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Effects of Porcine Relaxins upon Uterine Hypertrophy and Protein Metabolism in Mice[1] (42519)

JOHN E. BYLANDER, EDWARD H. FRIEDEN,[2] AND WALTER C. ADAMS

Departments of Chemistry and Biological Sciences, Kent State University, Kent, Ohio 44242

Abstract. Two variant forms of porcine relaxin (B and C) are active in producing relaxation of the guinea pig pubic symphysis and in effecting uterine growth in rats. Only relaxin B, however, is active in the mouse pubic ligament assay. These two hormones were compared in mice for their effects upon uterine growth and incorporation of radioactively labeled proline into soluble protein and collagen *in vitro* and *in vivo*. Both relaxin B and relaxin C produced an early (3-hr) elevation in *in vitro* protein synthesis and a later (6-hr) increase in collagen incorporation of proline at the time when the uterotrophic effect was maximal. *In vivo* effects of relaxin C on the uterus were in some cases greater than relaxin B in contrast to the complete inactivity of the former upon the pubic ligament of the mouse. These findings suggest a high degree of tissue specificity for relaxin stimulation, a variability in responsiveness among tissues in the same animal, and perhaps a primary role of relaxin in uterine function with pelvic relaxation representing a secondary function which has developed in certain species.

In order to accommodate the growing fetus, the gravid uterus undergoes an increase in size and distensibility and more than triples its mass during the latter half of gestation. In rodents, the coincidence of the appearance of relaxin in plasma and its increase during the period of rapid uterine growth (1, 2) as well as the reported occurrence of uterine relaxin receptors in mice and rats (3) suggest a significant role for relaxin in uterine growth and maintenance during gestation. Porcine relaxin exhibits trophic and glycogenic effects in the uteri of ovariectomized, estrogen-primed mice and rats (4, 5) and also induces protein anabolic effects in rat uteri (6). Collagen synthesis has been implicated as a major target of the latter response, since proline incorporation into rat uterine collagen is stimulated by relaxin treatment to a significantly greater degree than is the incorporation of phenylalanine or proline into noncollagen (soluble) protein (6).

The major forms of relaxin which can be isolated from pregnant sow ovaries (CM-B, CM-a', CM-a) differ in the number of amino acid residues (from 28 to 31) at the C-terminal end of the B chain (7). In addition, acid–acetone extracts of pregnant porcine ovaries contain another relaxin species, relaxin C, which differs both chemically and biologically from the others (8, 9). Relaxin C contains one proline residue and a single tryptophane residue. It is about half as active as CM-B (or CM-a) in the guinea pig palpation assay, is very active in the rat uterine motility assay, but is essentially completely inactive in the mouse pubic ligament assay. Preliminary experiments in this laboratory have shown that relaxin C exerts trophic and glycogenic effects in uteri of ovariectomized, estrogen-primed rats, and dose response curves for relaxin C with respect to these two parameters are comparable to those observed for CM-B.

The work reported here had three principal objectives: (a) to determine whether the effects of relaxin upon uterine protein metabolism, previously demonstrated in rats, are also demonstrable in mice; (b) to determine whether relaxin C, in view of its inability to affect the mouse pubic ligament, possesses uterotrophic and protein anabolic activity in this species; and (c) to compare the effects of relaxin upon proline incorporation into collagen and other uterine proteins *in vivo* in order to confirm those observed *in vitro*.

[1] From a dissertation submitted by J. E. Bylander to Kent State University in partial fulfillment of the requirements for the Ph.D. degree. Present address: Department of Medicine, Medical University of South Carolina, Charleston, SC 29425.

[2] To whom requests for reprints should be addressed.

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Materials and Methods. L-[5-^{3}H]Proline (9.9 Ci/m*M*) was purchased from New England Nuclear. Relaxins B (ca. 2000 GPU/mg and electrophoretically indistinguishable from CM-B) and C were prepared by acid–acetone extraction of ovaries frozen at the time of collection from pregnant sows (Cyrgus Co., Omaha, NE) and purified by gel chromatography on Sephadex G-50 followed by ion-exchange chromatography on CM-52. On carboxymethylcellulose columns developed with ammonium acetate (pH 5.0), relaxin C emerges at 6.4 mS (B = 12.4 mS). On polyacrylamide slab gels buffered to pH 5.0 with Tris–acetate, relaxin C migrates about half as rapidly toward the cathode as does relaxin B.

White Swiss mice (strain CD-1) obtained from Charles River were ovariectomized at age 32 days and 10 days later were primed with a subcutaneous injection of 5 μg estradiol benzoate in 0.1 ml sesame oil. Six and one-half days later the animals received a single, subcutaneous injection of either 3, 10, or 30 μg of relaxin B or C in 0.2 ml of 1% benzopurpurine 4B (BP) or BP alone (controls). Food was withheld from the time of relaxin administration until the animals were sacrificed, 3, 6, or 12 hr later. After the indicated treatment period for the *in vitro* experiments, the uteri were removed, slit longitudinally, blotted on moist filter paper on an ice bath, and weighed. They were then incubated for 1 hr at 37°C, with constant agitation, in 2.0 ml Krebs–Ringer phosphate solution containing L-proline (1.0 m*M*, 6.2 μCi L-[5-^{3}H]proline/ml). For *in vivo* experiments estrogen-primed, ovariectomized mice received 20 μg relaxin B or C, a dose which was within the limits of effective doses in the previous experiments. After 3 or 9 hr the animals were weighed and given an intraperitoneal injection of 0.10 mCi L-[^{3}H]proline/100 g body wt together with an excess dose (0.7 m*M*/100 g body wt) of unlabeled L-proline (10). Preliminary experiments indicated that under these conditions proline incorporation into uterine protein increases linearly for at least 3 hr; accordingly, the animals were sacrificed 3 hr later and uterine and lung tissue were removed.

Soluble protein and collagen were separated and isolated as previously described (6). Soluble protein was determined by the Lowry method; the collagen fraction was hydrolyzed and its hydroxyproline content was determined (11). The collagen content was calculated as 8.27× hydroxyproline. Portions of the hydrolysate and the soluble protein fraction were counted in a liquid scintillation counter in Bray's solution (12). Specific activities of soluble protein and collagen were calculated as disintegrations per minute per milligram protein and are expressed as a percentage of control values. Assuming unequal variance, Student's *t* tests were performed on the original data, and *P* values of 0.05 or less were considered significant.

Results. As little as 3 μg relaxin C produced a significant uterotrophic response within 3 hr (Fig. 1); by 6 hr this increment was no longer significant, although the effects of large doses (10 and 30 μg) persisted for at least 6 hr. In contrast, the maximum uterotrophic response to relaxin B did not appear until 6 hr after administration. Larger doses (30 μg) of either hormone were required to maintain uterine hypertrophy during the entire 12 hr of the experiment.

Stimulation of soluble uterine protein synthesis by either form of relaxin was maximal 3 hr after injection. After 6 hr, relative specific activities of soluble protein had markedly decreased; after 12 hr, incorporation rates for animals treated with either form of relaxin were significantly less than that for controls. The incorporation of labeled proline into the collagen fraction of mouse uteri was not notably affected by relaxin within the first 3 hr after injection, but significant increases of 40 to 100% were evident by 6 hr at all doses utilized. The effects of 3 and 10 μg of relaxin C were attenuated by 12 hr, but relaxin B stimulation of collagen incorporation continued through this latter period. The peak of collagen synthesis occurred at 6 hr postinjection and more closely resembled the pattern of uterine weight stimulation rather than that of soluble protein synthesis.

In vivo experiments with ovariectomized, estrogen-primed mice (Table I) show that both relaxins increased uterine size as well as proline uptake into uterine proteins during a 3-hr period beginning 3 or 9 hr after relaxin treatment. As expected, the uterotrophic response to 20 μg of either relaxin was most evident 6 hr after treatment; by 12 hr a definite decline in this parameter had set in. Increases in sol-

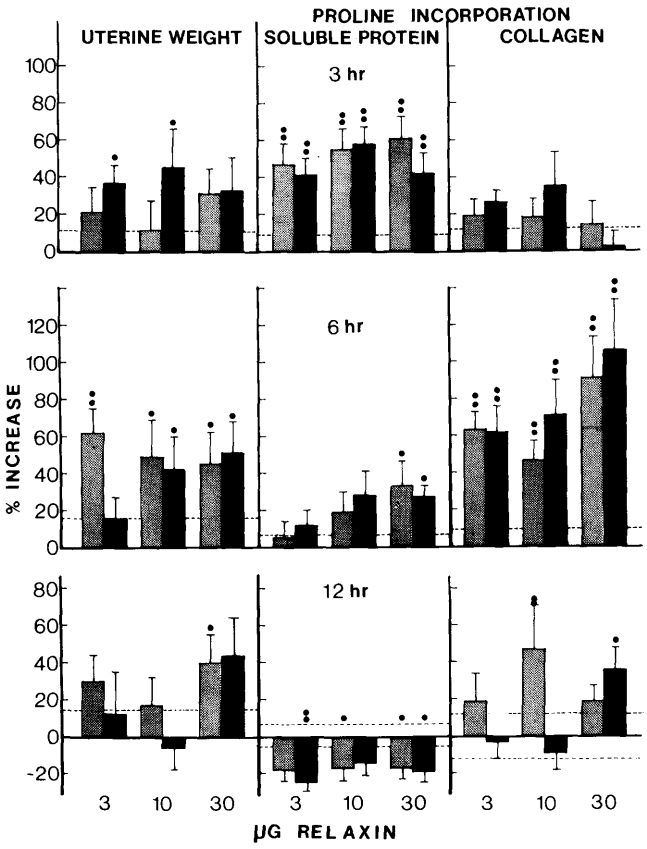

FIG. 1. Effects of relaxins B (shaded bars) and C (filled bars) upon parameters of uterine growth in ovariectomized, estrogen-primed Swiss mice. Experimental animals were sacrificed at 3, 6, or 12 hr after injection of 3, 10, or 30 μg relaxin in 1% BP. Uterine weight and *in vitro* incorporation of L-[^{3}H]proline during 1 hr are represented as a percentage increase (or decrease) of the relevant parameter compared to controls receiving only BP. Each bar represents the mean ± SE (vertical lines) for five to nine animals. Broken horizontal lines represent limits of standard errors of means for controls. ●; $P < 0.05$; ⁘; $P < 0.01$. For other details, see text.

uble protein synthesis were most evident during the 3- to 6-hr post injection period and declined somewhat 6 hr later. Rates of collagen synthesis remained elevated by relaxin during the entire period of the experiment. Relaxin C produced significantly greater stimulation of collagen synthesis than did relaxin B, during the 3- to 6-hr interval after relaxin administration. Neither preparation of relaxin had any effect upon incorporation into either soluble protein or collagen of the lung (data not shown) during any of the experimental periods examined.

Discussion. Both forms of relaxin used in the work reported here were potent stimulators of uterine growth and uterine protein synthesis in mice, despite the fact that one of them (relaxin C) does not promote elongation of the murine pubic ligament.[3] The qualitative and quantitative conformity of the results of *in vivo* and *in vitro* incorporation studies indicates that the conclusions reached earlier relating to the general anabolic effects of relaxin in rat uteri (6) were fully justified. However, the

[3] The failure of relaxin C to effectively elongate the pubic ligament in mice, first reported in 1981 (9), has since been confirmed in two laboratories using doses of 2–40 μg/animal.

TABLE I. *IN VIVO* INCORPORATION OF L-[^{3}H] PROLINE DURING 3 hr *IN UTERI* OF ESTROGEN-PRIMED, OVARIECTOMIZED MICE TREATED WITH 20 μg RELAXIN B OR C 3 OR 9 hr EARLIER

Treatment[a]	*n*	Uterine weight		Uterine soluble protein		Uterine collagen		Lung soluble protein
		mg	Percentage change	dpm/mg	Percentage change	dpm/mg	Percentage change	dpm/mg
Controls	16	101.0 ± 10.2		6520 ± 220		6510 ± 540		4460 ± 60
6 hr after relaxin								
Relaxin B	9	150.8 ± 14.2[b]	+49	8420 ± 410[b]	+29	8610 ± 510[b]	+32	4450 ± 150
Relaxin C	7	181.7 ± 19.2[b]	+80	8880 ± 920[b]	+36	11770 ± 700[b]	+81	4650 ± 210
12 hr after relaxin								
Relaxin B	8	122.9 ± 16.2	+21	7930 ± 350[b]	+22	9170 ± 1240[b]	+41	4360 ± 150
Relaxin C	8	118.2 ± 11.3	+17	7410 ± 280[b]	+14	10350 ± 850[b]	+59	4410 ± 80

[a] Swiss mice were ovariectomized at 32–33 days. Ten days later they were primed with 5 μg estradiol benzoate in 0.1 ml sesame oil. After another week, the animals were given a subcutaneous injection of 20 μg relaxin B or C in 0.2 ml benzopurpurine solution (BP) or BP alone (controls). Food was withdrawn at the time of injection; 3 or 9 hr later (and 3 hr before autopsy) each animal was given an intraperitoneal injection of L-[5-^{3}H] proline with a large dose of "cold" L-proline (0.10 mCi [^{3}H]-proline and 0.7 mmole L-proline/100 g body wt). Values for uterine weights and specific activities are means ± SEM.

[b] $P < 0.01$.

conclusion that collagen synthesis in the rat uterus is the primary target of relaxin action may be an artifact of the time period (12 hr) selected for those experiments since effects upon soluble protein were observed earlier.

The finding that a variant form of porcine relaxin, a form which is incapable of modifying the mouse pubic ligament, retains uterotrophic potency in the mouse uterus is consistent with the hypothesis that the primary and perhaps original function of relaxin is more closely related to its effect upon metabolic and structural adaptations of the reproductive tract necessary for viviparity than to its role in symphyseal relaxation. It has been suggested that the relaxin gene may share a common evolutionary origin with the insulin gene, in which case subsequent evolution of relaxin may have been toward an insulinlike function restricted to the reproductive tract—specifically to the uterus (13). In this regard it is notable that both porcine relaxin and bovine insulin exhibit abilities to suppress blood glucose and to stimulate the reproductive tract of the spiny dogfish (*Squalus acanthias*) (14). And although relaxin isolated from ovaries of the sand tiger shark (*Odontaspis taurus*) is ineffective in stimulating uterine or pubic ligament relaxation in the mouse, it will induce symphyseal and uterine relaxation in the guinea pig (15). It is not known, however, whether sand shark relaxin will affect uterine growth and metabolism in mammals (or, indeed, in the shark reproductive tract). An examination of such effects should provide information that would help define the original role of relaxin.

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