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Stimulation of Rat Uterine Collagen Synthesis by Relaxin[1] (42140)

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Abstract. Immature, ovariectomized, estrogen-primed rats respond to the administration of porcine relaxin by an increase in the incorporation of labeled amino acids ([^{14}C]leucine, [^{14}C]phenylalanine, [^{3}H]proline) into uterine proteins *in vitro*. The maximum response occurs about 12 hr after a single injection of 0.1 mg relaxin in benzopurpurine 4B solution; subsequently, the relaxin effect declines but is still apparent after 24 hr. Smaller, but still significant increases in incorporation rates can be induced by relaxin in the absence of estrogen priming. Uterine collagen synthesis, as indicated by the incorporation of [^{3}H]proline and its conversion to hydroxyproline, appears to be a primary target of the relaxin stimulus, since the effect of relaxin upon proline incorporation into uterine collagen is significantly greater than its effect upon labeling of noncollagen protein.

The immature, ovariectomized, estrogen-primed rat has been shown to provide a useful model for studies of the metabolic and biochemical effects of relaxin (1–3). In such animals, the administration of 0.1 mg (200 GPU) of porcine relaxin in 1% benzopurpurine 4B (BP) solution results in rapid increases in uterine weight, protein content, and glycogen content; responses of all three parameters can be detected within 6 hr of the administration of the hormone. The uterotrophic and glycogenic effects of relaxin are diminished, but not eliminated, in the absence of estrogen priming. Responses similar to those seen in ovariectomized animals can also be observed in the nongravid horns of unilaterally pregnant animals when relaxin is given early in the second half of gestation (4).

The work reported here was undertaken in order to define more precisely the protein anabolic properties of relaxin. To this end, we have studied the incorporation of several amino acids (leucine, phenylalanine, proline) into uterine proteins of relaxin-treated and control animals. Relaxin administration is accompanied by increased rates of incorporation (*in vitro*) of all three amino acids into uterine protein. With [^{14}C]phenylalanine as substrate, maximum stimulation occurs approximately 12 hr after a single injection of relaxin in BP. Furthermore, uterine collagen biosynthesis appears to be especially sensitive to relaxin, since enhancement of [^{3}H]proline incorporation into uterine collagen by relaxin significantly exceeds its effect upon uptake of either [^{14}C]phenylalanine or [^{3}H]proline into noncollagen (soluble) protein.

Materials and Methods. Radioactive amino acids were purchased from New England Nuclear Corporation or from Amersham, Inc. Porcine relaxin was isolated from NIH relaxin concentrate R-P-1[3] or from an acid–acetone extract of pregnant sow ovaries (5). Relaxin from either source was first separated from higher molecular weight contaminants by filtration through Sephadex G-50, then further purified either by column electrophoresis on Sephadex G-25 in pH 9.0 ammonium acetate buffer (6) or ion-exchange chromatography on carboxymethylcellulose; of the several relaxin species isolated, relaxin B, which has a specific activity of 2000 GPU/mg and is electrophoretically indistinguishable from CM-B, was used for the experiments described here.

Sprague–Dawley rats from the colony maintained in the Kent State University Bio-

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logical Sciences vivarium were ovariectomized when 30 days of age. Estrogen-primed animals received 5 μg estradiol benzoate in sesame oil 1 week after ovariectomy. Fourteen days postoperatively, relaxin-treated animals received 0.1 mg relaxin in 0.1 ml BP; controls received only BP. Rats were sacrificed 4–24 hr later, the uteri were removed, placed on filter paper on an ice bath, slit longitudinally, weighed, and incubated, with constant agitation, in 2.0 ml Krebs–Ringer phosphate buffer containing the appropriate amino acid. No more than 2 min elapsed between sacrifice of the animal and transfer of the tissue to the incubation medium. The temperature was maintained at 37°C. At the end of the incubation period, the tissue was removed, rinsed in distilled water, and frozen on dry ice.

After thawing, each tissue sample was homogenized in 0.15 *M* NaCl solution. For experiments requiring no separation of uterine proteins, 5 mg of "cold" amino acid was added to the homogenate and the protein precipitated by the addition of trichloroacetic acid (TCA) to 6.7%. The precipitate was redissolved in 0.2 *M* NaOH and the cycle of precipitation and solution repeated until the radioactivity of the protein-free filtrate was negligible. The residual protein was dissolved in 0.2 *M* NaOH; aliquots were taken for protein determination by the Lowry method (7) or total nitrogen and for radioactivity by scintillation counting in 5 ml Bray's solution (8).

For either relaxin-treated or control animals, incorporation of labeled phenylalanine into uterine protein *in vitro* was found to be linear for at least 3 hr; accordingly, incubation periods of 1 hr were used routinely.

For experiments requiring separation of soluble protein and collagen, the 0.15 *M* NaCl homogenate was centrifuged, washed with saline, and soluble protein isolated from the combined supernatants as described above. The NaCl-insoluble residue was washed repeatedly in the presence of "cold" amino acid (proline) then subjected to two 30-min extractions with 0.30 *M* TCA solution at 90°C to solubilize collagen (9); as estimated from determinations of hydroxyproline content, less than 3% of rat uterine collagen is soluble in 0.15 *M* NaCl, while 98% is extracted by hot TCA. The hydrolysate was evaporated to dryness, redissolved in water, and its radioactivity and hydroxyproline content determined, the latter by the Stegemann and Stalder method (10). In some experiments, hydroxyproline and proline were individually estimated after fractionation of the hydrolysate on Dowex 50 (10, 11); the proline:hydroxyproline ratio averaged 1.25, indicating minimal (less than 10%) contamination of collagen with other proline-containing proteins (12). The collagen content of the TCA extract was calculated as 8.27 × its hydroxyproline content.

The tissue specificity of the effect of relaxin upon protein synthesis was assessed by incubating uteri and diaphragms of relaxin-

TABLE I. INCORPORATION OF [1-^{14}C]LEUCINE INTO RAT UTERINE PROTEIN *IN VITRO*

Expt.	Estradiol benzoate[a] (μg)	Relaxin B (mg)	Time[b]	No. rats	Uterine weight (mg)	CPM/mg protein	% Increase
I	5	—	0	10	108 ± 13	630 ± 29	—
	5	0.1	4.5	9	168 ± 22	960 ± 48[c]	53
	5	0.1	12.0	9	127 ± 23	1040 ± 65[c]	66
II	—	—	0	7	45 ± 2	370 ± 17	—
	—	0.1	13.0	7	60 ± 4[c]	495 ± 25[c]	33

Note. Uteri were incubated 1 hr in Krebs–Ringer phosphate containing 1.0 m*M* L-[1-^{14}C]leucine (0.2 μCi in experiment I, 0.25 μCi in experiment II). Values for uterine weights and specific activities are means ± SEM.

[a] Priming dose administered 1 week before relaxin.

[b] Time (hr) between relaxin administration and autopsy.

[c] $P < 0.010$ as compared to untreated controls.

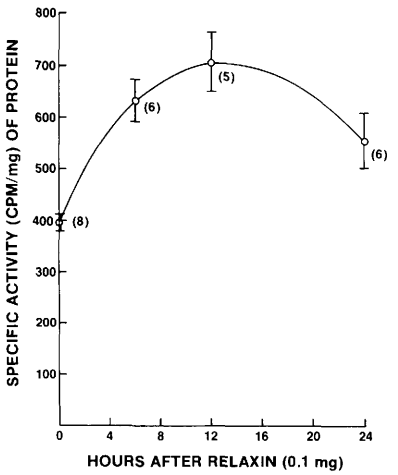

FIG. 1. Time dependence of incorporation of [^{14}C]phenylalanine into uterine protein. Ovariectomized, estrogen-primed, immature rats were used. Relaxin-treated animals received 0.1 mg relaxin B in BP and were sacrificed 4–24 hr later. Uteri were incubated for 1 hr in Krebs–Ringer phosphate buffer containing L[U-^{14}C]Phe (0.8 m*M*, 0.2 μCi/ml). Bracketed figures indicate the number of animals in each group. Vertical bars indicate range of SE about the mean.

treated and control animals with tritiated proline; specific activities of soluble protein and collagen were subsequently determined for both tissues.

The significance of differences between means was evaluated by Student's *t* test, with probability values of 0.05 or less considered significant.

Results. The data summarized in Table I indicate that a significant increase (53%) in the rate of incorporation of labeled leucine into uterine protein of ovariectomized, estrogen-primed rats may be detected within 4–5 hr after the administration of a single dose of relaxin; after 12 hr the difference was increased to 66%. In unprimed animals, a reduced, but still significant increase was also evident (Table I, experiment 2). Figure 1 indicates that a maximum increase in the rate of phenylalanine incorporation into unfractionated uterine protein occurs approximately 12 hr after its administration (in BP); after 24 hr the relaxin-induced enhancement was reduced to approximately one-half that observed at 12 hr.

The data in Tables II and III indicate that relaxin stimulated the synthesis of uterine collagen, as indicated by incorporation of tritiated proline, to a greater extent than the synthesis of noncollagen (soluble) protein, whether the latter was measured by phenylalanine or proline uptake. In contrast, protein synthesis in nonreproductive tract tissue (e.g., diaphragm) was insensitive to the action of relaxin.

Discussion. It is clear that relaxin can induce a rapid and sizeable increase in the synthesis of uterine protein. While the protein anabolic effect of relaxin is enhanced by prior estrogen treatment (in ovariectomized animals) a significant effect may be observed in the absence of estrogen priming. As suggested earlier (1), the action of estrogen is likely due

TABLE II. INCORPORATION OF [^{14}C]PHENYLALANINE AND [^{3}H]PROLINE INTO UTERINE PROTEIN[a]

Relaxin B (mg)	No. rats	Uterine weight (mg)	Specific activity (cpm/mg) Soluble protein (^{14}C)	Specific activity (cpm/mg) Collagen (^{3}H)
—	7	122 ± 7	1065 ± 75	800 ± 87
0.1	8	135 ± 17	1413 ± 56	1315 ± 130
		Percentage increase	33	65
		P	<0.005	<0.010

[a] Ovariectomized, estrogen-primed rats were used. Vehicle or relaxin was administered in 0.1 ml 1% BP. Uteri were removed 11.5–13.5 hr later; one horn was incubated in Krebs–Ringer phosphate buffer containing L-[^{14}C]phenylalanine (1.6 m*M*, 0.19 μCi/ml); the other in buffer containing L-[^{3}H]proline (1.6 m*M*, 2.5 μCi/ml). Soluble protein was isolated from the first group; collagen from the second. Values for uterine weights and specific activities are means ± SEM.

TABLE III. INCORPORATION OF [^{3}H]PROLINE INTO UTERINE AND DIAPHRAGM PROTEIN[a]

	Uteri				Diaphragms		
			Specific activity (CPM/mg)			Specific activity (cpm/mg)	
Relaxin B (mg)	No.	Weight (mg)	Soluble protein	Collagen	No.	Soluble protein	Collagen
—	14	209 ± 16	2685 ± 117	1815 ± 126	7	2070 ± 90	1585 ± 80
0.17	16	264 ± 27	3345 ± 316	2830 ± 135	8	2150 ± 95	1470 ± 70
		Percentage increase	25	56		4	−7
		P	<0.10	<0.001		N.S.	N.S.

[a] Ovariectomized, estrogen-primed rats were used. Vehicle or relaxin were administered in 0.15 ml BP. Uteri and diaphragms were removed 12–12.5 hr later. Each uterine horn and diaphragm (165–265 mg) was incubated in Krebs–Ringer phosphate buffer containing L-[5-^{3}H]proline (1.0 *m*M, 5.8 μCi/ml). Values for uterine weights and specific activities represent means ± SEM.

to the fact that estrogen appears to be an effective modulator of the population of relaxin receptors in the uterus (13).

Our data indicate that uterine collagen synthesis represents an important (perhaps the most important) facet of the relaxin response. Cullen and Harkness (14) first suggested 20 years ago that relaxin may be involved in uterine collagen metabolism; their hypothesis rested upon the observation that relaxin, in combination with estrogen and progesterone, increased uterine distensibility in a manner similar to that observed in rats during gestation. In unilaterally pregnant rats, the weights and collagen contents of both the gravid and nongravid horns increase during the latter half of pregnancy (4, 15). While changes in plasma estrogen and progesterone concentrations seem insufficient to account for such differences (16, 17) the period of rapid uterine growth coincides more closely with the rise in plasma relaxin which begins around the 10th day of gestation in the rat (18).

We have found that uterine involution and collagenolysis in the postparturient rat are inhibited by relaxin, either alone or acting synergistically with estrogen (data to be published). Relaxin thus appears to act as an anticatabolic, as well as an anabolic modulator of uterine collagen metabolism. Whether these two facets of the effects of relaxin upon uterine physiology share a common biochemical basis, or whether different mechanisms are involved, must await more detailed studies of uterine collagen metabolism in appropriate animal models.

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