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REVIEW

Nongenomic Action of Steroids in Myometrial Contractility

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Steroid hormones are involved in several fundamental aspects of all living beings, with a few slight chemical differences among steroids being enough to give them the extraordinarily diverse biologic specificities that are important in animal physiology and medical therapeutics. Indeed, in the uterus, they have a remarkable action on uterine contractility with physiologic significance in the important reproductive processes of mammalian pregnancy and parturition. The regulation of progesterone on the myometrial contractile activity and related wider subjects of the endocrinology of pregnancy and parturition have been reviewed many times during the twentieth century. However, new data indicate that several progesterone metabolites and some synthetic steroids induce a progesterone-like uterine-relaxing effect. Experimental evidence from our laboratory has shown that 5-reduced progestins and androgens are more potent than progesterone itself in decreasing uterine contractility. The purpose of this review is to update current knowledge of endogenous and exogenous steroids on the phenomenon of uterine contractility, by summarizing their structural differences to induce changes on this process and discussing the possible mechanism of steroids to regulate uterine muscle activity.

Key Words: Steroids; nongenomic effects; progesterone metabolites; 5-reduced metabolites; myometrium; uterine relaxation.

Introduction

Progesterone plays an important role in the maintenance of pregnancy in mammals, exerting a relaxing effect directly in the uterine muscle. Pioneering experiments *(1,2)* demonstrated that the corpus luteum is essential for the maintenance of pregnancy in the rabbit. These findings established, for the first time, the importance of a hormone as an inhibitory factor *(1–5)*. This observation was supported by further studies performed both in vitro *(6)* and in vivo *(7,8)*.

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Immediately after the isolation and characterization of progesterone *(9)*, the uterine contraction inhibitory effect of progesterone was confirmed in vitro and in vivo *(10–16)*. In general, the in vitro studies pointed to an inhibitory effect of progesterone on the oxytocin-induced uterine contractile response, whereas in vivo experiments revealed the inhibitory effect of progesterone to be on spontaneous uterine activity.

These series of findings on the ability of progesterone to replace the corpus luteum in maintaining pregnancy within a quiescent myometrial environment emphasized that the role of progesterone during pregnancy has an inhibitory effect on uterine activity. This effect was described by Csapo *(17)* as the "progesterone block." This gave birth to the predominant conceptualization of progesterone as the defense mechanism of pregnancy *(18)*, and the use of the name *progestation steroid* to describe it.

Numerous observations in the late 1950s and early 1960s by Csapo and coworkers, as well as others, clarified the inhibitory effect of progesterone and led to a search for an explanation of its action *(16–22)*. The experimental results confirmed that progesterone induces an inhibitory effect on the myometrial response to oxytocin in rabbits *(18,19)*. The inhibitory effect induced by progesterone was also reported on electrical stimulation of contractions in rabbits *(18)*. Collateral works qualified the rat uterus as a good model for the studies of progesterone action. It was observed that spontaneous and electrical activity were decreased in vivo and in vitro by this hormone in pregnant and nonpregnant rats *(20,21)*, with a diminution in frequency and amplitude of oxytocin-elicited contractions. This is in contrast to the effect induced by estrogen *(21,22)*. In addition, it was confirmed that the administration of progesterone may maintain pregnancy in ovariectomized rats *(23)*. In summary, these 30 yr of research characterized the effects of progesterone as an uncoupling of the excitation-contraction process *(24)*.

Regarding the effect of progesterone on human uterine contractility, studies in pregnant and nonpregnant myometrial strips in vitro have shown that this steroid hormone induces a diminution in the duration of the contraction cycle in oxytocin-stimulated uterus, and on spontaneous contractions *(25,26)*. These findings resulted in the conclusion that the sensitivity of myometrium to oxytocin was greatly reduced in the presence of progesterone. However, the in vivo studies did not entirely agree with the in vitro results;

the im administration of progesterone at high doses (400 mg daily for 4 d) to pregnant women at term failed to reduce significantly the spontaneous contractility or the uterine response to oxytocin *(27)*. Intravenous administration of progesterone as a single dose (100 mg) did not significantly modify the progression of oxytocin-induced labor *(28)*, while some partial suppression of uterine activity by progesterone administered intraamniotically was reported *(29)*. However, synthetic progesterones such as 17α-hydroxyprogesterone caproate, injected intravenously, or medroxyprogesterone acetate, administered intramyometrially, produced an inhibitory effect *(30,31)*.

The lack of inhibitory response in vivo could possibly result from an insufficient amount of administered progesterone (considering that 280 mg/d of progesterone is produced in late pregnancy), or the route of administration led to a decrease in progesterone bioavailability. In addition, many variables could be influencing the absorption of progesterone owing to its physicochemical properties, i.e., its low dissolution rate. In fact, in some of these reports, the steroid was administered in a milky solution or colloidal suspension.

Effect of Other Steroids on Myometrial Activity

Estrogens

Reynolds *(32)* found the effect of estrogen on the in vivo uterine activity of rabbits to increase contractility. Later studies in vitro *(15)* and in vivo *(16)*, using rabbits, revealed that the estrogen-dominated uterus has a longer contraction cycle and, regarding the relation of stimulus frequency to tension, exhibits a positive staircase phenomenon. The uteri of ovariectomized animals in vitro, on the other hand, exhibit no staircase phenomenon. However, studies in vitro with pregnant rats demonstrated a biphasic effect induced by estradiol; at high concentrations (10–15 μg/mL) there is an inhibitory effect, but lower concentrations (0.2 μg/mL) favor the action of oxytocin *(33)*. By contrast, it has been reported that the spontaneous activity in vitro of nonpregnant rats is inhibited in a concentration-dependent manner by estrogens *(34,35)* and catecholestrogens *(36)*.

The importance of estrogens in human pregnancy is also controversial. Intravenous infusion of estradiol-induced spontaneous labor required significantly lower doses of iv oxytocin for labor induction *(37)*, while Järvinen et al. *(38)* reported a facilitation of onset of labor by estrogens. By contrast, Klopper and Dennis *(39)* found no effect of estrogen on the duration of labor, while estradiol, at high concentrations (10–15 μg/mL), reduced the frequency of contractions in vitro *(40)*. It is important to consider that all of these studies have reported the effect of estradiol at high doses, when compared with its plasma levels (16 ng/mL) in late gestation.

In summary, characterization of the effect of estrogens on uterine contractility remains unclear. However, from the divergent observations reported, one can conclude that estrogens, in particular 17β-estradiol, induce a relaxing effect in vitro, whereas in vivo the effect could be stimulating (oxytocic effect). The oxytocic response could be the result of an indirect effect on the uterine smooth muscle. For instance, evidence indicates that estrogens are involved in controlling the increase in receptor concentration for several uterotonic agents including oxytocin *(41,42)*, prostaglandins *(43–45)*, serotonin *(46)*, and catecholamines *(47,48)*. In particular, estrogens increase the α-adrenoceptors' concentration, which results in a consequent decrease in sensitivity to the relaxing effects of β-adrenergic agonists *(49–51)*. Moreover, it is known that estrogen acts in vivo and in vitro to promote a number of events that are temporally related to the onset of labor in women and other mammalian species; these include the stimulation of prostaglandin production in decidua/endometrium, the formation of gap junctions, and the synthesis of oxytocin receptors *(52,53)*.

Progestins, Androgens, and Corticosteroids

With respect to testosterone, in 1937, Robson *(54)* reported an inhibitory effect induced by this hormone in the isolated rabbit uterus. However, apart from progesterone and estradiol, the potential effect of other steroids on myometrial contractility received little attention.

It is only recently that in vitro studies have established a progesterone-like effect on the spontaneous uterine contractility of rats induced by a wide series of steroids. Progestins such as progesterone, 20α-hydroxy-4-pregnen-3-one, and dihydro- and tetrahydroprogestins, induced relaxation in nonpregnant *(55)* and pregnant *(56)* rats. Androgens, including testosterone and its 5-reduced metabolites, are able to produce relaxation in nongravid rat uterus *(57)*. Moreover, corticosteroids (17α-hydroxyprogesterone, deoxycorticosterone, and 11-deoxycortisol) cause some inhibition of contractility in nonpregnant rat uterus *(58)*.

In summary, the analysis of these data indicates that some 5-reduced metabolites such as 5β-progestins (5β-pregnane-3,20-dione, 3β-hydroxy-5β-pregnan-20-one, 3α-hydroxy-5β-pregnan-20-one) and 5α- and 5β-androgens (5α- and 5β-dihydrotestosterone, androsterone, and androstanediol) are markedly more potent relaxants than their Δ4,3-keto precursors (progesterone or testosterone), while some 5α isomers of progestins (5α-pregnane-3,20-dione and 3β-hydroxy-5α-pregnan-20-one) are ineffective or have only slight potency. Corticosteroids inhibit some contractility at millimolar concentrations, when compared with the potent relaxing effect induced by progestins and androgens at micromolar concentrations. Interestingly, deoxycorticosterone produces a concentration-dependent biphasic effect; that is, high concentrations (millimolar range) elicit inhibition of contractility, whereas low concentrations (nanomolar range) produce excitation *(58)*. These and other results *(59,60)* determined the importance of 5-reduced metabolites on uterine contractility, in which progesterone plays a role as a pro-

hormone. In addition, these studies have established an interesting chemical structure–biologic activity relationship.

Progesterone and testosterone possess a Δ4,3-keto structure (progestins, androgens, and adrenal steroids), but the potency of their metabolites to induce uterine relaxation seems to be variable depending on their α/β configuration at C5 and subsequently 3α- or 3β-hydroxylation. Therefore, the 5β-reduction in the A-ring gives an optimal activity, whereas the relaxant potency of the 5α configuration is highly dependent on the subsequent 3-reduction. This interesting correlation has also been verified by the relaxing efficacy of these metabolites on uterine contractions induced by potassium *(59,60)*, oxytocin *(61)*, prostaglandins *(62)*, serotonin *(63)*, and acetylcholine *(64)*. The high relaxing potency on spontaneous contractions induced by some 5-reduced progestins was also confirmed in the rabbit by 3α-hydroxy-5α-pregnan-20-one, called by others allotetrahydroprogesterone or tetrahydroprogesterone *(65)*.

Recently, the relaxing effect of some 5-reduced progestins has been studied using human myometrium in vitro. The spontaneous contractility was not significantly inhibited by two 5α-progestins *(66,67)*, whereas 5β-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one were more potent relaxants than progesterone *(68)*. It has also been reported that 5β-pregnan-3,20-dione (5β-dihydroprogesterone) is able to prevent both the spontaneous and the oxytocin-induced contractions in human myometrium in vitro *(69)*. These data, taken together with the results from rat studies, raise the possibility that the progestins with a 5β configuration are very effective in inducing uterine relaxation.

On the other hand, the relaxing effect of progesterone and several of its metabolites has been shown in nonuterine smooth muscle. In this respect, contractile inhibition is induced by both progesterone and estrogens in the urinary tract and regional gastrointestinal tissues *(70–72)*, and by estrogens in different vascular beds including the uterine vasculature *(73–75)*. The 5-reduced progestins and androgens are able to reduce the spontaneous contractility of guinea pig ileum *(76)*, as well as the contractile response produced by barium in the rat epididymis and seminal vesicles *(77)*, by histamine or carbachol in guinea pig trachea *(78)*, and by norepinephrine *(79)*, potassium, and calcium *(80)* in rat aorta. Indeed, all these studies have shown the ability of 5-reduced metabolites to induce smooth muscle relaxation.

The 5-reduced natural progestins, pregnanolones, and pregnanediones have long been recognized by their anesthetic effects *(81)*. Certainly, the 5β-reduced compounds are more potent than the 5α-epimers in eliciting changes in brain electrical activity *(82)* and relaxing smooth muscle *(55,57,59,60,68,76,78,79,82)*.

All of these in vitro studies have shown the effect of steroids in smooth muscle tissues at the micromolar range, as a consequence of their low solubility in the bath solution (estimated from their physicochemical properties). However, it is also important to consider that in human late gestation the total secretion of placental progesterone is about 280 mg/d (approx 158 μ*M*/d), even higher than the effective concentrations of steroids to induce inhibition of uterine contractility.

Synthetic Steroids and Antihormones

In addition to the relaxing effect induced by endogenous steroids, a few studies have examined the potential relaxing effect induced by synthetic steroids on uterine contractility as it correlates with the uterine quiescence promoted by progesterone. For instance, some synthetic progestins (17α-hydroxyprogesterone caproate and medroxyprogesterone acetate) have been reported to have a uterine-relaxing effect in humans *(30,31),* as do some aminoestrogens *(34)*.

Althesin (alphaxolone), an anesthetic steroid whose active component is 3α-hydroxy-5α-pregnane-11,20-dione, and its 5β isomer (3α-hydroxy-5β-pregnan-11,20-dione) also have relaxing properties in vitro on spontaneous rat uterine contractions *(83)*. These anesthetic steroids (neuroactive steroids) are related chemically to pregnanolones and differ only in the 11-oxo radical.

Perusquía and Kubli-Garfias *(84)* have reported that the antihormone mifepristone (RU 486, 17β-hydroxy-11β-4-dimethylaminophenyl-17α-[1 propynyl]-estra-4,9-dien-3-one) produces an instantaneous relaxing effect, higher than that induced by progesterone at the same concentration, on uterine contractility of rat, subsequently followed by an increase in contractility several minutes later. Their study suggests that the relaxing effect observed could take place before the action at the receptor-genome level (genomic action) through a nongenomic mechanism.

On the other hand, it is generally accepted that RU 486 elicits uterine contractions, since in humans RU 486 enhances uterine contractions in early pregnancy *(85)*, and its use for favoring labor in delayed cases has been successful, shortening the time to complete delivery, decreasing the amount of oxytocin needed and the amount of pain *(86)*. Nevertheless, there are some contradictions. For example, clinical use of RU 486 for pregnancy interruption, and evacuating the product of conception, requires a small dose of prostaglandins subsequent to the development of antiprogesterone action *(85–87)*. This type of treatment in women indicates that RU 486 requires the assistance of a uterotonic agent to complete the process owing to the fact that the antihormone might, as a first step, be inducing uterine relaxation. In agreement with this is the report that, in monkeys, RU 486 produces only light contractions, insufficient to induce labor *(88)*. Other research has demonstrated that RU 486 treatment during late pregnancy in the rhesus macaque resulted in a transient decrease in uterine activity that occurred during the early morning hours immediately following the first dose of RU 486. Eight hours after treatment, uterine contractility increased progressively (long duration, low frequency, and high amplitude) but remained as a type of contraction pattern that was insufficient to induce delivery *(89)*.

To explain the uterotonic effect of RU 486, it has been demonstrated that RU 486 increases the formation of gap junctions between myometrial cells in rats *(90)* and guinea pigs *(91)*, which is in direct contrast with the inhibition in the formation of gap junctions induced by progesterone *(92)*. Therefore, a relaxing (nongenomic) effect may be the primary mechanism of RU 486 and an increase in gap junctions may be the secondary mechanism. However, more studies are required to clarify the complex role that this compound plays in uterine contractility.

It is known that some antiestrogens decrease uterine tone *(93,94)*. Tamoxifen in vitro reduces oxytocin-induced contractions in the rat uterus *(95)*. In addition to tamoxifen and its quaternary analog (tamoxifen ethyl bromide), other nonsteroidal antiestrogens such as clomiphene, nafoxidine, and ethamoxytriphetol, as well as the steroidal antiestrogen ICI 164,384, have been studied on rat uterine contractions *(96)*. These drugs inhibited contractions induced by oxytocin, calcium, methacholine, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and KCl. Therefore, the inhibitory effect of antiestrogens on uterine contractility has been described as independent of their binding to the intracellular estrogen receptor (ER), which could be mediated by an action to block calcium entry. In addition, tamoxifen may induce other actions at different levels that indirectly lead to uterine contractility; in fact, antiestrogens may reduce the release of prostaglandins *(97)*.

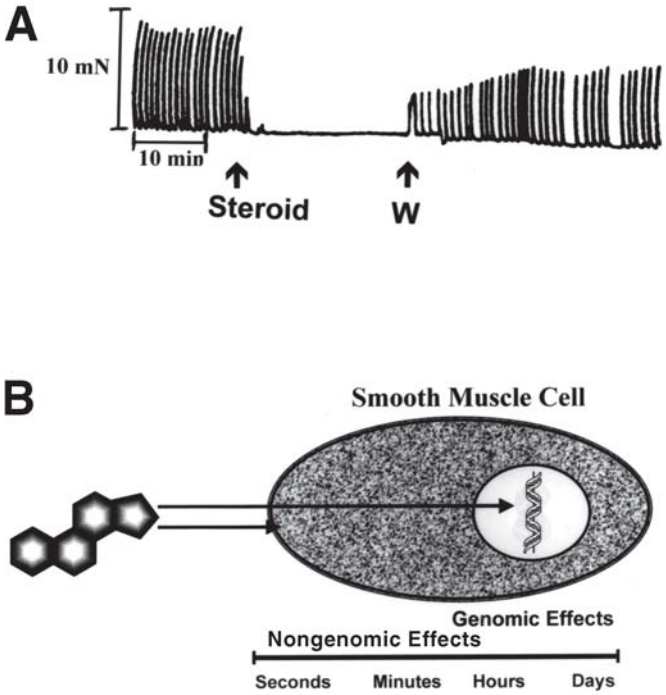

Fig. 1. (**A**) Typical tracing of spontaneous contractility in isolated rat uterus. Note that the immediate relaxing effect of steroid (5β-reduced metabolite at 4.6 μ*M*) is reversed after washout (W), showing that the steroid effect was reversible. (**B**) Characteristics of steroid action in the uterine smooth muscle cell. Nongenomic Effects: rapid in onset (seconds, minutes), short in duration, following disappearance of steroid from the tissue, not modified by antihormones or inhibitors of protein synthesis. Genomic Effects: slower in onset (minutes, hours), longer in duration, and persistence of effects after steroid is removed from tissue.

Mechanism of Action

Much of our understanding of the regulation of uterine contraction by progesterone and its metabolites, as well as their mechanism of action, comes from studies of other excitable tissue, also the target of steroids, such as the central nervous system (CNS). The classic studies from Selye *(81, 98)* indicated that a wide series of steroids may decrease the excitability of CNS by inducing anesthesia. Moreover, they are able to produce, by a nongenomic action, sedative/hypnotic and anticonvulsant effects *(99–101)*. There have since been numerous observations of steroid-induced changes in neuronal activity occurring with a latency from seconds to a very few minutes; these are direct actions and not mediated by intracellular receptor occupancy *(102–104)*.

In this respect, it has been shown that the latency for the relaxing effect of steroids on uterine contractility is instantaneous (<1 min), reversible (the effect disappears after the steroid is removed from the tissue), not modified by inhibitors of protein synthesis and transcription, and can be explained as a nongenomic (membrane) action *(59,105)* (Fig. 1). Nevertheless, the cellular or molecular basis of steroid action on uterine smooth muscle contractility has not yet been satisfactorily explained.

The mechanism presumably involved in the nongenomic relaxing effect of progesterone and its metabolites on uterine contractility is related to the blockade of extracellular calcium entry by inactivating the calcium channels (voltage- and receptor-activated calcium channels), the interaction of steroids with some membrane receptors ($GABA_A$ and oxytocin receptors), the interaction of steroids with plasma membrane steroid receptor, and the interaction with intracellular steroid receptors (genomic action through steroid receptor) (Fig. 2).

Does the Nongenomic Relaxing Action of Steroids Involve an Interaction with Calcium Channels?

It has been postulated that some ions are involved in the mechanism of action of steroids. For instance, it was described that estrogens are able to reduce the uptake of calcium by myometrial mitochondria, and progesterone decreases the calcium retention by the myometrium *(106,107)*. In recent years, it has been proposed, concurrently, that the mechanism of action of Δ4,3-keto and the 5-reduced metabolites to induce uterine relaxation might be achieved by blocking the external calcium influx into the smooth muscle cell. This hypothesis is supported by the fact that steroids relax the KCl-induced tonic contraction in a concentration-dependent manner *(59,60,108)*. In the depolarized myometrium, the addition of calcium to the media causes contraction and is reduced in a concentration-response manner by the presence of steroids. This shows an effect resembling that pro-

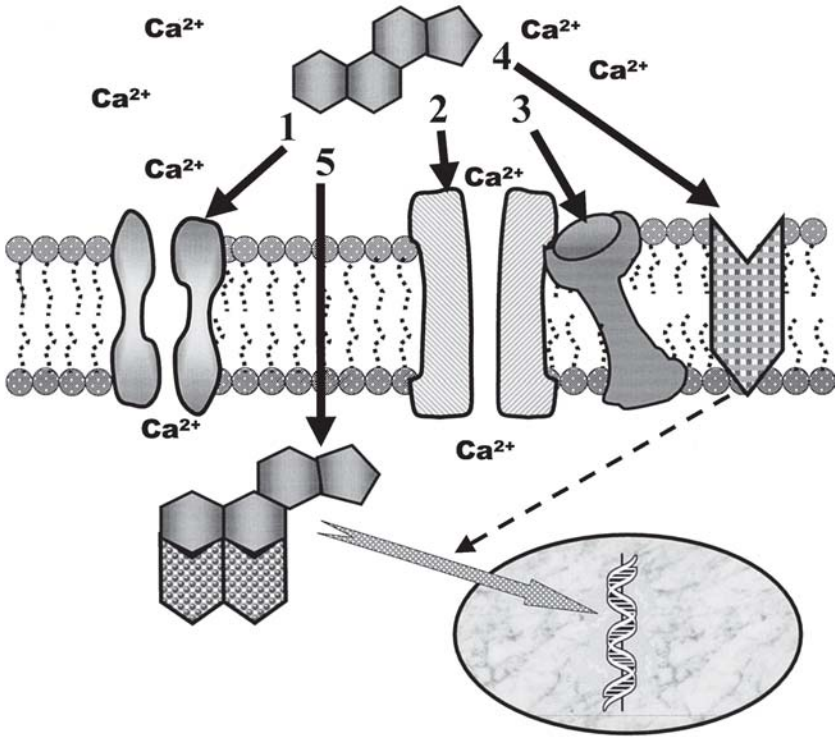

Fig. 2. Schematic model of the possible mechanism of action hypothesized for steroids to induce uterine relaxation. Steroid as a calci um blocker by inhibiting the extracellular calcium influx into the cell to inactivate (1) voltage-operated calcium channels and (2) receptor-operated calcium channels. (3) Steroids bind to distinct membrane receptors to inhibit the agonist function, (4) steroids bind to plasma membrane steroid receptor to complement the transcriptional actions of the intracellular receptor, and (5) steroids diffuse ins ide the cell to bind the intracellular progesterone receptor (PR) and activate transcription.

duced by a typical calcium-entry blocker (verapamil) and suggests that steroids might have a calcium-antagonistic property *(59)*. Steroid-induced relaxation is reversed by calcium *(60,108)* and calcium ionophores (A-23187 and X-537A) *(59)*. These findings suggest that the relaxing effect of steroids on uterine contractility is a consequence of calcium-entry blockade by inactivation of voltage-operated calcium channels (VOCs) *(59)*.

Apart from the VOCs that may be directly involved in the steroid action, other channels may play a significant role in determining the relaxing effect of steroids by regulating the external calcium influx. For instance, the interaction of excitatory agonists with their specific membrane receptors is able to open the receptor-operated calcium channels (ROCs) to induce calcium influx in smooth muscle cells *(109,110)*. It has been reported that endogenous steroids (Δ4,3-keto and the 5-reduced metabolites) with significant relaxant potency may also prevent or inhibit the contractions induced by serotonin *(63)*, acetylcholine *(64)*, PGE_2 and $PGF_{2\alpha}$ *(62)*, and oxytocin *(61)* in the isolated rat uterus (Fig. 3). Recently, it has been demonstrated that a 5β-reduced progestin (5β-pregnane-3,20-dione) antagonizes the oxytocin-induced contractions in human myometrium in vitro *(69)*. In summary, the referenced works in rat uterus have suggested that potent relaxation induced by steroids in uterine tissue precontracted by the aforementioned uterotonic agents implies a reduction in extracellular calcium influx by inactivation of ROCs.

Indeed, pharmacologic evidence indicates that steroids have similar relaxant effects on contractions induced by activation of both ROCs and VOCs, and this may imply that steroids are blocking the calcium entry of both (Fig. 2, steps 1 and 2).

In support of this view, it has been proposed that the relaxing effect of steroids in other smooth muscle preparations is also associated with the prevention of calcium inflow *(78,80,111)*. Furthermore, it has been reported that estrogens are blocking the VOCs in the pig *(112,113)* and gilt *(114)* uterine vasculature, and in rabbit isolated coronary preparations *(115)*. Additionally, a direct inhibitory effect of estradiol on voltage-dependent calcium currents has been demonstrated in A7r5 vascular smooth muscle cells line *(116)*, and in coronary and tail artery myocytes *(117, 118)*. It has also been found that some neurosteroids can rapidly and reversibly suppress voltage-gated calcium currents in hippocampal neurons *(119)*.

Is the Nongenomic Relaxing Action of Steroids Related to an Interaction with Membrane Receptors?

The mechanism of action of progesterone and its metabolites to induce their nongenomic effect in depressing cellular excitability has been the subject of controversy. A large body

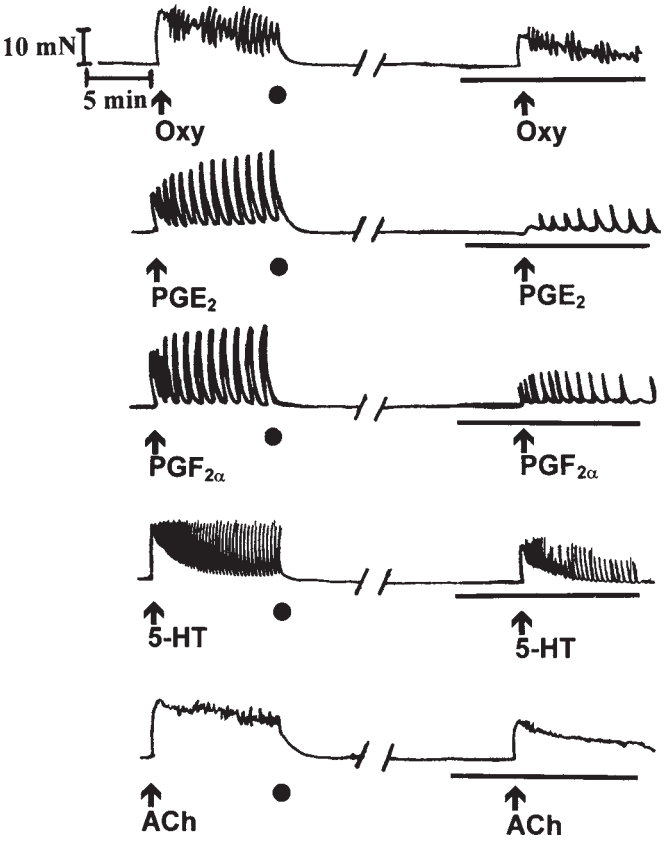

Fig. 3. Contractile responses induced by uterotonic agents: oxytocin (Oxy, 100 p*M*), PGE_2 (1.1 μ*M*) and $PGF_{2\alpha}$ (0.8 μ*M*), serotonin (5-HT, 10 μ*M*), and acetylcholine (ACh, 100 μ*M*) in the isolated rat uterus. The contractions were antagonized by steroid in a concentration-dependent manner. The solid black line indicates the incubation time of 5β-progestin, added at its IC_{50} calculated for each uterotonic agent (12.5 μ*M* Oxy, 4.8 μM 5-HT, 9.0 μ*M* ACh, 25 μ*M* PGE_2 and $PGF_{2\alpha}$). Circles represent the time of washout. (Adapted from refs. *61-64*.)

of data has shown that in the CNS, some neurosteroids and neuroactive steroids interact with $GABA_A$ receptors and modulate the function of associated chloride channels since the steroids are able to bind and potentiate the $GABA_A$ receptor in the brain. This interaction has been proposed as the mechanism of the anesthetic effect of those steroids *(120–122)*.

Consequently, it has been suggested that the uterine-relaxing effect induced by 3α-hydroxy-5α-pregnan-20-one in rabbit *(65)*, by this progestin and 3β-hydroxy-5β-pregnan-20-one in rat *(123)*, is mediated through the $GABA_A$ system. By contrast, other results *(60)* do not support an interaction of steroids with $GABA_A$ receptors since GABA and muscimol (a $GABA_A$ receptor agonist) from 1 μ*M* to 1 m*M* failed to produce any response on uterine contractility in pregnant, nonpregnant, and estrogenized rats, suggesting that the $GABA_A$ receptors are not involved in the physiologic modulation of contractility in the rat uterine musculature. Collaterally, in this study *(60)* it was also determined that bicuculline and picrotoxin ($GABA_A$ receptor antagonists) failed to block the uterine-relaxing effect of progesterone, testosterone, and a wide series of their 5-reduced metabolites, including the two steroids previously associated with the $GABA_A$ receptor as the locus of their action (see *refs. 65,123*). However, this finding is in partial agreement with the study of Putnam et al. *(123)*, who found that picrotoxin did not block the relaxation induced by progesterone and 5β-pregnane-3,20-dione. Probably the controversy over these finding is owing to species difference (rabbit and rat) or to the different experimental conditions. Likewise, bicuculline and picrotoxin also failed to block relaxation induced by progestins and androgens in vascular *(79)* and airway *(78)* smooth muscle.

On the other hand, it has been proposed that the effect of progesterone on uterine sensitivity to oxytocin involves direct nongenomic action of progesterone on the uterine oxytocin receptor (OTR). In a recent study, Grazzini et al. *(124)* showed that progesterone inhibits oxytocin binding to OTR-containing membranes in vitro and suppresses oxytocin-induced inositol phosphate production and calcium mobilization in the rat. However, oxytocin binding to the human OTR was unaffected by progesterone but was altered by its metabolite 5β-pregnane-3,20-dione. Their report suggested a direct interaction between a steroid hormone and a member of the large class of G-protein-coupled receptors (OTR).

Taken together, these findings infer the possibility of direct interaction of steroid hormones with specific membrane receptors to GABA or oxytocin (Fig. 2, step 3). However, this hypothesis is little supported by the evidence that steroids also antagonize uterine contractions induced by KCl *(59,60)*, serotonin, prostaglandins, and acetylcholine *(62–64)*. This is indicative of steroids not acting selectively on any kind of receptor as they inhibit contractions induced by different uterotonic agents, related or not, to membrane receptors. In fact, because of chemical differences among the variety of excitatory agonist receptors and steroids, the steroids probably do not bind at the same site. Decreased binding of oxytocin in the presence of steroids may be owing to conformational changes, i.e., membrane stabilization in myometrial plasma membranes induced by steroids. Thus, they are acting at the same time on different membrane proteins (Fig. 2). In addition, the possibility of an interaction of steroid hormones with inhibitory H_2-histaminergic or β_2-adrenergic receptors can be dismissed since their specific antagonists did not block the relaxing effect of steroids *(59)*.

Nevertheless, there may be other mechanisms by which steroids interact with cell membrane proteins to effect cellular actions. Pietras and Szego *(125)* identified a plasma membrane ER in uterine cells, which responded to estradiol with the increased production of the second-messenger cyclic adenosine monophosphate. The actions of the membrane ER in some ways are unique but, in other instances, precede and complement the transcriptional actions of the nuclear ER (Fig. 2, step 4). Paralleling this, evidence has emerged that other steroid receptors may exist in the membrane and exert rapid cellular effects. These include proges-

terone, mineralocorticoids, glucocorticoids, and 1,25 vitamin D; however, the plasma membrane receptor has not been isolated *(126)*.

Is the Uterine-Relaxing Effect of Steroids Related to Intracellular Receptor Occupancy?

Another line of evidence demonstrating the role of progesterone and its metabolites on uterine activity is based on progesterone antagonist action. In a single previous study on the combination of RU 486 and steroid administration in rat uterus in vitro, the effect of only two progestins (5β-pregnane-3,20-dione and progesterone) was blocked, suggesting that their action was mediated through the PR *(123)* (Fig. 2, step 5). This suggestion is inconsistent with the nongenomic action indicated by the uterine-relaxing effect of the steroid, in which the effect is instantaneous, terminates when the steroid is removed from the tissue, and is not blocked by inhibitors of protein synthesis, as well as by a complementary report that RU 486 did not block the relaxing effect of progesterone and several 5-reduced progestins in isolated human myometrium at term *(68)*. Thus, the possibility that steroids may act at the genomic level to inhibit uterine contractility has not yet been raised.

By contrast, PRs are present in abundance in uterine tissue from nonpregnant women and women in early pregnancy and are decreased with the advance of pregnancy to undetectable concentrations at term *(127)*. Proportionally inverse, there is a progressive increase in progestin plasma levels throughout gestation (Fig. 4). In this context, it is interesting that the steroid genomic way is not a necessary step in their mechanism of action to induce uterine quiescence.

In the present review, we have seen that natural steroids, particularly 5β-pregnane-3,20-dione, have been associated with different sites of action (calcium channels, membrane receptors, and intracellular PR (see Fig. 2). These findings are consistent with the possibility that progesterone and its metabolites have more than one effect on uterine smooth muscle. Therefore, steroid action involves setting into motion a chain of events that culminate in specific responses. Theoretically, steroids are modulating uterine contractility by nongenomic action, before the genomic actions that induce another physiologic response in the uterus (Fig. 1).

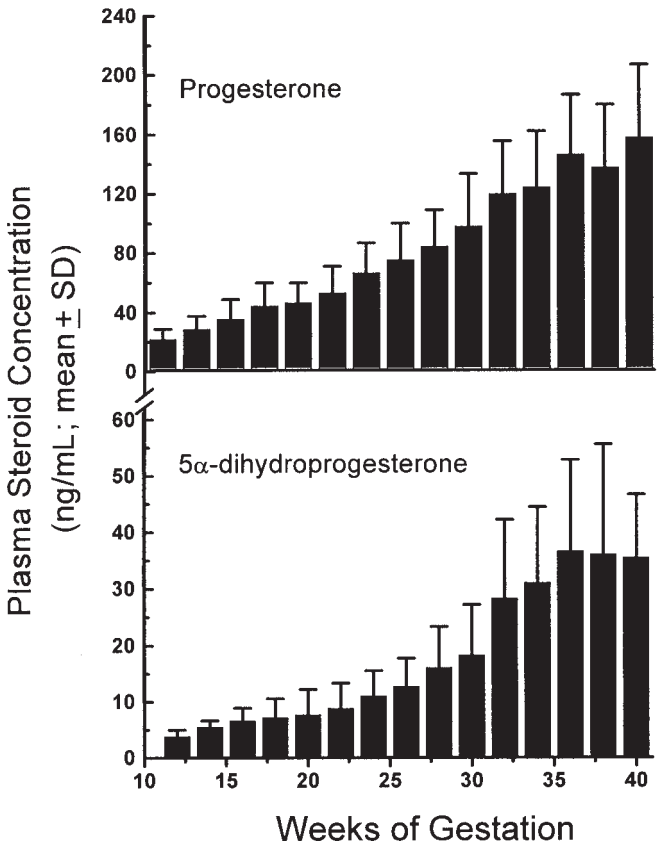

Fig. 4. Progesterone and 5α-pregnane-3,20-dione (5α-dihydroprogesterone) mean plasma levels ± SD during human pregnancy. (Data reprinted from ref. *131*.)

Progesterone Metabolism

Progesterone is secreted from the adrenal, the corpus luteum, and the placental trophoblast. Part of progesterone formed in the placenta can be transported to the fetus, where it is metabolized to a number of products, including 5-reduced metabolites such as 3α-hydroxy-5β-pregnan-20-one and 3β-hydroxy-5α-pregnan-20-one *(128)*.

The development of Δ4-5α-reductase and 3α- and 3β-hydroxy steroid dehydrogenase in rat fetuses and placentas has been detected. The activities of these enzymes were greater in the placenta than in the fetus. As pregnancy progresses, the activities increase in the fetus but diminish in the placenta. Although the placenta secretes only marginal amounts of progesterone, it has a notable capacity to metabolize progesterone to 5α-reduced metabolites. The fetus has the ability to utilize progesterone as early as d 13, and its potential to convert progesterone into A ring–reduced products increases during gestation *(129)*. Some studies have determined that the main enzymatic activities are 5α-reductase and 20α-hydroxysteroid dehydrogenase, and a small amount of 5β-reductase in the rat and human uterus in vitro *(130)*. Thus, the major metabolites of progesterone in pregnant women are 5α-pregnane-3,20-dione (5α-dihydroprogesterone) and 20α-dihydroprogesterone, and it has also been shown that the concentration of progesterone and its main 5-reduced metabolite (5α-dihydroprogesterone) in blood increase greatly during pregnancy *(131)* (Fig. 4).

Moreover, evidence that the 5β metabolites are found in urine during pregnancy as 5β-pregnane-3α,20α-diol (pregnanediol), 3α-hydroxy-5β-pregnan-20-one and its 3β-isomers *(128,132,133)*, as well as the presence of 5β-pregnane-3,20-dione in myometrium *(134)* implies that progesterone was metabolized toward 5β compounds, which indicates the presence of 5β-reductase, identified in rat *(135)* and human uterus *(136,137)*.

Thereafter, the 5-reduction of the A ring allows the subsequent 3α- or 3β-reduction of the 3-oxo group by the 3α- or 3β-hydroxy steroid oxidoreductase. However, the distribution of each enzyme and their different isoforms regarding hormonal status in the uterus and other tissues needs to be examined.

The fact that the 5-reduced steroids are detected in the female reproductive system and their serum levels are progressively increased throughout gestation *(131)* is consistent with the possibility that these metabolites are inducing uterine relaxation during pregnancy. Therefore, uterine contractility might be modulated by progesterone accompanied by those 5-reduced metabolites, negating the concept that they are just hormonally inactive excretion products. In addition, the metabolites from progesterone possess structural similarities (cyclopentenophenanthrene), which may explain their relaxing progesterone-like action, in some cases with higher potency than their precursor, progesterone, owing to certain chemical alterations discussed previously.

Conclusion

The old concept that parturition is solely effected by progesterone withdrawal is too simple to account for the precision in which the process occurs. It still clearly forms the basis of our thinking for different species of mammals, including human. We have seen that progesterone and its 5-reduced metabolites may modulate uterine activity by inducing uterine relaxation. From pharmacologic evidence, one could suggest a nonspecific membrane (nongenomic) effect, which may have a mechanism involving calcium fluxes. However, the reports indicate that multiple mechanisms exist in the uterus for inhibiting uterine contractility by progesterone and its metabolites. Future systematic studies will have to consider both the nongenomic and genomic effects of steroids. New synthetic derivatives should allow their therapeutic use as tocolytic agents with significant relevance in gynecology and obstetrics.

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