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Unusual Steroid Specificity of the Cell Surface Progesterone Receptor on Human Sperm

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SUMMARY

The steroid specificity of the cell surface progesterone receptor in human sperm was examined with the use of progesterone, testosterone, and androstane analogues. Many compounds were shown to be more effective than progesterone at increasing intracellular free calcium concentration, e.g., 2α-methyl-17β-methoxy-5α-androstan-3-one. Several testosterone analogues were demonstrated to be antagonists of progesterone, e.g., 9(11)-dehydro-2α,17α-dimethyltestosterone. The synthetic potent progestigens, norethynodrel, cyproterone acetate, norethindrone, and megestrol acetate, were found to be only weak stimulators of the sperm cell surface receptor. Furthermore, these compounds were shown to antagonize the effect of progesterone to elevate intracellular free calcium concentration in sperm. It is known that progesterone and some of its analogues bind to the intracellular progesterone nuclear receptor via the α-face of the steroid molecule. In stark contrast, it was concluded from the analysis of the steroid analogues examined on human sperm in this study that intimate contact exists between the effective progesterone analogues and the sperm cell surface progesterone receptor across the β-face of the steroid C/D-ring "upper" edge (C11, C12, and C17). Positioning of the C21 methyl group is also critical for efficacy, and recognition of the steroid A-ring seems not to be involved.

It has been shown recently that progesterone interacts with a cell surface steroid receptor on human sperm, causing an immediate increase in $[Ca^{2+}]_i$, which results in the acrosome reaction (1–8). The acrosome reaction is required to occur before the sperm can successfully fertilize the oocyte (9, 10). Characterization of this cell surface progesterone receptor is justified not only because of the potentially important function of this receptor in fertilization but also because similar receptors may exist in brain and other tissues (e.g., Ref. 11 and references therein). The presence of a cell surface steroid receptor on the head of human sperm is inferred from the following data. Progesterone covalently bound to albumin, a high molecular weight protein that does not enter the cell (4), is able to mimic the effects of unbound progesterone to elevate $[Ca^{2+}]_i$ (4) and the acrosome reaction (6), albeit with less potency (4, 6). The binding of the progesterone/albumin complex to the sperm head was directly visualized with the additional coupling of fluorescein-isothiocyanate to the albumin complex (5, 12, 13).

Preliminary studies indicated that the sperm progesterone receptor was very different from the nuclear progesterone receptor. For example, the very potent anti-progestin RU486 was a very weak antagonist of the sperm cell surface progesterone receptor (4, 14, 15). Also, several potent progestigens were shown to be ineffective agonists at the sperm receptor (4). In this study, we examined the effects of several progesterone analogues and related steroids on the sperm cell surface progesterone receptor. This receptor is also referred to as a nongenomic progesterone receptor (11) because stimulation of this receptor initiates a rapid calcium influx (≤1 sec) that is not mediated by a nuclear (or genomic) progesterone receptor. The objective of the current study was to identify the structural requirements for steroids to be either effective agonists or antagonists at the cell surface progesterone receptor on human sperm.

Materials and Methods

Human sperm, obtained through masturbation from proven fertile healthy donors, were loaded with Fura-2 as described previously (3). $[Ca^{2+}]_i$ was measured in suspensions of sperm by monitoring changes in Fura-2 fluorescence with a SPEX ARMC spectrofluorimeter (3, 4). In some experiments, concentration-response relationships were examined for progesterone and several progesterone analogues. The efficacy of many steroid analogues to increase $[Ca^{2+}]_i$ was examined with the use of a 10 μM concentration, and the effect was compared with 10 μM progesterone (a maximally effective concentration). The data shown for each steroid are the mean ± standard error of three to six separate experiments and are represented as percentage of the effect observed with progesterone. The concentration of progesterone in follicular fluid after ovulation is 3–6 μM (16).

Steroids were obtained from The Upjohn Company (Kalamazoo,

ABBREVIATION: $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration.

MI), Steraloids, Inc. (Wilton, NH), Sigma Chemical Co. (St. Louis, MO), and Schering A.G. (Berlin, Germany). The reference data for the steroids are as follows (numbers in brackets are Chemical Abstracts Registry numbers): **1**, [57–83-0]; **2**, [1162–54-5]; **3**, [1995–23-9]; **4**, [2636–91-1]; **5**, [566–65-4]; **6**, [15981–49-4]; **7**, [903–71-9]; **8**, [83603–61-6]; **9**, [17652–16-3]; **10**, [80–75-1]; **11**, [2268–98-6]; **12**, [600–57-7]; **13**, [24377–08-0]; **14**, [472–54-8]; **15**, [434–18-4]; **16**, $C_{22}H_{34}O_2$; **17**, [34184–77-5]; **18**, [68–96-2];**19**, [17308–02-0]; **20**, [630–56-8]; **21**, [14772–75-9]; **22**, [520–85-4]; **23**, [71–58-9]; **24**, [3562–63-8]; **25**, [595–33-5]; **26**, [2098–66-0]; **27**, [427–51-0]; **28**, [68–22-4]; **29**, [51–98-9]; **30**, [68–23-5]; **31**, [797–63-7]; **32**, $C_{21}H_{28}F_2O_2$; **33**, [2300–07-4]; **34**, $C_{21}H_{29}ClO_2$; **35**, [95160–20-6]; **36**, [604–28-4]; **37**, [516–55-2]; **38**, [72654–84-3]; **39**, [128–20-1]; **40**, [2243–08-5]; **41**, [2300–03-0]; **42**, [2300–02-9]; **43**, [2300–06-3]; **44**, [604–20-6]; **45**, [604–19-3]; **46**, [2640–71-3]; **47**, [15981–54-1]; **48**, [600–57-7]; **49**, [26137–58-6]; **50**, [339–02-6]; **51**, [378–38-1]; **52**, $C_{21}H_{30}O_4$; **53**, [14918–33-3]; **54**, [600–48-6]; **55**, $C_{29}H_{42}O_4$; **56**, $C_{28}H_{36}O_5S$; **57**, [14418–07-6]; **58**, [2701–48-6]; **59**, [1582–62-3]; **60**, $C_{22}H_{30}O_3$; **61**, [3642–85-1]; **62**, [14529–56-7]; **63**, [600–59-9]; **64**, [565–99-1]; **65**, $C_{22}H_{28}O_3$; **66**, [22614–40-0]; **67**, [20757–34-0]; **68**, [4031–30-5]; **69**, [599–14-4]; **70**, [600–73-7]; **71**, [83583–32-8]; **72**, [83583–31-7]; **73**, [16031–66-6]; **74**, [595–67-5]; **75**, [2525–60-7]; **76**, [83583–33-9]; **77**, [1096–38-4]; **78**, [438–07-3]; **79**, [1424–09-5]; **80**, [596–69-0]; **81**, [570–59-2]; **82**, [2477–61-4]; **83**, [80966–18-5]; **84**, $C_{29}H_{39}NO_3$; **85**, $C_{22}H_{32}O_3$; **86**, [641–77-0]; **87**, $C_{22}H_{32}O_4$; **88**, [603–98-5]; **89**, [17652–16-3]; **90**, [1882–82-2]; **91**, [595–77-7]; **92**, [29371–92-4]; **93**, [1097–51-4]; **94**, [378–36-9]; **95**, $C_{24}H_{33}FO_5$; **96**,[145–14-2]; **97**, [15137–31-2]; **98**, $C_{22}H_{34}O_3$; **99**, [64–85-7]; **100**, [298–65-7]; **101**, [50–22-6]; **102**, [600–67-9]; **103**, [640–39-1]; **104**, [599–17-7]; **105**, [152–58-9]; **106**, [548–97-0]; **107**, [566–35-8]; **108**, $C_{25}H_{36}O_6$; **109**, [13990–32-4]; **110**, [68374–18-5]; **111**, [61478–49-7]; **112**, [17925–66-5]; **113**, [13900–29-3]; **114**, [68374–20-9]; **115**, [75442–50-1]; **116**, [68374–16-3]; **117**, [70503–91-2]; **118**, [61478–48-6]; **119**, [75246–12-7]; **120**, [75246–13-8]; **121**, $C_{20}H_{30}O_2$; **122**, $C_{21}H_{32}O_2$; **123**, $C_{23}H_{34}O_2$; **124**, $C_{20}H_{32}O_2$; **125**, [75246–08-1]; **126**, [75246–07-0]; **127**, $C_{20}H_{28}O_2$; **128**, $C_{20}H_{32}O_2$; **129**, [75246–01-4]; **130**, $C_{21}H_{34}O_2$; **131**, $C_{21}H_{32}O_2$; **132**, [1452–40-0]; **133**, [58–19-5]; **134**, [3704–07-2]; **135**, [2066–31-1]; **136**, [2233–69-4]; **137**, [13886–37-8]; **138**, [58–18-4]; **139**, [5197–22-8]; **140**, [1605–89-6]; **141**, [13611–00-2]; **142**, [1039–17-4]; **143**, [5287–58-1]; **144**, [17021–26-0]; **145**, [1605–89-6]; **146**, [17021–26-0]; **147**, [1093–99-8]; **148**, [3704–09-4]; **149**, [19990–39-7]; **150**, [855–19-6]; **151**, [13452–06-7]; **152**, $C_{22}H_{32}O_3$; **153**, $C_{23}H_{29}F_3N_2O_2$; **154**, $C_{21}H_{32}O_2$; **155**, $C_{21}H_{32}O_3$; **156**, $C_{21}H_{32}O_3$; **157**, $C_{22}H_{32}O_2$; **158**, $C_{21}H_{26}O_3$; and **159**, $C_{22}H_{30}O_4$.

Approximately one half of this steroid collection was examined directly [silica thin layer chromatography in the two solvent systems: hexane/ethylacetate (1:1) and toluene/acetone (2:1)] for purity. In general, the purity of these samples was found to be excellent. Of the steroids examined, those lacking adequate purity were excluded. Nevertheless, as discussed recently in connection with a quite different biological study, it is possible for biological efficacy to arise from even trace impurities within a steroid sample (17). For this reason, virtually all of the key conclusions within this report derive from self-consistent structure-efficacy correlations across a subgroup of the steroids. Conclusions for which this is not the case are identified clearly.

Sources for the other reagents have been described previously (3). A cytosolic fraction that contained progesterone receptors was prepared from uteri of rabbits 5 days after treatment with Depo-estradiol cypionate as described (18). Aliquots of this cytosolic fraction were incubated for 20 hr at 4° with [^{3}H]progesterone (1.4 nM, ~66 Ci/mmol) and competing steroids. Bound and free steroids were separated with the use of a dextran/charcoal method (18), and the relative binding affinity of each steroid was determined at the 50% competition level according to the method of Korenman (19). Progesterone was assigned a relative binding affinity value of 100%.

Results

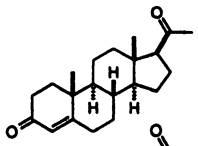
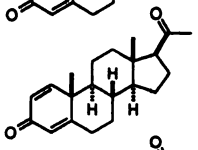
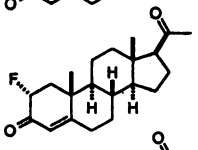
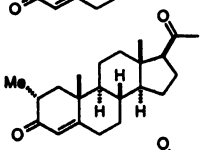
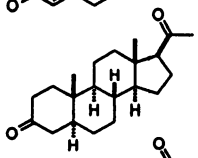
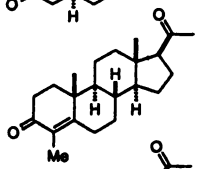
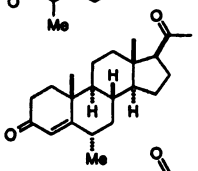
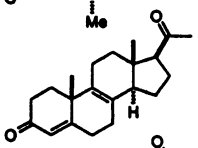
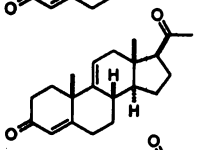
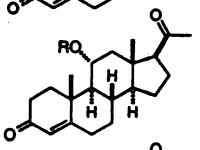
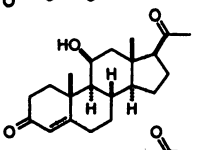
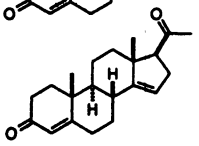
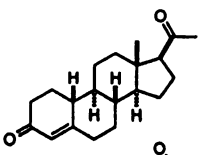
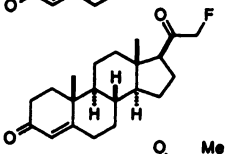
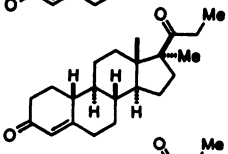
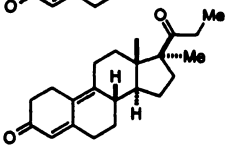
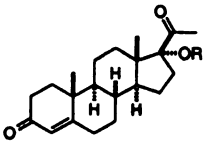
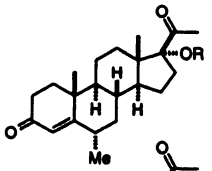
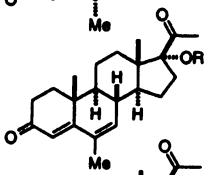
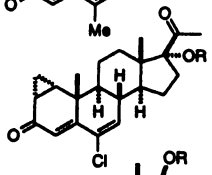
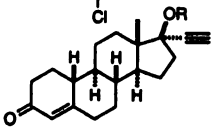
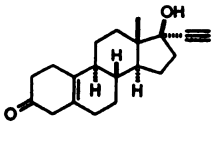
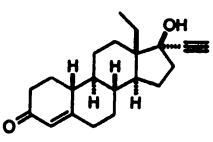
Unique structure-efficacy relationships for the sperm and uterine progesterone receptors. More than 100 progesterone analogues were used to delineate the structural requirements for the human sperm surface progesterone receptor-dependent acute increase in $[Ca^{2+}]_i$. This set was augmented by additional sets of androstanone and testosterone steroids and a smaller set of miscellaneous steroids. As is to be anticipated from this breadth of structure, a diversity of efficacy (including both agonist and antagonist behavior) was found. With regard to $[Ca^{2+}]_i$ increase (agonist behavior), many of the analogues were less effective than progesterone (**1**) (Table 1), but some were both more potent and more effective. Furthermore, it became evident that the structure-efficacy relationship for the sperm cell surface receptor was quite different from that of the "classic" nuclear progesterone receptor, which elicits actions at the level of the genome. The set of 30 progesterone analogues that demonstrated the structure-efficacy divergence for these two progesterone receptors is given in Table 1. The data given in Table 1 are the relative (compared with progesterone, set at 100%) sperm Ca^{2+} influx and relative binding to the genomic uterine receptor.

Although the contrasting structure-efficacy requirements for the two receptors are evident across the entire set, particular attention is drawn to the following analogues. Steroids **2–6** of Table 1 are progesterone analogues with steroid A-ring substitution. Although it will be shown subsequently that the core A-ring structure is actually unnecessary for sperm receptor agonist efficacy, it can be substituted advantageously. Each of these steroids show improved relative efficacy compared with **1**. In contrast, relative binding to the uterine receptor was diminished in each case. The 6α-methyl of **7** reduced efficacy somewhat for both receptors. The introduction of additional unsaturation in the steroid B/C/D-rings (by placing a double bond at positions 8,9 gives **8**; 9,11 gives **9**; and 14,15 gives **13**) diminished the relative uterine receptor binding but for **8** and **9** improved sperm Ca^{2+} influx. Two additional substitutions to the progesterone structure that improved sperm Ca^{2+} influx, but diminished uterine binding, were those of the 11β-hydroxyl (**12**) and the 21-fluorine (**15**). Conversely, the genomic progestins 19-*nor*-methyl-17,21-dimethylprogesterone (**16**), promegestone (**17**), and norethindrone (**28**) bound to the uterine receptor equally well (or better than progesterone) yet were poor at increasing sperm Ca^{2+} influx. A plot of the two activities for the steroids of Table 1 gave no correlation ($r = -0.28$).

Clearly, the extensive structure-efficacy data established for the uterine progesterone receptor are of no predictive value for understanding the structure-efficacy relationships for the sperm cell surface progesterone receptor. Therefore, a comprehensive examination of the basic sperm progesterone receptor structure-efficacy relationships was undertaken, using the additional sets of analogues in Figs. 1, 6, 7, and 12 (see later). Fig. 1 shows the effect of substituents on many of the carbon atoms of the progesterone structure. In general, substituents at C2, C4, C11, C12, C21, and especially C17 affected the sperm receptor, whereas there was a tolerance for substitution (little effect) at C6 and C9α. The structure-efficacy observations are presented sequentially (stepwise from C2 to C21).

TABLE 1

Comparison of select progesterone analogues for their relative Ca^{2+} ion influx in human sperm and for relative binding to the rabbit uterine cytosolic progesterone receptor

Steroid		Relative sperm Ca^{2+} ion influx[a]	Relative uterine receptor binding
	1	(100)	(100)
	2	114 ± 13	23
	3	144 ± 18	51
	4	117 ± 19	17
	5	117 ± 25	9
	6	142 ± 31	17
	7	85 ± 24	72
	8	129 ± 20	29
	9	139 ± 15	79
	10 R = H 11 R = Ac	35 ± 6 9 ± 6	4 <1
	12	183 ± 33	42
	13	66 ± 19	88
	14	95 ± 13	156
	15	149 ± 38	65
	16	1 ± 1	162
	17	1 ± 1	163
	18 R = H 19 R = Formyl 20 R = Acetyl 21 R = nCaproyl	96 ± 5 55 ± 8 16 ± 8 21 ± 3	3 21 89 34
	22 R = H 23 R = Ac	75 ± 5 14 ± 3	6 70
	24 R = H 25 R = Ac	79 ± 8 2 ± 2	ND[b] 89
	26 R = H 27 R = Ac	60 ± 7 0	ND[c] ND[c]
	28 R = H 29 R = Ac	8 ± 4 49 ± 5	100 59
	30	12 ± 2	20
	31	1 ± 1	78

[a] Mean (at least three determinations) ± standard error.
[b] N.D. = not determined.
[c] The relative binding affinities of these steroids for the rabbit uterine progesterone receptor have been reported to be cyproterone acetate **27** (85%) >> cyproterone **26** (28).

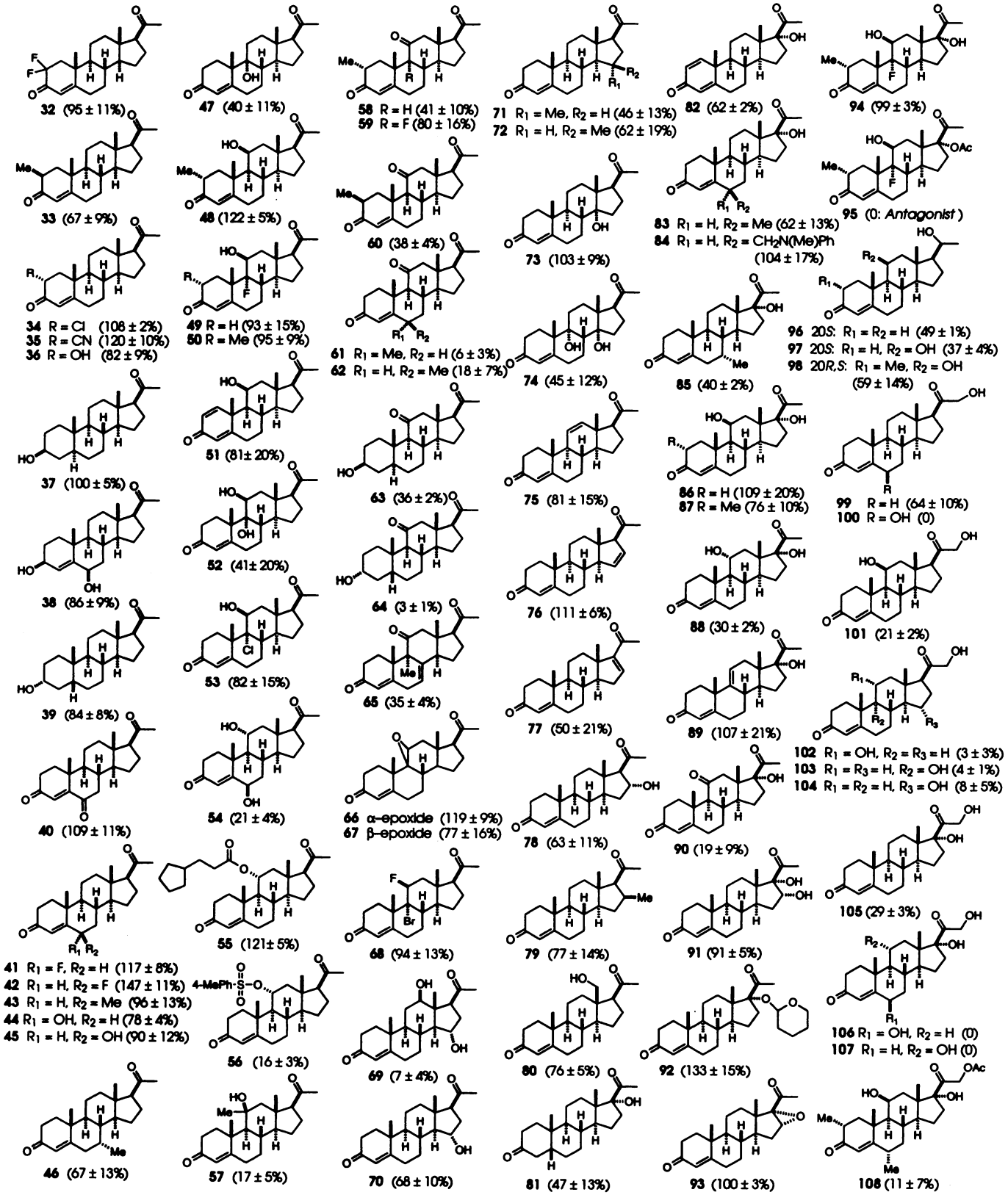


Fig. 1. The remaining set of progesterone analogues with relative sperm Ca^{2+} ion influx (percentage relative to **1**). The arrangement follows the approximate order of the position of the substituent, beginning with C2 (structure **32**) and ending with C21 (**99–108**).

Consequences of progesterone substitution on agonist efficacy and potency. When comparing the efficacy of the steroid analogues to increase $[Ca^{2+}]_i$, the following approximate terms were used (Fig. 1): full or equal efficacy (75–105%), good efficacy (40–75%), and poor efficacy (≪40%). The initial structure-efficacy assessments of Table 1 showed the 2α-fluoro (**3**) and 2α-methyl (**4**) progesterones to be more effective than progesterone. Accordingly, the dose-response effect of 2α substitution, including both **3** and **4**, and the 2α-chloro (**34**) and 2α-cyano (**35**) progesterones were examined in a potency assay with **1**. Each of these four analogues was more potent (5–10-fold) than progesterone (**1**).

Curiously, the 2,2-difluoroprogesterone (**32**) was 10-fold less potent than progesterone (Fig. 2). Geminal difluoro substitution, which in the example of fatty acids alters the molecular structure much more than monofluoro substitution (20), may unfavorably distort the steroid. The 2β-methylprogesterone (**33**) was approximately one third as potent [and thus 30-fold less than its 2α-methyl (**4**) steroisomer] and 67% as effective as **1** (Fig. 3). As noted above, methyl placement at C4 gives **6**, with a relative sperm Ca^{2+} influx of 142% of that of progesterone.

The unimportance of the progesterone 3-ketone was established by the full efficacy of the 4,5α-dihydro-3β-ol (**37**). Neither the 4,5-double bond (compare **1** and **5**) nor the 3-ketone was required for efficacy. Even more astonishing was the good efficacy of the 4,5β-dihydro-3α-ol (**39**). The 5β configuration imparts a *cis* relationship between the steroid A- and B-rings, resulting in a downward (rather than outward) projection of the A-ring. Both C2 and C3 of **39** occupy different regions when bound within the receptor compared with C2 and C3 of **37**. Thus, the 3-ketone of **1** cannot contribute to sperm receptor recognition, and likewise the 2α substituents noted above contribute primarily to potency rather than to efficacy. This contrasts with the potent genomic progestins, all of which contain a 3-ketone. The progestin desogestrel does not contain a 3-ketone; however, it is a prodrug without direct genomic efficacy until it undergoes conversion in the liver to the active 3-keto-desogestrel metabolite (21). In the present sperm assay, it is very unlikely that steroids devoid of 3-oxygenation are converted to 3-keto steroids. This is because (i) the time courses of the steroids to increase $[Ca^{2+}]_i$ were identical regardless of whether there was a C3 ketone (data not shown) and (ii) the increase in $[Ca^{2+}]_i$ induced by all of the steroids occurred without delay (≤1 sec).

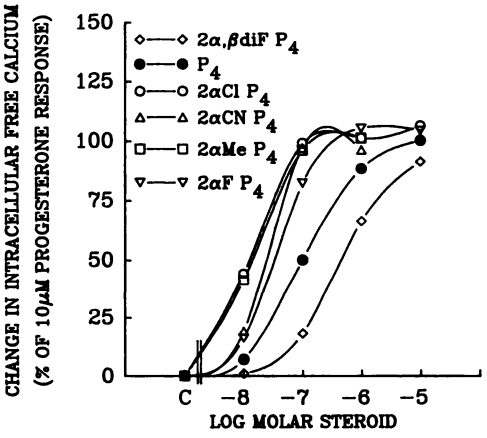


Fig. 2. Dose-response relationship of various progesterone derivatives (**1**, **3**, **4**, **32**, **34**, and **35**) with groups in the 2α position to increase $[Ca^{2+}]_i$. Progesterone derivatives at various concentrations were added to Fura-2-loaded human sperm. The response at each concentration was determined as the difference between the basal $[Ca^{2+}]_i$ (before steroid addition) and the maximum level of $[Ca^{2+}]_i$ (usually 15–20 sec after steroid addition). Each value is the mean from three separate experiments.

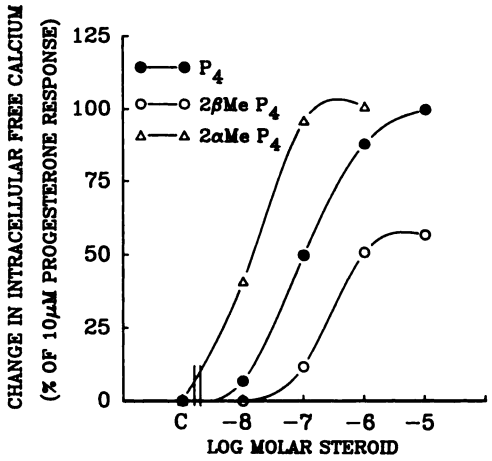


Fig. 3. Dose response of progesterone (**1**), 2β-methylprogesterone (**33**), and 2α-methylprogesterone (**4**) to elevate $[Ca^{2+}]_i$. See legend for Fig. 2 for other details.

Several potent synthetic progestins have C6 substitutions, such as medroxy progesterone acetate (**23**), megestrol acetate (**25**), and cyproterone acetate (**27**); the effect of C6 substituents on sperm receptor Ca^{2+} influx was therefore of particular interest. Several C6-substituted progesterones were evaluated in an identical efficacy assay to that reported in Table 1. Both C6 fluoroprogesterones were relatively more effective than **1** [6α-fluoro (**41**), 117%; 6β-fluoro (**42**), 147%]. The 6-keto (**40**), 6α- and 6β-methyl derivatives (**7** and **43**), and 6-hydroxyl diastereomers (**44** and **45**) were essentially equally effective as **1**. The full agonist efficacy of the 3β,6β-diol (**38**) is in complete accord with the structure-efficacy patterns discussed so far. The overall data are suggestive of a relative unimportance of the C6 substituent to the sperm receptor response, as if these small C6 substituents are not in contact with the receptor. The good agonist efficacy of the 7α-methyl (**46**) is consistent with the possibility that this open space extends to C7.

The presence of an 11β-hydroxyl (**12**) but not an 11α-hydroxyl (**10**) substituent increased significantly the effectiveness, but not the potency, of sperm Ca^{2+} influx relative to **1** (Fig. 4). The combination of a 2α-methyl and an 11β-hydroxyl (**48**) resulted in increased effectiveness and potency (Fig. 4). The disubstituted 9α-fluoro-11β-hydroxyl (**49**) and the trisubstituted 2α-methyl-9α-fluoro-11β-hydroxyl (**50**) were of equal effectiveness (93% and 95%, respectively). Although these data confirm a receptor binding acceptance of the 11β-hydroxyl, no simple conclusion can be made concerning 11α substitution. Substitution of the 11α-hydroxyl of **10** with an acetyloxy (**11**) or large, rigid tosyl (**56**) reduced efficacy (9% and 16%, respectively), whereas the conformationally flexible (3-cyclopentyl)propionyl (**55**) retained effectiveness (121%). The substantial decrease in efficacy for the 11α-methyl-11β-hydroxylprogesterone (**57**) to 17% is further suggestive of an unfavorable receptor sensitivity to certain 11α substituents (possibly not a steric effect, given the favor-

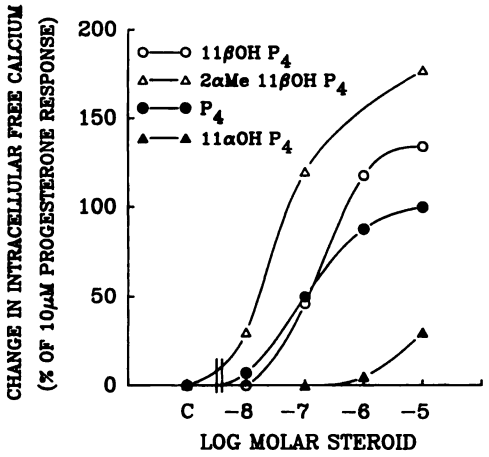


Fig. 4. Dose response of progesterone (**1**), 11β-hydroxylprogesterone (**12**), 2α-methyl-11β-hydroxylprogesterone (**48**), and 11α-hydroxylprogesterone (**10**) to increase sperm $[Ca^{2+}]_i$. See legend for Fig. 2 for other details.

able binding of **55** with its large 11α substituent). Both epoxide diastereomers of the active 9,11-dehydro steroid (**9**) were effective agonists, with the 9,11α-epoxide (**66**) possessing increased effectiveness (119%), whereas the 9,11β-epoxide (**67**) was slightly less efficacious (77%).

A tolerance for substitution, as was seen for C6 analogues, was seen also for 9α substitution, including fluorine (**49** and **50**), hydroxyl (**52**), chloro (**53**), and bromo (**68**) substitution. This latter 9α-bromo-11β-fluoroprogesterone retains full efficacy (**68**). A fluorine atom, as found in **49** and **50**, is a sterically small but strongly dipole active (in this respect, similar to an hydroxyl) substituent, but the 9α-bromo of **68** is a substantial functional group, projecting downward in the center of the α-face of the steroid A,B-rings. The general tolerance for 9α substitution and the particular efficacy of **68** argue for the entire A,B-ring α-face region of the steroid as being unimportant to sperm receptor recognition (see Discussion).

The receptor data for the set of eight 11-keto progesterones (**58-65** and **90**) are complex but suggestive of a moderately disadvantageous effect for the solitary 11-ketone (perhaps similar to that of the 11α-hydroxyl). However, each structure of this set combines the 11-ketone with other substituents. A small decrease in agonist efficacy was seen when the 11-one was combined with the favorable 2α-methyl substituent (**58** and **59**) and when combined with the less favorable 2β-methyl (compare **33** with **60**) and equipotent 3β-ol (compare **37** with **63**). In contrast, the combination of the 11-ketone with a 6-methyl (regardless of configuration) (compare **7** with **61**) (**43** and **62**) or 3α-ol (compare **39** with **64**) resulted in a significant loss in agonist efficacy. The behavior of the 6-methyl-11-one set (**61** and **62**) is particularly difficult to explain, as the generally good agonist efficacy of the 6-methylprogesterones (**7** and **43**) can be interpreted only in terms of relative steric freedom for the 6-methyl of the receptor-bound 6-methylprogesterones. An examination of the most stable steroid conformation (to inquire, for example, whether a substantial conformational alteration is transmitted to the 6-methyl by the 11-ketone) is sometimes of value in these circumstances. A comparison of the energy-minimized conformations of **7** and **61** (Fig. 4) was, however, rewarding.

The effect of the 11-ketone was localized to hardly perceptible differences at C12, the C18 methyl, and the steroid D-ring; no effect was detectable at C6 (Fig. 5). The large differences in biological efficacy between these steroids, despite similar structures, may be interpreted in terms of a presentation to the receptor of a steroid conformation other than that of the most stable solution conformation. Thus, two possibilities exist. 6α-Methylprogesterone-3,11-dione (**61**) may bind in a low-energy conformation (different than that shown in Fig. 5) that indeed results in a substantial relative movement of the 6-methyl compared with **7**. Alternatively, the conformation of **7** recognized by the steroid may indeed be similar to that shown in Fig. 5, but as a result of unfavorable steric or electrostatic interactions between **61** and the receptor, the analogous conformation of **61** (shown in Fig. 5) is energetically unfavored. These represent intrinsic limitations to the comparison of energy-minimized solution conformations to bound ligand structure-efficacy interpretation. This uncertainty pertains also to the several additional conformations of other steroids discussed below. Nevertheless, the overall data with the 11-keto steroids are consistent with the previous observations in reinforcing a focus on the C/D-ring region as particularly critical to progesterone agonist efficacy.

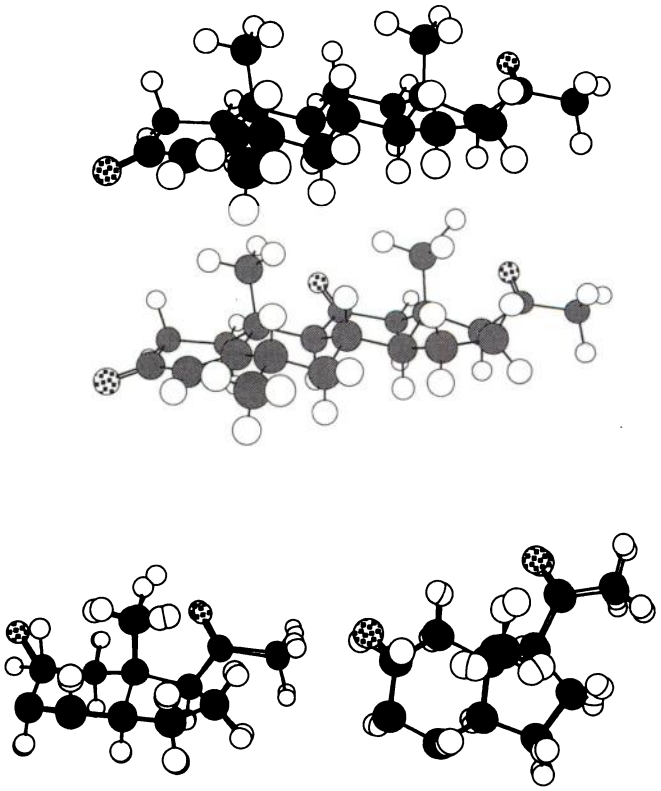


Fig. 5. Most stable conformations of the effective 6α-methylprogesterone agonist (**7**) (*top*) and the weakly effective agonist 6α-methyl-11-ketoprogesterone (**61**) (*middle*) in ball-and-stick perspective. The computation of the most stable conformation (MM2 energy minimization) and rendering of the conformational structure were performed with Chem3D (Cambridge Scientific). Steroids are shown with the A-ring to the *left* and with carbons C4 and C6 in closest view. *Bottom*, overlay of the C/D-rings of (**7**) and (**61**) in edge (*left*) and top (*right*) perspectives. The significant loss in agonist efficacy on 6α-methyl-11-keto substitution must derive from an extraordinarily subtle conformational and electronic effect. Atoms: *black*, carbon; *white*, hydrogen; *dotted*, oxygen.

A substantial majority of the progesterone analogues in Table 1 and Fig. 1 retain agonist efficacy. Although the overall sense of these data is in agreement with the opinion that the sperm receptor is a receptor for progesterone, it is remarkable that substitution on only two of the progesterone carbon atoms is disabling (e.g., immediate loss of agonist efficacy on introduction of a single, preferably small, substituent). Disabling substituents are of particular value in the visualization of the receptor surface as they identify points where the agonist contact is intimate. The first of these two disabling substitutions was encountered in the 12β,15α-diol (**69**) with a 7% efficacy. This poor efficacy cannot be attributed to the 15α-alcohol as its presence alone (**70**) retained good efficacy (68%). It may be inferred, therefore, that close contact exists between the steroid and the receptor at C12β (no steroid of these sets probes C12α). Among the proximal carbons to C12, only C11α substitution (**10** and **54**) had poor activity and therefore may also be forbidden. The remaining carbons of the C-ring may be substituted with retention of full agonist efficacy: substitutions at C11β, full agonist efficacy for *inter alia* **48**, **51**, and **53**; C18, full agonist efficacy for **80**; and C14, full agonist efficacy for **73**. Unfortunately, the argument of an intimate contact between the receptor and the 12β locus rests entirely on one compound (**69**), and therefore this conclusion remains tentative.

There does not seem to be close contact between the receptor and most portions of the progesterone D-ring. Both C15α (noted previously as permissive for hydroxyl substitution) and C15β accommodate methyl substitution (**71** and **72**) with good efficacy (46% and 62%, respectively). Substitution with either 16α-hydroxyl (**78**) and 16β-methyl (**79**) retains good efficacy (63% and 77%, respectively). Although, as discussed subsequently in some detail, the C17 substitution is critical to the agonist/antagonist transition, the 17α substituent does not impede steric contact with the receptor, as progestins with both small 17α-hydroxyl (**18**) and large tetrahydropyranyl (**92**) substituents retain full agonist efficacy (96% and 133%, respectively).

Agonist/antagonist control of the sperm receptor by C17 substitution. Three steroid families are distinguished by the C17 substituent [androstanone, no substitution; testosterone, 17β-OH; progesterone, 17β-C(O)CH_3]. It is inconceivable that the sperm progesterone receptor not be acutely sensitive to functional group interchange at this position, and (not surprisingly) the biological data for the sperm receptor support this supposition.

As reported previously (3), 17α-hydroxylprogesterone (**18**) is inactive as a stimulator of the progesterone nuclear receptor but is an effective agonist at the sperm cell surface progesterone receptor (96%). A broader examination of 17α-hydroxylprogesterones supports the conclusion that the 17α-hydroxyl is well tolerated by the sperm receptor but not the uterine progesterone receptor (Table 1 and data below). Furthermore, other α-hydroxyl substituents, such as 11α-hydroxyl (**10**), also greatly reduced uterine progesterone receptor binding. It has been proposed that α-hydroxylation in general disrupts binding to this receptor, due to α-face participation in binding (22). In contrast, α-hydroxyl progesterone substitution preserves sperm receptor effectiveness [14α-hydroxyl (**73**), 103%; 6α-hydroxyl (**44**), 78%; 2α-hydroxyl (**36**), 82%]. Acetylation of the 17α-hydroxyl on **18** gave analogue **20** diminished sperm receptor efficacy, but uterine receptor binding was drastically increased (Table 1). Interestingly, substitutions elsewhere that alone increased sperm receptor efficacy for Ca^{2+} influx lost the increased effect when combined with a 17α-hydroxyl [compare 4,5-dihydro- (**5**), 117% with 4,5α-dihydro-17α-hydroxyl (**81**), 47%; 1,2-dehydro- (**2**), 114% with 1,2-dehydro-17α-hydroxyl (**82**), 62% and 11β-hydroxyl (**12**), 183% with 11β,17α-dihydroxyl (**86**), 109%].

Substitution, by acylation, on the oxygen molecule of the C17α hydroxyl is the second disabling substitution. Although in the case of the compact formyl substituent (**19**) the agonist efficacy is merely impaired (55%), the larger acetyl substituent (**20**, **23**, **25**, **27**, and **95**) effectively abolishes agonist efficacy (16%, 14% 2%, 0%, and 0%, respectively). Progesterone analogue **95** is of particular interest in addressing the structural basis for the loss of agonist efficacy in that it possesses four substituents (2α-methyl, 9α-fluoro, 11β-hydroxyl, and 17α-hydroxyl) that are well tolerated (**94**) (99% efficacy). Yet, all agonist efficacy of **95** is lost on mere acetylation at a position proved to be sterically unencumbered. A hypothesis for the remarkable consequence of acetylation (indeed, **95** has antagonist efficacy) is developed after presentation of the entire set of steroids.

The remaining progesterones of Fig. 1 can be used to address the importance of the integrity of the progesterone C17β-C(O)CH_3 substituent. Reduction in this carbonyl is the primary metabolic event abolishing uterine progesterone agonist efficacy. The carbonyl itself is of secondary importance with respect to the sperm receptor, as each of the three C20 alcohols (**96** and **97**, 20*S*; **98**, 20*RS* mixture) retained good agonist efficacy (~50%). It is shown below that a C20 substituent is inconsequential to agonist efficacy. It may be the combination of the change from trigonal to tetrahedral bonding on C20 reduction and the secondary presence of the hydroxyl which achieve a suboptimal placement of the more critical C21 methyl (see below). Hydroxyl substitution of **1** at C21 yields **99**, with reduced agonist efficacy (64%), but the addition of other hydroxyl substituents is profoundly disadvantageous [6β,21-dihydroxyl (**100**), 0%: compare with **45**, 90%; 11β,21-dihydroxyl (**101**), 21%: compare with **12**, 183%; 11α,21-dihydroxyl (**102**), 3%: compare with **10**, 35%; 9α,21-dihydroxyl (**103**), 4%: compare with **47**, 40%; 15α,21-dihydroxyl (**104**), 8%: compare with **71**, 46%; 17α,21-dihydroxyl (**105**), 29%: compare with **18**, 96%]. Continuing this trend, neither of the trihydroxylprogesterones **106** and **107** possessed agonist efficacy. The effect of substitution, by an acetyl, on the unfavorable C21 hydroxyl was examined with one example (**108**). Its substituents (other than the C21 acetyloxy) should be compatible with agonist efficacy (compare with **41** and **87**). That **108** is in a poor agonist implies that a C21 acetyloxy is as disadvantageous a substituent as is the C21 hydroxyl.

Functional agonist equivalence of the 17β-methoxy and 17β-acetyl substituents. Surprisingly, many of a series (Fig. 6) of 17β-methoxyandrostenone (compare **1** with **109**) and 17β-methoxyandrostanone derivatives proved to be exceedingly effective agonists but not more potent than **1** (data not shown). Almost all of the entire set of 23 were active. Moreover, the effect of substitution in this series replicated (or enhanced) the effect of the same substituent in the progesterone series. At the 10 μM test concentration, the following steroids gave a larger effect relative to **1** (also at 10 μM): 2α-methyl-17β-methoxy-5α-androst-4-en-3-one (**110**),

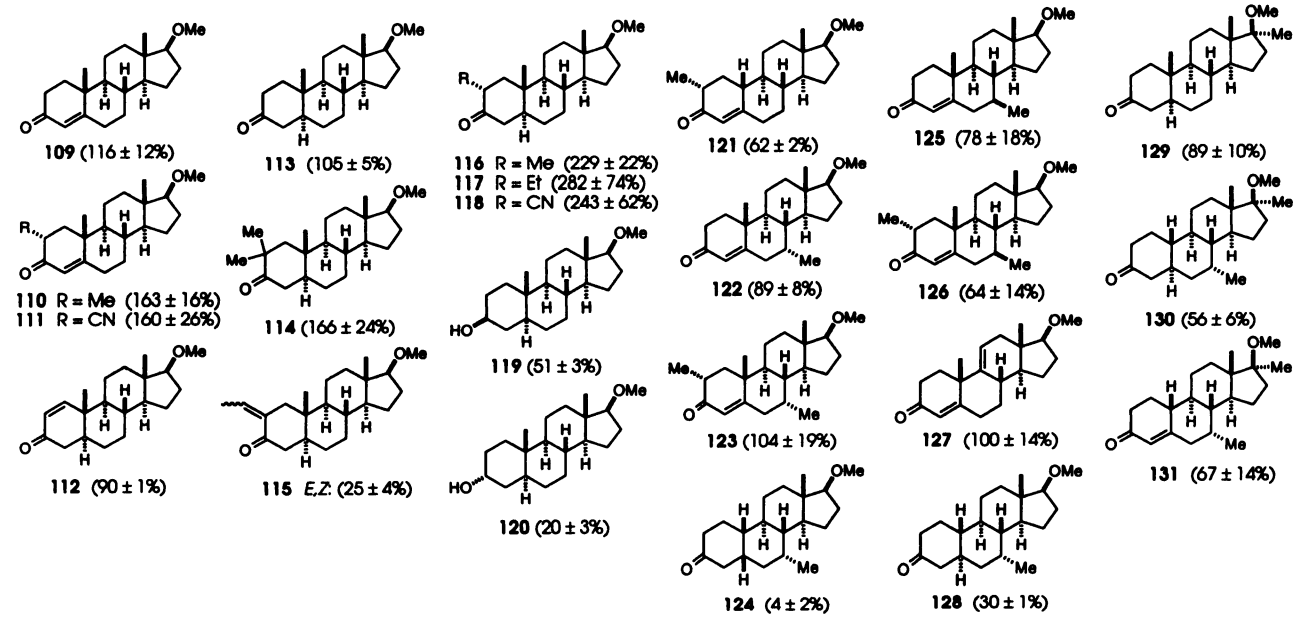


Fig. 6. The set of 17β-methoxy androstanol, androstanone, and androstenone analogues, with relative efficacy for inducing Ca^{2+} ion influx into human sperm (percentage relative to **1**).

163%; 2α-cyano-17β-methoxy-5α-androst-4-en-3-one (**111**), 160%; 2,2-dimethyl-17β-methoxy-5α-androstan-3-one (**114**), 166%; 2α-methyl-17β-methoxy-5α-androstan-3-one (**116**), 229%;2α-ethyl-17β-methoxy-5α-androstan-3-one(**117**),282%; and 2α-cyano-17β-methoxy-5α-androstan-3-one (**118**), 243%. The remaining members of the set confirm the unessential involvement of the 3-ketone (**119** and **120**) and C19 methyl, exemplified by **14**, **121**, **128**, **130**, and **131**, and the lack of close receptor contact at C7α and 7β, illustrated by **122**, **123**, **125**, and **126**. C17α-methyl substitution is tolerated (**129**-**131**). The poor efficacy of the 5β-dihydro-7α-methyl (**124**) may be rationalized in terms of an unfavorable combination of the less desirable *cis* A,B-ring and 7α-methyl.

Two androstanes were examined for relative binding affinity to the uterine receptor. Both **110** and **116** were essentially incapable of binding (<1%). Several conclusions concerning the sperm receptor structure-efficacy relationship may be made. The 17β-methoxy is an effective replacement for the 17β-C(O)CH_3 but not for binding to the progesterone nuclear receptor. The C20 carbonyl of progesterone is unimportant for sperm receptor agonist efficacy. Beneficial 2α substituents for the sperm receptor include the methyl, ethyl, and cyano groups. The 5α-A,B-ring-saturated steroids achieve a conformation as effective, or more effective, than the 4,5-A,B-ring-unsaturated ring of the progesterones.

Sperm progesterone receptor antagonists that contain 17β-hydroxyl groups. A complex relationship was found for the set (Fig. 7) of 17β-hydroxyl and 17-keto androstanes (testosterones). In general, these were ineffective agonists but capable antagonists (preventing progesterone-dependent $[Ca^{2+}]_i$ elevation). Of the 17 structures of this set, only one (**132**), with the advantageous 2α-methyl, had good

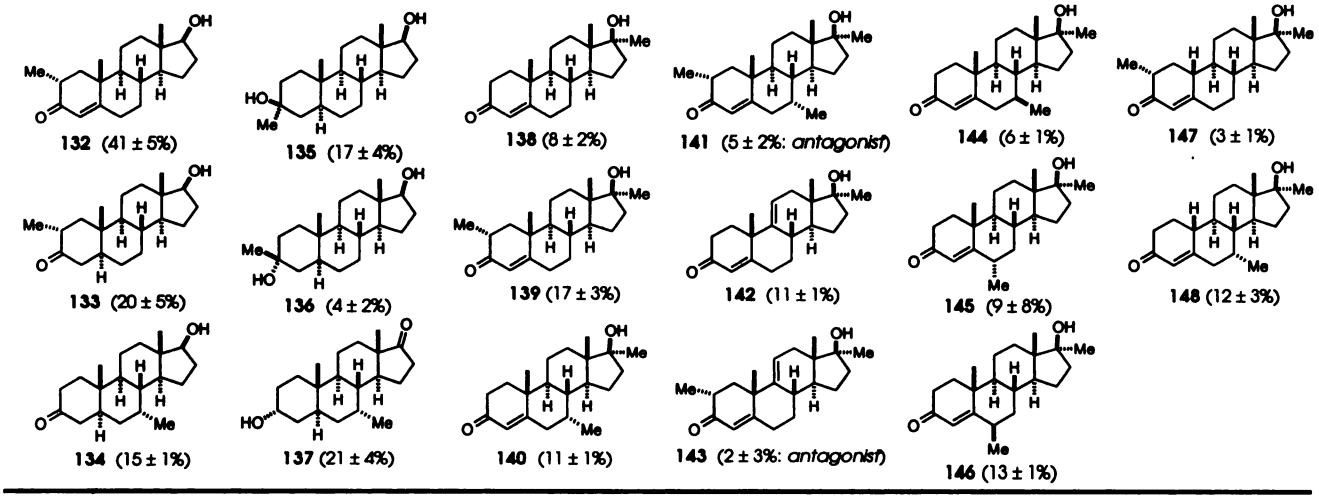


Fig. 7. The set of the testosterone analogues, with relative efficacy for inducing Ca^{2+} ion influx into human sperm (percentage relative to **1**).

efficacy (41% at 10 μM). The antagonist efficacy and potency were examined in some detail with two of the set. When added to the sperm receptor 35 sec before **1**, both 10 μM 2α,7α,17α-trimethyl-testosterone (**141**) and 10 μM 9(11)-dehydro-2α,17α-dimethyl-testosterone (**143**) blocked the 1 μM progesterone-mediated $[Ca^{2+}]_i$ increase (Fig. 8). An identical outcome was seen at shorter (10 sec) and longer (60 sec) preincubations (data not shown). The dose dependence for this inhibition was measured (Fig. 9). At 10 μM **141** or **143**, there was almost complete inhibition of the agonist efficacy of **1** (0.1–10 μM). Even at 0.01 μM **143**, a substantial inhibition was observed at all progesterone concentrations tested. Further analysis of the potency of these inhibitors was hampered by the fact that at >10 μM **1**, there was an apparently receptor-independent $[Ca^{2+}]_i$ increase. For example, **1** at 100 μM increased $[Ca^{2+}]_i$ to ~1 μM and caused a loss of viability, assessed by trypan blue uptake. This result may therefore be a consequence of a nonspecific membrane perturbation by **1**. The unexpected antagonist potency of both of these testosterones may derive in part from the same favorable interaction of their 2α-methyl substituent, as was also seen in the progesterones and androstanones.

Antagonists of progesterone-induced Ca^{2+} influx. Four potent nuclear progesterone receptor agonists [megestrol acetate (**25**), cyproterone acetate (**27**), norethindrone (**28**), and norethynodrel (**30**)], previously demonstrated to be ineffective at eliciting sperm Ca^{2+} influx (Table 1), also were sperm progesterone receptor antagonists (Fig. 10). Cyproterone acetate was a particularly potent inhibitor of progesterone-stimulated Ca^{2+} influx, effective over the entire concentration range examined (0.01 μM–10 μM, Fig. 11). Based on the cumulative structure-efficacy data, it seems improbable that two of the three points of structural difference between **1** and **27** (the 1α,2-fused cyclopropane and the 6-chloro) account for the change from agonist to antagonist. As was seen for other 2α substituents, the 1α,2-cyclopropane may better account for the excellent potency. A chloro substituent is sterically and electronically similar to a methyl,

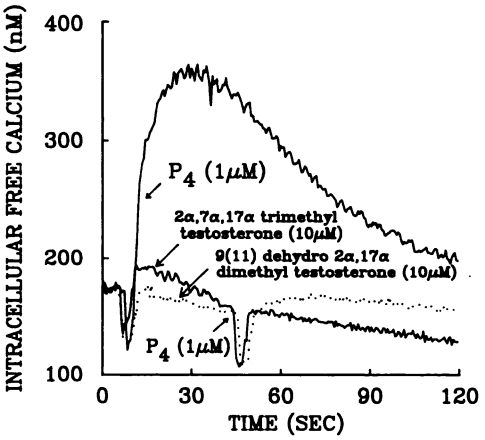


Fig. 8. Effect of 2α,7α,17α-trimethyltestosterone (**141**) (10 μM) and 9(11)-dehydro 2α,17α-dimethyltestosterone (**143**) (10 μM) on the ability of progesterone (1 μM) to increase $[Ca^{2+}]_i$. At 10 sec, 2α,7α,17α-trimethyltestosterone (10 μM) or 9(11)-dehydro 2α,17α-dimethyltestosterone (10 μM) was added to a suspension of Fura-2-loaded sperm. After 45 sec, 1 μM progesterone was added. Both testosterone analogues attenuated the ability of 1 μM progesterone to increase $[Ca^{2+}]_i$. The effect of 1 μM progesterone, alone, is also shown. Representative traces are shown.

thus matching the agonist structure of 6-methyl-6,7-dehydro-17α-hydroxylprogesterone (**24**). Rather, the lack of agonist efficacy of **27** must derive from the 17α-acetyloxy, and indeed cyproterone (**26**) had good agonist efficacy. Emphatic supporting evidence for a critical ability of the 17α-acetyloxy (but not 17α-hydroxyl) to induce an agonist to antagonist transition is found in the progressive structural series composed of 9α-fluoro-11β-hydroxylprogesterone (**49**) (agonist, 93%), 2α-methyl-9α-fluoro-11β-hydroxylprogesterone (**50**) (agonist, 95%), 2α-methyl-9α-fluoro-11β,17α-dihydroxylprogesterone (**94**) (agonist, 99%), and 2α-methyl-9α-fluoro-11β-hydroxy-17α-acetyloxyprogesterone (**95**) (agonist efficacy, 0%; full antagonist efficacy as discussed below).

Observations from a set of miscellaneous steroids. A final set of 12 steroids, not closely related to the preceding structures, was examined (Fig. 12). The most noteworthy steroids (**149-153**), each with good agonist efficacy, possessed a common 17β-acetyloxy (acetyl derivatives of the 17β-hydroxyltestosterones). The appearance of this agonist efficacy clearly coincides with the acetyl substitution. If the hypothesis is correct that the progesterone 17β-C(O)CH_3 is critical to eliciting agonist efficacy, then the 17β-acetyloxy found in this set of agonists must be an effective mimic of the progesterone 17β-C(O)CH_3. The 2α-methyl (**149**), 4-chloro (**150**), and 4-hydroxyl (**151**) have good efficacy; remarkably, the 1,2-dehydro-4,5α-dihydro-7α-methyl (**152**) is more active than progesterone. Steroid (**153**) possesses a trifluoromethyl-1*H*-pyrazole ring fused to the steroid A-ring. Although only modestly active, it confirmed previous observations that the 3-ketone is unnecessary for agonist efficacy and that there is available space at the receptor along the steroid C2,3 A-ring edge. Of the three 17-(*Z*)-ethylidene D-ring steroids (**154–156**), only the first had excellent efficacy. No simple structural correlation exists between the 21-methyl groups of **1** and **154** and the agonist efficacy; the respective methyl groups occupy different spaces. The good efficacy (46%) of the 16β-methyl (17α-progesterone) (**157**), a diastereomer of **79**, was surprising. An attractive, although simplistic, explanation is that agonist efficacy requires the additive combination of the B/C/D-ring conformation and the proper placement of the C21 methyl within the sperm progesterone receptor. Receptor binding, but only partial agonist stimulation, may occur when just the former feature is present. Steroid **157**, and possibly **158** (with a constrained C21 placement), has the former but not the latter feature; presumably, the 17α-acetyl of **157** projects into empty space. The lack of efficacy of 3,6,11-trione (**159**) can be ascribed to substantial and unfavorable conformational deformation.

Discussion

The sperm progesterone receptor is one of the most studied cell surface steroid receptors (e.g., Refs. 4–6, 23). Another cell surface progesterone receptor has been characterized on the *Xenopus* oocyte (24–26). This receptor also displays steroid binding characteristics different than those of the progesterone nuclear receptor. The studies of Li and Patino (26) showed that the potent progestin R5020 or promegestone (**17**) bound very poorly to the *Xenopus* oocyte cell surface receptor. Promegestone (**17**) is also a very poor stimulator of Ca^{2+} influx in sperm (Table 1), demonstrating that both of these cell surface progesterone receptors are recognized

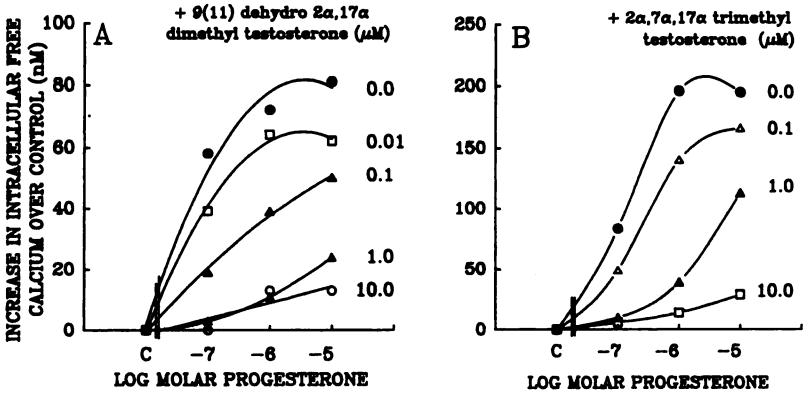


Fig. 9. Dose response of progesterone to increase $[Ca^{2+}]_i$ in the presence of either 9(11)-dehydro-2α,17α-dimethyltestosterone (**143**) or 2α,7α,17α-trimethyltestosterone (**141**) at various concentrations. Sperm were preincubated with either 9(11)-dehydro-2α,17α-dimethyltestosterone or 2α,7α,17α-trimethyltestosterone at various concentrations for 35 sec; then, progesterone at various concentrations was added. See Fig. 8 for representative traces. Each value is the mean from three separate experiments (different donors).

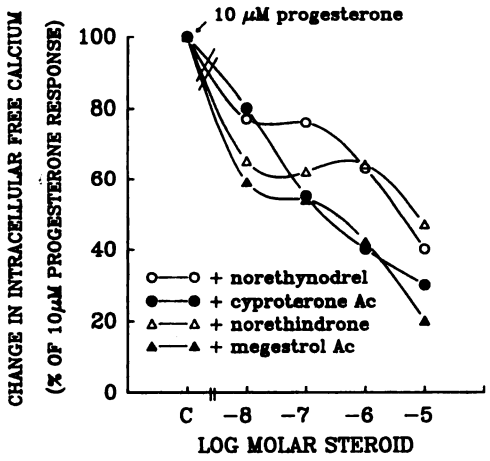


Fig. 10. Dose response of norethynodrel (**30**), cyproterone acetate (**27**), norethindrone (**28**), and megestrol acetate (**25**) to inhibit the ability of 10 μM progesterone to increase $[Ca^{2+}]_i$. Sperm were preincubated with the various progestigens as detailed in legend to Fig. 8. Each value is the mean from three separate experiments.

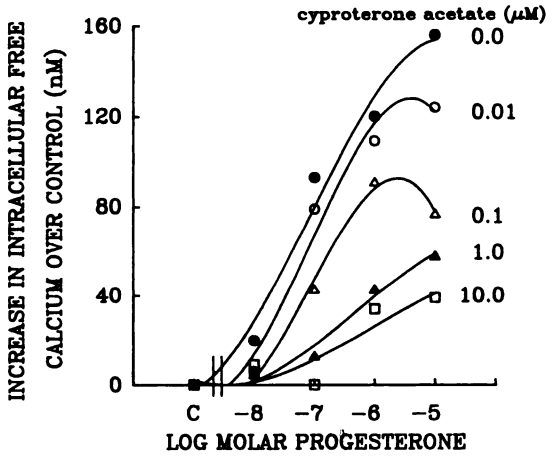


Fig. 11. Dose response of progesterone to increase $[Ca^{2+}]_i$ in the presence of cyproterone acetate (**27**) at various concentrations. Sperm were preincubated with cyproterone acetate before progesterone addition as in the legend to Fig. 8. Each value is the mean from three separate experiments.

poorly by **17**, which binds potently to the nuclear progesterone receptor (Table 1).

On stimulation by progesterone, the sperm receptor elicits a very rapid increase in $[Ca^{2+}]_i$ (2, 3) and the acrosome reaction (2). The first structure-efficacy studies showed that in addition to progesterone, 17α-hydroxylprogesterone and 11β-hydroxylprogesterone were very good stimulators of $[Ca^{2+}]_i$ (3). In contrast, β-estradiol, testosterone, corticosterone, and estrone were very poor stimulators of $[Ca^{2+}]_i$ (3). These studies were subsequently confirmed with the use of direct radioligand binding studies (7). The fact that 17α-hydroxylprogesterone was a receptor agonist in sperm strongly suggested a dissimilarity between this receptor and the progesterone nuclear receptor because 17α-hydroxyl progesterone was bound poorly to this latter receptor (27, 28). This presumption was further strengthened by the finding that several classic synthetic progesterone agonists (norethindrone, promegestone, cyproterone acetate) were poor $[Ca^{2+}]_i$ stimulators; furthermore, the very potent anti-progestigens RU486 and ZK98.299 were not very potent antagonists of the action of progesterone on sperm (4, 14, 15). The current study confirms this presumption and defines the basic structure-efficacy relationship for steroid binding to the human sperm progesterone receptor.

Although presenting by no means a complete set of possible permutations, this collection of steroids has been used to identify the effect on agonist efficacy of substitution at, in particular, C2, C6, C9α, C11, C12β, C17, C20, and C21. The visualization of the stereochemical and steric effects of these substitutions was assisted by an inspection of the energy-minimized, most stable conformation of the progesterone analogue (**94**) and the androstanone (**117**) agonist (Fig. 13). Together, these illustrate substituent incorporation at all carbons except C6, C12, and C21. As discussed previously, even a single modification at one of these positions may affect agonist efficacy. A number of C2α groups (including methyl, ethyl, and cyano) preserved effectiveness, and their presence seemed to correlate with improved potency (for both agonists and antagonists). In contrast, 2β-methyl substitution was deleterious (Fig. 3). With reference to the conformations of **94** and **117**, the C2α substituent projects outward from the steroid, approximately within the A,B-ring plane. The 2β, not present in **94** and **117**, and 11β (as found in **94**) substituents project above the β-face. An interpretation consistent with the biological data is that C2α and C11β substituents may engage favorable binding pockets on the receptor, whereas C2β and C11α substituents provoke an unfavorable steric interaction. The combination of the C11β-hydroxyl and C2α substituents synergize to achieve increased potency and effectiveness relative to **1**. The excellent agonist efficacy shared by the 2α-substituted 17β-methoxyandrostenones, exemplified by **110** and **111**, and 5α-reduced 17β-methoxyandrostanones, exemplified by **116–118**, offer circumstantial evidence

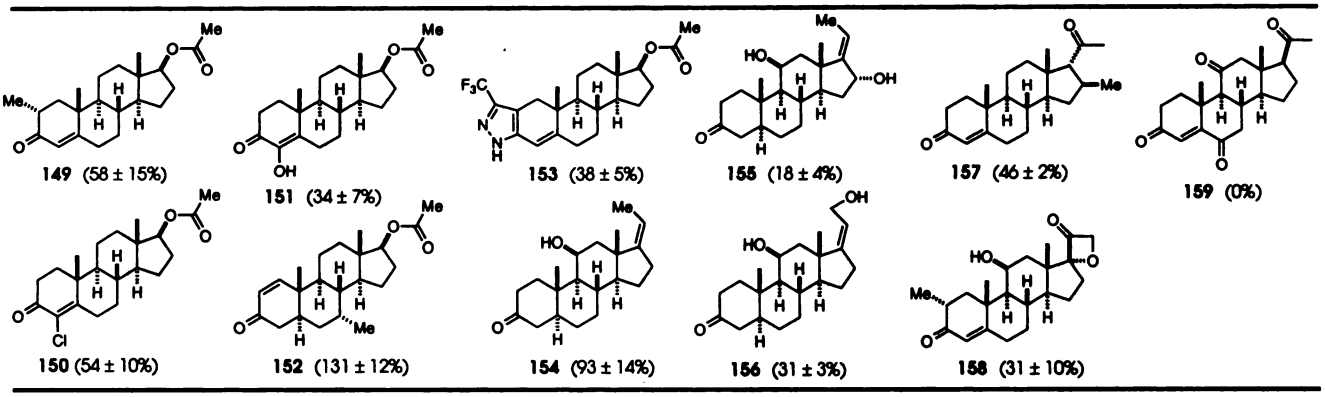


Fig. 12. The set of miscellaneous analogues, with relative efficacy for inducing Ca^{2+} ion influx into human sperm (percentage relative to **1**).

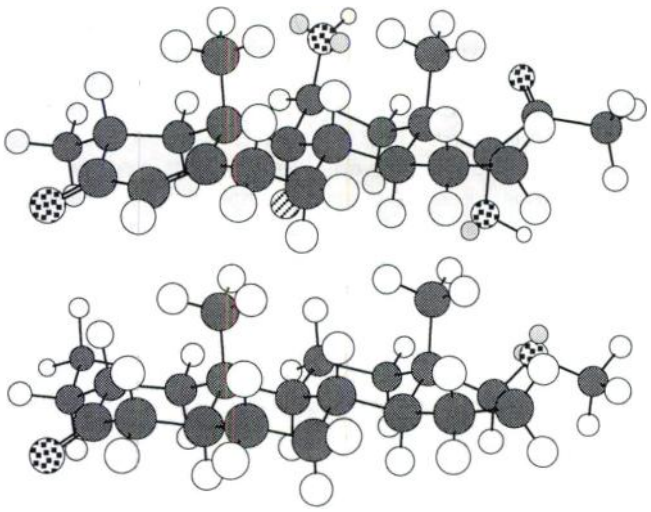


Fig. 13. Most stable conformations of progesterone agonist (**94**) (*top*) and androstanone agonist (**117**) (*bottom*) in ball-and-stick perspective. The lowest energy conformation of **94** was obtained directly. The lowest energy conformation of **117** was obtained by minimization of 2α-ethyl-11β,17α-dihydroxylprogesterone followed by substitution of the 9α-hydrogen with fluorine. The steroids are shown with the A-ring to the *left* and with carbons C4 and C6 in closest view. Particular attention is directed to the nearly identical A-ring conformations and orientation of the C17 side chain. Atoms: *black*, carbon; *white*, hydrogen; *dotted*, oxygen (*light gray*, nonbonding electrons); *striped*, fluorine.

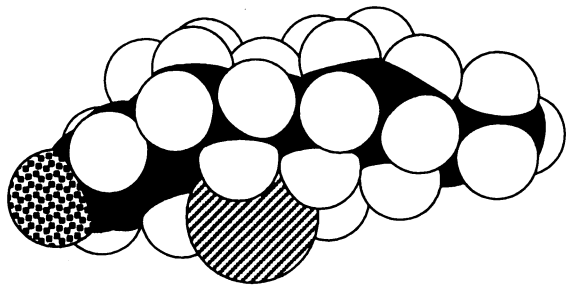


Fig. 14. Most stable conformation of progesterone agonist (**68**) in space-filling perspective. The lowest energy conformation was obtained by minimization of 11β-fluoroprogesterone, followed by substitution of the 9α-hydrogen with bromine. The view of this space-filling perspective (reduced in scale by 20% relative to the ball-and-stick perspectives of the other figures) approaches the plane of the steroid edge-on to emphasize approach to the steroid α-face (C3 carbonyl of the steroid A-ring at the extreme *left* with C6 and C7 closest in view). The large volume occupied by the bromo substituent blocks effectively all contact to the α-face of the A,B-rings. Atoms: *black*, carbon; *white*, hydrogen; *dotted*, oxygen; *striped*, bromine (fluorine is not in view).

that the steroid conformation recognized by the sperm receptor resembles that of the low energy solution conformation, approximated by the computational structures shown in the figures. In these solution conformations, both the 2α and 17β substituents can project to the same region of the receptor, which may correlate with their common increased agonist efficacy.

Little or no decrease in agonist efficacy occurs on 9α-bromo substitution (**68**; 94%). In the most stable conformation of **68** (Fig. 14), this substituent projects directly downward from the steroid A,B-ring plane, shielding the steroid A,B-ring α-face. The agonist behavior was retained in general for 9α-substituted steroids (**50**, **52**, **53**, **65**, and **74**) and implies an absence of contact between the steroid A,B-ring α-face and the sperm receptor. It is possible, therefore, that in contrast to the progesterone nuclear receptor (for which the structure-efficacy data show binding to the steroid α-face; e.g., Refs. 27 and 28), the sperm receptor binds (*inter alia*) preferentially to the steroid β-face.

The critical importance of the C17 substituent has been emphasized. The sperm receptor clearly recognizes a properly placed 17β-methoxy and 17β-$C(O)CH_3$ as equivalent functional groups. It also accepts the combination of a 17α-hydroxyl and 17β-acetyl within the generic agonist structure. In contrast, the biological data implicate the presence of either a 17β-hydroxyl (testosterone) or the 17α-acetyloxy/17β-acetyl combination with an abrupt change from agonist to antagonist. The lack of agonist efficacy in both instances is suggested to correlate to the absence, or the improper positioning, of an effective 17β substituent. A comparison of the computed energy-minimized solution conformation of antagonist **95** (0.01 μM achieves a ~50% inhibition of the $[Ca^{2+}]_i$ stimulation by 10 μM progesterone; Fig. 15) with the conformations of agonists **1** and **94** (Fig. 13) shows a significant effect due to the 17α-acetyloxy of **95** (Fig. 16).

This effect of the 17α-acetyloxy substituent was examined further by the higher level MM2 structural and conformational energy minimization (Mosaic; Upjohn, Kalamazoo, MI). Virtually identical conformers to those shown in Fig. 16 were obtained for both **1** and **95**. For **1**, two low energy conformations were obtained. The computed energy difference between the two (2 kJ mol^{-1}) likely is insignificant (the next stable conformer was 6 kJ mol^{-1} less stable). The two conformers have C16-C17-C20-C21 dihedrals of 170° and 42° (similar to the 41° dihedral shown for **1** in Fig. 16), respec-

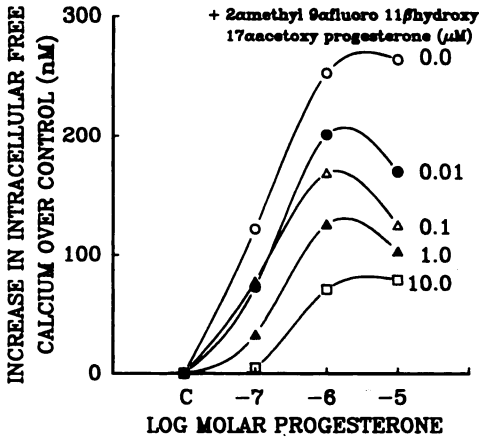


Fig. 15. Dose response of progesterone (**1**) to increase $[Ca^{2+}]_i$ in the presence of 2α-methyl-9α-fluoro-11β-hydroxyl-17α-acetyloxy progesterone (**95**) at various concentrations. Sperm were preincubated with 2α-methyl-9α-fluoro-11β-hydroxyl-17α-acetyloxy progesterone before progesterone was added, as described in the legend to Fig. 8.

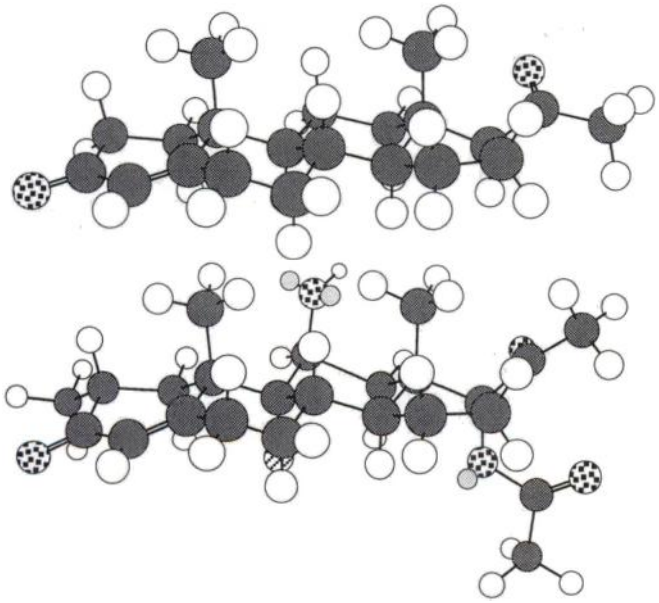


Fig. 16. Most stable conformations of progesterone (**1**) (*top*) and the progesterone antagonist (**95**) (*bottom*). The lowest energy conformation of **1** was obtained directly. The lowest energy conformation of **95** was obtained by minimization of 2α-methyl-11β-hydroxyl-17α-acetyloxy progesterone, followed by substitution of the 9α-hydrogen with fluorine. Although 17α-hydroxyl substitution has no effect on the D-ring orientation of the C21 methyl (compare **1** with **94**; Fig. 13), the 17α-acetyloxy of **95** results in a significant movement of C21 that may be associated with its loss of efficacy. Atoms: *black*, carbon; *white*, hydrogen; *dotted*, oxygen (light gray, nonbonding electrons); *striped*, fluorine.

tively. For **95**, two low energy conformations also were obtained (2 kJ mol^{-1} energy difference) with C16-C17-C20-C21 dihedrals of 1° (similar to the 2.5° dihedral of Fig. 16) and 165°. The nonbonded interactions in **95** result in a C20-C17-O17α bond angle for the two conformations (average, 111.5°) larger than the C20-C17-H17α bond angle for the two conformations of **1** (average, 104.5°). Thus, although the conformations of **1** and **95** with the 165–170° C16-C17-C20-C21 dihedral are similar, the remaining two clearly are not (Fig. 16). Although these dissimilar conformations cannot be interpreted as visualizing the favored agonist (or antagonist) conformations, given the limitations of computational structures, as discussed previously, it may certainly be concluded that the 17α-acetyloxy results in a significant perturbation of the conformational energies and preferences of the 17β-acetyl side chain. This change, alone or in combination with the electrostatic demands introduced by the 17α-acetyloxy substituent, represents a reasonable hypothesis for the transition from agonist (**1**) to antagonist (**95**) behavior. The acetyloxy does not seem to impose a steric demand, as it binds well as a receptor antagonist (Fig. 15). It is within this factual framework, and with attention to the potent agonist efficacy of the 17β-methoxyandrostanes, that the hypothesis is presented that the proper placement of the C21 methyl is critical to the expression of agonist activity.

The computational energy-minimized conformations for three agonists with altered D-ring bonding [**76**, a 15,16-ene, (111%); **77**, a 16,17-ene, (50%); and **93**, a 16,17-epoxide, (100%)] are shown in Fig. 17. In comparison to antagonist **95**, the 17β-acetyl position is less perturbed, which is consistent with a relationship between its proper receptor placement and agonist efficacy. Thus, the structure-efficacy patterns at C17 clearly establish a divergence for receptor recognition of the steroid between the sperm and uterine progesterone receptors, and, through the spatial placement of the methyl group of the 17β substituent, offer a structural hypothesis for the agonist-antagonist transition.

Conclusions. Human sperm cell surface progesterone receptor-dependent acute increase in $[Ca^{2+}]_i$ is accomplished by an array of progesterone derivatives and progesterone mimetics. The structure-efficacy relationships for agonist di-

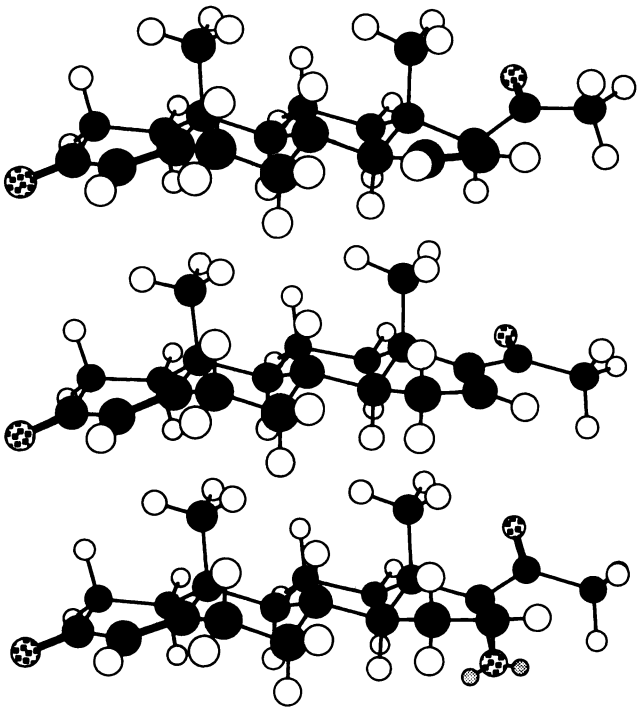


Fig. 17. Lowest energy conformations of agonists **76** (*top*) and **77** (*middle*) and an unminimized representation of a conformation of **93** (*bottom*). Although it must be realized that there will be several low-energy conformations resulting from rotation about the C17/C20 single bond, the overall orientation of the acetyl substituent in **1** (see Fig. 16) and these three is similar (common agonist efficacy). These conformations contrast with that of **95** (antagonist; see Fig. 16).

verge markedly from those established previously for the nuclear progestin receptor. For the sperm receptor, the simplest interpretation of these data is that full agonist efficacy occurs when the steroid is bound and positions properly its C21 methyl. Steroid binding seems to not involve recognition of the steroid A-ring (although small 2α substituents improve binding significantly), the A,B-ring α-face, C19 methyl, the B/C/D-ring "lower" edge (C6, C7, C14, and C15, although the receptor may be sensitive to C6 substitution in some cases), or the C17α substituent. The data set tested show an intimate contact between the steroid and receptor across the C/D-ring "upper" edge (C11, C12, and C17). The proposition that the structural requirements for steroid activation of the sperm cell surface progesterone receptor and the nuclear progesterone receptor are vastly different is completely substantiated (27). A number of structural features that serve to define relative potency and agonist or antagonist efficacy have been defined. The data also show that modulation of Ca^{2+} influx in sperm by steroids is more sensitive to changes on the β-face relative to the α-face of the steroid analogues. This implies that progesterone (and the active analogues) binds predominantly to the cell surface receptor via the β-face. Steroids that are either more potent and effective than progesterone and steroids that are potent antagonists have been identified. The data support the conclusion that it may be feasible to design steroids to interact specifically with the sperm cell surface receptor (and perhaps cell surface progesterone receptors of other tissues; Ref. 11) without perturbation of the progesterone nuclear receptor.

Acknowledgments

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