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Progesterone receptor characterized by photoaffinity labelling in the plasma membrane of *Xenopus laevis* oocytes

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R 5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) is a synthetic analogue of progesterone, which is the physiological hormone that reinitiates germinal vesicle breakdown in *Xenopus laevis* oocytes. U.v.-driven photoaffinity labelling experiments were conducted with [^{3}H]R 5020 in oocyte subcellular fractions, and covalently bound radioactivity was analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. In P-10000 (the pellet sedimenting between 1000 and 10000*g* and which contains plasma membrane), a major radioactive band migrating as a 30kDa peptide was found. Non-radioactive progesterone competed with the [^{3}H]R 5020 labelling of this fraction, but not with the labelling of minor [^{3}H]R 5020-binding fractions. It displayed the required characteristics of a specific progesterone-binding membrane 'receptor', postulated from previous studies with intact oocytes and with cell-free P-10000 preparations of membrane-bound adenylate cyclase. The apparent K_i of approx. 4μM for progesterone was compatible with the active concentration of the hormone. Binding specificity, as determined in competition studies, was highly correlated with the germinal vesicle breakdown activity of the steroids and analogues tested. The receptor was not found in the vitelline envelope, in vitelline platelets, in melanosome-enriched or microsomal fractions, in cytosol, nor in germinal vesicles of oocytes. The properties of this membrane steroid receptor are different from those of the already known soluble intracellular steroid receptors, in particular regarding ligand binding specificity and subcellular distribution.

Progesterone, the steroid that reinitiates meiosis in arrested full grown *Xenopus laevis* oocytes, acts at the surface membrane level (Smith & Ecker, 1971; Godeau *et al.*, 1978; reviewed by Baulieu & Schorderet-Slatkine, 1983). Recently, in cell-free experiments, progesterone and other agonist and antagonist steroids were found to modulate membrane-bound adenylate cyclase activity, in precise correlation with their effect on germinal vesicle breakdown in intact oocytes (Finidori-Lepicard *et al.*, 1981). We have used the same membrane-containing subcellular fraction as for this enzymic study, but failed to demonstrate specific steroid binding by conventional methodology based on reversible interaction; this was probably due to the relatively low affinity of the receptor (see below) and to the considerable amount of non-specific binding. Therefore we took advantage of the two-double-bond system of the synthetic progestin R 5020, an agonist of germinal vesicle breakdown, in order to obtain affinity labelling of a putative receptor after u.v. activation.

R 5020 binding has been described previously in *Rana pipiens* oocyte cytosol (Kalimi *et al.*, 1979) and *X. laevis* oocyte membrane (Sadler & Maller, 1982), but in neither case was the binding of physiological progesterone convincingly demonstrated (see the Discussion). This points to the difficulty of using a synthetic derivative which, besides binding to the same site as the natural hormone, may also bind to other sites that are not physiologically significant.

Abbreviations used: R 5020, 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione; R 2341, 17β-hydroxy-4,9,11-oestratriene-3-one; SDS, sodium dodecyl sulphate.

Materials and methods

Preparation of subcellular fractions

Oocytes from *X. laevis* were defolliculated with collagenase as described (Schorderet-Slatkine &

Drury, 1973). These experiments were carried out with full grown (stage V–VI) oocytes, and germinal vesicle breakdown was scored as usual (Schorderet-Slatkine, 1972).

Oocytes were homogenized in 10 vol. of buffer {83 mM-NaCl, 1 mM-$MgCl_2$, 10 mM-Hepes [4-(2-hydroxy-ethyl)-1-piperazine-ethanesulphonic acid], pH 7.9 (Sadler & Maller, 1982) in a Dounce homogenizer (20 strokes, pestle A). The pellet obtained at 100*g* for 10 min (P-100) contained mainly yolk platelets. Centrifugation of the supernatant at 1000*g* for 10 min gave a melanosome-enriched pellet (P-1000). The plasma-membrane-containing fraction P-10000 was obtained by centrifugation of the supernatant at 10000*g* for 20 min (Finidori-Lepicard *et al.*, 1981). This fraction contained adenylate cyclase, 5'-nucleotidase and alkaline phosphodiesterase (Finidori *et al.*, 1982). Centrifugation of the supernatant at 165000*g* for 90 min yielded a microsomal P-165000 pellet. All pellets were resuspended in buffer and kept in liquid N_2 until utilization. In specific experiments, the endoplasm of oocytes was obtained by aspiration with a glass needle. A yolk platelet pellet was obtained after centrifugation at 1000*g* for 10 min, and further centrifugation of the supernatant at 10000*g* for 20 min gave a 'cytoplasmic particle fraction'.

Germinal vesicles were obtained by manually opening oocytes in Barth medium. Vitelline envelopes were manually dissected with watchmaker's forceps. These fractions were Dounce homogenized, pelleted at 10000*g* for 20 min and resuspended in buffer. Denuded oocytes were obtained by manually removing the vitelline envelope (Hirai *et al.*, 1983) in buffer containing 35% (v/v) glycerol.

Photolabelling experiments

[^{3}H]R 5020 (50 Ci/mmol) given by Roussel-Uclaf, alone or in combination with unlabelled steroids, was dissolved in buffer containing glycerol and ethanol. Biological fractions were added just before irradiation so that the final concentrations were 35% (v/v) glycerol and 1% (v/v) ethanol in 800 μl. The protein concentration (Lowry method) of the P-10000 was 0.6 mg/ml. Samples were placed in a glass Petri dish (4 cm diameter), the temperature being maintained at −15°C.

The centre of a 150 W mercury lamp (Hanau TQ 150, Heraeus, France) was positioned 2.5 cm above the sample. A water-cooled Pyrex jacket prevented the transmission of light of $\lambda < 300$ nm. Routinely, the samples were irradiated for 20 s by moving an opaque screen, and were thereafter transferred to centrifuge tubes that were kept at 0°C in the dark.

SDS/polyacrylamide-gel electrophoresis

Irradiated P-1000 and P-10000 were centrifuged at 27000*g* for 10 min and washed twice with buffer. P-165000 was washed by centrifugation at 100000*g* for 60 min. Cytosolic proteins were precipitated by 10% (w/v) trichloroacetic acid, and the precipitate was washed twice in 5% (w/v) trichloroacetic acid and then in diethyl ether.

Washed pellets were dissolved in sample buffer [10% (w/v) glycerol, 5% (v/v) mercaptoethanol, 3% (v/v) SDS, 62.5 mM-Tris, pH 6.8], boiled for 3 min and electrophoresed on polyacrylamide rod gels (0.6 cm × 8 cm) (Laemmli, 1970). Unless specified, 10% (w/v) acrylamide gels were used. Samples (0.3 mg of protein corresponding to 70 oocytes in the case of P-10000) were layered on a 7 mm stacking gel (3.75% acrylamide) and electrophoresed at a constant current of 3 mA/gel using Bromophenol Blue as tracking dye. Frozen gels were cut in 1.25 mm slices. Paired slices were shaken overnight in 1 ml of Soluene 350 (Packard) and the radioactivity was determined by liquid-scintillation counting.

Results

Specific photolabelling of the 30 kDa peptide

Electrophoresis of P-10000 previously irradiated in the presence of 0.1 μM-[^{3}H]R 5020 showed radioactivity migrating near the dye front on SDS/8.5% polyacrylamide gels (Fig. 1*a*). However, on 10% polyacrylamide gels, a radioactive peak was observed corresponding to a peptide of approx. 30 kDa (Fig. 1*b*). On an 11.5% polyacrylamide gel, an additional bound radioactivity peak was seen in the region of approx. 15 kDa (Fig. 1*c*).

Competition with 50 μM unlabelled R 5020 abolished labelling of all macromolecules (Fig. 1*c*). In contrast, 50 μM unlabelled progesterone selectively reduced by 60% the 30 kDa peak, when added simultaneously with the radioactive R 5020, indicating progesterone interaction with the corresponding macromolecules. Further experiments were conducted uniquely with 10% gels, and dealt with the major 30 kDa peak.

Omission of mercaptoethanol from the sample buffer did not change radioactivity migration, whereas the 30 kDa peak was abolished by pretreatment of P-10000 by Pronase (50 μg/ml) for 1 h at 20°C (results not shown). No change, and in particular no heavier peak, was observed when P-10000 was prepared in the simultaneous presence of soya bean trypsin inhibitor (50 μg/ml), phenylmethanesulphonyl fluoride (0.5 mM) and EDTA (1.5 mM) (results not shown). The presence of 0.1 mM-*p*-hydroxymercuribenzoate strongly inhibited photolabelling (results not shown). Additional controls showed that [^{3}H]R 5020 irradiation in absence of P-10000 or in presence of a P-10000 fraction prepared from *X. laevis* liver did not yield a 30 kDa labelled peak (results not shown).

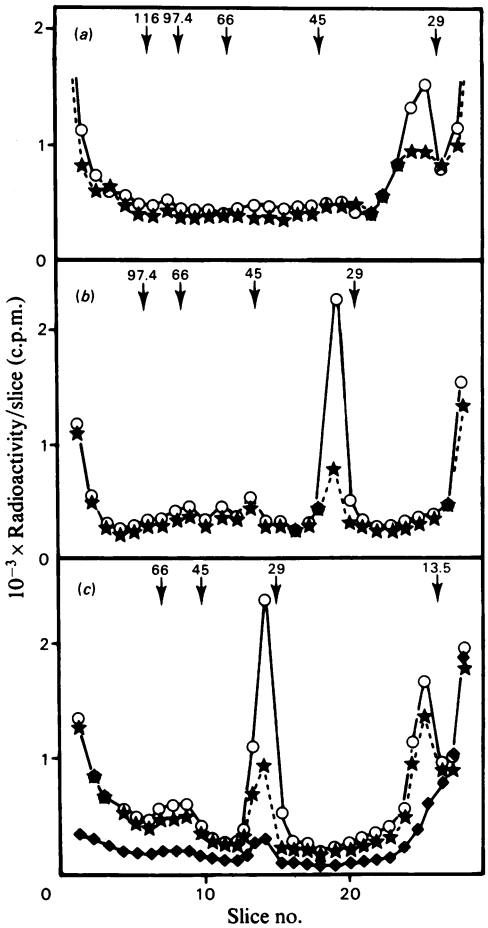

Fig. 1. *SDS/polyacrylamide-gel electrophoresis of P-10000 photolabelled with [^{3}H]R 5020*

Samples were irradiated, as described in the Materials and methods section, for 20s at −15°C with 0.1 μM-[^{3}H]R 5020 in the absence (○) or presence (★) of 50 μM unlabelled progesterone or in the presence of 50 μM unlabelled R 5020 (♦). Unbound steroids were removed by centrifugation washings and samples (corresponding to 70 oocytes) were electrophoresed. The concentration of polyacrylamide was 8.5% (*a*), 10% (*b*) or 11.5% (*c*). The arrows indicate M_r ($\times 10^{-3}$) values.

Irradiation kinetics

We found that photolabelling of the 30kDa peak was biphasic as a function of time. Maximum covalent binding was observed after 1–5min of irradiation, and was followed by a slow decrease (Fig. 2). When photoaffinity labelling was performed in the presence of 50 μM-progesterone and the residual ('non-specific') bound radioactivity was subtracted, the secondary decrease of the progesterone-depressible [^{3}H]R 5020 binding ('specific') was faster, suggesting that two or more binding sites with different stability might be involved in total [^{3}H]R 5020 binding.

Pre-irradiation (1min) of [^{3}H]R 5020 in the absence of the P-10000 fraction suppressed the 30kDa peak after secondary irradiation in the presence of P-10000 (Table 1). Thus the decrease of velocity after 15s and the plateau observed at 1–5min may not correspond to saturation or equilibrium, but to a rapid inactivation of [^{3}H]R 5020 available for covalent coupling. A 1min irradiation of P-10000 alone before exposure to [^{3}H]R 5020 and photolabelling, yielded a small decrease of the 30kDa peak (Table 1).

The decrease of binding described in Fig. 2 was found to be time- and temperature-dependent (Table 1). To work under initial velocity conditions and to minimize degradation of the radioactive complexes, short exposure time and low temperature had to be used, and for systematic studies we chose 20s irradiation at −15°C. Preincubation of P-10000 at 0 or 20°C in the dark with [^{3}H]R 5020 with or without 50 μM-progesterone for up to 15min did not change the amount of 30kDa peak formed. This indicated that the reversible association between R 5020 and binding sites was rapid, compared with the covalent coupling step. This allowed the use of classical representation of enzymology to determine apparent K_d and K_i values for photoreactive and non-photoreactive steroids, respectively, provided initial velocity was used (Hanstein, 1979).

Subcellular distribution

After treatment of P-100 with [^{3}H]R 5020, most of the covalently bound radioactivity migrated as a broad peak in the 80–120kDa region (Fig. 3*a*), corresponding to a large protein band seen in a companion gel stained with Coomassie Blue. This binding was not inhibited by 50 μM-progesterone. Additionally, two other 45 and 30–35kDa radioactive peaks, not depressed by progesterone, were observed. Similar results were obtained with yolk platelets prepared by aspiration of the endoplasm (results not shown).

The same results as with P-100 were obtained with P-1000, which is contaminated by small yolk platelets. Melanin did not enter the gel (results not shown).

In addition, when the cytoplasmic particle fraction was prepared (see the Materials and methods section), carefully avoiding contamination by the oocyte cortex, the 30kDa receptor was not seen (Fig. 3*b*). Moreover, neither the P-165000 nor the cytosol fractions contained any covalently labelled peak (Fig. 3*c*). Finally, in germinal vesicle and vitelline envelope fractions, only the progesterone

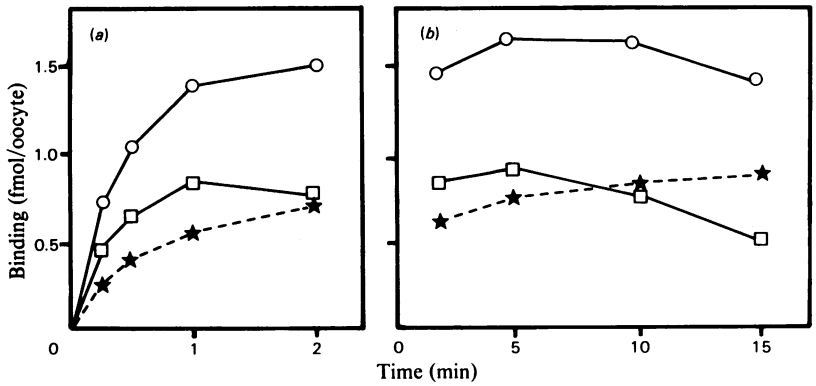

Fig. 2. *Time course of covalent binding of [^{3}H]R 5020 to P-10000*
Samples were irradiated for the indicated times with 0.1 μM-[^{3}H]R 5020 and analysed on SDS/10% polyacrylamide gels. The amount of [^{3}H]R 5020 covalently bound to the 30 kDa receptor was obtained by summing the radioactivity of each point of the peak and subtracting the base-line value. Progesterone-suppressible binding (□) was determined by difference between the data obtained in the absence (○) or presence (★) of 50 μM unlabelled progesterone. (*a*) and (*b*) represent two experiments conducted with different P-10000 preparations.

Table 1. *Effect of pre-irradiation and time and temperature of irradiation on [^{3}H]R 5020 covalent binding to the 30 kDa receptor*
P-10000 samples were irradiated for the times and at the temperatures indicated in the table in the presence of 0.1 μM-[^{3}H]R 5020 alone (total binding) or together with 50 μM unlabelled progesterone (residual binding). Progesterone-suppressible binding was obtained by difference between total and residual binding. Pre-irradiation of R 5020 alone or membranes alone was for 1 min at −15°C. Results are expressed as the percentage of total binding obtained by irradiation for 20 s at −15°C and in the absence of pre-irradiation.

Pretreatment	Time of photolabelling	Temperature (°C)	Total binding (%)	Residual binding (%)	Progesterone-suppressible binding (%)
R 5020 pre-irradiated	20 s	−15	40	32	7
Membranes pre-irradiated	20 s	−15	83	40	43
No pre-irradiation	20 s	−15	100	35	65
	20 s	0	100	43	58
	20 min	−15	65	53	12
	20 min	0	65	66	0

non-suppressible 100 kDa and 45 kDa peaks were observed, in variable amounts, suggesting contamination by yolk (Fig. 3*b*).

When intact oocytes were irradiated in the presence of 0.1 μM-[^{3}H]R 5020, and the P-10000 fraction subsequently prepared, the 30 kDa region of the gels was poorly labelled (results not shown), whether oocytes were preincubated (16 h) with the radioactive ligand or not. Removal of the vitelline envelope slightly increased the labelling, which nevertheless remained only approx. 10% of the corresponding amount labelled in the isolated P-10000 fraction.

Binding constants of R 5020 and progesterone in P-10000

Various concentrations of [^{3}H]R 5020 (0.1–50 μM) were used in the presence or absence of 50 μM-progesterone (20 s irradiation). An Eadie plot revealed two categories of sites (Fig. 4). The first sites ('A') had apparent $K_d = 1.2$ μM and $V_{max.} = 17$ fmol/min per oocyte, and their labelling by [^{3}H]R 5020 was abolished completely by 50 μM-progesterone. The sites corresponding to [^{3}H]R 5020 binding not depressed by progesterone ('B') had apparent $K_d = 13$ μM and $V_{max.} = 140$ fmol/min per oocyte.

When 0.1 μM-[^{3}H]R 5020 was used at several concentrations of progesterone (0–250 μM), the Dixon plot confirmed that radioactive binding inhibition by progesterone was complex (Fig. 5*a*). Assuming independent binding sites and fully competitive mechanisms, an apparent K_i of approx. 300 μM was determined from values obtained with progesterone concentrations $\geqslant 50$ μM, corresponding to the B sites. Data for A sites, obtained

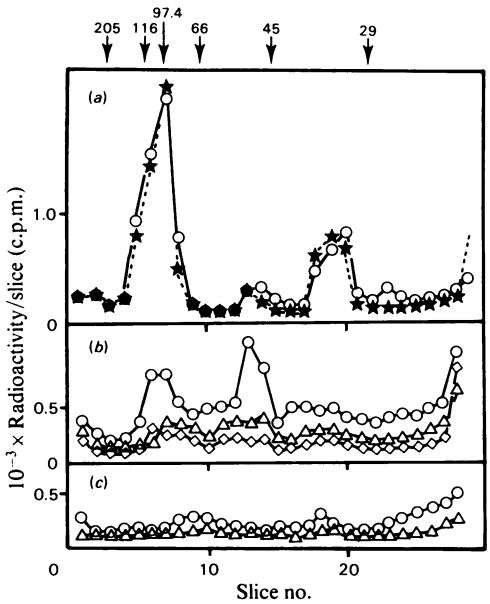

Fig. 3. *Covalent binding of [³H]R 5020 to various subcellular fractions*

Fractions were prepared as described in the Materials and methods section, irradiated with 0.1 μM-[³H]R 5020 for 20s at −15°C and electrophoresed on SDS/10% polyacrylamide gels. (*a*) P-100 (vitelline platelets) irradiated in the absence (○) or presence (★) of 50 μM-progesterone; (*b*) germinal vesicles (○), vitelline envelopes (△) and cytoplasmic particle fraction (◇); (*c*) cytosol (○) and P-165000 (microsomes) (△). Arrows show M_r ($\times 10^{-3}$) values.

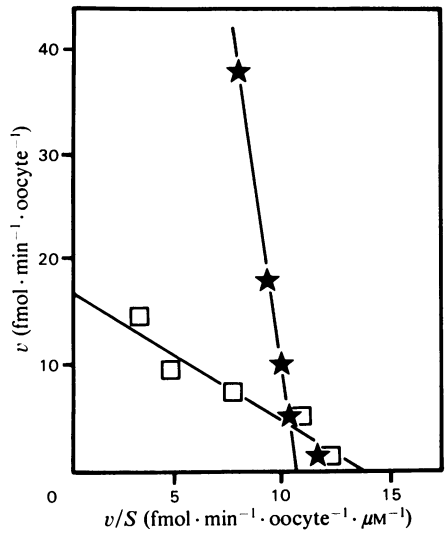

Fig. 4. *Eadie plot of [³H]R 5020 photolabelling of the 30kDa receptor*

Initial velocities (v) of photolabelling were determined after irradiation of P-10000 for 20s at −15°C, as described in the legend to Fig. 2. The concentration of [³H]R 5020 (S) ranged from 0.1 to 50 μM. ★, Progesterone-nonsuppressible covalent binding (B sites) measured in the presence of 50 μM-unlabelled progesterone; □, progesterone-suppressible covalent binding (A sites) obtained by difference between total binding and binding to B sites.

by subtracting the 'non-specific' binding measured in the presence of 50 μM-progesterone, gave a straight Dixon plot (Fig. 5*b*) with apparent $K_i = 3.8$ μM (S.D. = 0.5, $n = 4$).

Steroid binding specificity

A variety of steroids (50 μM) were tested for their inhibition of the u.v.-driven covalent attachment of [³H]R 5020 (0.1 μM), in the presence or absence of 50 μM-progesterone. The fraction of residual [³H]R 5020 binding to sites A and B was determined. Dose–response curves for germinal vesicle breakdown were determined in parallel for each steroid and results were normalized by defining an arbitrary value of 1 for the progesterone concentration producing 50% germinal vesicle breakdown. The results are summarized in Table 2. Progesterone was found to be the best binder to A sites, closely followed by biologically very active steroids such as testosterone, deoxycorticosterone and 4-androstenedione. Conversely, the non-agonist cholesterol was a weak competitor. Rank correlation between the fraction of residual covalent binding to A sites and the relative concentration inducing 50% germinal vesicle breakdown was highly significant ($P \ll 0.01$), since $r = 0.80$ for $n = 17$. R 2341, and to a lesser extent oestradiol, both antagonists of progesterone-induced oocyte maturation (Baulieu *et al.*, 1978), were good competitors for A sites. Forskolin, which inhibits progesterone-induced germinal vesicle breakdown by an activation of membrane-bound adenylate cyclase (Schorderet-Slatkine & Baulieu, 1982), was a weak competitor. No correlation between binding and action was observed for B sites.

Discussion

The reported data demonstrate the u.v.-driven covalent attachment of [³H]R 5020 to a *X. laevis* oocyte homogenate fraction sedimenting between 1000 and 10000*g* (P-10000), known to contain plasma membrane and adenylate cyclase that is specifically inhibited by progesterone (Finidori *et al.*, 1982). A 30kDa photolabelled fraction was detected by SDS/polyacrylamide-gel electrophoresis under denaturing conditions. The 30kDa material seemed to involve a protein component

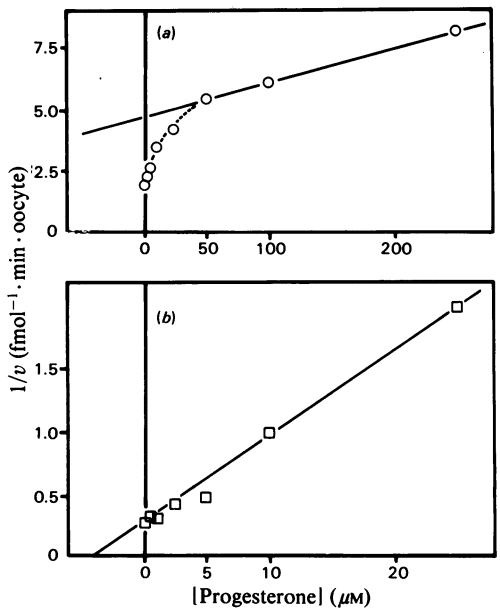

Fig. 5. *Dixon plot of inhibition by progesterone of* [^{3}*H*]*R 5020 photolabelling of the 30kDa receptor*
Initial velocities (v) of photolabelling were determined as described in the legend to Fig. 4. (*a*) Total binding (A + B sites) obtained when the progesterone concentration was varied from 0 to 250 μM; (*b*) data obtained by subtracting the value of v obtained in the presence of 50 μM-progesterone.

(Pronase experiment), of yet undefined characteristics. It was not observed in other subcellular fractions which do not contain plasma membrane (Fig. 3). It was not seen in the corresponding P-10000 fraction of liver cells. Although principally studied on 10% polyacrylamide gels, the 30kDa peak was also examined on 11.5% polyacrylamide gels, which revealed in addition an approx. 15kDa bound radioactivity peak. This did not seem to be a proteolytic or a subunit fragment of the 30kDa peptide under our experimental conditions (see the Results section); this 15kDa component, which is not progesterone-depressible, has not been further studied.

A fundamental result was the inhibition of the covalent labelling by [^{3}H]R 5020 of the 30kDa receptor by 50 μM-progesterone. The K_i of the inhibition was calculated to be approx. 3.8 μM. This has not been found for the reported 'steroid receptor' described by Sadler & Maller (1982) as a 110kDa peptide. The [^{3}H]R 5020 labelling that they described was augmented rather than inhibited by progesterone. However, they used SDS/8.5% polyacrylamide gels, with which the 30kDa peak is difficult to observe (Fig. 1*a*). In our P-10000 fraction, we never observed any photolabelling in the 110kDa area. In all other fractions, either enriched in or contaminated by yolk vitelline proteins, we detected a 80–120kDa component that bound [^{3}H]R 5020 covalently. This binding, as well as the smaller one occasionally seen in the 45kDa area, was not inhibited by progesterone.

Inhibition by *p*-hydroxymercuribenzoate suggested that one SH group is essential in binding and/or covalent interaction of [^{3}H]R 5020 with the 30kDa receptor, since competitive inhibition of steroid binding is unlikely. This observation also may be related to the effect of organomercurials, reinitiating maturation by interaction at the oocyte surface level (Pays *et al.*, 1977), although there are several potential targets for *p*-hydroxymercuribenzoate in the membrane.

Quantitative studies and binding kinetics have revealed the rapid inactivation of [^{3}H]R 5020 by irradiation, preventing the use of classical representations for reversible binding such as the Scatchard plot, and thence determination of the number of binding sites. However, the use of a very short irradiation time is compatible with a measure of the initial velocity of photolabelling. Since the latter is relatively slow as compared with the reversible binding of R 5020 to the 30kDa receptor, this permitted the treatment of the data according to Michaelis–Menten kinetics to determine the K_d of R 5020 and the K_i for progesterone (assuming that there is competitive inhibition of the photolabelling reaction). The kinetics of inactivation (Fig. 2) suggest that the 30kDa peak could be the sum of two independent binding systems for [^{3}H]R 5020, differentially inhibited by progesterone and of different stability. The Eadie plot (Fig. 4) confirmed the presence of two binding systems. The 'high' affinity binding sites (A sites) had $K_d = 1.2$ μM, compatible with the concentration of steroid inducing 50% germinal vesicle breakdown (Sadler & Maller, 1982). The lower-affinity B sites have an apparent affinity 10-fold less. The measured $V_{max.}$ values for A and B sites were 17 and 140fmol/min per oocyte, respectively, but the difference in binding site number is not known, since the proportionality factor may be different for A and B sites. Inhibition data obtained with progesterone also demonstrate binding heterogeneity. Assuming that the A and B sites are independent and that [^{3}H]R 5020 binding to both sites is competitively inhibited by progesterone, K_i values were respectively 3.8 and 300 μM (Fig. 5). The ratio of the K_d of R 5020 for A and B sites is approx. 10, whereas the ratio of the K_i for progesterone is approx. 100. This may mean that only A sites have a biological significance related to the action of progesterone. The fact that the affinity of R 5020 seems higher than that of progesterone whereas the germinal vesicle breakdown activity

Table 2. *Inhibition of* [^{3}*H*]*R 5020 (0.1 μM) binding by various steroids or steroid analogues*

The inhibition was measured for a single concentration (50 μM) of each compound, in the absence (A and B sites) or presence (B sites) of 50 μM-progesterone and expressed as the fraction of residual [^{3}H]R 5020 binding to A and B sites. The relative ability of each compound to induce germinal vesicle breakdown (except for the antagonists oestradiol, R 2341 and forskolin) was estimated from dose–response curves (doses range from 0.01 to 50 μM) and expressed as a maturation index (ratio of the compound to progesterone concentrations that induce 50% vesicle breakdown). Oocytes were prepared from human choriogonadotropin-treated (125 units) females and percentage germinal vesicle breakdown was recorded after a 10 h incubation, on groups of 30 oocytes. Rank correlation (antagonist excluded) between the fraction of R 5020 residual binding to A sites and the maturation index gives $r = 0.80$ for 15 degrees of freedom ($P \ll 0.01$).

Steroid	Fraction of R 5020 residual binding			Maturation index	(Rank)
	A sites	(Rank)	B sites		
Progesterone	0	(1.5)	0.87	1	(2.5)
4-Androstene-3,17-dione	0	(1.5)	0.65	2.6	(4)
Testosterone	0.05	(3)	0.89	1	(2.5)
3α-Hydroxy-5β-pregnan-20-one	0.08	(4)	0.63	8	(6)
Deoxycorticosterone	0.14	(5)	0.75	0.5	(1)
Corticosterone	0.28	(6)	0.76	13	(8.5)
5α-Dihydrotestosterone	0.32	(7)	0.84	10	(7)
20β-Hydroxy-4-pregnen-3-one	0.35	(8)	0.87	26	(12)
11α-Hydroxyprogesterone	0.41	(9.5)	0.70	20	(10.5)
5α-Androstane-3α,17β-diol	0.41	(9.5)	0.97	13	(8.5)
5α-Dihydrocorticosterone	0.42	(11)	0.93	22	(10.5)
3β-Hydroxy-5-pregnen-20-one	0.44	(12)	0.69	32	(13)
Diethylstilboestrol	0.63	(13.5)	0.67	400	(16)
5α-Pregnane-3β,20α-diol	0.63	(13.5)	0.88	80	(14)
Cortisol	0.65	(15)	0.75	6.3	(5)
Ouabain	0.81	(16)	0.73	400	(16)
Cholesterol	0.84	(17)	0.88	400	(16)
Oestradiol	0.24	–	0.74		–
R 2341	0.29	–	0.46		–
Forskolin	0.59	–	0.80		–

of progesterone is greater than that of R 5020 is unclear. Differences of metabolism under the conditions used, also the fact that the affinity was determined by a method involving brief exposure to the steroid, whereas activity takes hours to be recorded, may be involved. In addition, even if the Dixon plots obtained with 50 μM-progesterone-suppressible velocities intersect above the abscissa (results not shown), in a way compatible with fully competitive inhibition, a more complex, partially mixed inhibition is yet possible.

The apparent affinity of several hormones for A sites is highly correlated with their ability to provoke germinal vesicle breakdown. The only discrepancy involves cortisol, which is not as good a competitor as its inducing potency suggests. The same was observed for cyclase inhibition (Finidori-Lepicard *et al.*, 1981), and cortisol is the only steroid very active after injection into oocytes (Drury & Schorderet-Slatkine, 1975). Cortisol may act at a level and by a mechanism different from other steroids, or alternatively there might be an activating metabolism in intact oocytes not observed in cell-free experiments. It is very interesting that the progesterone antagonists oestradiol and R 2341 were relatively good competitors, confirming the concept of receptor-mediated antagonism (Baulieu *et al.*, 1978). It would have been structurally conceivable for the diterpene forskolin, an activator of adenylate cyclase in the membrane (Schorderet-Slatkine & Baulieu, 1982), to bind to the steroid site, but it did not show competition for [^{3}H]R 5020. Ouabain, a steroid which binds to ($Na^+ + K^+$)-dependent ATPase, also did not compete for [^{3}H]R 5020.

The relatively low labelling of the 30 kDa receptor in intact oocytes, even after denudation of the vitelline envelope, may be due to poor efficiency of u.v. irradiation under the conditions used. Alternatively, this result might be related to the possibility that the binding site is located in the inner part of the plasma membrane (Tso *et al.*, 1982).

The progesterone receptor here described differs from steroid binding to melanin (Ozon & Bellé, 1973; Jacobelli *et al.*, 1974) and R 5020 binding to the cytosol fraction of *Rana pipiens* oocytes (Kalimi *et al.*, 1979). In fact, we could not obtain covalent binding of [^{3}H]R 5020 in melanosomes or in cyto-

sol of *X. laevis* oocytes. Finally, a molecular mass of 30kDa is far from what is found for intracellular steroid hormone receptors in somatic cells, including oestrogen receptor in *X. laevis* (Bergink & Wittlif, 1975). That the 30kDa receptor displays the appropriate characteristics qualifying it for being a receptor in this system does not indicate that it is the whole of the receptor: the definition of receptor adopted in the present work is specifically related to specific steroid binding. If the intact receptor is a multiunit structure, the 30kDa component might have been separated from other components present.

In conclusion, the binding sites 'A' of the progesterone receptor described here satisfy accepted criteria applied to receptor characterization, in terms of rapid binding, apparent saturability, affinity related to biological activity of ligands, whether they are the natural hormone or related compounds. Moreover, its exclusive localization in a cell-free fraction where hormone activity can be demonstrated (Finidori-Lepicard *et al.*, 1981) concurs to establish its biological significance. However, its purification, and further reconstitution experiments, will be necessary to a better understanding of progesterone action in *X. laevis* oocyte maturation.

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