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STRUCTURE–FUNCTION RELATIONSHIPS OF VARIOUS STEROIDS RELATIVE TO INDUCTION OF NUCLEAR BREAKDOWN AND OVULATION IN ISOLATED AMPHIBIAN OOCYTES

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SUMMARY

Progesterone and certain other steroids induce the resumption of meiotic division in amphibian oocytes *in vitro*. This system permits the examination of structure–function relationships of various steroids relative to their capacity to induce two specific physiological responses, nuclear breakdown and ovulation. The results indicate that the spatial arrangement of substituents on the upper, β surface of the steroid molecule is of critical importance in the induction of nuclear breakdown; the 3, 20-dione, 21-ol configuration being the most active. This must include an angular methyl group at carbon 19. The most potent inducers have an unsubstituted α surface, and any introduction of a polar group diminishes or abolishes biological activity. Ovulation demonstrates similar α and β surface requirements, but ovulation is much more sensitive to substitution in the β surface at carbon 11. The results suggest that progesterone interacts with a receptor in such a way that the β surface is specifically bound to a protein and the α surface exibits non-specific binding to an adjacent hydrophobic region.

INTRODUCTION

Progesterone has been considered to be the primary inducer of meiotic maturation [1–3] or ovulation [4] in amphibians. Progesterone is believed to exert its primary action at the oocyte surface, since exogenous but not injected progesterone initiates the resumption of meiotic maturation [3]. However, a variety of nonestrogenic steroids have been shown to be potent ovulators [4] or initiators of nuclear breakdown [2, 3, 5–11], which is an early morphological event in meiotic maturation. These include cortisol, corticosterone and testosterone, as well as progesterone. The steroids previously studied have, in general, been examples of naturally occurring steroids (estrogens, androgens, etc.) and involve multiple differences in the steroid nucleus. There has been no systematic study of the possible structure–function relationships for either the resumption of meiosis or of ovulation. An important question is whether there is a single meiotic and ovulatory steroid for the amphibian, or whether several steroids may be equally effective as long as they incorporate the required active functional groups.

The present study examines the structure–function relationships for steroids which induce both ovulation and nuclear (germinal vesicle) breakdown. *Rana pipiens* follicles have been chosen for this study since each sexually mature female contains 1–3 thousand fully grown oocytes, and these can be induced to undergo synchronous maturation and ovulation *in vitro*. In addition, induction of meiotic maturation can be readily studied in oocytes free of follicle cells. The results presented here indicate that there are unique structural requirements for inducing both meiotic maturation and ovulation and suggest a possible mechanism of interaction between steroid and the oocyte surface.

MATERIALS AND METHODS

Adult *Rana pipiens* females were obtained from Vermont and maintained in artificial hibernation at 4°C. All experiments were carried out during the months of November–May, and were replicated over the course of two winter and spring seasons. Except as noted, all studies were carried out in Ringer's solution containing 111 mM NaCl, 2.0 mM KCl, 2.0 mM $NaHCO_3$, 1.1 mM $CaCl_2$, and 0.08 mM NaH_2PO_4. This solution differs from classical Ringer's solution in that it contains inorganic phosphate. Previous studies in this laboratory also used phosphate containing Ringer's solution [10, 11]. Steroids were obtained from Steraloids (Pawling, N.Y.) and Sigma Chemical Co. (St. Louis, Mo.); purity was confirmed by thin-layer chromatographic analysis.

Ovaries were removed from a sexually mature frog, rinsed with full strength Ringer's solution, and cut into sections, each section containing a cluster of 40–50 fully grown intact oocytes, held together by a thin sheet of connective tissue. All oocytes were surrounded by serosa and an epithelium of follicle cells. In parallel experiments, oocytes free of follicle cells were prepared by a modification of the method of Masui [1] as follows: clusters of oocytes were incubated at room temperature in Ca-free Ringer's solu-

tion for 0.5–1.0 h, and the epithelial layer was carefully peeled off using watch-makers' forceps under a 25 power binocular microscope. Both intact oocyte-follicle cell preparations and denuded oocytes were equilibrated in Ringer's solution at least 60 min prior to transfer to steroid-containing solutions.

Individual clusters or denuded oocytes were blotted on filter paper, and transferred to a glass scintillation counting vial containing 10 ml of Ringer's solution to which had been added varying concentrations of different steroids. Steroids were dissolved in 95% ethanol and the concentrations adjusted so that the final alcohol concentration in the Ringer's solution was 0.1%. Steroids were routinely put into Ringer's solution by vigorous shaking and heating on a water bath. Steroid concentrations are expressed in μmolar units; 10.0 μg/ml steroid is 28–35 μmol. Since each female contains 1–3 thousand fully-grown oocytes, it was possible to screen 10–15 different steroids, each at several concentrations, with sibling oocytes.

All incubations were carried out at 18–22° under a hood to reduce airborne contamination. At the times indicated, the number of oocytes released from the total follicle population were noted, and the vials were capped and placed in a boiling water bath for 10 min. Twenty-five oocytes from each sac were then examined for germinal vesicle breakdown by dissection under a binocular microscope. Care was taken to choose a representative population from both ovulated and unovulated eggs.

It should be emphasized that these studies were carried out in Ringer's solution containing 80 μM inorganic phosphate ion. Incubation in phosphate-free medium produced a more varied response and generally required higher steroid concentrations for an equivalent response. It should be noted that other investigators have variously used phosphate-free Holtfreter's solution [2], phosphate-free Ringer's solution [1, 3, 6, 7], or phosphate-free Barth's medium [5] for studies of steroid induction of germinal vesicle breakdown. Serum albumin has also been added to the incubation medium in one previous study [1]; we have found no significant effect from adding 0.5% serum albumin to Ringer's solution and have omitted it from all media to avoid steroid binding to the serum albumin.

RESULTS

Induction of germinal vesicle breakdown

A total of 40 steroids were tested for their ability to induce germinal vesicle breakdown. The results presented here represent a composite of findings with more than 20 females. Unless otherwise stated, studies were carried out with oocytes with intact follicle cells.

A representative time course for germinal vesicle breakdown is shown in Fig. 1. The onset of germinal vesicle breakdown was essentially identical for various steroids when compared using sibling oocytes. In the example shown, onset of germinal vesicle breakdown occurred about 10 h after initial exposure to inducing levels of deoxycorticosterone, progesterone, and the 5α- and 5β-pregnanediones. The onset of germinal vesicle breakdown varied from about 8–13 h between different females. Furthermore, onset of germinal vesicle breakdown was essentially the same for both submaximal and maximal inducing levels of each of the active steroids. In contrast to the time of onset of breakdown, the time required for 80% germinal vesicle breakdown varied from less than 1 h for deoxycorticosterone to about 4 h for progesterone or pregnanedione. Maximum breakdown was reached after a similar time interval with all four steroids, i.e., about 4 h.

The relative ability of increasing concentrations of several pregnene derivatives to induce germinal vesicle breakdown is illustrated in Fig. 2. As can be seen, the 21-hydroxy derivative of progesterone (deoxycorticosterone) was much more effective than progesterone. The 11β-hydroxy progesterone was somewhat less active than progesterone. In contrast, 17α-hydroxyprogesterone was only slightly active and 11α-hydroxyprogesterone was completely inactive.

The relative inducing ability of the active steroids can be best estimated from the linear portions of the dose response curves (see Fig. 2). Since the progesterone response varied with the season [2, 10], the steroid concentration required to produce 50% GVBD has been related to an arbitrary value of 1.0 for progesterone in each experiment. As shown in Table 1, the response is reproducible; steroids with values less than 1.0 are more effective than progesterone whereas

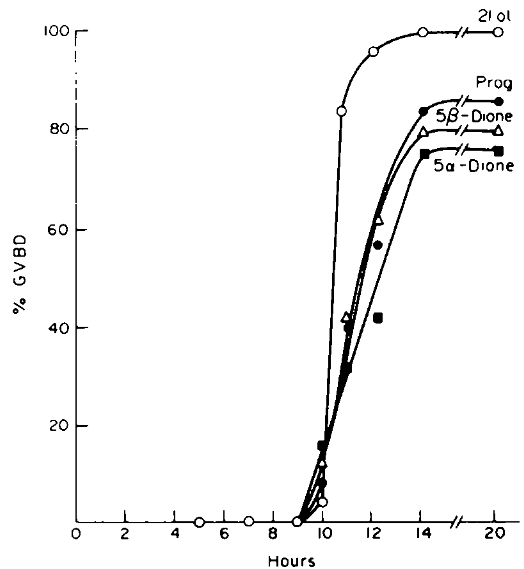


Fig. 1. Time course of germinal vesicle breakdown (GVBD). Clusters of 40–50 follicles containing fully grown oocytes were incubated at 18°C in Phosphate Ringer's solution containing 15 μM concentrations of the steroid indicated: 21-ol, deoxycorticosterone; Prog, progesterone; 5β-dione, 5β-pregnanedione; and 5α-dione; 5α-pregnanedione. Values shown are for sibling oocytes and are representative of three such experiments.

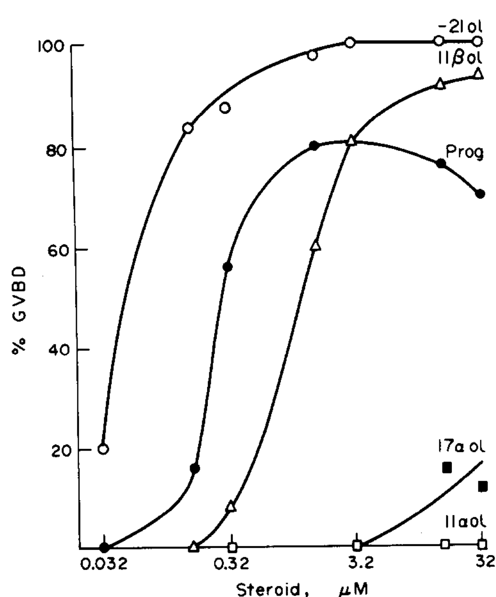


Fig. 2. Maximal germinal vesicle breakdown (GVBD) as a function of steroid concentration in the medium. Clusters of 40–50 follicles with fully grown oocytes were incubated for 24 h at 18°C in 10 ml Phosphate Ringer's solution containing the steroid concentration indicated on the abscissa. Values shown are for sibling oocytes and are typical for May.

those with values greater than 1.0 are less effective. The values shown in Table 1 will be discussed in greater detail in connection with subsequent tables.

The experiments outlined in Figs. 1 and 2 were carried out with isolated ovarian follicles, i.e., oocytes with a layer of follicle cells closely applied to the oocyte surface. Since follicle cells could be a selective barrier to steroids such as the 11α- or 17α-hydroxyprogesterone, parallel experiments to those in Figs. 1 and 2 were repeated with isolated follicles and denuded oocytes (Table 2). Again the 11α- and 17α-hydroxyprogesterones were essentially inactive, whereas progesterone and deoxycorticosterone demonstrated similar activity in both systems. Germinal vesicle breakdown was never seen in isolated follicles or denuded oocytes incubated in Ringer's solution without steroid.

The results of experiments presented above (Figs. 1 and 2, Table 1) emphasize the importance of the

Table 1. Relative steroid concentrations required to induce 50% germinal vesicle breakdown

Steroid	Relative concentration required to induce 50% GVBD
Deoxycorticosterone	0.18 ± 0.05*
Cortisol	0.53 ± 0.03
17α-Methyltestosterone	0.77 ± 0.09
Progesterone	1.0
Testosterone	1.4 ± 0.2
11β-Hydroxyprogesterone	3.3 ± 0.5
5α-Pregnanedione	3.3 ± 0.9

* S.E., 4 experiments.

Table 2. Comparison of steroid induction of germinal vesicle breakdown in intact follicles and denuded oocytes

		% GVBD*	
Steroid	μM	Intact follicles	Denuded oocytes
None	—	0	0
Progesterone	32	88	80
11α-Hydroxyprogesterone	32	0	0
17α-Hydroxyprogesterone	32	8	10
Deoxycorticosterone	3.2	100	100

* Based on examination of 25 follicles or 10 denuded oocytes for each steroid. Values shown are for sibling oocytes and are typical of three experiments.

position of the substituents in the steroid molecule in determining biological activity. For that reason a series of experiments have been carried out comparing the effect of selective and systematic changes in the steroid molecule.

Effect of axial and equatorial hydroxyl group substitutions

The general pattern of structure–function relationships can be most easily seen by comparing the effects of axial or equatorial substitutions on induction of germinal vesicle breakdown. A comparison of the effect of selective substitution of a single hydroxyl group on various carbon atoms of the 4-pregnene nucleus (Table 3) indicates that an OH in an equatorial conformation greatly reduces (2α and 6α positions) or completely inhibits (11α) induction of germinal vesicle breakdown. Similarly, introduction of an OH group into a quasi-axial position below the plane of the ring system (17α) also markedly inhibits. In contrast, OH groups in an axial position above the plane of the ring system (6β, 11β, or 16α) or on an angular methyl group above the ring system (position 18) has little effect on activity. These findings indicate a requirement for a hydrophobic, non-polar α surface of the progesterone molecule.

Effect of changes in ring D and C-17 side chain

A second phase of this study has been to compare the effect of selective changes in the D ring and in the side chain at carbon 17. As shown in Table 4, reduction of the 20-keto group to a 20β-hydroxy derivative has no effect on maximal inducing activity, although the results indicate that a higher concentration of 20β-dihydroprogesterone is required for a response equivalent to that of progesterone. In contrast, the 20α isomer has greatly reduced activity with only 30–40% GVBD occurring at its limit of solubility. Examination of a Dreiding model indicates that the β configuration is above the plane of the ring system while the α hydroxyl is slightly below. This is again consistent with the general findings in Table 3. Loss of the 20-oxygen function (as in 4-pregnen-3-one) completely abolished activity. Thus, a 20-car-

Table 3. Effect of hydroxylation of 4-pregnene-3, 20-dione (progesterone) on its ability to induce germinal vesicle breakdown in isolated *R. pipiens* oocytes

Hydroxylation				Relative concentration* required to induce 50% GVBD	Maximal GVBD %
Ring	Carbon	Isomer	Conformation		
A	2	α-ol	equatorial		16
B	6	α-ol	equatorial	~20.0	48
	6	β-ol	axial (above ring)	5.5	88
C	11	α-ol	equatorial		0
	11	β-ol	axial (above ring)	3.3	88
D	16	α-ol	axial† (above ring)	2.3	76
	17	α-ol	axial (below ring)		12
	18	-ol	......(above ring)	3.2	92

* Relative to progesterone as 1.0 (see Table 1). Values shown are from a representative experiment.
† With respect to ring C.

bonyl function appears to be essential for maximum biological activity.

An interesting pattern emerges from a comparison of several progesterone derivatives with hydroxyl or keto functions at carbons 17, 20, and/or 21. Introduction of a hydroxyl group at carbon 21 enhances activity. As shown in Table 4, the 20-keto, 21-hydroxy derivative (deoxycorticosterone) was the most active steroid tested. Reduction of the 20-keto to a 20β hydroxy function markedly reduced the relative activity but the derivative still produced maximum germinal vesicle breakdown. Introduction of a hydroxyl group at carbon 21 can activate the otherwise inactive 17α-hydroxyprogesterone.

The apparent requirement for a carbonyl dipole on carbon 20 is further supported by the finding (Table 5) that the introduction of a double bond (16-dehydroprogesterone) between carbons 16 and 17 of the D ring also totally abolished activity. Introduction of the 16-double bond into the progesterone molecule produces an α,β-unsaturated ketone and would largely abolish the carbonyl dipole.

The results in Table 5 also indicate that the replacement of the sidechain with a 17-keto function (androstenedione) largely abolishes activity. However, the 17β-hydroxyandrostene derivative (testosterone) is nearly as active as progesterone (Table 2). Furthermore, as also shown in Table 1, introduction of a 17α-methyl group (axial, below plane of ring) greatly enhances testosterone activity and 17α-methyltestosterone becomes more active than progesterone.

Table 4. Effect of selective changes in the side chain at carbon 17 of the pregnene nucleus on induction of germinal vesicle breakdown

Oxygen function at carbon:			Relative concentration required to induce 50% GVBD	Maximal GVBD %
17	20	21		
None	Keto	None	1.0	92
None	β-ol	None	1.7	88
None	α-ol	None		36
None	Keto	ol	0.2	100
None	β-ol	ol	3.3	84
α-ol	Keto	None		8
α-ol	Keto	ol	1.1	96
None	None	None*	--	0

* 4-Pregnen-3-one

Table 5. Effect of selective changes in the D ring on induction of germinal vesicle breakdown

Steroid	Maximal GVBD %
16-Dehydroprogesterone	0
17β-Hydroxy-4-androsten-3-one (Testosterone)	77
17α-Methyl-testosterone	86
4-Androstene-3, 17-dione	12

Effect of changes in ring A

A third phase of this work has been concerned with the effects of changes in ring A on the ability of oocytes to undergo maximal germinal vesicle breakdown. Table 6 demonstrates that reduction of the carbon 4-5 double bond to either the α or β isomer of pregnanedione has little effect on maximal inducing activity. In contrast, reduction of the 4-double bond in testosterone to form the 5α-isomer (dihydrotestosterone) greatly diminishes inducing activity. Reduction of the 3-keto function of progesterone or of 5α-pregnanedione to either the 3α or 3β configuration produced compounds that were maximally active. However, activity may have been due to the 3-keto form of these steroids, produced through oxidation by follicle cells or oocytes. In contrast, the 3α- or β-hydroxy derivatives of 5β-pregnanedione were completely inactive.

Finally, an interesting finding was that loss of the angular methyl group projecting above the plane of the ring at carbon 19 (19-Nor) abolished inducing activity of testosterone and androstenedione. Since estrogens are inactive as inducers [4, 5], it would appear that the C-19 methyl is required for activity.

Induction of ovulation

Figure 3 compares the % ovulation in isolated amphibian follicles as a function of the concentration

Table 6. Effect of reductive changes in the A ring and loss of 19-methyl on the ability to induce germinal vesicle breakdown

Steroid	Maximal GVBD %
Changes in pregnene series	
5α-pregnane-3, 20-dione	89
5β-pregnane-3, 20-dione	83
3α-hydroxy-5α-pregnan-20-one*	52
3β-hydroxy-5α-pregnan-20-one*	93
3α-hydroxy-5β-pregnan-20-one	0
3β-hydroxy-5β-pregnan-20-one	0
3β-hydroxy-4-pregnen-20-one*	77
Changes in androstene series	
17β-hydroxy-5-androstan-17β-ol-3-one (dihydrotestosterone)	8
Changes at C-19	
19-Nortestosterone	0
19-Norandrostenedione	0
Norethindrone	0

* Possible conversion to the 3-keto steroid (active).

of steroid in the incubation medium. As can be seen, a rather narrow range of progesterone concentrations will induce ovulation in *in vitro* follicles. Furthermore, in the experiment shown, deoxycorticosterone reaches inhibitory levels before threshold levels are reached for progesterone. 5α- or 5β-Pregnanedione, on the other hand, required higher concentrations to induce maximal ovulation and reaches its limit of solubility before an inhibitory effect is seen. Thus, broad dose response curves must be examined to adequately measure steroid specificity in ovulation.

As one approach to understanding structure–function relationships for induction of ovulation, steroids have been listed in order of decreasing activity in Table 7. All steroids tested for maturation inducing activity were examined and if not listed were not active in inducing ovulation. The right hand columns indicate the steroid concentration and the maximal ovulatory response in a typical preparation that demonstrated steroid induction of ovulation. As shown, deoxycorticosterone was most effective, with progesterone, 20β-dihydroprogesterone, 5α- and 5β-pregnanedione, and testosterone demonstrating similar maximal activity. 17α-Methyltestosterone and 5α-dihydrotestosterone were successively less active. Inactive steroids included all those with equatorial or axial hydroxyl groups below the plane of the ring as well as a number of steroids that were very effective in inducing germinal vesicle breakdown. This latter group included those steroids with substituents in the 11-position (e.g. 11β-hydroxyprogesterone, cortisol, and corticosterone).

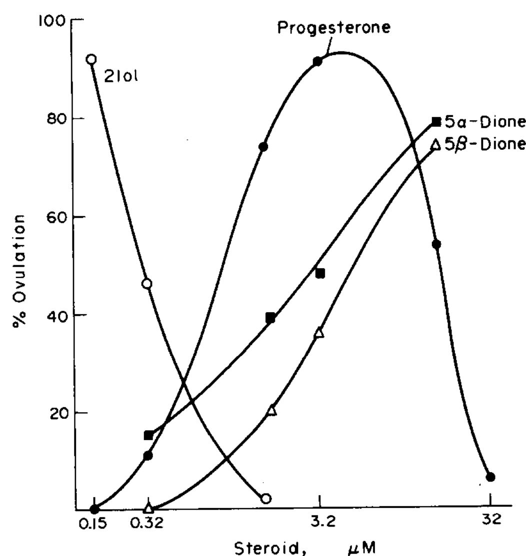


Fig. 3. Maximal ovulation as a function of steroid concentration in the medium. Clusters of 40–50 follicles with fully grown oocytes were incubated for 24 h at 18°C in 10 ml Phosphate Ringer's solution containing the steroid concentration indicated on the abscissa. Values shown are for sibling oocytes and the relative response is typical of 5 such experiments (see text).

It should be noted that the ovulatory response is subject to more variation than maturation. For example, there were differences in ovulatory response between females that seems to be unrelated to seasonal variations. Follicles from a given female may undergo maximal germinal vesicle breakdown but fail to ovulate. This may be due to a requirement for additional pituitary factors as suggested by Wright [4], or be a function of prior estrogen priming. In addition, as reported by Subtelny *et al.* [12], and confirmed here, high steroid concentrations block ovulation but not maturation.

Inhibitors of maturation and ovulation

A number of *in vivo* antiovulatory compounds as well as progesterone analogues found to be inactive in inducing germinal vesicle breakdown were tested for their ability to inhibit progesterone induction of germinal vesicle breakdown and/or ovulation. As shown in Table 8 the antiovulatory compound 17α-chloroethynyl-17β-hydroxy-19-nor-4, 9 (10)-androstadiene (MK 665) was effective in blocking both germinal vesicle breakdown and ovulation but relatively high concentrations were required. In contrast, the antiovulatory compound ethynyl estradiol appeared

Table 7. Effect of various steroids on the *in vitro* induction of ovulation in *Rana pipiens* follicles

Steroid	μg/ml	Maximum % ovulation*
Deoxycorticosterone	0.5	96
5α-Pregnanedione	5.0	80
Progesterone	5.0	79
20β-Dihydroprogesterone	1.0	74
5β-Pregnanedione	10.0	73
Testosterone	5.0	72
17α-Methyltestosterone	5.0	54
5α-Dihydrotestosterone	5.0	19

* Values for sibling follicles after 24 h continuous exposure to steroid concentrations at 20–24°C.

Table 8. Inhibition of progesterone induced germinal vesicle breakdown and ovulation by specific steroids

Inhibitory steroid*	μg/ml	% GVBD	% Ovulation
None		100	86
MK-665†	11	92	62
	55	4	0
11α-Hydroxyprogesterone	5	68	0
Plant steroids (strophanthidin)	400	16	0
Ethynyl estradiol	1.6	62	100

* Follicles were preincubated in the appropriate steroid for 1 h and transferred to Ringer's solution containing 5.0 μg/ml progesterone plus the inhibitory steroid for 24 h. Values are for sibling follicles.

† 17α-chloroethynyl-17β-ol-19nor-4,6(10)-androstadiene.

to block germinal vesicle breakdown and facilitate ovulation. In fact, when progesterone levels are used that approach the ovulatory threshold (0.1–0.5 μg/ml), the further addition of ethynyl estradiol produced a near maximal ovulatory response (60–70%). 11α-Hydroxyprogesterone, a steroid without GVBD inducing activity (Table 3), inhibited progesterone induced GVBD by about 30% but completely blocked ovulation. Thus, ovulation and GVBD have differing sensitivities to inhibition by 11α-hydroxyprogesterone and suggests independent processes for the two events.

Finally, the cardiac aglycone strophanthidin (19-CHO) was effective in blocking both germinal vesicle breakdown and ovulation. However, it is not clear whether the cardiac glycoside is competing with progesterone for an initial site of action, or whether it acts as a Na, K-ATPase inhibitor and blocks a subsequent membrane event [13].

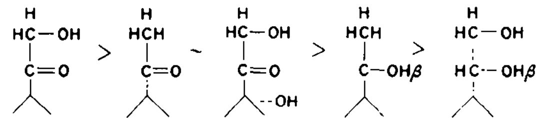
DISCUSSION

The results presented here indicate that the spatial arrangement of the polar substituents on the upper or β surface of the steroid molecule is of critical importance in the induction of both germinal vesicle breakdown and ovulation. In both events, the 3, 20-dione, 21-ol configuration is the most active. A second requirement for activity is an unsubstituted α or lower surface, and any introduction of a polar group diminishes or abolishes biological activity. Steroids with a hydroxyl group in the 11-position above the plane of the ring system (11β-hydroxyprogesterone, cortisol) which were maximally active in germinal vesicle breakdown were completely inactive in inducing ovulation. Thus, the structural requirements are more restricted for ovulation than for maturation.

Evidence has accumulated suggesting that progesterone initiates germinal vesicle breakdown at the surface of the oocyte [6, 9, 14, 15] and that subsequent metabolism of progesterone plays little role in nuclear breakdown. This interpretation is supported by our finding that the time of onset of germinal vesicle breakdown is the same for a wide range of steroids (e.g. progesterone, deoxycorticocosterone, 17α-methyltestosterone, etc.) and is independent of steroid concentration. This suggests a common initial stimulus with minimal effects of subsequent metabolites.

The optimal inducing activity of 20-keto steroids such as progesterone could be due to hydrogen bonding between the carbonyl dipole and a region on or in the oocyte plasma membrane. Such bonds are highly directional and would project above the β plane of the steroid. Introduction of a hydroxyl group on carbon 21 (deoxycorticosterone) would increase hydrogen bonding and a secondary ring system could form between the side chain at carbons 20 and 21 and membrane constituents. On the other hand, introduction of a 17α hydroxyl in the progesterone molecule could have a dual effect, either of which would reduce biological activity. One, as a polar substituent in the apolar plane of the steroid it would inhibit, and two, the 17 hydroxyl group would reduce the C-20 carbonyl dipole moment and largely abolish hydrogen bonding with the oocyte surface. Further substitution of a 21 hydroxyl group would again allow hydrogen bonding by the C-17 sidechain.

The predicted order of decreasing biological activity based on hydrogen bonding above the β plane



is in good agreement with the observed activity.

An interesting finding was that the introduction of an α methyl group in the 17-position greatly enhances the inducing activity of testosterone (Table 2). The non-polar character of the 17-methyl group may shield the 17β hydroxyl group from below and further supports the general requirement of a non-polar, hydrophobic surface of the steroid nucleus. These findings further suggest that the postulated requirement of dipole interaction between membrane and steroid for biological activity is equally well satisfied by an appropriate oxygen function on carbon 17, 20 or 21.

In the 5α (trans) configuration the junction of the A:B rings is slightly less planar than in the parent compound, while formation of the 5β configuration induces a marked bend in the molecule. Ring A in the β-configuration would project almost vertically below the plane of the ring system. In most steroid target tissues the 5α-reduced compounds retain at least some of the biological activity of the parent compound, cf. [11], in contrast to the 5β-reduced compounds which are without activity. As discussed elsewhere [11] the *R. pipiens* ovary appears to be unique in that both the 5α and 5β reduced isomers are nearly as effective as the parent compound. It should be noted, however, that the 5β isomer is active only as the 3-keto form; the inactivity of 3-hydroxy

5β-pregnane-20-dione would further support the requirement for non-polar substituents projecting below the ring system. In other words, ring A may project below the plane of the steroid nucleus (β configuration) as long as there are no polar constituents attached.

In steroid target tissues studied to date, the relative biological activity of the hormone can be correlated with its ability to bind to specific receptors. In terms of binding potential a spatial requirement (as shown here for the β surface) is usually associated with specific steroid–protein interaction. The most potent inducers of germinal vesicle breakdown or ovulation have an unsubstituted α surface, and any introduction of a polar group diminishes or abolishes biological activity. In terms of binding potential, the α surface thus follows the "polarity rule", i.e. any increase in polar substituents decreases steroid–protein interaction. This type of interaction is generally of a hydrophobic type and is associated with nonspecific binding. Thus, in the oocyte we have an apparent steroid receptor interaction in which one surface of the steroid hormone displays specific (high affinity) binding, whereas the other surface displays nonspecific (low affinity) binding. Furthermore, it is important to recognize that both surface interactions are essential for biological activity.

If a strict geometric fit is believed to exist between the shape of the steroid and receptor, then the requirement for exogenous steroid in inducing maturation and the structure–function restrictions outlined here suggest that the steroid hormone is inserted into the oocyte plasma membrane with the β face conforming to a high affinity binding protein and the α face closely associated with a hydrophobic region, possibly with phospholipid at protein–lipid interface. An analysis of steroid affinities for the oocyte surface and for isolated membranes could provide further insight into the mechanism of steroid initiation of meiotic maturation.

It should be emphasized that in this paper we have examined only two of the complex events which occur in the oocyte in response to steroid hormone. The action of progesterone on the oocyte has been considered to be an exception to the current concept that steroids act by evoking new mRNA transcription [14]. Actinomycin D, even when injected into the egg, does not block progesterone induction of germinal vesicle breakdown, meiosis, or the ability of the egg to be activated. Enucleated eggs, exposed to progesterone, become capable of being activated.

Progesterone does, however, initiate a diversity of changes in the amphibian oocyte. The earliest response which we have observed is a negative-going membrane hyperpolarization [16] followed by a transient increase in RNA synthesis [10]. An increase in protein synthesis is necessary for germinal vesicle breakdown [2, 10] and ovulation [10], and changes in intracellular Na^+ and K^+ concentrations can be correlated with a progressive membrane depolarization which begins after the first meiotic division, and continues during the completion of the second meiotic division and the beginning of the first mitotic division [16, 17]. The fact that cortisol, testosterone, and progesterone are effective inducers of germinal vesicle breakdown and ovulation, whereas only progesterone stimulated the early RNA synthesis [10], suggests that there may be more than one site for steroid action. Multiple or "pleiotypic" responses are initiated by many, and possibly all other hormones that stimulate growth and development (for an interesting discussion of this point, see [18]). The possibility exists, therefore, that the complete expression of steroid hormone effect on the oocyte may result from a cooperative action initiated by simultaneous or sequential but independent interactions with one or more extranuclear and nuclear sites.

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