Progesterone Inhibits the Uterotrophic Effect of Relaxin in Immature Rats (42902)

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Abstract. **Injection of progesterone for 3 days before treatment with relaxin inhibited the trophic effect of the peptide in both estrogen-primed and unprimed uteri. The depression in collagen concentration and increase in apparent rate of proline incorporation into collagen induced by relaxin alone were also eliminated, indicating a fundamental blockade of the effect of relaxin in this experimental design as well as a close association of changes in collagen concentration with tissue hypertrophy. The effect of relaxin on incorporation of proline into soluble protein was not blocked by progesterone, however, suggesting a separate mechanism for this anabolic effect of relaxin.**

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There is considerable evidence to support the conclusion that the ovarian peptide hormone relaxin is uterotrophic in addition to its capability in a number of species to promote symphyseal relaxation, softening of the cervix, and suppression of spontaneous myometrial contractility (1). Normal gestation, parturition, and a high incidence of live births can occur in rats ovariectomized early in pregnancy when maintenance with progesterone and estrogen is supplemented with porcine relaxin (1–3). Yet, it has been difficult to demonstrate more than a modest effect upon the uterus of exogenous relaxin administered to the intact pregnant rat during the latter half of gestation (4).

The demonstration of uterotrophic effects of relaxin in the rat commonly utilizes ovariectomized, immature rats primed 1 week earlier with a single dose of estradiol (4, 5). The limitations of this as a general model for the role of relaxin in uterine accomodation include the fact that progesterone dominates the uterine environment in the second half of gestation. Increases in the concentration of progesterone in the rat during gestation coincide with the early period of uterine growth (6) and precede the rise in relaxin levels occurring during the final phase of uterine hypertrophy (7). This suggests that progesterone might potentiate the later effect of relaxin upon the uterus. In early studies using relatively impure preparations of porcine relaxin (8), 1 mg of progesterone administered simultaneously with daily doses of relaxin did not alter the uterine weight response in estrogen-primed animals but enhanced uterine responsiveness in unprimed animals. In order to test the role of progesterone conditioning in a simplified model, we have used sequential exposure to estrogen, progesterone, and a highly purified preparation of porcine relaxin to assess the potentiating effect of each hormone upon the one following. In this protocol, progesterone, rather than augmenting the effect of relaxin, eliminates nearly all of its uterotrophic actions.

Materials and Methods

Animals. Female Sprague-Dawley rats were obtained at 30 days of age, from the Department of Biological Sciences Animal Facility and were bilaterally ovariectomized under ether anesthesia (Day 1) and maintained in a 14:10 light:dark environment (lights on 0700 to 2100 hr). One half were injected sc with 5 μg of estradiol benzoate in corn oil 1 week later (Day 8). Two days later one-half of the estrogen-primed animals and one-half of the unprimed animals were injected sc with 1 mg of progesterone in corn oil every 2 days for a total of three injections (Days 10, 12, and 14). At 2100 hr on Day 14, one-half of the animals in each of the four groups described above were injected sc with 0.05 mg of procine relaxin B (9) in 0.1 ml of 1% Benzopurpurine-4B (Aldrich Chemical Co., Milwaukee, WI) and the remainder were injected with 0.1 ml of Benzopurpurine-4B solution. Animals were killed by cervical dislocation 12 hr after relaxin injection (0900 hr, Day 15), body weights were obtained, and the

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reproductive tract removed. The uteri were placed in a petri dish on ice, dissected free of connective tissue, and separated at the cervical junction. The tissues were split lengthwise, opened to provide optimum exposure to incubation medium, and weighed to the nearest 0.1 mg.

Incorporation of [^{3}H]Proline. Media were prepared consisting of Krebs-Ringer phosphate buffer containing 0.119 mg of L-Pro/ml and 5 μCi [^{3}H]Pro/ml (26 Ci/m*M*; ICN Radiochemicals, Irvine, CA). Immediately after weighing, tissues were placed in 2.0 ml of medium and incubated for 1 hr at 37°C (8). Tissues were removed from the medium, placed in vials in dry ice, and then frozen at −20°C until processing.

Tissue Analysis for [^{3}H]Proline Incorporation into Soluble Protein and Collagen. Tissues were removed from frozen storage and whole uteri were quickly homogenized in 0.15 *M* NaCl. Homogenates were left in the cold room overnight after which they were centrifuged at 2500 rpm for 10 min at 4°C. Soluble protein and collagen were separated as previously described (9). Total soluble protein was measured according to the Lowry method (10), and an aliquot of each sample was counted in Bray's solution (11). Specific radioactivity was calculated as dpm/mg of soluble protein.

Collagen was determined following hydrolysis in 12.2 *M* HCl at 110°C for 25 hr. The hydrolysates were evaporated to dryness and the residues redissolved in 3.0 ml of water. Aliquots of 0.2 ml were counted in Bray's solution, and others were analyzed for hydroxyproline content by the method of Stegemann and

Table 1. Body weights and uterine weights in immature, ovariectomized rats treated with estradiol, progesterone, and relaxin

Group[a]	Control	Relaxin[b]	Progesterone[c]	Progesterone[c] + relaxin[b]
Unprimed				
Body weight (g)	184.2 ± 9.8	186.2 ± 5.7	177.1 ± 2.2	166.0 ± 4.2
Uterine weight (mg)	61.2 ± 1.7	86.7 ± 4.2[d]	57.8 ± 7.9	57.8 ± 3.8
Estrogen-primed				
Body weight (g)	164.6 ± 2.8	167.2 ± 2.6	160.0 ± 2.5	157.6 ± 1.4
Uterine weight (mg)	151.8 ± 16.6	255.7 ± 17.4[d]	157.2 ± 5.0	165.4 ± 9.7

Note. Data are presented as means ± SEM for six to seven animals per group.
[a] Five micrograms of estradiol benzoate administered on Day 8 after ovariectomy.
[b] Five-tenths milligrams of relaxin administered in 1% Benzopurpurine-4B at 1700 hr (Day 14) to animals killed at 0900 hr (Day 15).
[c] One milligram of progesterone administered on Days 10, 12, and 14.
[d] $P < 0.05$ compared with other means in group.

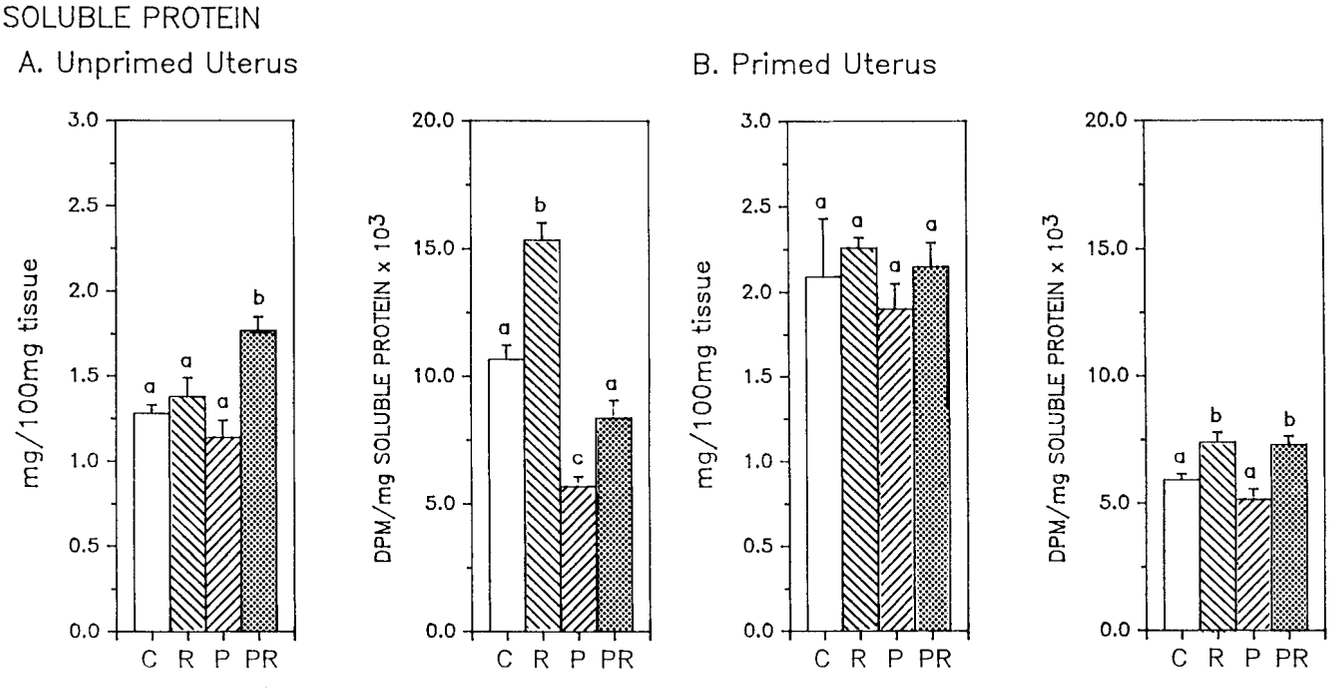

Figure 1. Concentration of soluble protein fraction and its uptake of [^{3}H]proline in uteri obtained from six to seven rats per group, unprimed (A) or estrogen-primed (B), treated with 1 mg of progesterone (P) 1, 3, and 5 days previously, 0.05 mg of porcine relaxin (R) 12 hr previously, or both (RP). Uteri were incubated for 1 hr in Krebs-Ringer phosphate buffer containing L-[^{3}H]proline (0.119 mg, 5 μCi/ml). Vertical bars indicate range of SE about the means; significant ($P < 0.05$) differences are indicated by different letters.

Stalder (12). Hydroxyproline values were converted to milligram collagen by multiplying by a factor of 8.27 (13).

Statistical Analysis. Data were analyzed using two-way analysis of variance. ANOVA significant to a P of <0.01 were followed by an *a posteriori* Tukey HSD test of significance among means with a P of <0.05 considered significant.

Results

Relaxin administration to unprimed, ovariectomized rats produced a 40% increase in uterine weight (Table I), whereas priming with 5 μg of estradiol benzoate 1 week earlier increased uterine response to relaxin to 68% above those not receiving relaxin. Administration of three injections of progesterone in a 1-week period produced no effect on uterine weight in primed or unprimed animals, but the same regime of progesterone administration for 1 week prior to relaxin administration completely eliminated the uterotrophic effect of relaxin treatment.

Uterine-soluble protein concentration was not affected by relaxin, although the incorporation of labeled proline into this protein fraction was enhanced in concert with the increase in weight of the tissue (Fig. 1). Despite suppression of the relaxin-induced uterine weight increase by progesterone, however, incorporation of labeled proline into soluble protein was in-

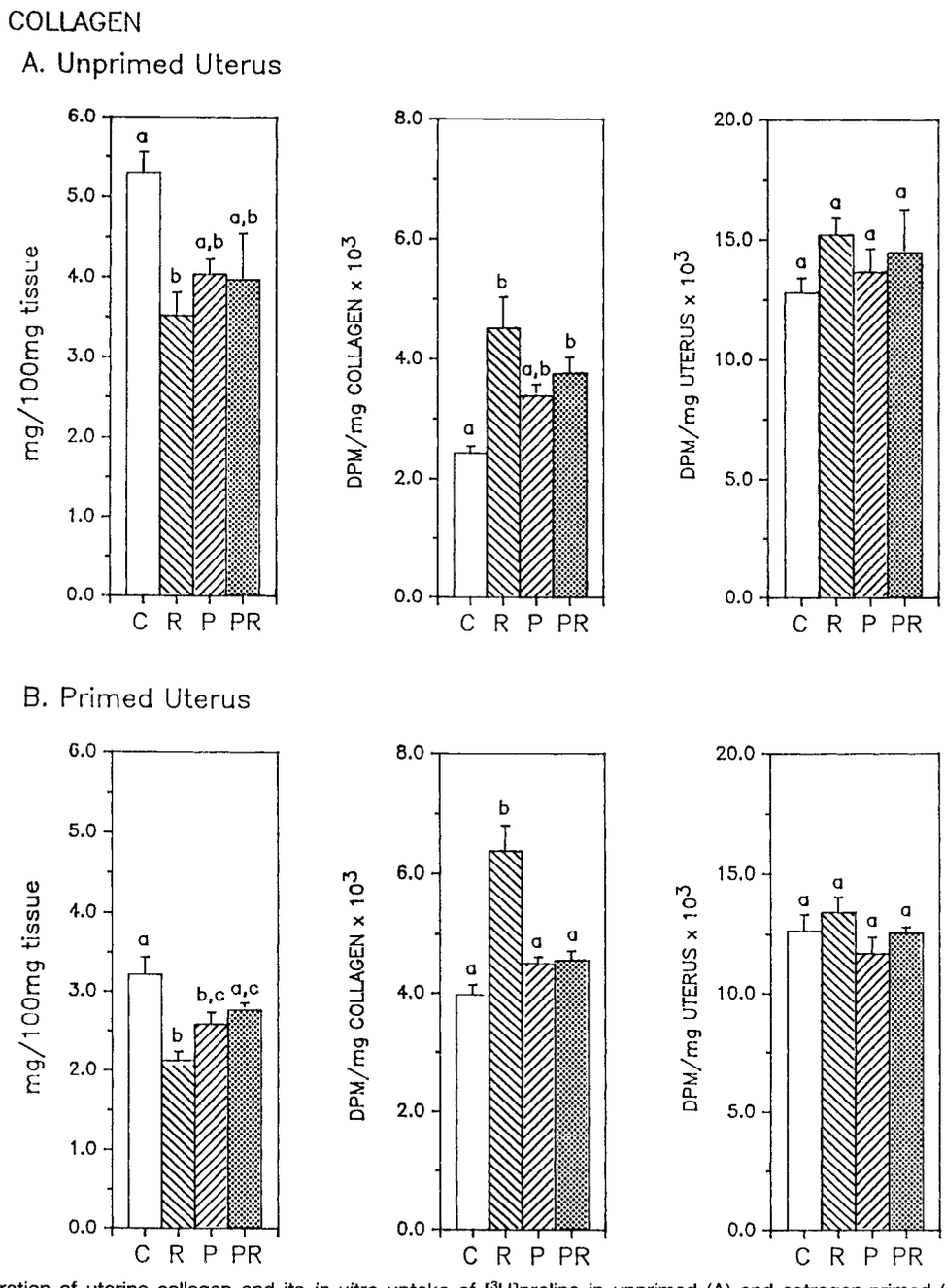

Figure 2. Concentration of uterine collagen and its *in vitro* uptake of [^{3}H]proline in unprimed (A) and estrogen-primed (B) rats treated as described in Figure 1. Significant ($P < 0.05$) differences are indicated by different letters.

creased significantly by relaxin in progesterone-treated animals, and in unprimed animals this led to an increased soluble protein concentration following treatment with progesterone plus relaxin.

Coincident with its stimulation of uterine growth, relaxin induced a 30% decrease in the uterine collagen concentration while increasing its specific radioactivity (Fig. 2) in both estrogen-primed and unprimed animals. Both the decrease in collagen concentration and the increase in its specific radioactivity were abolished by prior progesterone treatment. The reduction in collagen concentration mirrored the increase in specific radioactivity such that the incorporation of the amino acid per milligram of tissue was unchanged among all of the groups.

Discussion

Previous studies (5) demonstrated that progesterone did not modify the uterotrophic effect of relaxin when both were given simultaneously for 7 days to estrogen-primed rats. In this study, where progesterone preceeded a single injection of relaxin, the uterine weight increase was completely blocked. However, progesterone still permitted an increased incorporation of labeled proline into soluble protein in both primed and unprimed animals without affecting protein concentration. Thus, with relaxin alone, newly synthesized protein accumulated as the tissue grew, whereas in the presence of progesterone, relaxin apparently increased soluble protein turnover with no net increase in tissue protein content.

The one-third decrease in collagen concentration following relaxin administration was associated with tissue enlargment and with an apparent increase in proline incorporation; but the net collagen labeled per milligram of tissue during the 1-hr incubation was the same in all groups. These measures were not specifically affected by progesterone, except in that the hypertrophy of the tissue was suppressed; collagen concentration and proline incorporation were otherwise unaltered.

Thus, it appears that relaxin induces a rapid growth of the uterus in 12 hr which is not accounted for by water uptake but rather results from a net accumulation of protein (4, 5). Although *in vitro* incorporation of proline into uterine collagen in increased by relaxin (9), total uterine collagen content is unaffected by this brief exposure, and changes in its concentration reflect this as it becomes diluted with newly synthesized protein and other tissue components. These effects occur in both unprimed and estrogen-primed tissues, although the estrogen-priming produces a tissue much more responsive to relaxin.

Administration of progesterone between estrogen priming and relaxin treatment interferes with the ability of the peptide to stimulate uterine growth. This is consistent with observations in the gravid uterus in which exogenous porcine relaxin does not appreciably affect uterine size or metabolism late in pregnancy and suggests that in the progesterone-dominated uterus relaxin effects are attenuated (4). Yet, from the effect of relaxin on soluble protein synthesis, despite progesterone inhibition, it is possible that the peptide may still participate in tissue growth and remodeling under physiologic conditions but that the full expression of its effects are limited by steroid hormone concentrations. A likely focus of the effect of progesterone could be upon relaxin receptors; but although progesterone has been found to suppress uterine estrogen receptors (14, 15) we have no information at this time on its effect upon relaxin receptors.

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