Uterine Oestrogen-Receptor Binding to Oligo(dT)–Cellulose

AN INHIBITOR FROM HYPOTHALAMIC CYTOSOL

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The binding of rat uterine cytosol oestrogen receptor *in vitro* to oligo(dT)–cellulose is mediated by an activating factor in the cytosol [Thrower, Hall, Lim & Davison (1976) *Biochem. J.* 160, 271–280]. A potent inhibitor of this binding is present in hypothalamic cytosol. This inhibitor may have a role *in vivo* in regulating receptor translocation in the hypothalamus.

The oestrogen receptor present in rat uterine cytosol will bind *in vitro* to chromatography columns containing DNA–cellulose or oligo(dT)–cellulose; the binding to the two matrices is the same (Hall *et al.*, 1976; Thrower *et al.*, 1976). If this bound receptor is eluted from the column by using 0.4–0.6M-KCl and subjected to sucrose-density-gradient centrifugation, it behaves as a 5S species distinct from the 4S receptor present in untreated cytosol. Nuclear oestrogen receptor extracted from preparations of uterine nuclei by 0.4–0.6M-KCl also behaves as a 5S species on sucrose density gradients, and there is increasing evidence that the binding of cytosol receptor *in vitro* to DNA–cellulose or oligo(dT)–cellulose mimics the binding of the receptor in uterine nuclei *in vivo* (Yamamoto & Alberts, 1972; Fox, 1978) and *in vitro* (Haselbacher & Eisenfeld, 1976).

We have previously shown that the binding to oligo(dT)–cellulose involves an activation factor present in limiting amounts in uterine cytosol preparations (Thrower *et al.*, 1976). Rat hypothalamic oestrogen receptor will also bind to oligo(dT)–cellulose (White *et al.*, 1978) or DNA–cellulose (J. O. White & L. Lim, unpublished work), and hypothalamic receptor changes from 4S to 5S after translocation to the nucleus *in vivo* (Linkie, 1977). In the course of studies to see if the binding to oligo(dT)–cellulose involved a similar activation factor, we have discovered the presence in hypothalamic cytosol of a potent inhibitor of the action of the uterine activation factor.

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Experimental

Adult female Wistar rats [Charles River (U.K.), Margate, Kent, U.K.] were used at 60–80 days of age, without regard to the phase of the oestrous cycle.

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17 β -[2,4,6,7-³H]Oestradiol (sp. radioactivity 85Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Oligo(dT)–cellulose was from Collaborative Research, Koch–Light Laboratories, Colnbrook, Bucks., U.K.

Tissue from eight rats was pooled for each experiment. Preparation of uterine and hypothalamic cytosols in TED buffer [10mM-Tris/HCl (pH7.6)/1mM-EDTA/1mM-dithiothreitol] and measurement of specific oestrogen receptor (total bound [³H]-oestradiol minus non-specific-bound [³H]oestradiol) content and binding to oligo(dT)–cellulose were made essentially as previously described (White *et al.*, 1978). Incubation of cytosol preparations with [³H]oestradiol (1nM) in the absence or presence of a 100-fold excess of diethylstilboestrol was performed for 18–24h at 4°C. All assays were carried out in duplicate, and agreement was closer than 5% for both assay techniques.

To have approximately equal concentrations of receptor in the hypothalamic and uterine preparations, uterine cytosol [17–18% (w/v) wet tissue] was routinely diluted 1:30 [0.6% (w/v) with TED buffer]. This produced a considerable decrease in apparent content of receptor, and a small decrease in receptor binding to oligo(dT)–cellulose. However, in separate experiments (results not shown) we established that addition of either bovine serum albumin (5mg/ml), or hypothalamic cytosol in amounts used in these experiments, did not significantly increase the apparent content of uterine receptor in the diluted cytosol.

Preliminary experiments established that at the concentration of [³H]oestradiol used over 80% of the high-affinity hypothalamic and uterine binding sites were complexed to [³H]oestradiol. The use of 5nM-[³H]oestradiol, or the presence of a 100-fold excess of (unlabelled) oestradiol during incubation of labelled receptor with oligo(dT)–cellulose, did not alter the binding of hypothalamic or uterine receptor to the

column. All samples, even those where hypothalamic or uterine receptor had been masked with unlabelled oestradiol, contained 1 nM-[³H]oestradiol, so that non-specific binding of radioactive label would be unaffected by mixing of samples.

Results and Discussion

On a single incubation with oligo(dT)-cellulose, a much lower proportion (8–12%) of hypothalamic receptor-oestradiol complex bound to the matrix than did uterine complex (30–50%). We therefore expected that on mixing the radioactively labelled uterine cytosol with increasing amounts of hypo-

Fig. 1. Proportions of uterine receptor (a) and hypothalamic receptor (b) bound to oligo(dT)-cellulose

Each graph shows the combined results from three to four experiments. (a) After preincubation in 1 nM-[³H]oestradiol ± excess diethylstilboestrol, uterine cytosol [0.25 or 0.5 ml, 0.6% (w/v)] was mixed with various amounts of hypothalamic cytosol [0–0.5 ml, 17% (w/v)] in which the oestrogen receptor had been masked by preincubation in unlabelled 100 nM-oestradiol + 1 nM-[³H]oestradiol. The final volume of the mixture was made up to 1.0 ml with TED buffer containing 1 nM-[³H]oestradiol. From each mixture 2 × 0.2 ml was assayed for ³H-labelled receptor on Sephadex LH₂₀, and 2 × 0.2 ml was loaded onto 110 mg columns of oligo(dT)-cellulose, incubated for 1 h at 4°C, washed, and eluted in 0.6 M-KCl buffer. Specific bound radioactivity (receptor) was estimated as previously described (White *et al.*, 1978). (b) The same procedure as in (a) was followed, except that the hypothalamic receptor was radioactively labelled, and uterine receptor masked, and various amounts of uterine cytosol (0–0.5 ml) were added to the hypothalamic cytosol (0.25–0.8 ml) in a final volume of 1.0 ml before assay.

thalamal cytosol there would be a progressive decrease in binding of the labelled uterine receptor to the oligo(dT)-cellulose, if the uterine activation factor was compatible with both receptors; and no decrease in binding if the factor was specific for the uterine receptor only. Uterine oestrogen receptor was selectively measured by first masking the hypothalamic receptor with unlabelled oestradiol.

Fig. 1(a) shows that neither of the predicted results was obtained. Instead, a portion of hypothalamic cytosol equivalent to 15 mg of tissue produced over 80% inhibition of uterine-receptor binding, although at larger doses of the cytosol there was a significant recovery in the binding ($P < 0.001$). There was no change in the content of uterine receptor after mixing. Total non-specific binding increased linearly with added hypothalamic cytosol.

In similar experiments in which the hypothalamic receptor was radioactively labelled and uterine receptor masked with unlabelled oestradiol, we found no increase in hypothalamic receptor binding to oligo(dT)-cellulose (Fig. 1b), such as might have been expected if the hypothalamic receptor is sequestering the uterine activation factor. There is insufficient hypothalamic receptor for it to be lowering the observed binding of uterine receptor by complexing directly to the receptor.

Once uterine receptor was bound to oligo(dT)-cellulose, incubation with hypothalamic cytosol failed to remove it (results not shown). However, if the bound receptor, now in the 5S form (Thrower *et al.*, 1976), was eluted in 0.6 M-KCl and subsequently diluted with TED buffer, addition of 0.1 ml of hypothalamic cytosol again inhibited subsequent binding to oligo(dT)-cellulose, while 0.5 ml of hypothalamic cytosol again gave less inhibition (Table 1).

Table 1. Effect of hypothalamic cytosol on re-binding of 5S uterine receptor to oligo(dT)-cellulose

Uterine cytosol in TED buffer [1 ml, 17% (w/v)] was labelled with [³H]oestradiol and incubated on oligo(dT)-cellulose (300 mg) as previously described (White *et al.*, 1978), washed, and eluted with 1 ml of 0.6 M-KCl buffer. This '5S receptor' preparation was diluted to 6 ml with TED buffer, and 0.5 ml portions were mixed with 0, 0.1 or 0.5 ml of hypothalamic cytosol preincubated in unlabelled 10 nM-oestradiol, in a final volume of 1.0 ml. The mixtures were subsequently treated as described in the legend to Fig. 1(a). The number of determinations of binding is shown in parentheses. All values are within 10% of the mean.

Sample	Uterine receptor bound to oligo(dT)-cellulose (%)
5S receptor	57.8 (4)
5S receptor + 0.1 ml of hypothalamic cytosol	14.2 (2)
5S receptor + 0.5 ml of hypothalamic cytosol	31.2 (2)

The evidence points to the presence in the hypothalamus of an inhibitor of the uterine-receptor binding to oligo(dT)-cellulose. This inhibitor may bind the receptor itself, or it may bind the activation factor. The recovery of binding at higher concentrations of hypothalamic cytosol is probably due to the uterine receptor or receptor-inhibitor-complex binding to some other acceptor molecule from hypothalamus, which binds to oligo(dT)-cellulose.

It is unlikely that this inhibitor acts by blocking access to the oligo(dT), since this would also prevent binding of other proteins, but this remains to be tested. The presence of this inhibitor in hypothalamic cytosol provides a possible explanation of the poor binding of hypothalamic oestrogen receptor to oligo(dT)-cellulose, and also perhaps of the low extent of translocation of receptor in hypothalamus after oestradiol administration *in vivo* (White *et al.*, 1978).

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