraphs led to studies in the 1980s and 1990s investigating direct effects of progestins on brain excitability at the level of the single neuron. Smith and coworkers found that progesterone, via either systemic injection or local application, significantly enhanced inhibitory responses of rat Purkinje cells to gamma amino butyric acid (GABA), the principal inhibitory amino acid transmitter in the brain, while it significantly suppressed response to glutamate—

Table 2. Rapid effects of progestins

Reference	Organ studied	Effect observed	Latency	Specificity for hormone	Block by protein synthesis inhibitors	Other
Morrow *et al.*, [109]	Brain	3α,5α-reduced P_4 metabolites ↑ chloride ion flux both alone and with GABA	20 min	+	—	Also ↑ GABA agonist binding
Petitti and Etgen, 1992 [63]	Brain	P_4 suppresses norepinphrine induction of cAMP	5 min	-	—	—
Majewska *et al.*, 1987 [156]	Brain	3α,5α-reduced P_4 metabolites ↑ GABA, receptors	20 min	+	—	Also ↑ GABA agonist binding
Havens and Roase, 1988 [42]	Brain	P_4 ↓ electrophysiological excitability of golden hamsters dorsal midbrain neurons	10–20 min	+	—	—
Ross *et al.*, 1971 [36]	Brain	P_4 induces lordosis behavior in rats	3–4 min	+	—	—
Smith *et al.*, 1987 [39]	Brain	P_4 ↓ glutamate responsiveness of rat Purkinje neurons	3–10 min	-	—	—
Smith *et al.*, 1989 [41]	Brain	P_4 ↑ GABA responsiveness of rat Purkinje neurons	5–15 min	-	No	—
Kubli-Garfias *et al.*, 1976 [43]	Brain	3α-hydroxy-5β-pregnan-20-one ↓ neuronal activity in cats	10 s	+	—	—
Schumacker *et al.*, 1990 [157]	Brain	P_4 ↑ oxytocin receptor binding in the rat hypothalamic ventromedial nucleus	30 min	+	No	Effect may be important in the regulation of lodosis
Kato *et al.*, 1971 [158]	Brain	3α-hydroxy-5β-pregnan-20-one induces anesthesia in rats	<10 min	+	—	—
Putnam *et al.*, 1991 [52]	Uterus	P_4 and P_4 metabolites suppresses rat uterine contractility	5 min	+	—	—
Kubli-Garfias *et al.*, 1987 [57]	Smooth muscle	P_4 metabolites suppress contraction of jejunum, ileum and coronary artery	5–10 min	+	—	—
Meiri *et al.*, 1986 [159]	Frog muscle neuromuscular junction	P_4 increase synaptic activity	10–20 min	-	—	—
Osman *et al.*, 1989 [160]	Sperm	P_4 induces acrosomes reaction	10 s	-	—	—
Thomas and Meizel, 1989 [161]	Sperm	P_4 stimulates a rapid Ca^{2+} influx and hydrolysis of phosphoinosides in human sperm	10 s	-	—	—
Blackmore *et al.*, 1990 [64]	Sperm	P_4 stimulates Ca^{2+} influx in human sperm	1–5 s	+	—	17α-OH P_4 also active
Meizel and Turner, 1991 [65] Tesarik *et al.*, 1992 [66] Forestol *et al.*, 1993 [67]	Sperm	P_4 stimulates Ca^{2+} influx and the acrosome reaction in human sperm	10 s	-	—	P_4 BSA conjugate still active
Finidori-Lepicard *et al.*, 1981 [50]	Amphibian oocyte	P_4 inhibits membrane-bound adenylate cyclase	1–5 s	+	—	—

continued opposite

Table 2—continued

Other membrane effects of progestins which are not rapid

Reference	Organ studied	Effect observed	Latency	Specificity for hormone	Block by protein synthesis inhibitors	Other
Baulieu *et al.*, 1978 [162]	Amphibian oocyte	P_4 induces oocyte maturation	5–8 h	+	Yes	P_4 still effective after conjugation to macromolecule
Ke and Ramirez, 1987 [60]	Rat hypothyalami	P_4 stimulates LHRH release	2–4 h	+	—	P_4 conjugate-BSA still effective
Dluzen and Ramirez, 1991 [62]	Rat corpus striatum	P_4 stimulates Dopamine release	1–2 h	+	—	P_4 conjugate-BSA still effective
Brann *et al.*, 1990 [38]	Brain	3α-5α-THP increases LH release in the rat	3–5 h	+	—	Effect blocked by $GABA_A$ receptor blocker picrotoxin
Vincens *et al.*, 1989 [16]	Brain	3α-5α-THP decreases PRL release form rat pituitary cells *in vitro*	1 h	+	—	Effect blocked by picrotoxin

Table 3. Rapid effects of estrogens

Reference	Organ studied	Effect observed	Latency	Specificity	Blocked by protein synthesis inhibitors	Other
Smith *et al.*, 1987 [56]	Brain	E_2 ↑ Purkinje cells response to glutamate	<1 min	+	—	Effect not blocked by tamoxifen
Garcia-Secura *et al.*, 1987 [75] Garcia-Secura *et al.*, 1989 [77]	Brain	E_2 ↑ neuronal membrane exoendocytotic pits	<1 min	+	—	Effect was blocked by tamoxifen
Nabekura *et al.*, 1986 [74]	Brain	E_2 ↑ K^+ conductance of medial amygdala neurons and decreased neural excitability	2 min	+	No	—
Smith *et al.*, 1989 [164]	Brain	E_2 ↑ Purkinje cell response to movement	15 min	+	—	Effect was long term, lasting up to $1\frac{1}{2}$ h
Kelly *et al.*, 1976 [70]	Brain	E_2 ↓ preoptic-septal	1 min	+	—	—
Kelly *et al.*, 1977 [71, 72]		neurons depending on				
Kelly *et al.*, 1978 [73]		day of cycle administered				
Yagi *et al.*, 1973 [68]	Brain	E_2 ↑ and/or ↓ preoptic neuron unit firing rates	15 min	+	—	—
Dufy *et al.*, 1976 [69]	Brain	E_2 ↑ firing rate of electrically activated hypothalamic units	<5 min	+	—	—
Dufy *et al.*, 1979 [78]	Brain	E_2 induces action potentials in pituitary clonal cells	1 min	+	—	—
Zyzek *et al.*, 1981 [79]	Brain	E_2 ↑ PRL release from pituitary clonal cells	10 min	+	—	—
Pourreau-Schneider *et al.*, 1986 [165]	Breast cancer cells	E_2 ↑ density and length of microvilli on breast cancer cells	1 min	-	—	—
Rambo and Szego, 1983 [166]	Uterus	E_2 rapidly [uvua] microvilli on endometrial cells	30 s	+	—	—
Thompson and Moss 1994 [167]	Brain	E_2 rapidly [uvua] release in the nucleus accumbens	2 min	-	—	—

Table 4. Rapid effects of androgens, glucocorticoids and vitamin D metabolites

Reference	Organ studied	Effect observed	Latency	Specificity	Block by protein synthesis inhibitors	Other
Androgens						
Yamada *et al.*, 1979 [80]	Brain	T increased firing rates of hypothalamic neurons	2–30 s	+	—	—
Teyler *et al.*, 1980 [168]	Brain	T either ↑ or ↓ excitability of hippocampal cells depending on day of female cycle examined	5–10 min	+	—	—
Kubli-Garfiad *et al.*, 1982 [82]	Brain	5β-reduced T metabolites, but not T, inhibited brain electrical activity and inhibited seizure activity induced by convulsant drugs	<1 min	+	—	—
Orsini *et al.*, 1985 [81]	Brain	T ↑ Lateral hypothalamic neuronal activity	<4 s	+	—	—
Koenig *et al.*, 1985 [169]	Heart	T ↑ Ca^{2+} influx endocytosis, ornithine decarboxylase activity and polyamine synthesis in rat heart myocytes	<30–60 s	–	—	Effect blocked by Ca^{2+} chelator and Ca^{2+} antagonists
Kubli-Garfias *et al.*, 1980 [170]	Uterus	3α-5α-reduced androgens potently suppress rat myometrial contractibility	2–5 min	+	—	—
Sacks and Leipheimer, 1988 [171] Meisel *et al.*, [172]	Penis	T ↑ penile striated muscle activity	6–30 min	+	No	—
Glucocorticoids						
Hua and Chen, 1989 [173]	Coeliac ganglion	Cortisol rapidly suppresses spontaneous discharges of neurons	1–2 min	–	—	Cortisol BSA-conjugates still effective
Hall, 1982 [174]	Brain	Glucocorticoids modulate brain activity		(Review)		
French-Mullen *et al.*, 1994 [175]	Brain	Allotetra hydrocorticosterone inhibits calcium channels	2–20 min	+	—	Effect occurs via a pertussis toxin-sensitive G-protein coupled mechanism
Vitamin D metabolites						
Lieberherr *et al.*, 1989 [89]	Small intestine	1,25-dihydroxy-vitamin D_3 (1,25-$(OH)_2$-D_3) rapidly ↑ IP_3 and DAG release in rat enterocytes	<5 min	–	—	—
Bickle *et al.*, 1978 [176]	Small intestine	1,25-$(OH)_2$-D_2 ↑ intestinal calcium transport	—	–	No	—
De Boland and Norman, 1990 [85]	Duodenum	1,25-$(OH)_2$-D_3 ↑ duodendal Ca^{2+} transport	2–20 min	–	—	—
Civitelli *et al.*, 1990 [88]	Osteoblastic cells	1,25-$(OH)_2$-D_3 ↑ Ca^{2+} transport in osteoblast-like cells	<5 s	+	—	—
Barsony and Marx, 1988 [177]	Human skin fibroblasts	1,25$(OH)_2$-D_3 ↑ cGMP in fibroblasts	<1 min	–	—	—
De Boland and Boland, 1987 [86]	Chick skeletal muscle	1,25$(OH)_2$-D_3 ↑ muscle Ca^{2+} uptake	3–15 min	+	No	Effect blocked by Ca^{2+} antagonists
Barsony *et al.*, 1990 [178]	Human skin fibroblasts	1,25$(OH)_2$-D_3 induces rapid reorganization of vitamin D receptors	s to min	+	—	—
Norman *et al.*, 1992 [83]			(Review)			

Table 5. Evidence supporting steroid hormone receptors on plasma membranes

Reference	Organ	Steroid ligand	K_d (m)	Concentration of binding sites per protein	Specificity
Towle and Szego, 1983 [96]	Rat brain	Progesterone	$1\text{-}2\times10^{-8}$	550 fmol/mg	+
		17β-Estradiol	$1\text{-}2\times10^{-8}$	820 fmol/mg	+
		Corticosterone	1×10^{-7}	130 fmol/mg	+
		Testosterone	1×10^{-8}	180 fmol/mg	+
Bression *et al.*, 1986 [179]	Rat brain (anterior pituitary)	17β-Estradiol	0.041×10^{-9}	13.6 fmol/mg	+
Ke and Ramirez, 1990 [97]	Rat brain	Progesterone–BSA–^{125}I	$K_i=2.8\times10^{-8}$	—	+
Tischkau and Ramirez, 1993	Rat brain (anterior pituitary)	Corticosterone	4.4×10^{-8}	31 fmol/mg	+
Koch *et al.*, 1977, 1978 [180, 181]					
Schaeffer *et al.*, 1980 [182]	Rat brain (anterior pituitary)	2-Hydroxyestradiol	4×10^{-10}	13 fmol/mg	+
Suyemitsw and Terayama, 1975 [183]	Rat liver	Cortisol	$1.5\text{–}1.9\times10^{-9}$	3.4–4.6 pmol/mg	+
Pietros and Szego, 1980 [106]	Rat liver	17β-Estradiol	3.5×10^{-9}	526 fmol/mg	+
Berthois *et al.*, 1986 [105]	Breast cancer MCF-7 cells	17β-Estradiol	8×10^{-8}	8000–16000 sites per cell	+
Kostellow *et al.*, 1982 [45]	Rana oocytes	Progesterone	5.1×10^{-7}	70 fmol per oocyte	+
Sadler and Maller, 1982 [46, 47]	*Xenopus* oocyte	R5020	1×10^{-6}	—	+
Blondeau and Baulieu, 1984 [184]	*Xenopus* oocyte	R5020	1.2×10^{-6}	—	+
Tesarik *et al.*, 1992 [99] Blackmore and Lattanzio, 1991 [185]	Human sperm	Progesterone–BSA–Fluorescein Isothiocynate	—	—	-

the principal excitatory transmitter in the brain. Both effects were demonstrated to occur within 3–10 min of steroid treatment. The rapid neuronal response (within minutes) to progesterone would appear to preclude a genomic mechanism [39, 40 for a review]. Furthermore, because the animals were ovariectomized 3–5 weeks prior to the experiment, the intracellular progesterone receptors would be extremely low or absent. This would make the genomic effect even less likely. In a follow-up study, Smith *et al.* [41] found that prior treatment with a protein synthesis inhibitor did not prevent the ability of progesterone to decrease the glutamate response. This finding lends further support to the concept that the effect of progesterone on Purkinje cell responsiveness is exerted through a nongenomic mechanism.

Havens and Rose [42] demonstrated that in Golden hamsters, progesterone was able to produce two neurophysiological effects upon dorsal mid-brain neurons after s.c. injection: (1) it strongly suppressed transsynaptic activation; and (2) it, in most cases, suppressed (in antidromically invaded neurons) the amplitude of the soma-dendritic spike component. Both of these effects occurred rapidly (within 10–20 min) after progesterone injection. Kubli-Garfias *et al.* [43] noted that administration of progesterone metabolites through the carotid artery of cats depressed neuronal activity in mesencephalic tegmentum within 10 s. In this function 5β-reduced progestins were notably more potent than the 5α-reduced metabolites. Progesterone inhibited neuronal firing with significantly longer latencies than 5β-metabolites in the brain. In summary, the above studies demonstrate that progestins can exert rapid depressive type effects on single neurons in different regions of the brain, apparently through non-classical mechanisms.

Effects of progesterone on oocyte maturation. An extensive amount of work has been done on the effect, as well as the mechanism of action, of progesterone on *Xenopus laevis* oocyte maturation. Along these lines, progesterone has been shown to stimulate germinal vesicle breakdown (GVBD) in the Xenopus oocyte when applied to the outer surface of the oocyte but not when injected within the oocyte [44]. The authors, based on this finding, suggested that the effect of progesterone was mediated by an interaction with the plasma membrane. Subsequently, progesterone receptors were identified by several laboratories in the plasma membrane of amphibian oocytes [45–47]. That the effect of progesterone occurrred by interaction with the plasma membrane and not intracellularly was further supported by its effectiveness even when linked to a 20,000 molecular weight polymer to prevent entry into the cell [48]. The rapid membrane-mediated action of progesterone appears to be due to its regulation of calcium transport [49] and inhibition of adenylate cyclase mediated through the guanine nucleotide regulatory protein [50, 51].

Other non-classical effects of progesterone. Progesterone and its metabolites have also been demonstrated to be potent inhibitors of uterine smooth muscle contractility [52–56]. Recent work done in our laboratory [52] has demonstrated that this inhibition of contractility occurs with progesterone and 5β-dihydroprogesterone which bind to intracellular progesterone receptors as well as 3α-hydroxy-5α-pregnan-3,20-dione (3α,5α-THP) and 3β-hydroxy-5β-pregnane-3,20-dione (3β,5β-THP) which do not bind to intracellular progesterone receptors [52]. The non-classical action of 3α,5α-THP and 3β,5β-THP is supported by the demonstration that their action is not blocked by the progesterone receptor antagonist RU486 but is blocked by the $GABA_A$ receptor antagonist picrotoxin. Progesterone metabolites have also been shown to inhibit smooth muscle contractility in tissues other than the uterus such as rat ileum and jejunum and dog coronary artery [57]. Other non-classical effects of progesterone such as hypothalamic LHRH release and activation of the acrosomal reaction in sperm are described in the next section.

Evidence of progestin action without entry into the cell. Progesterone has been demonstrated to bring about the rapid release of LHRH from the hypothalamus *in vitro* and *in vivo* by a number of investigators [58, 59 for review]. The studies of Ramirez and coworkers [60, 61] have shown that progesterone conjugated to bovine serum albumin (BSA), an alteration which renders it incapable of entering the cell, is still capable of stimulating LHRH release from hypothalami *in vitro*. Further work by Ramirez and coworkers has demonstrated that progesterone can also stimulate dopamine release from the corpus striatum of ovariectomized estrogen-primed rats through a similar membrane-mediated mechanism as evidenced by steroid–BSA conjugate studies [62]. This effect was reported to be stereospecific, as deoxycorticosterone conjugated to BSA could not effect a similar release of dopamine. It has also been shown that the effect of progesterone on desensitization of the α_1-adrenergic receptor augmentation of c-AMP formation in the rat hypothalamus [63] can also be exerted with progeterone–BSA conjugate. This is also true for the effect of progesterone in the maturation of *Xenopus laevis* oocyte while linked to a 20,000 molecular weight polyethylene oxide polymer to prevent entry into the cell [48 for review]. Perhaps the best studied effect is the ability of progesterone and 17α-hydroxy progesterone in bringing about an increase in cytosolic free calcium due to calcium influx from extracellular calcium to induce the acrosome reaction [64–67]. This interaction of progesterone with the sperm is a membrane effect because it occurs even when the entry of progesterone into the cell is blocked by conjugating it with bovine serum albumin [65]. The above examples provide strong evidence for non-classical and non-genomic membrane effects of steroid hormones.

Estrogens

Rapid membrane effects of estrogens have been noted in a number of different sites within the brain and are summarized in Table 3. Yagi [68] noted that preoptic and hypothalamic units from ovariectomized rats responded to an i.v. injection of estradiol in two ways; some units had a decreased firing rate ranging up to 150 min, whereas other units responded with a transitory increase in firing rates. The remaining units were unresponsive to estradiol. The latency of the neuron response to estradiol was 15 min. Dufy *et al.*, [69] confirmed and extended these results in ovariectomized rabbits, reaching an identical conclusion that there exist two types of hypothalamic neurons sensitive to estrogen. The neurons respond very rapidly to systemic estrogen injection, and the effects of estradiol on hypothalamic neurons may be related to the positive and negative feedback capability of estrogens on gonadotropin secretion.

Rapid effects of estrogens on neuronal activity have also been reported by Kelly *et al.* to occur *in vivo* after electrophoretic administration of estrogen to medial preoptic neurons from intact female rats, and *in vitro* after application to arcuate, hippocampal and parvicellular ventromedial hypothalamic neurons [70–73]. Nabekura *et al.* [74] also demonstrated that estradiol caused a hypopolarization and concurrent decreased excitability of rat medial amygdala neurons upon superfusion of slices *in vitro*. The effect of estradiol was dose dependent, apparent within minutes, and not affected by prior treatment with cycloheximide or actinomycin D, which inhibit protein synthesis. Based on these findings, the authors suggested that the effects of estradiol were due to interaction with membrane receptor sites with an associated didactic regulation of membrane ionic conductance.

Smith *et al.* [39] recently examined the effect of estradiol on the extrahypothalamic CNS. In their study, they found that either iontophoretic or i.v. administration of 17β-estradiol resulted in a significant increase in cerebellum Purkinje cell excitatory responsiveness to glutamate. The effect occurred as early as 1 min after iontophoretic application and 10 min after i.v. injection. In all cases, recovery to control basal level response was not observed by 2 h post estradiol. The effects did not appear to involve the classical intracellular estradiol receptor based on two findings: (1) the rapidity of the effect; and (2) prior administration of the classical estrogen receptor antagonist, tamoxifen did not prevent any of the rapid effects of estradiol. These findings also point to a nongenomic mechanism of action for effects of estradiol within the CNS.

Estrogens can also exert rapid effects upon neuronal membrane ultrastructure in the brain. Naftolin and coworkers [75, 76] have shown that perifusion of physiological levels of 17β-estradiol, but not 17α-estradiol, causes an increase in arcuate neuronal membrane

exo-endocytotic pits within 1 min of perifusion of brain slices. This effect was dose related and could be blocked with the antiestrogen tamoxifen. Similar results were observed in developing rat cerebrocortical neurons in culture [77]. The authors suggest that the rapid increase in exo-endocytotic pits in neuronal membranes could be related to an increased endocytotic activity, which may reflect the preferential internalization of some membrane proteins induced by estradiol. This may translate into a mechanism for remodeling synaptic contacts in adult rats, as well as a mechanism for sexual differentiation of developing synaptic connections in young animals. The extreme rapidity of the effect clearly appears to preclude a genomic mechanism.

Finally, rapid effects of estradiol at the level of the anterior pituitary have been reported by Dufy *et al.* [78], in which 17β-estradiol application to GH3/B6 clonal pituitary cells in culture induced calcium-dependent action potentials within 1–2 min (lasting 5–6 min). Subsequent studies by Zyzek *et al.* [79] demonstrated that this increase in electrical activity in the pituitary cells was paralleled by an acute release of prolactin. Both effects were dose dependent and stereospecific, since 17α-estradiol had no effect on either parameter.

In summary, estrogens, similar to progestins, exert many rapid effects upon neuron excitability in the CNS which appear to be mediated by nonclassical, nongenomic mechanisms.

Androgens, glucocorticoids and vitamin D metabolites

The rapid and non-classical actions of androgens, glucocorticoids, and vitamin D metabolites are summarized in Table 4.

Androgens. Yamada [80], using adult male rats, found that electrophoretic application of testosterone to individual neurons in the anterior hypothalamus and septal nucleus resulted in an increase in their firing rates with a latency of response from 2–30 s. Using microiontophoretic application, Orsini *et al.* [81] found that testosterone increased within seconds the spike frequency of more than half of the neurons tested in the lateral hypothalamus in the male rat. This was thought to be specific since cholesterol did not cause any activation. The authors suggested a nongenomic effect since it occurred within seconds. Of the remaining neurons, some did not respond to testosterone (or any other steroid), while others were actually depressed by testosterone. In effect, there appear to be subpopulations of neurons which can respond differently to a given steroid. This suggests that the effect one observes is dependent on the neuronal population one chooses.

Kubli-Garfias *et al.* [82] found that testosterone metabolites such as androsterone and androstanediol, but not testosterone or 5α-dihydrotestosterone, suppressed brain activity in the cat within 1 min of i.v. injection. Garcia-Segura *et al.* [77] found that testosterone had about 50% of the potency of estradiol (see previous section) in exo-endocytotic pit density in the arcuate nucleus. Thus, testosterone, like estradiol, can also rapidly alter neuronal membrane ultrastructure.

Glucocorticoids. Work by Hua and Chen [173] demonstrated that glucocorticoids can hyperpolarize the membrane potential of hippocampal neurons in brain slice preparations and of coeliac ganglion neurons. The effect appears to be achieved through a non-genomic mechanism since bovine albumin–glucocorticoid conjugates (which cannot enter the cell) exhibit the same effect, and since the inhibition of neuron activity occurs within 2 min. Many other reports of glucocorticoid regulation of neuron activity have appeared in the literature. These have been summarized in previous reviews on the actions of glucocorticoids [69].

Vitamin D metabolites. Within the last decade an abundance of papers have appeared in the literature describing rapid, nongenomic effects of the active steroid hormone metabolite of vitamin D, 1,25-dihydroxy-vitamin D_3. This area was recently reviewed by Norman and coworkers [83, 84]. A major rapid effect of 1,25-dihydroxy-vitamin D_3 which occurs in seconds to minutes is the stimulation of calcium levels which has been reported in chick duodenum [85], chick muscle cells [86], mammary tissue [87] and osteoblasts [88]. In most of these tissues increased phospholipid metabolism has also been reported [88, 89]. Evidence has also accumulated suggesting that 1,25-dihydroxy-vitamin D_3 stimulates protein kinase activity [90] which in turns activates voltage sensitive calcium channels.

MECHANISMS OF NON-CLASSICAL ACTIONS OF STEROID HORMONES

Figure 1 illustrates the possible mechanisms underlying rapid non-classical actions of steroid hormones. Steroid hormones could produce rapid membrane effects through either: (1) a non-specific effect on membrane fluidity; (2) binding to specific steroid hormone receptors present in the cell membrane; or (3) binding to and modulation of neurotransmitter membrane receptors such as the $GABA_A$ receptor. The biological effect of steroids could be due to one of the above mechanisms or to a combination of more than one. The evidence for each mechanism is discussed below.

Rapid effects of steroid hormones—due to changes in membrane fluidity?

In 1961, Willmer [91] proposed that steroids could be inserted vertically in the phospholipid bilayers of membranes which would alter the fluidity of the membrane and block membrane ionic channels. Estradiol and progesterone have been reported to influence membrane fluidity in a variety of tissues including breast cancer cells [92], vaginal epithelial cells [93] and human spermatozoa [94]; both an increase and decrease in fluidity were reported in these studies. 1,25-Dihydroxy-vitamin D has been reported to increase calcium uptake by chick intestinal brush border

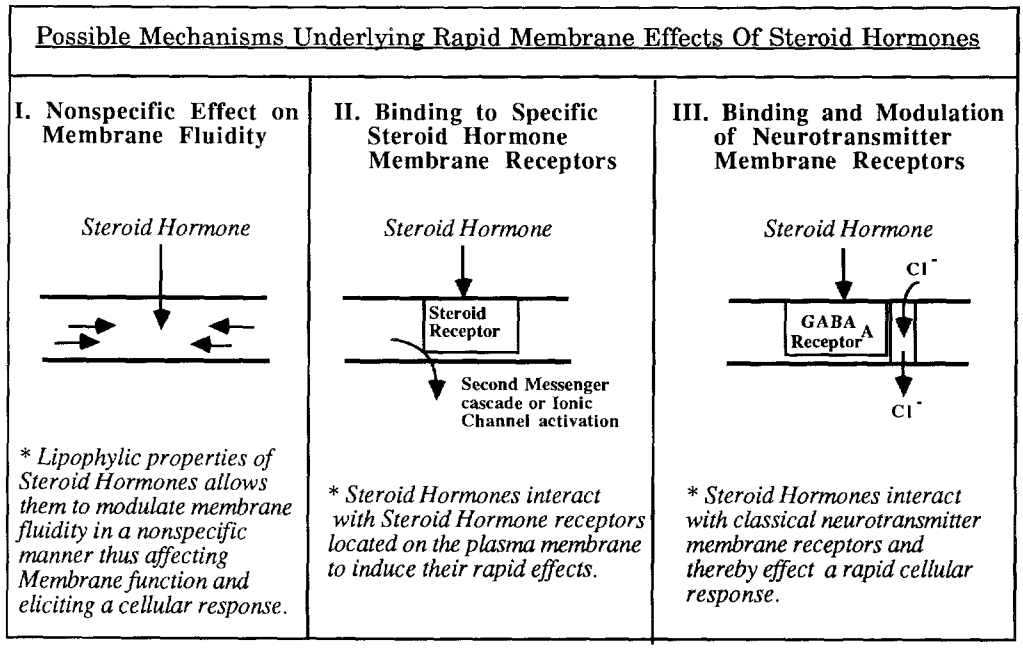

Fig. 1. Possible mechanisms underlying rapid membrane effects of steroid hormones.

membrane without affecting membrane fluidity [95]. Thus, while steroids may indeed alter membrane fluidity, it is unclear to what degree, if any, this effect would contribute to the overall biological action of the steroid. Furthermore, it is hard to reconcile how steroid hormones could exert tissue specific effects if regulation of cell membrane fluidity was a major mechanism of membrane effects of steroids since all membranes would be expected to be susceptible to such non-specific fluidity changes. The high specificity of many of the steroid effects is also difficult to reconcile with the major mechanism being a non-specific pertubation of membrane fluidity. Because of these questions, recent attention has turned to the search for membrane receptors for steroid hormones.

Steroid hormone receptors on plasma membranes as mediators of rapid effects of steroid hormones

Evidence supporting the presence of membrane receptors for steroid hormones has accumulated in the literature over the past 15 years and these are summarized in Table 5. Both neural and non-neural membrane steroid receptors have been reported, and in most cases the steroids bind to the membrane receptors with modest affinity and with specificity. Towle and Sze first described membrane binding sites for steroids in the brain in 1983 [96]. Ramirez and coworkers [97, 98] have recently reported that radioactive tagged progesterone conjugated to BSA at the 11-position binds to synaptosomal membrane preparations from the medial basal hypothalamus/preoptic area and the corpus striatum. This binding can be displaced by competition with unlabelled progesterone–BSA conjugates at the 11- or 3-position.

The membrane binding by progesterone-11-BSA appears to be regulated by estradiol as specific binding is decreased by 80% 14 days after ovariectomy and restored by estradiol replacement [98]. Progesterone has also been reported to bind to human spermatozoa and this effect appears to be important for the induction of calcium influx and the acrosomal reaction [99, 100]. Electron microscope autoradiographic studies have also revealed plasma membrane binding sites for estradiol in human spermatozoa although the importance and function of such sites remains to be determined [101, 102]. Other studies have reported membrane binding sites for estrogens in breast cancer cells [103–105], liver [106] and uterus [107, 108]. Thus, there is ample evidence in the literature for steroid binding sites in the plasma membrane of a variety of tissues which may play a role in mediating many of the rapid non-genomic effects of steroids discussed previously.

Steroid hormone regulation of $GABA_A$ receptors on plasma membranes

Recent studies have provided evidence that steroids, particularly 3α-hydroxy ring-A reduced steroids, are potent regulators of the $GABA_A$ receptor in the brain [109–112 and 113, 114 for review].

The $GABA_A$ receptor is a membrane-bound protein complex which mediates the action of GABA—the major inhibitory neurotransmitters in the brain. The $GABA_A$ receptor is composed of multiple homologous membrane-spanning subunits ($\alpha, \beta, \gamma, \delta$) that form a chloride channel [113, for review]. GABA produces its action by increasing chloride channel conductance.

The first evidence that steroids could regulate the $GABA_A$ receptor were derived from studies demonstrat-

ing that the steroidal anesthetic, alfaxalone, potentiated GABA effects and enhanced the binding of GABA ligands *in vitro* [111]. Subsequent studies by Simmonds *et al.* in 1984 [110] extended these findings to the endogenous steroid androsterone by demonstrating that it also potently potentiated GABA effects and enhanced the binding of GABA ligands *in vitro*. Other studies demonstrated that the progesterone metabolite, 3α,5α-THP, and the deoxycorticosterone metabolite, 3α,5α-tetrohydrodeoxycorticosterone (THDOC), exhibit nanomolar potency in stimulating $GABA_A$ receptor mediated chloride ion flux [109, 112, 113]. These endogenous steroids are approximately 10 times more potent than benzodiazepines and 200 times more potent than pentobarbital in potentiating GABA-mediated chloride uptake in rat cerebral cortical synaptoneurosomes [109, 112].

Other steroids such as pregnenolone sulfate and dehydroepiandrosterone sulfate have been shown to act as noncompetitive antagonists of the $GABA_A$ receptor [115, 116]. The physiological significance of steroid regulation of $GABA_A$ receptors is far-reaching as such interactions could have importance in stress, cycle related seizures, premenstrual syndrome anxiety, "post-partum blues", memory and cognition, and depression to name only a few [113, for review]. $GABA_A$ receptors can also modulate the effect of progesterone on gonadotropin secretion via the 3α,5α-THP metabolite [38]. The levels of 3α-hydroxysteroids in the brain can be as high as 10–30 nM after stress [117] and up to 100 nm in the plasma in the third trimester of pregnancy [117]. The high plasma levels of 3α,5α-THP during pregnancy may be important for inhibition of uterine contractility as suggested previously by us [52] and others [118].

Steroid regulation of other neurotransmitter receptors on the plasma membrane

It is also possible that steroids could act to regulate other neurotransmitter receptor complexes in the brain. In support of this possibility pregnenolone sulfate, which is in high concentrations in the brain and appears to be produced by the brain [119], has recently been reported to augment *N*-methyl-D-aspartate receptor mediated increasing intracellular calcium in cultured rat hippocampal neurons [120].

Activation of steroid hormone action in the absence of the ligand

Phosphorylation is an important step in the gene activation by steroid–receptor complexes and dibutryl cAMP can elicit mating behavior in female rats similar to the effects of progesterone [121]. Progesterone induced lordosis behavior is also facilitated by phosphodiesterase inhibitors [122]. 8-Bromo-cAMP has been shown to mediate progesterone receptor-dependent transcription in the absence of progesterone [123]. The expression of progesterone receptor-dependent transcription is also stimulated by okadiac acid, an inhibitor of protein phosphatases [123].

The activation of steroid receptor-mediated transcription by a variety of growth factors such as EGF [124, 125] and IGF-1 [126] has been demonstrated by the use of antiestrogens which were able to antagonize EGF-stimulated uterine growth and EGF and IGF-1 stimulated estrogen mediated transcription using estrogen receptor gene constructs in transfected cells. Furthermore, when progesterone receptor-negative monkey kidney cells were cotransfected with a chicken progesterone receptor expression vector and a reporter plasmid, dopamine increased the progesterone receptor-mediated transcription to that comparable with progesterone induced transcription [127]. These are a few examples of multiple pathways of steroid hormone receptor activation. The physiological significance of these pathways has not been established.

ROLE OF STEROID HORMONES IN THE CLASSICAL MECHANISM—THE LIGAND INSERTION HYPOTHESIS

As stated earlier, it is not the intention of the authors to review the classical genomic mechanism of action of steroid hormones. An excellent review on this subject by Tsai and O'Malley has been published recently which provides a thorough discussion including the role of phosphorylation, heat shock proteins, receptor homo and heterodimers, chromatin structure and the ever increasing importance of nuclear transcription factors [128]. The importance of transcription factors in explaining agonist and antagonist properties of compounds is also briefly discussed in this review. As yet unanswered questions are the fate of the steroid hormone after the occupied receptor binds to DNA, the relatively poor correlation between receptor binding and biological activity and enhancement of the biological activity of the truncated receptor by the steroid in the absence of a steroid binding domain. Thus, a new examination of steroid–DNA interaction is warranted.

The hypothesis that genes can be regulated by the direct interaction of small molecules with DNA in concert with chromosomal proteins was originally put forth in 1977 [129]. The hypothesis was based upon the discovery of stereochemical fit of hormonal steroids and other important natural molecules between base pairs in partially unwound double stranded DNA. The discoveries were prompted by a search for the raison d'etre for the structures of small natural product molecules. The underlying rationale for these studies was that although enzymes and receptors proteins were directly responsible for the synthesis and biological activity of molecular structures in nature, this structural information was ultimately present in the genes. Molecular models of molecules such as estradiol exhibited remarkable physicochemical complementarity with DNA in that each functional group, e.g. the 3 and 17β-hydroxyl

groups, formed stereospecific hydrogen bonds to phosphate groups on adjacent DNA strands and the hydrophobic core of the steroid conformed to the size and shape of the hydrophobic space between base pairs. That these findings were not fortuitous was supported by the lack of complementarity of non-naturally occurring steroid enantiomers (mirror images) and DNA.

The original observations of complementary stereochemical relationships between steroids and nucleic acids which resulted from studies with simple space filling models were later confirmed using Kendrew skeletal models as well as silastic polymer models constructed from computer generated X-ray coordinates of DNA [130, and references therein]. The latter revealed differences in the shapes of the spaces or "cavities" between base pairs resulting from different sequences in partially unwound DNA. The cavities accommodated different molecules, e.g. steroid/thyroid hormones fit best in the cavity derived from the sequence 5'-dTdG-3'·5'-dCdA-3' whereas certain plant hormones (gibberellic acid) fit best into the sequence 5'-dTdA-3'·5'-dTdA-3'. The cavities also revealed structure–activity relationships [130–132] in which the pattern of hydrogen bonding linkages and the degree of fit of various ligands into the cavities correlated with degree of hormonal activity. Studies using DNA cavities further suggested that as steroid precursors and cholesterol underwent biosynthetic steps to form natural steroid hormones, their fit in DNA increased progressively [133].

Many of the original observations of stereochemical complementarity of small molecule and nucleic acids were confirmed by computer modeling [133–141]. Computer modeling provides graphics to examine highly reliable structures derived from various spectroscopic methods and X-ray crystallography as well rigorous energy calculations which can measure interactions among molecules with considerable precision. A summary of the results include the following:

(1) Ligands in the steroid superfamily of hormones fit between base pairs and form unique stereospecific donor–acceptor linkages to the DNA. All of the ligands with the exception of retinoic acid form linkages to adjacent DNA backbones. Energy calculations demonstrated remarkable van der Waals and electrostatic interactions reflecting the steric and hydrogen bonding complementarity of the ligands with DNA.
(2) Structural alterations in the natural hormones structures generally resulted in a poor fit into DNA as measured by energy calculations. For example, in the case of steroids these included alterations in the chirality of the natural steroid, the cyclopentanophenanthrene pattern, and/or the locations of hydrogen bonding functional groups.
(3) Within a given class of hormones, the degree of fit of candidate ligands into DNA as measured by energy calculations correlated closely with degree of hormonal activity.
(4) Intermediates in the metabolic pathways to the hormonal ligands generally resulted in increasing fit into DNA measured by energy calculations. The natural hormones possessed the lowest energy of interaction (best fit) with DNA. Intermediates along the metabolic deactivation pathways of the hormones reflected decreasing fit into DNA. Interestingly, similar results were obtained with the plant hormone gibberellic acid.
(5) Molecules which had unfavorable interactions with DNA, e.g. stressed various sites as measured by energy calculations frequently had adverse side effects.

Recently, "pharmacophores" or three dimensional blueprints for biological activity were discovered using the above approach [142]. The pharmacophores were created by making a composite map of all active compounds based upon the orientation of fit of the structures into DNA. The composite structure was then used prospectively to correlate structure with activity. An example of the creation of a pharmacophore is depicted in Fig. 2 for estrogens. As shown in Fig. 3, a good correlation can be demonstrated between hormonal activity for various estrogen analogs and fit to the estrogen pharmacophore. The discovery of such pharmacophores has obvious implications in the development of new hormonal agonists and antagonists.

While the main focus of these studies was an attempt to understand why only certain molecules and not others exist in nature, the ability to develop predictive pharmacophores based upon the stereochemistry of DNA raises obvious questions about the mechanism(s) of hormone action. In previous studies [140], it was noted that fit of various estrogens into DNA measured by energy calculations correlated well with estrogenic activity whereas receptor binding for the same set of molecules exhibited poor correlation with estrogenic activity. The lack of correlation between receptor binding and hormonal activity thus appears to preclude the ability to develop a pharmacophore based upon the active binding site in the estrogen receptor. This is further supported by the lack of correlation between fit to the existing pharmacophore and receptor binding [Fig. 3]. Taken as a whole, these findings go well beyond an understanding of how various hormone structures evolved and provide strong circumstantial evidence that ligands in the steroid family may be inserted into DNA during their mode of action [129, 130, 138, 140]. Such a hypothesis is also consistent with the observations of Sluyser [144], X-ray crystallographic studies of Duax [145] and recent reports demonstrating that various steroid ligands can come in direct contact with DNA. With regard to the latter, *in vivo* experiments by Liehr [146] have found covalent linkages between estrogens and DNA. The recently reported genotoxic

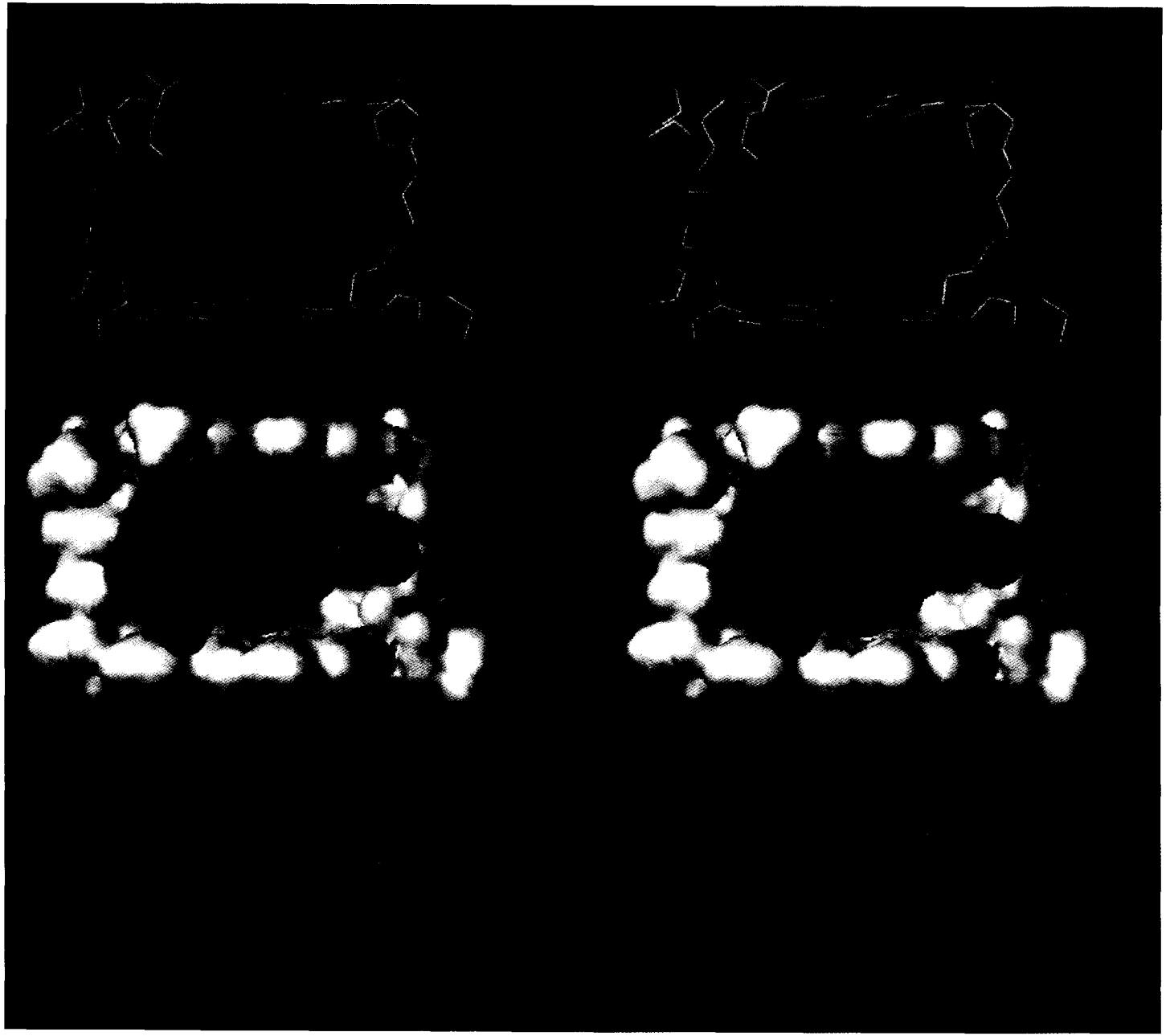

Fig. 2. Creation of the estrogen pharmacophore using computer modeling viewed in stereo (crossed eyes). Top: skeletal models of active estrogens (magenta) overlapped based upon optimum fit into DNA (yellow) viewed from the major groove; Middle: space filling model of the above showing the van der Waals surfaces of the DNA (yellow) with the composite surface of active estrogens (magenta); Bottom: the estrogen pharmacophore (magenta) resulting from the removal of the composite from the DNA.

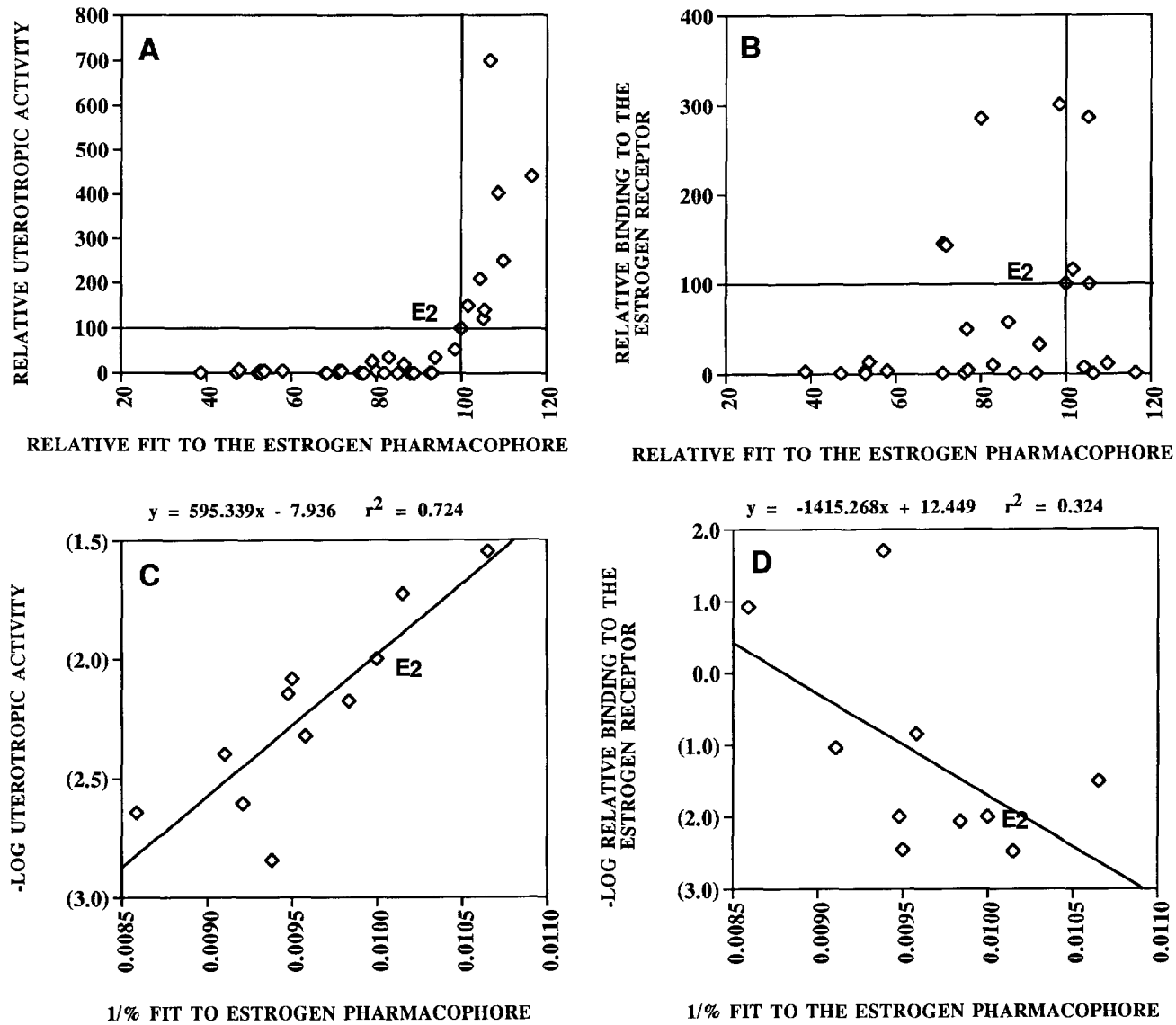

Fig. 3. Relative fit to the estrogen pharmacophore of various compounds correlated with varying estrogenic (uterotropic) activity (A) and binding to the estrogen receptor (B). All data were normalized to the values for estradiol (E_2). Statistical correlations for the ten most active estrogens are shown in panels (C) and (D), respectively. Measurements of fit were performed by assessing the relative differences in volumes between each compound and the pharmacophore and the electrostatic interactions between hydroxyl groups and an average point charge at the position of a phosphate oxygens originally used to orient the ligands during creation of the pharmacophore (Fig. 2). The values for uterotropic activity and estrogen receptor binding were obtained from literature sources [18–23, 132, 186–196].

damage and mammary tumor promoter activity caused by the estrogen metabolite 16α-hydroxyesterone [147] could also be explained by a direct interaction of the ligand with DNA. Similarly, studies by DeSombre *et al.* [148] suggest a close contact *in vivo* between DNA and estrogens labeled with radioactive isotopes of iodine. It is particularly intriguing that almost 40 years ago direct interactions between estrogens and nucleic acid components were studied by Munck [143, 149] prior to the discovery of receptor proteins.

In summary, the insertion of the ligand into DNA mediated in part by the receptor protein and perhaps other transcription factors would provide an answer to how the concentration and stereospecificity of various ligands are transmitted to hormonally responsive genes. Given that hormonal ligands have been shown to bind only weakly to naked DNA [150, and references therein], receptor proteins upon binding to hormone responsive elements may provide the precise environment necessary for the ligand to act. Such a mechanism would be consistent with the observations of DNA bending caused by steroid receptors [151] including the recent finding of Rainish *et al.* [152] demonstrating estrogen receptor induced DNA bending in the direction of the major groove. Modeling demonstrates that the direction of the insertion of each of the hormones would be from the major groove. Interestingly, Ding *et al.* [153] have also

recently demonstrated that certain chromosomal proteins can bend DNA thereby leading to the creation of preferred sites in DNA for the binding of certain small molecular weight DNA-reactive drugs. If these observations are also applicable to steroid hormone action, they would support the notion of Beekman *et al.* [154] that the ultimate role of the hormone lies in events after receptor–DNA binding, possibly in transcriptional activation. They also support the hypothesis of Fritsch *et al.* [155] that DNA allosterically modulates the steroid binding domain of the estrogen receptor and the conformational change is necessary for full transcriptional activity. Such allosteric changes may result in the release of the hormone into the site in DNA. Thus, the receptor would be largely responsible for the specificity of the response (e.g. estrogenic vs androgenic) whereas fit into DNA would govern the degree of hormonal response. In this manner, DNA would function as a secondary receptor with the ligand acting as a transcription factor. Future experimental approaches to test the insertion hypothesis will likely require techniques which can measure rapid, reversible interactions of the ligands with DNA in the presence of receptor proteins.

Finally, receptor mediated stereochemical insertion of steroid ligands into DNA may have other implications for how hormones regulate genes. It is well established that receptors in the steroid/thyroid superfamily contain regions that bind to specific DNA sequences (hormone response elements) thereby providing specificity in the ability of various ligands to regulate a given gene or set of genes [128]. The hormone response elements which are required for proper functioning of each receptor are highly conserved and fall into two major classes with progesterone, testosterone, mineralocorticoid and glucocorticoid receptors in one class and estrogens, vitamin D, thyroid and retinoic acid receptors in a second class. While the consensus hormone responsive elements and the amino acids of the domains of the receptors which bind to these elements are different between the classes, they are almost identical within each class. Why are there two classes of hormone response elements that modulate the activity of hormones with different structures and biological activities? Although a precise explanation is unknown, nevertheless, the answer must be related to the structure of the ligand. Members of the first class are all steroids with a common proton acceptor carbonyl group at the 3 position. Despite having widely different structures, ligands in the second class have a common proton donor functional group (i.e. hydroxyls including phenols or carboxylic acid groups). It is particularly striking that these ligands also fall into the same classes based upon the capacity of these functional groups to link stereospecifically to DNA upon insertion into the cavity [140]. These modeling observations which were reported independently and in many cases prior to the biological evidence for the two receptor classes cannot be coincidental [129–135, 138, 140 and references therein]. Taken as a whole, the relationships between the hormone response elements, the DNA binding domain of the receptor and the structure of the individual ligands can be explained in the following manner. The structural properties of the receptor upon binding to the hormone response elements in concert with other transcription factors impart the precise conformation and physicochemical properties of the DNA cavity (degree of unwinding, donor/acceptor properties of phosphate groups etc.) to facilitate insertion of the ligand. Receptors fall into two groups due to the similarity in the stereochemistry of the insertion of the ligands into the cavity created by the receptor–DNA interaction. Given that the cavity which accommodates all of the ligands, i.e. 5'-dTdG-3'·5'-dCdA-3', is invariant in all of the consensus hormone response elements, this sequence could be one of the sites where the hormones are inserted. Thus, the structure of ligand, the protein receptor and the regulatory sites in DNA are intimately connected. Further evidence that these observations cannot be fortuitous is provided by the finding that alterations of the structure of the DNA to configurations which do not occur naturally (e.g. ent-DNA, homo-DNA) abolish stereochemical insertion of any of the ligands into the DNA cavities [138, 140]. The insertion hypothesis also has important implications for how the regulatory genes, receptors and ligand structures evolved.

CONCLUSIONS AND PHYSIOLOGICAL SIGNIFICANCE

Figure 4 illustrates the many potential classical and nonclassical modes of action whereby steroid hormones could act to control cell responses. Mechanism #1, which represents the classical mode of action of steroid hormones, holds that steroids freely traverse the membrane to interact with intracellular steroid hormone receptors which in turn interact with DNA to induce transcription and initiate protein synthesis. The newly formed proteins then propagate the diverse actions of the steroid hormone on the cell. Mechanism #6 suggests a possible addendum to the classical mechanism in that it proposes that the steroid hormone ligand may interact with the receptor and DNA to ensure maximal transcriptional activity/efficiency and that the ligand is not just a casual observer to this event but actually aids and facilitates the event. In addition to the classical mechanism of action of steroid hormones, nonclassical mechanisms of steroid hormone action have been proposed (Mechanisms #2, 4 and 5) to explain rapid effects of steroids. These rapid effects of steroid hormones, which occur in seconds to minutes, appear to be mediated at the plasma membrane level as: (1) their rapidity would appear to preclude a genomic effect; (2) the rapid effect of the steroid hormone occurs even if the hormone is conjugated covalently to a large polymer and not permitted to enter the cell; (3) the rapid effects of the steroid hormones are not blocked by protein synthesis

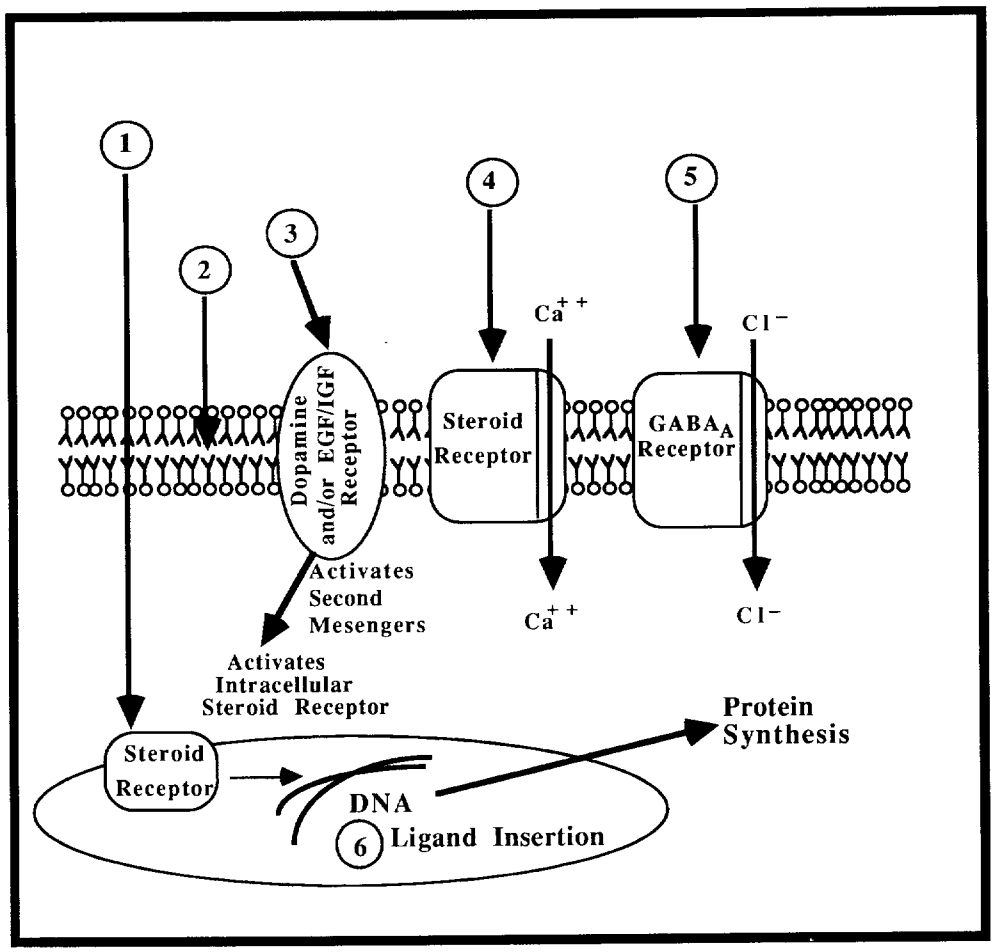

Fig. 4. Classical and non-classical modes of action of steroid hormones.

inhibitors; and (4) many of the rapid effects of steroid hormones are produced by hormones which do not bind the classical intracellular steroid hormone receptor. Steroid hormones could exert their rapid effects via a nonspecific effect to alter membrane fluidity as suggested by some investigators previously (Mechanism #2). However, this explanation fails to explain tissue and steroid specificity of the rapid effects of steroid hormones. Alternatively, steroid hormones could exert rapid effects via direct interaction with steroid hormone receptors located on the plasma membrane. A number of studies have provided evidence supporting the presence of such membrane receptors for a variety of steroid hormones in a variety of tissues (Table 5 and references therein). This mechanism would account for tissue and steroid specificity of the purported rapid effects. Metabolites of progesterone and deoxycorticosterone provide another tier to the diverse modes of action of steroid hormones based on their unique ability to regulate, at nanomolar potency, the activity of the $GABA_A$ receptor. Interaction of steroids with the $GABA_A$ receptor in the brain could have widespread physiological implications. For instance, elevation of steroids in the blood which regulate $GABA_A$ receptor activity has been shown to have a significant correlation with increased fatigue, confusion and decreased immediate recall [27]. Furthermore, progesterone metabolite levels were found to be attenuated in epileptic women as compared to a control group [38]. It has also been suggested that changes in $GABA_A$ receptor-active steroid levels during the cycle could account for the symptoms of anxiety and irritability during the premenstrual period as well as "postpartum depression" [117].

Other possible novel mechanisms of steroid hormone action have been derived from studies *in vitro* which have demonstrated that activation of second messenger systems directly, in the absence of the steroid hormone can lead to activation of steroid hormone receptor-mediated cellular responses [121–128]. This raises the possibility that biological activators other than the steroid could drive steroid hormone receptor mediated actions. Whether these admittedly *in vitro* findings actually occur *in vivo*, and to what degree, remains to be determined.

In conclusion, these proposed potential nonclassical mechanisms of action of steroid hormones should be viewed not as a challenge to the classical mechanism of

action but rather as an additional complementing layer of diversity. It is perhaps not surprising at all that such diverse modes of action for steroid hormones may exist as they could endow the individual with the ability to mount rapid and/or prolonged responses depending upon the specific stimuli and/or challenge presented. The integration of these diverse modes of action of steroid hormones ultimately allows for a fine-tuned multidimensional regulation of cellular, tissue and organ responses.

REFERENCES

1. Talalay P., Hurlock B. and Williams-Ashman H. G.: On a coenzymatic function of estradiol-17β. *Proc. Natn. Acad. Sci. U.S.A.* **44** (1958) 862–884.
2. Bush I. E. and Mahesh V. B.: Metabolism of 11-oxygenated steroids-2.2-methyl steroids. *Biochem. J.* **71** (1959) 718–742.
3. Jensen E. V. and Jacobson H. I.: Basic guides to the mechanism of estrogen action. *Recent Progr. Horm. Res.* **18** (1962) 387–414.
4. Pearlman W. H. and Pearlman M. R. J.: The metabolism *in vivo* of Δ^4-androstene-3,17-dione-7-^{3}H: its localization in the ventral prostate and other tissues of the rat. *J. Biol. Chem.* **236** (1961) 1321–1327.
5. Harding B. W. and Samuels L. T.: The uptake and subcellular distribution of C^{14}-labelled steroid in the rat ventral prostate following *in vivo* administration of testosterone-4C^{14}. *Endocrinology* **70** (1962) 109–118.
6. Mahesh V. B. and Greenblatt V. B.: Steroid secretions of the normal and polycystic ovary. *Rec. Progr. Horm. Res.* **20** (1964) 341–394.
7. Noteboom W. D. and Gorski J.: Stereospecific binding of estrogens in the rat uterus. *Archs Biochem. Biophys.* **111** (1965) 559–568.
8. Toft D. and Gorski J. A.: A receptor molecule for estrogens: isolation from the rat uterus and preliminary characterization. *Proc. Natn. Acad. Sci. U.S.A.* **55** (1966) 574–581.
9. Gorski J., Toft D., Shyamala G., Smith D. and Notides A.: Hormone receptors: studies on the interaction of estrogens with the uterus. *Rec. Progr. Horm. Res.* **24** (1968) 45–80.
10. Jensen E. V., Suzuki T., Kawashima T., Stumpf W. E., Jungblut P. W. and DeSombre E. R.: A two step mechanism for the interaction of estradiol with rat uterus. *Proc. Natn. Acad. Sci. U.S.A.* **59** (1968) 632–638.
11. Jensen E. V. and Desombre E. R.: Estrogen-receptor interaction: estrogenic hormones effect transformation of specific receptor proteins to a biologically active form. *Science* **182** (1973) 126–134.
12. Grody W. W., Schrader W. T. and O'Malley B. W.: Activation, transformation, and subunit structure of steroid hormone receptors. *Endocr. Rev.* **3** (1982) 141–163.
13. Savouret J. F., Misrahi M., Loosfelt H., Atger M., Bailly A., Perrot-Applanat M. T., Vu Hai M. T., Guiochon-Mantel A., Jolivet A., Lorenzo F., Logeat F., Pichon M. F., Bouchard P. and Milgrom E.: Molecular and cellular biology of mammalian progesterone receptors. *Rec. Progr. Horm. Res.* **45** (1989) 65–118.
14. Murdoch F. E. and Gorski J.: The role of ligand in estrogen receptor regulation of gene expression. *Molec. Cell. Endocr.* **78** (1991) C103–C108.
15. O'Malley B. W., Tsai S. Y., Bagchi M., Weigel N. L., Schrader W. T. and Tsai M.-J.: Molecular mechanism of action of a steroid hormone receptor. *Rec. Progr. Horm. Res.* **47** (1991) 1–26.
16. Rao G. S.: Mode of entry of steroid and thyroid hormones into cells. *Molec. Cell. Endocr.* **21** (1981) 97–108.
17. Tsai M. J. and O'Malley B. W.: *Mechanism of Steroid Hormone Regulation of Gene Transcription.* CRC Press, Boca Raton, FL (1994).
18. Raynaud J.-P., Bouton M.-M., Gallet-Bourguin D., Philibert D., Tournemine C. and Azadian-Boulanger G.: Comparative study of estrogen action. *Molec. Pharmac.* **9** (1973) 520–533.
19. Meyers C. Y., Kolb V. M. and Dandliker W. B.: Doisynolic acids: potent estrogens with very low affinity for estrogen receptors. *Res. Commun. Chem. Path. Pharmac.* **35** (1982) 165–168.
20. Rosser M. L., Muldoon T. G. and Hendry L. B.: Computer modeling of the fit of 11-beta-acetoxyestradiol into DNA correlates with potent uterotropic activity but not with binding to the estrogen receptor. *The Endocrine Society 73rd Annual Meeting* (1991) 122 (abstr. 368).
21. Korach K. S., Levy L. A. and Sarver P. J.: Estrogen receptor stereochemistry: receptor binding and hormonal responses. *J. Steroid Biochem.* **27** (1987) 281–290.
22. Korach K. S., Chae K., Levy L. A., Duax W. L. and Sarver P. J.: Diethylstilbestrol metabolites and analogs. *J. Biol. Chem.* **264** (1989) 5642–5647.
23. Brooks S. C., Wappler N. L., Corombos J. D., Doherty L. M. and Horwitz J. P.: Estrogen structure-receptor function relationships. In *Recent Advances in Steroid Hormone Action* (Edited by V. K. Moudgil). Walter de Gruyter, New York (1987) pp. 443–466.
24. Murdoch F. E., Meier D. A., Furlow J. D., Grunwald K. A. A. and Gorski J.: Estrogen receptor binding to a DNA response element *in vitro* is not dependent upon estradiol. *Biochem. J.* **36** (1990) 8377–8385.
25. Spanjaard R. A. and Chin W. W.: Reconstitution of ligand-mediated glucocorticoid receptor activity by trans-acting functional domains. *Molec. Endocr.* **7** (1993) 12–16.
26. Selye H.: Correlations between the chemical structure and the pharmacological actions of the steroids. *Endocrinology* **30** (1942) 437–453.
27. Child K. J., Currie, J. P., Davis B., Dodds M. G., Pearce D. R. and Twissell D. J.: The pharmacological properties in animals of CT1341—a new steroid anaesthetic agent. *Br. J. Anaesth.* **43** (1971) 2–13.
28. Iswari S., Colas A. E. and Karavolas H. J.: Binding of 5α-dihydroprogesterone and other progestins to female rat anterior pituitary nuclear extracts. *Steroids* **47** (1986) 189–203.
29. Saffran J., Loeser B. K., Bohnett S. A., Gray M. A. and Faber L. E.: The binding of 5α-pregnane-3,20-dione by cytosol and nuclear preparations of guinea pig uterus. *Endocrinology* **102** (1978) 1088–1097.
30. Strott C. A.: Metabolism of progesterone in the chick oviduct: relation to the progesterone receptor and biological activity. *Endocrinology* **95** (1974) 826–837.
31. Bäckström T.: Epileptic seizures in women related to plasma estrogen and progesterone during the menstrual cycle. *Acta Neurol. Scand.* **54** (1976) 321–347.
32. Rościszeska D., Buntner B., Guz I. and Zawisza L.: Ovarian hormones, anticonvulsant drugs, and seizures during the menstrual cycle in women with epilepsy. *J. Neurol. Neurosur. Psy.* **49** (1986) 47–51.
33. Bäckström T., Zetterlund B., Blom S. and Romano M.: Effects of intravenous progesterone infusions on the epileptic discharge frequency in women with partial epilepsy. *Acta Neurol. Scand.* **69** (1984) 240–248.
34. Luntz-Leybman V., Freund R. K. and Collins A. C.: 5α-Pregnan-3α-o1-20-one blocks nicotine-induced seizures and enhances paired-pulse inhibition. *Eur. J. Pharmac.* **185** (1990) 239–242.
35. Freeman E. W., Purdy R. H., Coutifaris C., Rickels K. and Paul S. M.: Anxiolytic metabolites of progesterone: correlation with mood and performance measures following oral progesterone administration to healthy female volunteers. *Neuroendocrinology* **58** (1993) 478–484.
36. Ross J., Claybaugh C., Clemens L. G. and Gorski R. A.: Short latency induction of estrous behavior with intracerebral gonadal hormones in ovarectomized rats. *Endocrinology* **89** 32–38.
37. Rodriguez-Manzo G., Cruz M. L. and Beyer C.: Facilitation of lordosis behavior in ovariectomized estrogen-primed rats by medial preoptic implantation of 5β, 3β, pregnanolone: a ring a reduced progesterone metabolite. *Physiol. Behav.* **36** (1986) 277–281.
38. Brann D. W., Putnam C. D. and Mahesh V. B.: γ-Aminobutyric $acid_A$ receptors mediate 3α-hydroxy-5α-preganan-20-one-induced gonadotropin secretion. *Endocrinology* **126** (1990) 1854–1859.
39. Smith S. S., Waterhouse B. D., Chapin J. K. and Woodward D. J.: Progesterone alters GABA and glutamate responsiveness: a possible mechanism for its anxiolytic action. *Brain Res.* **400** (1987) 353–359.
40. Smith S.: The effects of estrogen and progesterone on GABA an glutamate responses at extrahypothalmic sites. In: *Neuros-*

teroids and Brain Function (Edited by E. Costa and S. Paul). Thieme Medical Publishers Inc., New York (1991) pp. 87–94.
41. Smith S.: Progesterone administration attenuates excitatory amino acid responses of cerebellar Purkinje cells. *Neuroscience* **42** (1989) 309–320.
42. Havens M. and Rose J. D.: Estrogen-dependent and estrogen-independent effects of progesterone on the electrophysiological excitability of dorsal midbrain neurons in golden hamsters. *Neuroendocrinology* **48** (1988) 120–129.
43. Kubli-Garfias C., Cervantes M. and Beyer C.: Changes in multiunit activity and EEG induced by the administration of natural progestins to flaxedil immobilized cats. *Brain Res.* **114** (1976) 71–81.
44. Smith L. and Ecker R.: The interaction of steroids with *Rana pipiens* oocytes in the induction of maturation. *Devl. Biol.* **25** (1971) 232–247.
45. Kostellow A. B., Weinstein S. P. and Morrill G. A.: Specific binding of progesterone to the cell surface and its role in the meiotic divisions in rana oocytes. *Biochim. Biophys. Acta* **720** 356–363.
46. Sadler S. E. and Maller J. L.: Identification of a steroid receptor on the surface of *Xenopus* oocytes by photoaffinity labeling. *J. Biol. Chem.* **257** (1982) 355–361.
47. Sadler S. E., Bower M. A. and Maller J. L.: Studies of a plasma membrane steroid receptor in *Xenopus* oocytes using the synthetic progestin RU 486. *J. Steroid Biochem.* **22** (1985) 419–426.
48. Baulieu E.-E.: Cell membrane: a target for steroid hormones. *Molec. Cell. Endocr.* **12** (1978) 247–254.
49. Kostellow A. B. and Morrill G. A.: Calcium dependence of steroid and guanine 3',5'-monophosphate induction of germinal vesicle breakdown in rana pipiens oocytes. *Endocrinology* **106** (1980) 1012–1019.
50. Finidori-Lepicard J., Schorderet-Slatkine S., Hanoune J. and Baulieu E.-E.: Progesterone inhibits membrane-bound adenylate cyclase in *Xenopus laevis* oocytes. *Nature* **292** (1981) 255–257.
51. Morrill G., Schatz F., Kostellow A. and Poupko J.: Changes in cyclic AMP levels in the amphibian ovarian follicle following progesterone induction of meiotic maturation. *Differentiation* **8** (1977) 97–103.
52. Putnam C. D., Brann D. W., Kolbeck R. C. and Mahesh V. B.: Inhibition of uterine contractility by progesterone and progesterone metabolites: mediation by progesterone and gamma amino butyric acid$_A$ receptor systems. *Biol. Reprod.* **43** (1991) 266–272.
53. Perusquía M., García-Yañez E., Ibáñez R. and Kubli-Garfias C.: Non-genomic mechanism of action of Δ-4 and 5-reduced androgens and progestins on the contractility of the isolated rat myometrium. *Life Sci.* **47** (1990) 1547–1553.
54. Kubli-Garfias C., Hoyo-Vadillo C., López-Nieto E. and Ponce-Monter H.: Inhibition of spontaneous contractions of the rat pregnant uterus by progesterone metabolites. *Proc. West Pharmac. Soc.* **26** (1983) 115–118.
55. Kubil-Garfias C., Ponce-Monter H., Medrano-Conde L., López-Fiesco A. and Bondani A.: Role of calcium on the *in vitro* inhibition of the rat uterine contractility produced by androgens and progestins. *Arch. Invest. Méd. (Méx.)* **13** (1982) 219–224.
56. Kubli-Garfias C., Ortega-Suárez P., Hoyo-Vadillo C. and Ponce-Monter H.: Evidence that 5β-progestins produce uterine relaxation by diminishing cellular calcium permeability. *Drug Dev. Res.* **6** (1985) 103–107.
57. Kubli-Garfias C.: Modulatory action of 5-reduced androgens and progestins on the excitability of CNS and smooth muscle. *J. Steriod Biochem.* **27** (1987) 631–634.
58. Drouva S. V., Laplante E. and Kordon C.: Effects of ovarian steriods on *in vitro* release of LHRH from mediobasal hypothalamus. *Neuroendocrinology* **37** (1983) 336–341.
59. Ramirez V. D., Kim K. and Dluzen D.: Progesterone action on the LHRH and the nigrostriatral dopamine neuronal systems: *in vitro* and *in vivo* studies. *Rec. Progr. Horm. Res.* **41** (1985) 421–472.
60. Ke F.-C. and Ramirez V. D.: Membrane mechanism mediates progesterone stimulatory effect on LHRH release from superfused rat hypothalami *in vitro*. *Neuroendocrinology* **45** (1987) 514–517.
61. Ramirez V. D. and Dluzen D.: Is progesterone a pre-hormone in the CNS? *J. Steroid Biochem.* **27** (1987) 589–598.
62. Dluzen D. E. and Ramirez V. D.: Modulatory effects of progesterone upon dopamine release from the corpus striatum of ovariectomized estrogen-treated rats are stereo-specific. *Brain Res.* **538** (1991) 176–179.
63. Petitti N. and Etgen A. M.: Progesterone promotes rapid desensitization of α_1-adrenergic receptor augmentation of cAMP formation in rat hypothalamic slices. *Neuroendocrinology* **55** (1992) 1–8.
64. Blackmore P. F., Beebe S. J., Danforth D. R. and Alexander N.: Progesterone and 17α-hydroprogesterone. *J. Biol. Chem.* **265** (1990) 1376–1380.
65. Meizel S. and Turner K. O.: Progesterone acts at the plasma membrane of human sperm. *Molec. Cell. Endocr.* **11** (1991) R1–R5.
66. Tesarik J., Mendoza C., Moos J., Fénichel and Fehlmann M.: Progesterone action through aggregation of a receptor on the sperm plasma membrane. *FEBS* **308** (1992) 116–120.
67. Foresta C., Rossato M. and Di Virgilio F.: Ion fluxes through the progesterone-activated channel of the sperm plasma membrane. *Biochem. J.* **294** (1993) 279–283.
68. Yagi K.: Changes in firing rates of single preoptic and hypothalamic units following an intravenous administration of estrogen in the castrated female rat. *Brain Res.* **53** (1973) 343–352.
69. Dufy B., Partouche C., Poulain D., Dufy-Barbe L. and Vincent J. D.: Effects of estrogen on the electrical activity of identified and unidentified hypothalamic units. *Neuroendocrinology* **22** (1976) 38–47.
70. Kelly M. J., Moss, R. L. and Dudley C. A.: Differential sensitivity of preoptic-septal neurons to microelectrophoresed estrogen during the estrous cycle. *Brain Res.* **114** (1976) 152–157.
71. Kelly M. J., Moss R. L. and Dudley C. A.: The effects of microelectrophoretically applied estrogen, cortisol and acetylcholine on medial preoptic-septal unit activity throughout the estrous cycle of the female rat. *Expl Brain Res.* **30** (1977) 53–64.
72. Kelly M. J., Moss R. L., Dudley C. A. and Fawcett C. P.: The specificity of the response of preoptic-septal area neurons to estrogen: 17α-estradiol versus 17β-estradiol and the response of extrahypothalamic neurons. *Expl Brain Res.* **30** (1977) 43–52.
73. Kelly M. J., Moss R. L. and Dudley C. A.: The effects of ovariectomy on the responsiveness of preoptic-septal neurons to microlectrophoresed estrogen. *Neuroendocrinology* **25** (1978) 204–211.
74. Nakekura J., Oomura Y., Minami T., Mizuno Y. and Fukuda A.: Mechanism of the rapid effect of 17β-estradiol on medial amygdala neurons. *Science* **232** (1986) 226–228.
75. Garcia-Sergura L., Olmos G., Tranque P. and Naftolin F.: Rapid effects of gonadal steroids upon hypothalamic neuronal membrane ultrastructure. *J. Steroid Biochem.* **27** (1987) 615–623.
76. Naftolin F., Garcia-Segura L. M., Deefe D., Leranth C., Maclusky N. J. and Brawer J. R.: Estrogen effects on the synaptology and neural membranes of the rat hypothalamic arcuate nucleus. *Biol. Reprod.* **42** (1990) 21–28.
77. Garcia-Sergura L. M., Olmos G., Robbins R. J., Hernandez P., Meyer J. H. and Naftolin F.: Estradiol induces repia remodelling of plasma membranes in developing rat cerebrocortical neurons in culture. *Brain Res.* **498** (1989) 339–343.
78. Dufy B., Vincent J.-D., Fleury H., Pasquier P. D., Gourdji D. and Tixier-Vital A.: Membrane effects of thyrotropin-releasing hormone and estrogen shown by intracellular recording from pituitary cells. *Science* **204** (1979) 509–511.
79. Zyzek E., Dufy-Barbe L., Dufy B. and Vincent J. D.: Short-term effects of estrogen on release of prolactin by pituitary cells in culture. *Biochem. Biophys. Res. Commun.* **102** (1981) 1151–1157.
80. Yamada Y.: Effects of testosterone on unit activity in rat hypothalamus and septum. *Brain Res.* **172** (1979) 165–168.
81. Orsini J. C., Barone F. C., Armstrong D. L. and Wayner M. J.: Direct effects of androgens on lateral hypothalamic neuronal activity in the male rat: I. A microiontophoretic study. *Brain Res. Bull.* **15** (1985) 293–297.
82. Kubli-Garfias C., Canchola E., Arauz-Contreras J. and Feria-Velasco A.: Depressant effect of androgens on the cat brain electrical activity and its antagonism by ruthenium red. *Neuroscience* **7** (1982) 2777–2782.

83. Norman A. W., Nemere I., Zhou L.-X., Bishop J. E., Lowe K. E., Maiyar A. C., Collins E. D., Taoka T., Sergeev I. and Farach-Carson M. C.: 1,25(OH)$_2$-vitamin D$_3$, a steroid hormone that produces biologic effects via both genomic and nongenomic pathways. *J. Steroid Biochem. Molec. Biol.* **41** (1992) 231–240.
84. Nemere I. and Norman A. W.: Steroid hormone actions at the plasma membrane: induced calcium uptake and exocytotic events. *Molec. Cell. Endocr.* **80** (1991) C165–C169.
85. De Boland A. R. and Norman A. W.: Influx of extracellular calcium mediates 1,25-dihydorxyvitamin D$_3$-dependent transcaltachia (the rapid stimulation of duodenal Ca^{2+} transport). *Endocrinology* **127** (1990) 2475–2480.
86. De Boland A. and Boland R.: Rapid changes in skeletal muscle calcium uptake induced *in vitro* by 1,25-dihydroxyvitamin D$_3$ are suppressed by calcium channel blockers. *Endocrinology* **120** (1987) 1858–1864.
87. Mezzetti G., Monti M., Casolo L., Piccinini G. and Moruzzi M.: 1,25-dehydroxycholecalciferol-dependent calcium uptake by mouse mammary gland in culture. *Endocrinology* **122** (1988) 389–394.
88. Civitelli R., Kim Y. S., Gunsten S. L., Fujimori A., Huskey M., Aviolo L. V. and Hruska K. A.: Nongenomic activation of the calcium message system by vitamin D metabolites in osteoblast-like cells. *Endocrinology* **127** (1990) 2253–2262.
89. Lieberherr M., Grosse B., Duchambon P. and Drueke T.: A functional cell surface type receptor is required for the early action of 1,25-dihydroxyvitamin D$_3$ on the phosphoinositide metabolism in rat enterocytes. *J. Biol. Chem.* **34** (1989) 20,403–20,406.
90. De Boland A. and Norman A.: Evidence for involvement of protein kinase C and cyclic adenosine 3,5' monophosphate-dependent protein kinase in the 1,25-dihydroxyvitamin D$_3$-mediated rapid stimulation of intestinal calcium transport (transcaltachia). *Endocrinology* **127** (1990) 39–45.
91. Willmer E. N.: Steroids and cell surfaces. *Biol. Rev.* **36** (1961) 368–398.
92. Clarke R., van den Berg H. W. and Murphy R. F.: Reduction of the membrane fluidity of human breast cancer cells by tamoxifen and 17β-estradiol. *J. Natn. Cancer Inst.* **82** (1990) 1702–1705.
93. Reddy A. G., Shivaji S. and Gupta P. D.: Effect of estradiol on the membrane fluidity of the rat vaginal epithelial cells. *J. Steroid Biochem.* **33** (1989) 1229–1233.
94. Shivaji S. and Jagannadham M. V.: Steroid-induced perturbations of membranes and its relevance to sperm acrosome reaction. *Biochim. Biophys. Acta* **1108** (1992) 99–109.
95. Bikle D. D., Whitney J. and Munson S.: The relationship of membrane fluidity to calcium flux in chick intestinal brush border membranes. *Endocrinology* **114** (1984) 260–267.
96. Towle A. C. and Sze P. Y.: Steroid binding of synaptic plasma membrane: differential binding of glucocorticoids and gonadal steroids. *J. Steroid Biochem.* **18** (1983) 135–143.
97. Ke F. and Ramirez V. D.: Binding of progesterone to nerve cell membranes of rat using progesterone conjugated to ^{125}I-bovine serum albumin as a ligand. *J. Neurochem.* **54** (1990) 467–472.
98. Tischkau S. A. and Ramirez V. D.: A specific membrane binding protein for progesterone in rat brain: sex differences and induction by estrogen. *Proc. Natn. Acad. Sci. U.S.A.* **90** (1993) 1285–1289.
99. Tesarik J., Mendoza C., Moos J. and Carreras A.: Selective expression of a progesterone receptor on the human sperm surface. *Fertil. Steril.* **58** (1992) 784–792.
100. Mendoza C. and Tesarik J.: A plasma-membrane progesterone receptor in human sperm is switched on by increasing intracellular free calcium. *FEBS* **330** (1993) 57–60.
101. Hyne R. V. and Boettchre B.: The selective binding of steroids by human spermatozoa. *Contraception* **15** (1976) 163–174.
102. Cheng C. Y., Rose R. J. and Boettcher B.: The binding of oestradiol-17β to human spermatozoa-an electron microscope autoradiographic study. *Int. J. Andro.* **4** (1981) 304–313.
103. Berthois Y., Pourreau-Schneider N., Gandilhon P., Mittre H., Tubiana N. and Martin P. M.: Estradiol membrane binding sites on human breast cancer cell lines. Use of a fluorescent estradiol conjugate to demonstrate plasma membrane binding systems. *J. Steroid Biochem.* **25** (1986) 963–972.
104. Nenci I., Fabris G., Marzola A. and Marchetti E.: The plasma membrane as an additional level of steroid-cell interaction. *J. Steroid Biochem.* **15** (1981) 231–234.
105. Zänker K. S., Prokscha G. W. and Blümel G.: Plasma membrane-integrated estrogen receptors in breast tissue: possible modulator molecules for intracellular hormone level. *J. Cancer Res. Clin.* **100** (1981) 135–148.
106. Pietras R. J. and Szego C. M.: Partial purification and characterization of oestrogen receptors in subfractions of hepatocyte plasma membranes. *Biochem J.* **191** (1980) 743–760.
107. Pietras R. J. and Szego C. M.: Estrogen receptors in uterine plasma membrane. *J. Steroid Biochem.* **11** (1979) 1471–1483.
108. Müller R. E., Johnston T. C. and Wotiz H. H.: Binding of estradiol to purified uterine plasma membranes. *J. Biol. Chem.* **254** (1979) 7895–7900.
109. Morrow A. L., Suzdak P. D. and Paul S. M.: Steroid hormone metabolites potentiate GABA receptor-mediated chloride ion flux with nanomolar potency. *Eur. J. Pharmac.* **142** (1987) 483–485.
110. Simmonds M. A., Turner J. P. and Harrison N. L.: Interactions of steroids with the GABA-A receptor complex. *Neuropharmacology* **23** (1984) 877–878.
111. Majewska M. D., Harrison N. L., Schwartz R. D., Barker T. L. and Paul S. M.: Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* **232** (1986) 1004.
112. Belelli D., Lan N. C. and Gee K. W.: Anticonvulsant steroids and the GABA/benzodiazepine receptor-chloride ionophore complex. *Neurosci. Biobehav. Rev.* **14** (1990) 315–322.
113. Majewska M. D.: Neurosteroids: endogenous bimodal modulators of the GABA$_A$ receptor. Mechanism of action and physiological significance. *Prog. Neurobiol.* **38** (1992) 379–395.
114. Majewska M. D. and Schwartz R. D.: Pregnenolone-sulfate: an endogenous antagonist of the γ-aminobutyric acid receptor complex in brain? *Brain Res.* **404** (1987) 355–360.
115. Majewska M. D., Demirgoren S., Spivak C. E. and London E. D.: The neurosteroid dehydroepiandrosterone sulfate is an antagonist of the GABA$_A$ receptor. *Brain Res.* **526** (1990b) 143–146.
116. Paul S. M. and Purdy R. H.: Neuroactive steroids. *FASEB J.* **6,** (1992) 2311–2322.
117. Purdy R. H., Morrow A. L., Moore P. H. Jr. and Paul S. M.: Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc. Natn. Acad. Sci. U.S.A.* **88** (1991) 4553–4557.
118. Majewska M. D., Falkay G. and Baulieu E. E.: Modulation of uterine GABA$_A$ receptors during gestation and by tetrahydroprogesterone. *Eur. J. Pharmac.* **174** (1989) 43–47.
119. Baulieu E.-E. and Robel P.: Neurosteroids: a new brain function? *J. Steroid Biochem. Molec. Biol.* **37** (1990) 395–403.
120. Irwin R. P., Maragakis N. J., Rogawski M. A., Purdy R. H., Farb D. H. and Paul S. M.: Pregnenolone sulfate augments NMDA receptor mediated increases in intracellular Ca^{2+} in cultured rat hippocampal neurons. *Neurosci. Lett.* **141** (1992) 30–34.
121. Beyer C., Canchola E. and Larsson K.: Facilitation of lordosis behavior in the ovariectomized estrogen-primed rat by dibutyryl cAMP. *Physiol. Behav.* **26** (1981) 249–251.
122. Beyer C. and Canchola E.: Facilitation of progesterone induced lordosis behavior by phosphodiestrase inhibitors in estrogen primed rats. *Physiol. Behav.* **27** (1981) 731–733.
123. Denner L. A., Wiegel N. L., Maxwell B. L., Schrader W. T. and O'Malley B. W.: Regulation of progesterone receptor-mediated transcription by phosphorylation. *Science* **250** (1990) 1740–1748.
124. Ignar-Throwbridge D. M., Nelson K. G., Bidwell M. C., Curtis S. W., Washburn T. F., McLachlan J. A. and Korach K. S.: Coupling of dual signalling pathways: EGF action involves the estrogen receptor. *Proc. Natn. Acad. Sci.* **88** (1992) 4658–4662.
125. Ignar-Throwbridge D. M., Teng C. T., Ross C. A., Parker M. C., Korach K. S. and McLachlan J. A.: Peptide growth factors elicit estrogen receptor dependent transcriptional activation of an estrogen-responsive element. *Molec. Endocr.* **7** (1993) 992–998.
126. Aronica M. and Katzenellenbogen B. S.: Stimulation of estrogen receptor-mediated transcription and alteration in phosphorylation state of rat uterine ER by estrogen, cAMP and IGF-1. *Molec. Endocr.* **7** (1993) 743–752.
127. Power R. R., Mani S. K., Cordina J., Cannedy O. M. and O'Malley B. W.: Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science* **254** (1991) 1636–1639.

128. Tsai M. J. and O'Malley B. W.: Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *A. Rev. Biochem.* **63** 451–486.
129. Hendry L. B., Witham F. H. and Chapman O. L.: Gene regulation: the involvement of stereochemical recognition in DNA-small molecule interactions. *Perspect. Biol. Med.* **21** (1977) 120–130.
130. Hendry L. B.: Stereochemical complementarity of DNA and steroid agonists and antagonists. *J. Steroid Biochem.* **31** (1988) 493–523.
131. Hendry L. B., Bransome E. D. Jr, Hutson M. S. and Campbell L. K.: Method of predicting biological activity of compounds by nucleic acid models. *U.S. Patent* 4,461,619 (1984).
132. Uberoi N. K., Hendry L. B., Muldoon T. G., Myers R. B., Segaloff A., Bransome E. D. and Mahesh V. B.: Structure–activity relationships of some unique estrogens related to estradiol are predicted by fit into DNA. *Steroids* **45** (1985) 325–340.
133. Hendry L. B. and Mahesh V. B.: Stereochemical complementarity of progesterone and cavities between base pairs in partially unwound double stranded DNA using computer modeling and energy calculations to determine degree of fit. *J. Steroid Biochem. Molec. Biol.* **39** (1991) 133–146.
134. Hendry L. B. and Mahesh V. B.: Stereochemical complementarity of progesterone, RU486 and cavities between base pairs in partially unwound double stranded DNA assessed by computer modeling and energy calculations. *J. Steroid Biochem. Molec. Biol.* **41** (1992) 647–651.
135. Hendry L. B., Muldoon T. G. and Mahesh V. B.; The metabolic pathways for hormonal steroids appear to be reflected in the stereochemistry of DNA. *J. Steroid Biochem. Molec. Biol.* **42** (1992) 659–670.
136. Witham F. H. and Hendry L. B.: Computer modeling of gibberellin-DNA binding. *J. Theor. Biol.* **155** (1992) 55–67.
137. Hendry L. B.: Stereochemical complementarity of estradiol and DNA using a computer generated space filling model. *Am. J. Physiol.: Endocr. Metab.* **27** (1993) (Jan–June) cover.
138. Hendry L. B.: Drug design with a new type of molecular modeling based on stereochemical complementarity to gene structure. *J. Clin. Pharmac.* **33** (1993) 1173–1187.
139. Rowland M. J., Bransome E. D. Jr. and Hendry L. B.: Hypoglycemia caused by selegiline, an antiparkinsonian drug. Can such side effects be predicted? *J. Clin. Pharmac.* **34** (1993) 80–85.
140. Hendry L. B., Chu C. K., Rosser M. L., Copland J. A., Wood J. C. and Mahesh V. B.: Design of novel antiestrogens. *Steroid Biochem. Molec. Biol.* **49** (1994) 269–280.
141. Steinsapir J., Mora G., Muldoon T. G., Mahesh V. B. and Hendry L. B.: Androgenic activity of antiandrogens predicted by fit into DNA. *Am. J. Ther.* **1** (1994) 1–9.
142. Hendry L. B. and Mahesh V. B.: Gene regulation may involve receptor mediated insertion of hormonal ligands into DNA: Evidence based on computer modeling of nucleic acids and the discovery of steroid/thyroid pharmacophores. *The Endocrine Society (76th Annual Meeting)* (1994) 380 (abs. 719).
143. Munck A.: The interfacial activity of steroid hormones and synthetic estrogens. *Biochim. Biophys. Acta* **24** (1957) 507–514.
144. Sluyser M.: Interaction of steroid hormone receptors with DNA. *Trends Biochem. Sci.* **8,** (1983) 236–238.
145. Duax W. L., Griffin C. M. and Weeks D. C.: Models for steroid-receptor interactions derived from x-ray crystallographic investigations. In *Interaction of Steroid Hormone Receptors with DNA* (Edited by M. Sluyser). Ellis Horwood, England (1985) pp. 83–106.
146. Liehr J. G., Gladek A., Macatee T., Randerath E. and Randerath K.: DNA adduction formation in liver and kidney of male Syrian hamsters treated with estrogen and/or α-napthoflavone. *Carcinogenesis* **12** (1991) 385–389.
147. Telang N. T., Suto A., Wong G. Y., Osborne M. P. and Bradlow H. L.: Induction by estrogen metabolite 16α-hydroxyestrone of genotoxic damage and aberrant prolferation in mouse mammary epithelial cells. *J. Natn. Cancer Inst.* **84** (1992) 634–638.
148. DeSombre E. R., Shafii B., Hanson R. N., Kuivanen P. C. and Hughes A.: Estrogen receptor-directed radiotoxicity with Auger electrons: specificity and mean lethal dose. *Cancer Res.* **52** 5752–5758.
149. Munck A., Scott J. G. and Engel L. L.: The interaction of steroid hormones and coenzyme components. *Biochim. Biophys. Acta* **26** (1957) 397–407.
150. Lehner A. F., Muldoon T. G., Mahesh V. B., Bransome E. D. Jr. and Hendry L. B.: Initial studies of a phytoestrogen-deoxyribonucleic acid interaction. *Molec. Endocr.* **1** (1987) 377–387.
151. Nardulli A. M., Greene G. L. and Shapiro D. J.: Human estrogen receptor bound to an estrogen response element bends DNA. *Molec. Endocr.* **7** (1993) 331–340.
152. Rainish B. L., Greene G. L. and Nardulli A. M.: The human estrogen receptor bends DNA fragments containing consensus or imperfect EREs. *The Endocrine Society (76th Annual Meeting)* (1994) 318 (abs 470).
153. Ding Z-M., Harshey R. M. and Hurley L. H.: (+)-CC-1065 as a structural probe of Mu-transposase-induced bending of DNA: overcoming limitations of hydroxyl-radical footprinting. *Nucl. Acid Res.* **21** (1993) 4281–4287.
154. Beekman J. M., Allan G. F., Tsai S. Y., Tsai M.-J. and O'Malley B. W.: Transcriptional activation by the estrogen receptor requires a conformational change in the ligand binding domain. *Molec. Endocr.* **7** (1993) 1266–1274.
155. Fritsch M., Welch R. D., Murdoch F. E., Anderson I. and Gorski J.: DNA allosterically modulates the steroid binding domain of the estrogen receptor. *J. Biol. Chem.* **267** (1992) 1823–1828.
156. Majewska M. D., Harrison N. L., Schwartz R. D., Barker J. L. and Paul S. M.: Steroid hormone metabolites are barbituate-like modulators of the GABA receptor. *Science* **232** (1986) 1004–1007.
157. Schumacher M., Coirini H., Pfaff D. W. and McEwen B. S.: Behavioral effects of progesterone associated with rapid modulation of oxytocin receptors. *Science* **250** (1990) 691–694.
158. Kato R., Takahashi A. and Omori Y.: The mechanism of sex differences in the anethetic action of progesterone in rats. *Eur. J. Pharmac.* **13** (1971) 141–149.
159. Meiri H.: Is synaptic transmission modulated by progesterone? *Brain Res.* **385** (1986) 193–196.
160. Osman R. A., Andria M. L., Jones A. D. and Meizel S.: Steroid induced exocytosis: the human sperm acrosome reaction. *Biochem. Biophys. Res. Commun.* **160** (1989) 828–833.
161. Thomas P. and Meizel S.: Phosphatidylinositol 4,5-bisphosphate hydrolysis in human sperm stimulated with follicular fluid or progesterone is dependent upon Ca^{2+} influx. *Biochem. J.* **264** (1989) 539–546.
162. Baulieu E. E., Godeau J. F., Schorderet M. and Schorderet-Slatkine S.: Steroid induced meiotic division in *Xenopus laevis* oocytes: surface and calcium. *Nature* **275** (1978) 593–598.
162. Vincens M., Shu C., Moguilewsky M. and Philibert D.: A progesterone metabolite enhances the activity of the $GABA_A$ receptor complex at the pituitary level. *Eur. J. Pharmac.* **168** (1989) 15–21.
164. Smith S. S., Woodward D. J. and Chapin J. K.: Sex steroids modulate motor-correlated increases in cerebellar discharge. *Brain Res.* **476** (1989) 307–316.
165. Pourreau-Schneider N., Berthois Y., Gandilhon P., Cau P., Martin P. M. and Passerel M.: Early alterations at the plasma membrane of breast cancer cell lines in response to estradiol an hydorcytamoxifen. *Molec. Cell. Endocr.* **48** (1986) 77–88.
166. Rambo C. O. and Szego C. M.: Estrogen action at endometrial membranes: alterations in luminal surface detectable within seconds. *J. Cell Biol.* **97** (1983) 679–685.
167. Thompson T. L. and Moss R. L.: Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. *J. Neurochem.* **62** (1994) 1750–1756.
168. Teyler T. J., Vardaris R. M., Lewis D. and Rawitch A. B.: Gonadal steroids: effects on excitability of hippocampal pyramidal cells. *Science* **209** (1980) 1017–1018.
169. Koenig H., Fan C.-C., Goldstone A. D., Lu C. Y. and Trout J. J.: Polyamines mediate androgenic stimulation of calcium fluxes and membrane transport in rat heart myocytes. *Circul. Res.* **64** (1989) 415–426.
170. Kubli-Garfias C., López-Fiesco A., Pacheco-Cano M. T., Ponce-Monter H. and Bondani A.: *In vitro* effects of androgens upon the spontaneous rat uterine contractility. *Steroids* **35** (1980) 633–641.
171. Sachs B. D. and Leipheimer R. E.: Rapid effect of testosterone on striated muscle activity in rats. *Neuroendocrinology* **48** (1988) 453–458.

172. Meisel R. L., Leipheimer R. E. and Sachs B. D.: Anisomycin does not disrupt the activation of penile reflexes by testosterone in rats. *Physiol. Behav.* **37** (1986) 951–956.
173. Hua S.-Y. and Chen Y.-Z.: Membrane receptor-mediated electrophysiological effects of glucocorticoid on mammalian neurons. *Endocrinology* **124** (1989) 687–691.
174. Hall E. D.: Glucocorticoid effects on central nervous excitability and synaptic transmission. *Neurobiology* **23** (1982) 165–195.
175. Ffrench-Mullen J. M. H., Danks P. and Spence K. T.: Neurosteroids modulate calcium currents in hippocampal CA1 neurons via a pertussis toxin-sensitive G-protein-coupled mechanism. *J. Neurosci.* **14** (1994) 1963–1977.
176. Bikle D. D., Zolock D. T., Morrissey R. L. and Herman R. H.: Independence of 1,25-dihydroxyvitamin D_3-mediated calcium transport from *de novo* RNA and protein synthesis. *J. Biol. Chem.* **253** (1978) 484–488.
177. Barsony J. and Marx S. L.: Receptor-mediated rapid action of 1α,25-dihydroxycholecalciferol: increase of intracellular cGMP in human skin fibroblasts. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 1223–1226.
178. Barsony J., Pike J. W., DeLuca H. F. and Marx S. J.: Immunocytology with microwave-fixed fibroblasts shows 1α,25-dihydroxyvitamin D_3-dependent rapid and estrogen-dependent slow reorganization of vitamin D receptors. *J. Cell Biol.* **111,** (1990) 2385–2395.
179. Bression D., Michard M., Le Dafniet M., Pagesy P. and Peillon F.: Evidence for a specific estradiol binding site on rat pituitary membranes. *Endocrinology* **119** (1986) 1048–1051.
180. Koch B., Lutz-Bucher B., Briaud B. and Mialhe C.: Glucocorticoid binding to plasma membranes of the adenohypophysis. *J. Endocr.* **73** (1977) 399–400.
181. Koch B., Lutz-Bucher B., Briaud B. and Mialhe C.: Specific interaction of corticosteroids with binding sites in the plasma membranes of the rat anterior pituitary gland. *J. Endocr.* **79** (1978) 215–222.
182. Schaeffer J. M., Stevens S., Smith R. G. and Hsueh A. J. W.: Binding of 2-hydroxyestradiol to rat anterior pituitary cell membranes. *J. Biol. Chem.* **255** (1980) 9838–9843.
183. Suyemitsu T. and Terayama H.: Specific binding sites for natural glucocorticoids in plasma membranes of rat liver. *Endocrinology* **96** (1975) 1499–1507.
184. Blondeau J. P. and Baulieu E. E.: Progesterone receptor characterized by photoaffinity labelling in the plasma membrane of *Xenopus laevis* oocytes. *Biochem. J.* **219** (1984) 785–792.
185. Blackmore P. and Lattanzio F.: Cell surface localization of a novel non-genomic progesterone receptor on the head of human sperm. *Biochem. Biophys. Res. Commun.* **181** (1991) 331–336.
186. Axelson M., Sjovall J., Gustafsson B. E. and Setchell K. D. R.: Soya—a dietary source of the non-steroidal oestrogen equal in man and animals. *J. Endocr.* **102** (1984) 49–56.
187. Chernayaev G. A., Barkova T. I., Ergorova V. V., Sorokina I. B., Ananchenko S. N., Mataradze G. D., Sokolova N. A. and Rozen V. B.: A series of optical, structural and isometric analogs of estradiol: a comparative study of the biological activity and affinity to cytosol receptor of rabbit uterus. *J. Steroid Biochem.* **6** (1975) 1483–1488.
188. Crenshaw R. R., Jenks T. A., Luke G. M. and Bialy G.: Potential antifertility agents. 6. Synthesis and biological activities of optical isomers of 4β-(*p*-methoxyphenyl)-2β-methylcyclohexane-α-carboxylic acid and related compounds. *J. Med. Chem.* **17** (1974) 1258–1261.
189. Farnsworth N. R., Bingel A. S., Cordell G. A., Crane F. A. and Fong H. H. S.: Potential value of plants as sources of new antifertility agents. *J. Pharm. Sci.* **64** (1975) 717–754.
190. Gonzalez F. B., Neef G., Eder U., Wiechert R., Schillinger E. and Nishino Y.: Synthesis and pharmacological evaluation of 8α-estradiol derivatives. *Steroids* **40** (1982) 171–187.
191. Gordon S., Cantrall E. W., Cekleniak W. P., Albers H. J., Mauer S., Stolar S. M. and Bernstein S.: Steroid and lipid metabolism. The hypocholesteremic effect of estrogen metabolites. *Steroids* **4** (1964) 267–271.
192. Hilgar A. G. and Palmore J. Jr.: The uterotropic evaluation of steroids and other compounds-assay 2. In *Endocrine Bioassay Data, Part IV* (Edited by A. G. Hilgar and L. C. Trench) United States Department of Health Education and Welfare, National Institutes of Health (1985).
193. Hogg J. A. and Korman J.: Synthetic estrogens. In *Medicinal Chemistry, Vol. II.* John Wiley and Sons, New York (1956) pp. 34–217.
194. Kilbourn M. R., Arduengo A. J., Park J. T. and Katzenellenbogen J. A.: Conformational analysis of nonsteroidal estrogens. *Molec. Pharm.* **19** (1981) 388–398.
195. Korach K. S., Metzler M. and McLachlan J. A.: Estrogenic activity *in vivo* and *in vitro* of some diethylstilbestrol metabolites and analogs. *Proc. Natn. Acad. Sci. U.S.A.* **75** (1978) 468–471.
196. Raynaud J.-P., Ojasoo T., Bouton M. M., Bignon E., Pons M. and Crastes de Paulet A.: Structure–activity relationships of steroid estrogens. In *Estrogens in the Environment* (Edited by J. A. McLachlan) Elsevier, Amsterdam (1985) pp. 24–42.