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Effect of Purified Relaxin on Uterine Glycogen and Protein in the Rat (40756)

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Relaxin, a hormone of pregnancy discovered by Hisaw in 1926 (1), bears a considerable similarity to insulin in its constituent A and B chains, in the position of the disulfide cross-links, and in its conformation (2–4). Relaxin, however, is not known to possess systemic insulin activity, nor does it cross-react immunologically with insulin (4). Relaxin binds to various reproductive tissues, but not to liver and heart, tissues to which insulin characteristically binds (5). On the other hand, relaxin preparations have been shown to increase the glycogen, water, and nitrogen content, as well as tissue mass in the uteri of immature, estrogen-primed ovariectomized rats (6–12), whereas several studies have indicated that uterine glycogen content in rats is independent of fluctuating insulin levels (13, 14). Although estrogen has been implicated as the primary regulator of uterine growth and glycogen content, it is incapable, even in high doses, of producing the quantitative effects of relaxin on uterine composition (7).

Since most of the reports of the effects of relaxin on uterine composition have utilized low potency (10–150 GPU/mg) relaxin preparations, we have studied the effects of purified porcine relaxin upon the glycogen and protein contents of uteri in ovariectomized rats.

Materials and methods. Sprague–Dawley rats were bilaterally ovariectomized when 30 days old and 1 week later were given a single injection of either 5 μg estradiol benzoate in 0.1 ml sesame oil or 0.1 ml sesame oil alone. After another week they were given two injections 12 hr apart of either relaxin in 0.2 ml of 1% benzopurpurine 4-B or 0.2 ml of the benzopurpurine 4-B alone; the relaxin preparation used was an electrophoretically homogeneous preparation isolated by the method of Frieden *et al.* (15).[1] Food was withdrawn following the second injection; the animals were fasted 12 hr and then sacrificed by cervical dislocation. Blood samples were obtained at the time of sacrifice and the glucose content of the serum estimated using glucose oxidase (Sclavo glucinet). The uteri and diaphragm were removed and weighed; glycogen was extracted by a modification of the method described by Walaas (17) and determined by the anthrone method of Seifter *et al.* (18). In some experiments the uterine horns were divided with one horn used for glycogen determination and the second horn weighed and dried in a vacuum oven at 60° for a minimum of 72 hr in order to estimate tissue water content. The nitrogen content of the dried tissue was determined by a micro-Kjeldahl method.

Results. The effects of purified relaxin on uterine weight and glycogen content are summarized in Fig. 1. A total dose of 0.1 mg was found to increase significantly uterine weights in both untreated and estrogen-primed animals (21 and 48%, respectively). Relaxin also increased uterine glycogen concentration in both groups (36 and 86%, respectively). The combination of increased uterine weight and glycogen concentration resulted in a considerable increase in total uterine glycogen, especially in estrogen-primed animals (167%).

In a second experiment, relaxin doses of 1, 3, 10, and 30 μg were tested in estrogen-primed and unprimed rats in order to establish a dose-response relationship and to determine the sensitivity of the uterus to the glycogenic action of relaxin (Fig. 2). In estrogen-primed animals the glycogen concentration increased linearly with the log of the dose over the range of 3–30 μg. In unprimed animals the effect of 10 μg was essentially the same as that achieved with 30 μg. The uterotrophic response was similar to that for the glycogen response; 3 μg in

[1] Purification of porcine relaxin yields three electrophoretically distinct peptides with relaxin activity. The peptide used in this experiment was relaxin B (1750 GPU/mg) which appears to be identical with relaxin CM-B (16) and the preparation sequenced by Schwabe and McDonald (3).

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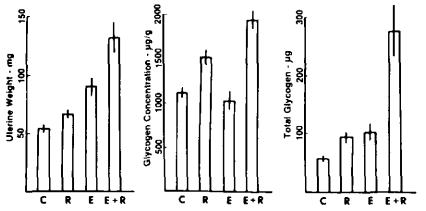

FIG. 1. The effect of relaxin (0.1 mg) on uterine weight, uterine glycogen concentration, and total uterine glycogen in unprimed and estrogen-primed ovariectomized rats. Estrogen priming consisted of 5 µg estradiol benzoate in sesame oil, injected s.c. 1 week following ovariectomy. Unprimed animals received sesame oil. Relaxin B (0.1 mg) was injected in two doses (0.05 mg in 0.2 ml benzopurpurine 4-B) 24 and 12 hr prior to sacrifice, 1 week following estrogen/oil treatment. The groups are: controls (C) received vehicles only; unprimed, relaxin-treated (R); estrogen primed plus benzopurpurine 4-B (E); and estrogen primed plus relaxin-treated (E + R). Vertical lines represent standard errors of the means. Each group included 14 rats.

both primed and unprimed animals were required to elicit a response. The dose–response relationship between relaxin and total uterine glycogen followed a similar pattern. For all three parameters measured, the slopes of log dose–response relationships for estrogen-primed animals appeared to be significantly greater than for animals receiving relaxin alone.

A third experiment was performed to establish whether the apparent uterotrophic effect of relaxin B involved true growth of the uterus. One-tenth milligram significantly increased dry weight proportional to the increase in wet weight with no change in water content (Table I). Total nitrogen content rose concomitantly with increased glycogen in uteri of both primed and unprimed animals.

Since these experiments have shown that relaxin has protein and carbohydrate anabolic effects on the uterus, it was of interest to determine whether relaxin B has systemic effects. Diaphragm glycogen levels in all groups were found not to differ significantly from controls, nor did relaxin have any significant effect on blood glucose (Table II).

Discussion. Using a relaxin preparation which contained 150 GPU/mg, Steinetz *et al.* (6) found a maximum uterine glycogenic effect at 24 hr after injection of 0.1 mg (15 GPU) relaxin in estrogen-primed animals. The glycogenic effect at 24 hr was confirmed using purified relaxin B (1750 GPU/mg). However, in contrast to previous reports (6, 19) the present experiments show that purified relaxin does not require estrogen pretreatment for its glycogenic effects on the uterus. Relaxin appears to affect uterine metabolism directly and does

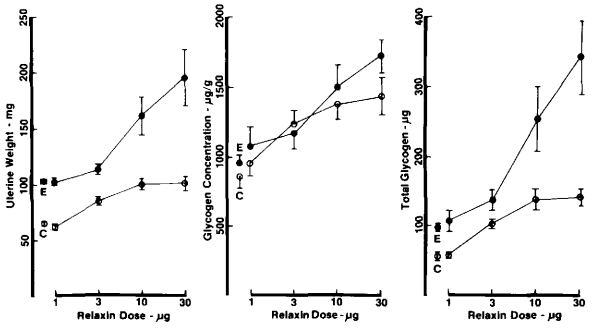

FIG. 2. Dose–response relationships between relaxin and uterine weight, uterine glycogen concentration, and total uterine glycogen in unprimed and estrogen primed ovariectomized rats. Estrogen priming consisted of 5 µg estradiol benzoate in sesame oil, injected s.c. 1 week following ovariectomy. Unprimed animals received sesame oil. Relaxin B (in benzopurpurine 4-B) was injected in split doses 24 and 12 hr prior to sacrifice, 1 week following estrogen/oil treatment. Unprimed, relaxin treated (○); estrogen primed plus relaxin (●); C, control-received vehicles only; E, estrogen primed plus benzopurpurine 4-B. Each point represents the mean of eight animals. Vertical lines represent standard errors of the means.

TABLE I. EFFECT OF 0.1 mg RELAXIN B ON UTERINE COMPOSITION[a]

Treatment	n	Uterine weight wet (mg)	Uterine weight dry (mg)	Uterine water (%)	Total uterine nitrogen (mg)	Total uterine glycogen (μg)
Control	(15)	47.5 ± 2.5	7.3 ± 0.4	84.5 ± 0.7	1.05 ± 0.04	44.5 ± 2.6
Relaxin	(15)	66.4 ± 3.2	11.8 ± 0.9	82.0 ± 1.3	1.38 ± 0.10	74.5 ± 5.8
Estrogen	(15)	88.9 ± 6.1	14.6 ± 1.1	83.6 ± 0.5	2.20 ± 0.13	73.9 ± 6.8
Estrogen + relaxin	(15)	123.8 ± 10.9	21.4 ± 1.8	82.7 ± 0.6	3.23 ± 0.38	143.3 ± 13.4

[a] Procedures are the same as those described in Fig. 1. Values represent means ± SE.

TABLE II. EFFECT OF 0.1 mg RELAXIN B ON DIAPHRAGM GLYCOGEN AND BLOOD GLUCOSE[a]

Treatment	Diaphragm glycogen (μg/g)	Blood glucose (mg/dl)
Control	1052.3 ± 133.0 (15)	75.5 ± 9.9 (10)
Relaxin	1029.2 ± 68.9 (15)	64.8 ± 6.8 (10)
Estrogen	824.4 ± 85.6 (15)	82.9 ± 3.0 (10)
Estrogen + relaxin	1003.6 ± 103.1 (15)	80.7 ± 5.4 (10)

[a] Procedures are the same as those described in Fig. 1. Values represent means ± SE. Numbers in parentheses indicate sample size.

not merely augment estrogen action despite the synergistic relationship which is observed.

In the late 1950s and early 1960s a number of investigators (6, 7, 11, 12) reported the uterine growth-promoting effects of low-potency relaxin preparations. They failed to elicit the response in unprimed animals, however, and concluded that estrogen priming was necessary for most relaxin effects. The results reported here show that relaxin B increases wet and dry weights in unprimed as well as primed animals. Furthermore, an increase in total uterine nitrogen similar to that of total uterine glycogen shows that relaxin causes a true increase in tissue mass regardless of whether estrogen is present although relaxin and estrogen do appear to be synergistic in this respect as well. The limited response to relaxin in unprimed animals (30 μg being no more effective than 10 μg for either the uterotrophic or glycogenic response) suggests that one effect of estrogen priming may be to increase the concentration of relaxin receptors in the uterus, although estrogen priming has been reported not to alter relaxin binding to the mouse pubic symphysis (5). Estrogen priming has also been found unnecessary for increases in cAMP levels in mouse pubic symphysis (20) or in ornithine decarboxylase content of mouse pubic symphysis and uterus (21).

Relaxin B did not increase uterine water content in contrast to earlier reports using less purified material (6, 7, 18, 19). However, the peak of uterine water imbibition in response to relaxin has been reported at 6

hr (6, 17, 18) and thus it is possible that the response had waned since our measurements were taken at 12 hr after the second injection.

It is evident that both the uterotrophic and glycogenic effects of relaxin, at least in the rat, are inherent properties of the hormone. Moreover, the fact that relaxin does not significantly alter diaphragm glycogen concentration or blood glucose levels indicates that the glycogenic action of relaxin is confined to the reproductive tract.

The glycogen content of the uterus has long been implicated as an important nutritional reserve for many energy-consuming processes that take place in this tissue, both during normal reproductive cycling and during pregnancy, including nidation (22), as well as parturition (23). During early pregnancy in the rat glycogen levels are reported to decline (22), and no relaxin is detectable in ovaries or serum of rats before Day 10 of gestation (24). Significant quantities of relaxin are detectable in ovaries from Day 16 of gestation to parturition (24) which corresponds to the periods of uterine accommodation during which its glycogen content increases, and the decline in uterine glycogen following parturition coincides with diminishing plasma relaxin (23). The evidence presented here suggests a direct role for relaxin in uterine growth during late stages of gestation and in the regulation of physiological processes in the female reproductive system, perhaps similar to insulin regulation of general body metabolism.

Summary. Purified, electrophoretically homogeneous porcine relaxin (1750 GPU/mg) increased uterine glycogen in both unprimed and estrogen-primed ovariectomized rats. In estrogen-primed animals the glycogenic response was linear with the logarithm of the dose from 3 to 30 μg/animal; in unprimed animals the effect of 10 μg was essentially the same as that achieved with 30 μg. Relaxin also increased wet and dry uterine weights and total nitrogen content, indicating true uterine growth. Uterotrophic, as well as glycogenic effects occurred without estrogen priming, although synergism of the two hormones was observed; this suggests that relaxin receptor concentrations in the uterus may be, to some degree, estrogen dependent. Relaxin did not affect the concentrations of diaphragm glycogen or plasma glucose; thus the direct effects of relaxin appear to be limited to control of metabolic events in the female reproductive system in a manner similar to insulin regulation of general body metabolism.

1. Hisaw, F. L., Proc. Soc. Exp. Biol. Med. **23**, 661 (1926).
2. Schwabe, C., Steinetz, B., Segaloff, A., McDonald, J. K., O'Byrne, E., Hockman, J., Carriere, B., and Goldsmith, L., Recent Prog. Hormone Res. **34**, 123 (1978).
3. Schwabe, C., and McDonald, J. K., Science **197**, 914 (1977).
4. Schwabe, C., and Harmon, S. J., Biochem. Biophys. Res. Commun. **84**, 374 (1978).
5. McMurtry, J., Kwok, S., and Bryant-Greenwood, G. D., J. Reprod. Fertil. **53**, 209 (1978).
6. Steinetz, B. G., Beach, V. L., Blye, R. P., and Kroc, R. L., Endocrinology **61**, 287 (1957).
7. Kroc, R. L., Steinetz, B. G., and Beach, V. L., Ann. N.Y. Acad. Sci. **75**, 385 (1959).
8. Zarrow, M. X., and Brennan, D. M., Proc. Soc. Exp. Biol. Med. **95**, 745 (1957).
9. Brennan, D. M., and Zarrow, M. X., Endocrinology **64**, 907 (1959).
10. Zarrow, M. X., and Brennan, D. M., Ann. N.Y. Acad. Sci. **75**, 981 (1959).
11. Brody, S., and Wiqvist, N., Endocrinology **68**, 971 (1961).
12. Wada, H., and Turner, C. W., Endocrinology **68**, 1059 (1961).
13. Swigert, R. H., Wagner, C. E., Herbener, G. H., and Atkinson, W. B., Endocrinology **70**, 600 (1962).
14. Leonard, S. L., and Schane, H. P., Endocrinology **77**, 209 (1965).
15. Frieden, E. H., Wu, L. C., and Rawitch, A. B., Fed. Proc. **38**, 636 (1979).
16. Sherwood, O. D., and O'Byrne, E. M., Arch. Biochem. Biophys. **160**, 185 (1974).
17. Walaas, O., Acta Endocrinol. **10**, 1975 (1952).
18. Seifter, S., Dayton, S., Novie, B., and Muntwyler, E., Arch. Biochem. **25**, 191 (1950).
19. Hall, K., J. Endocrinol. **20**, 355 (1960).
20. Braddon, S. A., Endocrinology **102**, 1292 (1978).
21. Braddon, S. A., Biochem. Biophys. Res. Commun. **80**, 75 (1978).
22. Demers, L. M., Yoshinaga, K., and Greep, R. O., Biol. Reprod. **7**, 297 (1972).
23. Chew, C. S., and Rinard, G. A., Biol. Reprod. **21**, 111 (1979).
24. Sherwood, O. D., and Crnedovic, V. E., Endocrinology **104**, 893 (1979).
25. Kostyo, J. L., Endocrinology **60**, 33 (1957).

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