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A POSSIBLE MECHANISM FOR THE ANTI-INDUCING EFFECT OF PROGESTERONE IN RAT LIVER

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The mechanism of the anti-inducing effect of progesterone has been explored in the glucocorticoid receptor system from rat liver. Sodium molybdate was used to stop the reaction at the level of the binding step.On the basis of our results we propose that progesterone binds to the receptor on a site (Pro-site) which is different from the one to which binds dexamethasone (Dex-site). Moreover we suggest that progesterone induces the transition to an "incompetent form" which is able to bind dexamethasone.

INTRODUCTION

Progesterone has been classified as an anti-inducer for glucocorticoid action (1). It was shown to bind specifically to the glucocorticoid receptor in intact cells and in cell-free systems, but no accumulation of the hormone receptor complexes was observed in the nucleus (2). Furthermore,an allosteric biconformational model has been proposed to support the anti-inducer effect of progesterone (1-3). More recently, Suther and al. (4) suggested the existence of a second site which might interfere with the occupancy of the glucocorticoid binding site. Jones and Bell (5) provided evidence of a negative cooperative interaction between the two binding sites. Svec and Rudis (6) demonstrated the existence of an antagonist progesterone-like binding site on the glucocorticoid receptors from various target tissues. However, because of the high concentrations of progesterone used, the physiological significance of this second site is not clear.

Our experiments allow us to suggest a mechanism for the anti-inducer effect of progesterone. Using molybdate as an inhibitor for the temperature dependent "activation" step, it was possible to gather evidence for progesterone-glucocorticoid receptor interactions on the "non activated form" without taking into account the activation process.

MATERIALS AND METHODS :

[^{3}H] dexamethasone (41-46 Ci/mM) in ethanol solution was obtained from the Radiochemical Centre - Amersham, UK. Non radioactive steroids were from Roussel Uclaf, Romainville, France and Sigma, London; other reagents from

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Merck, Darmstadt; activated charcoal and Dextran T_{70} from Pharmacia, Sweden. Preparation of the rat liver cytosol was performed as already described (7). Buffer I contained 20 mM Tris-HCl pH 7.4 , 25 mM KCl, 2.5 mM $MgCl_2$; 1 mM β- mercaptoethanol was added extemporaneously. Buffer II was the same as Buffer I but contained 20 mM sodium molybdate (assuming a final concentration of 12 mM in the incubations mixtures). The concentrations of proteins in the cytosol were adjusted to 15-20 mg/ml (8).

Steroid binding studies : Radioactive [^{3}H] dexamethasone was diluted in buffer and added to the cytosol at a final concentration of 2.10^{-8}M. Stock solutions containing 2.10^{-3}M non radioactive steroids were prepared in ethanol (a 1 % final concentration ethanol did not affect the binding parameters). Cytosol was routinely incubated in parallel with or without 2.10^{-5}M unlabelled dexamethasone in order to determine the non specific binding which was substracted from all measurements. The results given represent the specific binding. After incubation with the steroid, the cytosol was treated by the dextran-charcoal technique in order to eliminate free hormones and to determine protein binding (9).

- Steady-state assays were used to test the stability of the complexes : the cytosol was incubated in the presence of radioactive dexamethasone for 2 hours at 4°C before being transferred to 22°C. Samples of 0.2 ml were removed at different time intervals and treated by the dextran coated charcoal method. The radioactivity was measured in the supernatant.
- "Chase experiments" : after the 2 hour incubation at 4°C, the cytosol was incubated again during 5 min at 22°C ; then various concentrations of unlabelled dexamethasone were added and 0.2 ml samples were removed and treated like in steady-state assays.
- Competition assays : the same procedure as in "chase" experiments was used except unlabelled progesterone was added in place of unlabelled dexamethasone.
- Competitive association kinetics : cytosol was incubated at 4°C in the presence of [^{3}H] dexamethasone with or without unlabelled progesterone at various concentrations. 0.2 ml samples were tested for specific binding as a function of time.

Radioactivity was measured in a scintillation mixture (PPO 5g, diMePOPOP 0.5 g per 1 toluene) to which 0.2 volume Soluene was added in order to solubilize the aqueous sample (0.2 ml sample + 3 ml final mixture). Counting was performed in a Packard instrument with 35 % efficiency for tritium.

RESULTS AND DISCUSSION

In order to analyze the interaction of progesterone with the glucocorticoid receptor, we attempt to block the binding complex by using molybdate ions which have been shown to stop in vitro either the inactivation of the receptor or the activation process of the complex (10). Dissociation experiments were performed at 22°C since we noticed that at this temperature the stability of the complex was higher than at a temperature over 22°C.

Previous results obtained by Suther and al. (4) and by Jones and Bell (5) were confirmed by the "chase" experiments shown in figure 1. We observed that unlabelled progesterone (2.10^{-5}M) as compared to the same concentration of dexamethasone, enhances the dissociation of [^{3}H] dexamethasone. However, we observed, after a while, a significant reassociation of [^{3}H] dexamethasone with the receptor in the presence of progesterone.

When progesterone was added at various concentrations during "chase" experiments with radioactive complexes, we observed that the magnitude of the dissociation period was dependent on the concentration of progesterone added

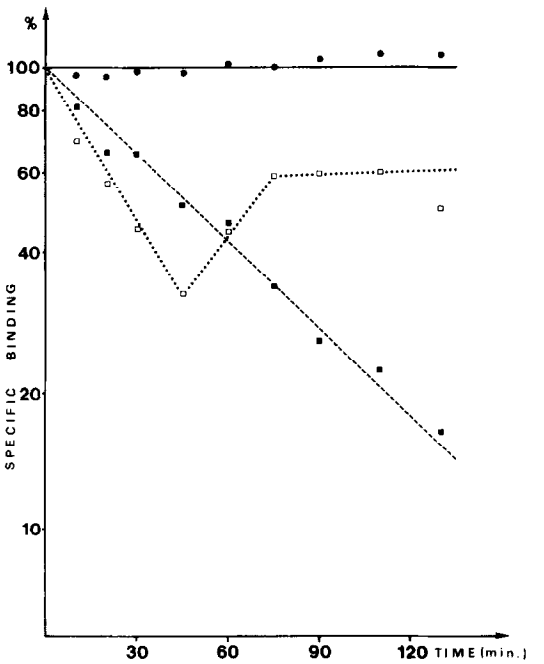

Fig.1 Steroid effect on the dissociation of [^{3}H] dexamethasone. The cytosol was incubated in the presence of [^{3}H] dexamethasone (2.10^{-8} M) ; after a 5 min preincubation at 22°C, 0.2 ml samples were tested for specific binding at different time intervals either in the presence of dexamethasone (2.10^{-5} M) (■-----■) or progesterone (2.10^{-5} M) (□·····□). In the control (●———●), binding was measured in the absence of non radioactive steroid. The results are expressed as a percentage of the specific binding measured at time 0 (100% : 0.18 pmoles [^{3}H] dexamethasone bound/mg protein).

(pannel A, fig.2). These results were confirmed by experiments shown in pannel B (fig.2) when unlabelled dexamethasone receptor complexes were submitted to exchange with [^{3}H] dexamethasone ($2 \cdot 10^{-8}$M) ; the labelled hormone was introduced in the incubation mixture in place of unlabelled dexamethasone with various concentrations of progesterone. In the control where progesterone was omitted, the exchange reached 60 % of the optimal binding after 130 min . These data are compatible with the dissociation rate.

In the presence of progesterone, a delayed exchange occurred depending on the concentration of progesterone, associated with an enhanced late reassociation for the lowest concentrations tested. High concentrations of progesterone inhibit the reassociation and finally do not allow dexamethasone to bind anymore. Jones and Bell (5) did not observe any reassociation, probably because of the high concentration of progesterone present (50 µM) during their kinetic studies. Moreover they worked at 4°C which temperature is too low to allow such an effect during the period of time chosen.

In another set of experiments, we studied the association of the hormone over a period of 24 hours (fig.3). We observed that the inhibition of dexa-

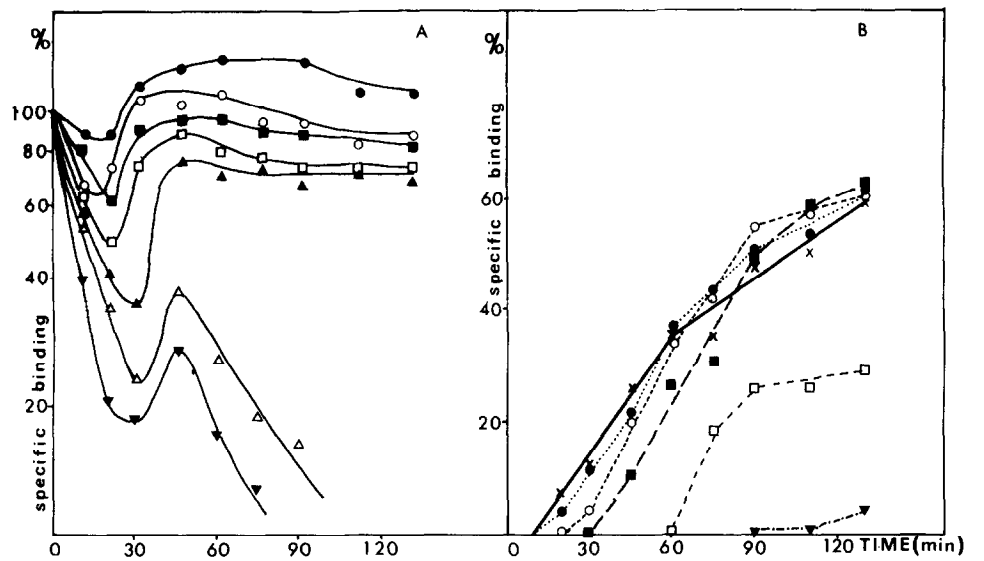

Fig.2 Effect of various concentrations of progesterone on the specific binding of [^{3}H] dexamethasone to the glucocorticoid receptor.
Pannel A : The cytosol was incubated successively (1) at 4°C with [^{3}H] dexamethasone (2.10^{-8} M) during 2.5 hours, (2) at 22°C for 5 min, before progesterone was added at time 0 at the following concentrations :
●—● 2.10^{-6} M, ○—○ 10^{-5} M, ■—■ 2.10^{-5}M, □—□ 3.10^{-5}M,
▲—▲ 4.10^{-5} M, △—△ 5.10^{-5}M, ▼—▼ 10^{-4}M . 0.2 ml samples were removed and tested for specific binding.

Pannel B : The cytosol was incubated at 4°C with unlabelled dexamethasone (2.10^{-8}M) during 2.5 hours. Free hormone was removed (by the dextran charcoal technique) and cytosol preincubated at 22°C for 5 min . [^{3}H] dexamethasone (2.10^{-8}M) and progesterone at various concentrations (x—x 0M, ●·····● 2.10^{-6}M, ○- - -○ 10^{-5}M, ■— —■ 2.10^{-5}M, □·---□ 3.10^{-5}M, ▼— —▼ 10^{-4}M) were added at time 0 in order to allow an exchange between unlabelled and labelled hormone. Samples (0.2 ml) were removed and tested for specific binding. Results are expressed as a percentage of total specific binding (100 % : 0.20 pmoles [^{3}H] dexamethasone bound/mg proteins).

methasone binding in the presence of molybdate ions was time dependent and varied as a function of the concentration of progesterone added (fig 3). After approximately 4 hours the binding was almost not prevented by the lowest concentrations of progesterone tested (2.10^{-6} - 10^{-5}M). Also 20 hours later no more inhibition of the binding was observed in the presence of less than 5.10^{-5}M progesterone.

On the basis of our results we propose a model for the anti-inducer activity of progesterone (fig.4) which may also explain the experiments recently reported in the litterature. When the anti-inducer binds to the progesterone site (Pro-site) it might provoke an allosteric transition during which the inducer is ejected from tis own site (dexamethasone site or Dex-site). This conformation of the receptor might be rather unstable and give rise to a more stable form when a new site becomes available for the binding

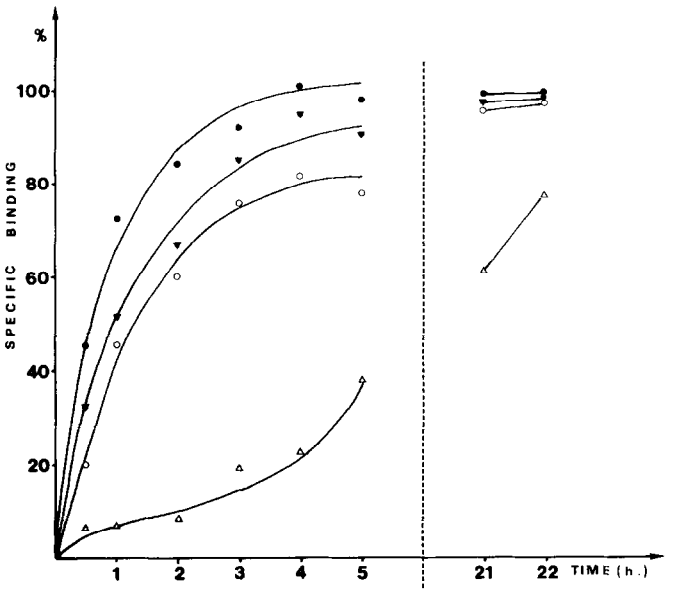

Fig. 3 Association of [^{3}H] dexamethasone (2.10^{-8}M) to the glucocorticoid receptor in the presence of progesterone (●——● 2.10^{-6}M, ▼——▼ 5.10^{-6}M, O——O 10^{-5}M, Δ——Δ 5.10^{-5}M). The experiment was performed at 4°C in the presence of molybdate (12mM). The results are expressed as a percentage of the total specific binding measured in the control (cytosol incubated without progesterone).

of the inducer ; we propose to call this form which would not express any biological activity : the "incompetent form". The existence of such a form is conceivable since in vivo experiments (11) suggest that the continuous presence of the anti-inducer is not required to inhibit the biological response.

Our system might have been particularly favorable to evidence this transitional step to the incompetent form by using both molybdate ions to block the receptor system and a convenient temperature (22°C).

We would also suggest that both sites are not strictly specific for inducers or anti-inducers. The Dex-site would have a much larger affinity for dexamethasone than for progesterone ; in this way it is possible to explain the very weak inducer effect of high concentrations of progesterone measured in vivo on TAT induction (1 - 11). The Pro site would have a much higher affinity for progesterone than for dexamethasone. This could be an explanation of the competitive effect of high concentrations of dexamethasone on the Pro site (5). Direct interaction of molybdate in that system cannot be ruled out but is almost improbable because in our association competitive system (fig.3) at 4°C, similar results were observed in the presence or in the absence of molybdate (results not shown). Degradation of progesterone during

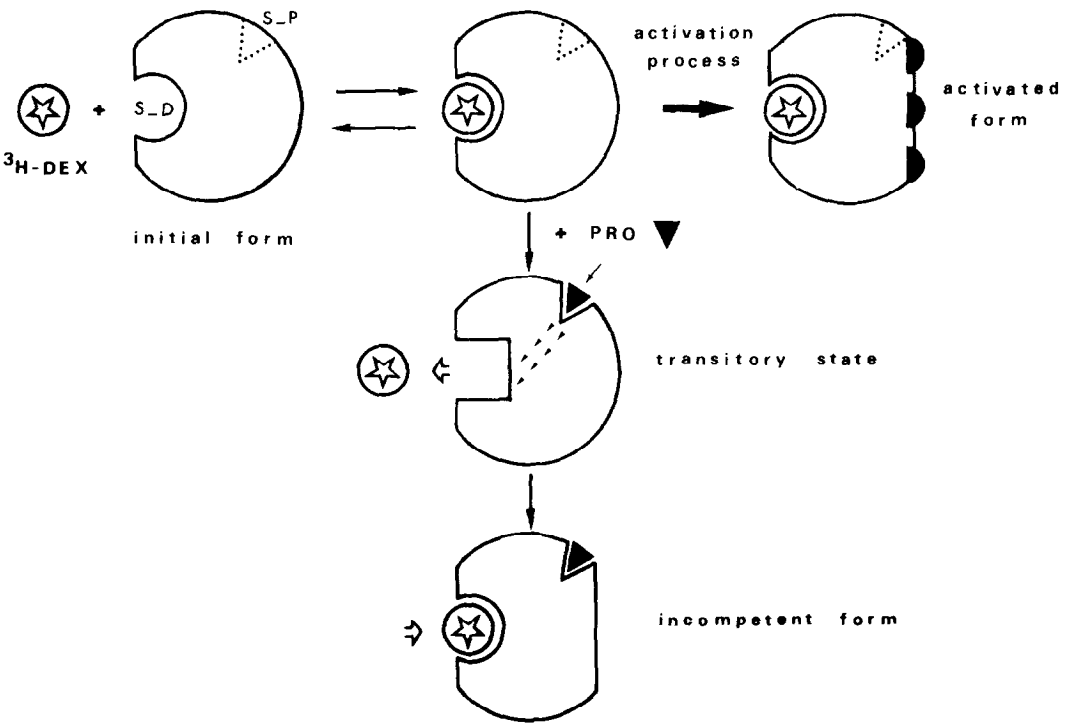

Fig.4 A possible model for the deviation of [^{3}H] dexamethasone receptor complex to an "incompetent form" by progesterone. S-D : dexamethasone-site S-P : progesterone-site.

the experiment is improbable since no labelled metabolite was visualized by chromatography on silica slab (technique provided by Amersham).

As already suggested (1-3), one could assume that the inability of some inducers to exert a full biological response is probably due to the different kinetics in the inducer and anti-inducer associations to their respective sites. In addition our model suggests that probably both sites are inter-dependent and exert a cross reactivity.

Other compounds should be tested, especially synthetic steroids, in order to determine if they act in a manner comparable to that found for progesterone in the present study.

ACKNOWLEDGEMENTS

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