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PROGESTERONE-INDUCED ESTROGEN RECEPTOR-REGULATORY FACTOR IN HAMSTER UTERINE NUCLEI: PRELIMINARY CHARACTERIZATION IN A CELL-FREE SYSTEM

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ABSTRACT: *In vitro* studies have demonstrated a progesterone-induced activity associated with the uterine nuclear fraction which resulted in the loss of nuclear estrogen receptor. Uterine nuclear suspension or nuclear KCl (0.5 M) extract from control and progesterone-treated (30 min or 2h) hamsters were incubated at 37 C for 0, 15, or 30 min in Tris-glycerol buffer. Preparations from progesterone-treated hamsters showed an accelerated reduction of total estrogen receptor which was primarily due to preferential loss of occupied receptor. This progesterone-dependent stimulation of estrogen receptor loss was absent when nuclear extract was prepared in phosphate buffer rather than Tris buffer. In addition, sodium molybdate and sodium metavanadate (both at 10 mM) inhibited this activity in nuclear extract. These observations support the hypothesis that progesterone modulation of estrogen action may be accomplished by induction (or activation) of an estrogen receptor-regulatory factor (Re-RF), and this factor may in turn act to eliminate the occupied form of estrogen receptor from the nucleus, perhaps through a hypothetical dephosphorylation-inactivation mechanism.

Interaction of the steroid hormone-receptor complex with target cell chromatin triggers the macromolecular events leading to the cellular response characteristic of the hormone (1,2). Our laboratory has shown recently that progesterone, a physiological modulator of estrogen action (3), regulates nuclear estrogen receptor (Re) retention in the hamster (4) and rat (5) uterus. This effect of progesterone was also shown to be selective for the estrogen-Re complex as compared to unoccupied nuclear Re (6). Thus, the mechanism by which progesterone modulates estrogen action may in part be a consequence of progesterone-induced loss of estrogen-Re complex from the nucleus. Furthermore, we found that this effect of progesterone can be blocked by inhibitors of RNA or protein synthesis (7). Collectively, these results support the hypothesis that progesterone induces a factor (estrogen receptor-regulatory factor, Re-RF) which acts on the occupied form of Re in the target cell nucleus.

The objective of the present study was to determine if a progesterone-induced Re-RF could be demonstrated in a cell-free system. Our findings indicate the presence of an activity which may qualify as Re-RF in the uterine nuclear fraction of progesterone-treated proestrous hamsters. This is the first demonstration that one hormone (progesterone) can induce a factor which controls the nuclear retention of receptors for a second hormone (estrogen).

METHODS

Adult female golden hamsters (Engle Labs, Farmersburg, IN) were treated with progesterone (5 mg/100 g BW) or corn oil vehicle on the morning of proestrus (cycle day 4) and killed at various times following treatment. Uteri were removed rapidly, stripped of fat and mesentery, slit longitudinally, blotted, weighed and placed in ice-cold buffered saline (pH 7.4). Uterine tissue was minced and homogenized in A_{30} buffer (50 mM Tris-HCl, 1 mM EDTA, 12 mM monothioglycerol, 30% glycerol (v/v), pH 7.5) by three 5-sec bursts with a Polytron Pt-10 homogenizer. Nuclear suspension, nuclear extract and cytosol were prepared as described previously (8), and samples were incubated for 0, 15, or 30 min at 37 C. Following incubation, total Re was determined by incubating (30 C, 1h) aliquots (300 µl) of nuclear extract or cytosol in a total volume of 500 µl with a saturating concentration (3 nM) of [^{3}H]-estradiol. Nonspecific binding was measured in a parallel incubation done with a 250-fold excess of diethylstilbestrol. Unoccupied Re was determined in a similar fashion by incubation for 18 h at 4 C (4). Occupied Re was the difference between total and unoccupied receptor. We have previously demonstrated that high salt preferentially extracts Type I estrogen-binding sites from hamster uterine nuclei, whereas Type II binding sites were shown to remain associated with the nuclear debris following extraction and to lack estrogen-binding specificity (9). For these reasons, only Type I Re sites were measured in this study.

RESULTS

When uterine nuclear suspensions from control and progesterone-treated hamsters were incubated at 37 C, total nuclear Re was lost more rapidly in nuclear suspension prepared 2 h after progesterone treatment than at 30 min or in control (Fig. 1A). In addition, the occupied form of nuclear Re was lost preferentially (Fig. 1B). Thus, a progesterone-induced activity capable of reducing Re was associated with the nuclear fraction *in vitro*.

To determine if this activity could be extracted from uterine nuclei, 0.5 M KCl extract was prepared from proestrous hamsters as described elsewhere (8). Figure 2 shows that the pattern of Re loss (total and occupied) was similar to that observed in a nuclear suspension. However, following progesterone treatment there appeared to be a greater reduction of occupied Re in nuclear KCl extract (Fig. 2B) as compared to suspended nuclei (Fig. 1B).

Uterine cytosol Re prepared from control and progesterone-treated hamsters was incubated in a manner similar to that described above for nuclear fractions. Although there was a substantial loss of cytosol Re under these assay conditions, no association between progesterone treatment and cytosol Re degradation was evident (data not shown).

The presence of both a progesterone-dependent activity which stimulates Re loss (Re-RF) and its substrate, Re, in the nuclear KCl extract permitted a preliminary characterization of this activity. We first tested the effects of buffer constituents on measurement of Re-RF activity (Table 1). When phosphate was substituted for Tris in the presence of EDTA and thioglycerol, Re-RF was both extractable by 0.5 M KCl and demonstrable in the nuclear extract from progesterone-treated hamster uteri. Removal of thioglycerol and EDTA from buffer A30 (i.e., Tris alone) had little effect on detection of Re-RF activity. However, Re-RF activity was inhibited in

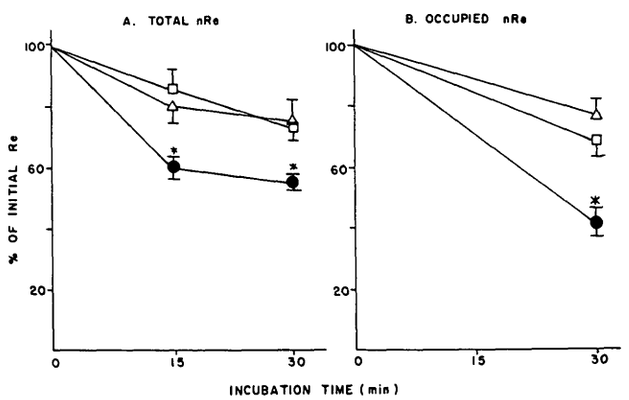

Figure 1. Uterine nuclear suspension from control or progesterone (P)-treated proestrous hamsters (5 mg/100g BW, sc) was incubated for 0, 15, or 30 min at 37 C. Nuclear receptor (nRe) was determined in 0.5M KCl extract following incubation as described in Methods. Values are expressed as a percent of the initial nRe level (0 time, 0 C). Control (□), 30 min after P (Δ), 2h after P (●). Each value represents the mean ± SEM (n = 5 or 6), * P <0.05.

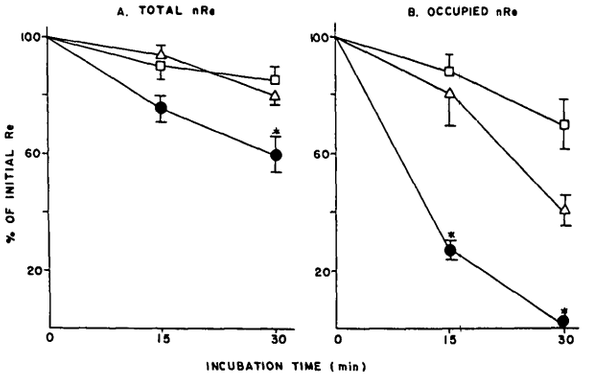

Figure 2. Uterine nuclear extract from control or progesterone (P)-treated proestrous hamsters (5 mg/100g BW, sc) was incubated for 0, 15, or 30 min at 37 C. Estrogen receptor (nRe) was determined following incubation as described in Methods. Values are expressed as percent of the initial level (0 time, 0 C). Control (□), 30 min after P (Δ), 2h after P (●). Each value represents the mean ± SEM (n = 5 or 6), * P <0.05.

phosphate alone as compared to Tris alone. Thus, phosphate inhibition of Re-RF activity was overcome by inclusion of EDTA and monothioglycerol.

Next we examined the influence of the phosphatase inhibitors, molybdate and vanadate, on progesterone-dependent Re loss in nuclear extract prepared in A_{30} buffer (Fig. 3). Sodium molybdate (10 mM) blocked Re loss at 37 C in both control and progesterone-treated nuclear extract, having a more pronounced effect on the control. On the other hand, 10 mM sodium vanadate had a small effect on Re loss in the control extract but completely inhibited the progesterone-induced increase in Re loss.

DISCUSSION

Progesterone down-regulates the estrogen receptor system, but the mechanism for this is not known. Our previous studies (5-8) suggested that progesterone may induce a receptor regulatory factor (Re-RF) which controls nuclear Re retention. The present results provide support for the existence of such a factor, and show that Re-RF activity can be demonstrated in a cell-free system. This factor appears to be present in the uterine nuclear fraction of progesterone-treated hamsters, to act preferentially on the occupied form of nuclear Re, and to be extractable from nuclei with 0.5 M KCl. Moreover, the onset of appearance of this activity in nuclear extract following progesterone treatment closely parallels the time course of Re loss from uterine nuclei observed *in vivo* (6).

Using cell-free assay conditions, we have begun to probe the biochemical properties of this putative receptor-inactivating factor. It is pertinent that Re-RF activity is diminished by phosphate in the absence of EDTA and thioglycerol, and that it is blocked by the phosphatase inhibitors molybdate and vanadate. It appears unlikely that Re-RF is a protease because various protease inhibitors do not prevent progesterone-induced nuclear Re loss (MacDonald RG, Okulicz WC, and Leavitt WW, in preparation).

Table 1. Effect of Buffer Composition on Re-RF Activity in Uterine Nuclear Extract

Buffer Composition	Treatment	Re, cpm/0.3 ml 0 C	Re, cpm/0.3 ml 37 C	Total Re Loss at 37 C (%)	Re-RF* Activity
50 mM Tris·HCl, 1 mM EDTA, 12 mM thioglycerol	C	7235 ± 192	4015 ± 343	45 ± 2	11
	P	5997 ± 223	2665 ± 256	56 ± 1	
25 mM phosphate, 1 mM EDTA, 12 mM thioglycerol	C	7990 ± 11	4721 ± 260	41 ± 3	12
	P	6828 ± 91	3217 ± 66	53 ± 2	
50 mM Tris·HCl	C	6090 ± 145	4469 ± 161	27 ± 3	13
	P	4721 ± 91	2848 ± 211	40 ± 6	
25 mM phosphate	C	6894 ± 164	5449 ± 89	21 ± 3	2
	P	5739 ± 66	4428 ± 186	23 ± 4	

All buffers contained 30% (v:v) glycerol, 0.5 M KCl and were adjusted to pH 7.5. Uterine nuclear extract was prepared from control (C) or progesterone (P)-treated hamsters (2h) in either 50 mM Tris·HCl or 25 mM phosphate. One half-hour prior to the assay, EDTA and thioglycerol were added to a portion of each extract from 100X stocks (in H_2O at pH 7.5). Triplicate aliquots (0.8 ml) of each nuclear extract were then incubated at 0 C or at 37 C for 30 min. Total Re was determined as described in Methods. Values are mean ± SEM. *Re-RF activity represents the net percentage increase in total Re loss due to P treatment.

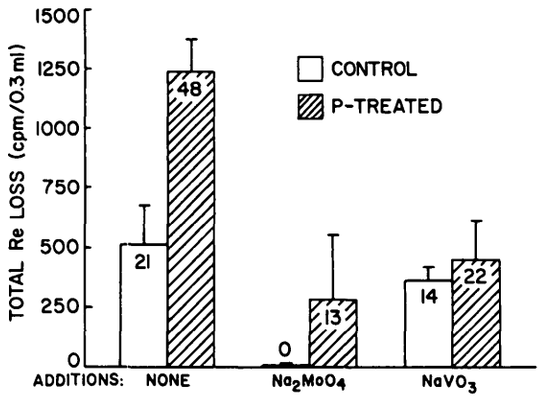

Figure 3. Uterine nuclear extract was prepared from hamsters treated for 2h with progesterone (P) or from controls in A_{30} buffer. Portions of each extract were pretreated for 30 min at 0 C with 10 mM concentrations of sodium molybdate or sodium vanadate. Triplicate 0.8 ml aliquots of each extract were then incubated at 0 C or at 37 C for 30 min. Total Re was measured as described in Methods. Values are mean ± SEM. The number within each bar represents the percent of Re lost at 37 C relative to that measured at 0 C in the same extract.

However, the inhibitory effect of phosphate, molybdate and vanadate on Re-RF activity suggests that this factor could be a phosphatase or an activator of phosphatase activity (10,11). Phosphate and molybdate prevent Re loss in the control in addition to the progesterone-treated preparation, whereas vanadate seems to selectively inhibit progesterone-dependent receptor degradation (Fig. 3). Thus, it is possible these agents could influence Re-RF action by inhibition of phosphatase activity or by direct stabilization of Re. Additional study is needed to distinguish between these possibilities.

Enzymes have been described which are capable of altering Re (12,13). We have shown for the first time under physiological conditions that progesterone stimulates an enzymatic mechanism responsible for controlling nuclear Re retention. The present study demonstrates a unique progesterone-induced activity which acts upon the occupied form of nuclear Re. It is not known whether steroid receptors are phosphoproteins, but this has been suggested (13,14). If true, then Re function may be regulated by the extent of receptor phosphorylation. In explanation for progesterone antagonism of estrogen action, we propose that nuclear retention of the Re-estrogen complex, which is responsible for estrogen-dependent gene expression, is controlled by a phosphorylation-dephosphorylation mechanism. Progesterone may induce an enzyme or enzyme activator which rapidly and selectively dephosphorylates nuclear Re. Receptor dephosphorylation would be a key step in the process of nuclear Re inactivation and attenuation of estrogen-dependent gene expression.

Our ability to extract Re-RF from uterine nuclei and monitor its activity in a cell-free system will permit the further characterization and study of this factor and its hormonal regulation.

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