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Effect of Relaxin on Mammary Gland Growth and Lactation in the Rat[1] (38663)

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Studies done previously in this and other laboratories (1–3) have demonstrated the importance of estrogen, progesterone and relaxin in growth and lobulation of the mammary glands of rats. In this species relaxin has been associated with increased lobulation of the mammary system.

Recent work done by Knox and Griffith (4) showed that relaxin administration to already lactating rats results in definite decreases in milk yield. It was suggested that relaxin acts in conjunction with estrogen and progesterone to inhibit the action of oxytocin and therefore decrease milk yield.

The purpose of the research reported herein was to test the effect of relaxin upon DNA and RNA levels of the mammary gland in order to evaluate any possible direct effect upon the secretory cells themselves.

Materials and Methods. Mature female rats of a Sprague–Dawley strain, weighing from 190 to 210 g were maintained on Purina Lab Chow and water *ad libitum* under artificial daylight conditions (14 hr) at a constant room temperature of 78 ± 1°F. All groups (N = 10) except one received bilateral ovariectomies 7–10 days prior to the start of the experiments. Daily injections of relaxin[2] (R) alone in doses of 20 and 90 guinea pig units (GPU), in combination with 1 μg estradiol benzoate or 1 μg estradiol benzoate (EB) plus 3 mg progesterone were administered for 20 days in a sesame oil plus 5% beeswax carrier. Groups receiving no treatment, EB only or EB plus progesterone (P) served as controls along with a normal 20-day pregnant group. All animals were sacrificed on day 21 with subsequent removal of the 6 abdominal–inguinal mammary glands. Fat and water extraction was accomplished using ethyl alcohol and diethyl ether. Dry fat-free tissue (DFFT) was then analyzed for DNA and RNA content according to Webb and Levy (5) and Albaum and Umbreit (6), respectively.

Changes in mammary gland DNA and RNA levels in response to treatments already mentioned were evaluated statistically using Duncan's New Multiple Range Test (7) at $\alpha = 0.05$.

Results. Twenty guinea pig units of relaxin administered for 20 days significantly increased DNA mammary gland content (mg/100 g body wt) over control values in the absence of ovarian hormones (Table I). The magnitude of this increase was 25%. A significant increase was also noted following 90 GPU compared to controls. However, no difference was established between DNA values of the two relaxin groups.

Even though relaxin, at both 20 and 90 GPU levels, significantly decreased the amount of RNA per mg of dry fat-free tissue compared to the control, the observed reduction based on RNA per 100 g body wt was not significant (Table I). As was the case with DNA values, no detectable difference in RNA values between 20 and 90 GPU levels could be observed.

Both 20 and 90 GPU levels of relaxin in conjunction with 1 μg estradiol benzoate significantly increased mammary gland DNA content (mg/100 g body wt) above control values (Table II). Estradiol benzoate alone showed no significant alteration in DNA content compared to controls. Increases of 14%, 20% and 27% in DNA content over control values resulted from estradiol, estradiol plus 20 GPU relaxin, and estradiol plus 90 GPU treatments, respectively. No significant increase in DNA could be attributed to relaxin when compared to estradiol treatment even though the addition of 90 GPU relaxin increased DNA content 16% over estradiol values.

Administration of 20 GPU relaxin and 1

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[2] Relaxin (Releasin) preparation supplied by Warner-Chilcott Laboratory, Morris Plains, N. J.

TABLE I. EFFECT OF RELAXIN ALONE ON DNA AND RNA IN RAT MAMMARY GLANDS (Mean ± SEM[a]).

Treatment	DNA* (μg/mg DFFT[b])	Total DNA* (mg)	DNA* (mg/100g bw[c])
Control	18.10 ± 0.57	11.39 ± 0.86	3.56 ± 0.22
20R[d]	23.86 ± 0.48[e]	15.42 ± 1.64[f]	4.70 ± 0.48[g]
90R	23.99 ± 2.36[e]	14.91 ± 1.60[f]	4.61 ± 0.51[g]
Treatment	**RNA (μg/mg DFFT)**	**Total RNA (mg)**	**RNA (mg/100g bw)**
Control	20.78 ± 0.34	12.99 ± 0.81	4.07 ± 0.23
20R[d]	17.94 ± 1.75[h]	11.62 ± 1.26	3.53 ± 0.37
90R	18.34 ± 0.44[h]	11.44 ± 0.62	3.54 ± 0.22

* Column values with different superscripts are significantly different ($\alpha = 0.05$).
[a] SEM—Standard error of the mean.
[b] DFFT—Dry fat-free tissue.
[c] BW—Body weight.
[d] Guinea pig units relaxin.

TABLE II. EFFECT OF RELAXIN IN CONJUNCTION WITH ESTROGEN ON DNA AND RNA IN RAT MAMMARY GLAND (MEAN ± SEM[a]).

Treatment	DNA* (μg/mg DFFT[b])	Total DNA* (mg)	DNA* (mg/100g bw[c])
Control	18.10 ± 0.57[f]	11.39 ± 0.86	3.56 ± 0.22[h]
EB[d]	22.13 ± 0.60[f, g]	10.62 ± 0.45	4.14 ± 0.14[h, i]
EB + 20R[e]	23.64 ± 1.00[g]	11.80 ± 0.37	4.46 ± 0.17[i]
EB + 90R	25.04 ± 0.69[g]	12.85 ± 0.60	4.90 ± 0.24[i]
Treatment	**RNA (μg/mg DFFT)**	**Total RNA (mg)**	**RNA (mg/100g bw)**
Control	20.78 ± 0.34	12.99 ± 0.81[k]	4.07 ± 0.23[m]
EB[d]	23.47 ± 0.96[j]	11.27 ± 0.57[k]	4.39 ± 0.19[m]
EB + 20R[e]	23.46 ± 0.79[j]	11.94 ± 0.83[k]	4.50 ± 0.30[m]
EB + 90R	16.37 ± 0.22	8.39 ± 0.31	3.20 ± 0.13

* Column values with different superscripts are significantly different ($\alpha = 0.05$).
[a] SEM—Standard error of the mean.
[b] DFFT—Dry fat-free tissue.
[c] BW—Body weight.
[d] EB—1 μg estradiol benzoate.
[e] R—Guinea pig units relaxin.

μg estradiol benzoate did not significantly change RNA (mg/100 g body wt) content compared to either control or estradiol only (Table II). A significant reduction in RNA did result from 90 GPU relaxin administered with estradiol. This reduction was significantly different from values of all three of the other groups involved in the experiment. The value obtained for estradiol plus 90 GPU relaxin was not significantly lower from the values attributed to relaxin alone.

Relaxin activity was altered somewhat in the presence of both estradiol and progesterone. The two steroids plus 90 GPU relaxin did not exhibit a significant increase in DNA values over the estradiol plus progesterone control (Table III). Relaxin at the 20 GPU level in combination with both estradiol and progesterone displayed significantly lower DNA values than either the steroid control or the steroid plus 90 GPU relaxin. The DNA values of the day 20 pregnant rats were also significantly lower than the control and 90 GPU animals involved in this experiment.

Ribonucleic acid levels showed a response to relaxin in conjunction with estradiol and progesterone similar to the response seen in

TABLE III. EFFECT OF RELAXIN IN CONJUNCTION WITH BOTH ESTROGEN AND PROGESTERONE ON DNA AND RNA IN RAT MAMMARY GLAND (MEAN ± SEM[a]).

Treatment	DNA* (μg/mg DFFT[b])	Total DNA* (mg)	DNA* (mg/100g bw[c])
EB + P[d]	32.29 ± 0.90	20.49 ± 0.69[f, g]	8.16 ± 0.27[h]
EB + P + 20R[e]	32.14 ± 0.97	18.30 ± 1.03[g]	7.50 ± 0.35[i]
EB + P + 90R	33.33 ± 1.43	21.91 ± 1.22[f]	8.67 ± 0.45[h]
Day 20 Pregnant	34.91 ± 1.70	19.34 ± 0.93[g]	7.52 ± 0.21[i]
Treatment	**RNA (μg/mg DFFT)**	**Total RNA (mg)**	**RNA (mg/100g bw)**
EB + P[d]	28.34 ± 0.85	18.18 ± 0.72[k]	7.23 ± 0.28[m]
EB + P + 20R[e]	23.58 ± 0.65[j]	13.33 ± 1.37	5.47 ± 0.16
EB + P + 90R	24.40 ± 0.52[j]	16.51 ± 0.59[k]	6.55 ± 0.24[m]
Day 20 Pregnant	37.42 ± 3.90	21.05 ± 2.96	8.13 ± 0.66

* Column values with different superscripts are significantly different ($\alpha = 0.05$).
[a] SEM—Standard error of the mean.
[b] DFFT—Dry fat-free tissue.
[c] BW—Body weight.
[d] EB + P — 1 μg estradiol benzoate plus 3 mg progesterone.
[e] R—Guinea pig units relaxin.

the first two experiments. Relaxin at both levels reduced RNA content (mg/100 g body wt) below estradiol plus progesterone values (Table III). However, the reduction was only significant at the 20 GPU dose. Values for all three treatment groups were significantly lower than the day 20 pregnant level.

Using an RNA to DNA ratio as an index of mammary gland activity (Table IV), further verification of nucleic acid alteration was expressed. Except for relaxin at the 20 GPU level in conjunction with estradiol, both levels of relaxin administration showed a significant decrease in the ratio when compared to their respective controls. The decrease in this ratio indicated an increase in DNA or a decrease in RNA or both.

Discussion. Relaxin administration to rats receiving simultaneous injections of 1.0 μg estradiol and 1.0 mg progesterone daily resulted in increased lobular alveolar growth (2). Fifteen and 45 GPU levels of relaxin administered with 1 μg estradiol benzoate to intact and ovariectomized rats also elicited significant growth of the lobular–alveolar system (8). Relaxin alone or in combination with progesterone (2) was only slightly effective. No combination of these three hormones was effective in causing mammary gland growth of hypophysectomized animals.

TABLE IV. RELAXIN EFFECTS ON RAT MAMMARY GLAND USING RNA/DNA RATIO AS A MEASURE (MEAN ± SEM[a]).

Treatment	RNA/DNA*
Control	1.15 ± 0.04[e]
20R[b]	0.75 ± 0.01[g, h]
90R	0.77 ± 0.03[g]
EB	1.06 ± 0.04[e, f]
EB + 20R	1.01 ± 0.06[f]
EB + 90R	0.66 ± 0.02[h, i]
EB + P[d]	0.89 ± 0.03
EB + P + 20R	0.74 ± 0.02[g, i]
EB + P + 90R	0.77 ± 0.04[g]
Day 20 Pregnant	1.08 ± 0.05[e, f]

* Values with different superscripts are significantly different ($\alpha = 0.05$).
[a]SEM—Standard error of the mean.
[b]R—Guinea pig units relaxin.
[c]EB—1 μg estradiol benzoate.
[d]P—3 mg progesterone.

Thyroidectomized rats treated with estrogen, progesterone and relaxin displayed greater lobular–alveolar development than nonthyroidectomized animals (2). In this case, however, no difference could be determined between groups receiving the steroids with or without relaxin. Maximal lobulation was nearly attained using estrogen alone in thyroidectomized animals.

Removal of the adrenal glands did not alter mammary gland appearance resulting from estrogen, progesterone and relaxin treatment; however, glands were reduced in weight and size (2). Replacement therapy utilizing deoxycorticosterone acetate restored the glands to a normal state.

Decreased milk yield resulting from relaxin administration has been reported in both goats (9) and rats (4). Knox and Griffith (4) speculated relaxin's role under these conditions to be antioxytocic in terms of milk letdown. Results presented in this thesis demonstrate relaxin's ability to decrease mammary gland RNA levels. It is therefore implied that relaxin slows cellular activity which, in turn, would decrease milk synthesis and result in lower yields.

Another plausible concept would be that relaxin acts as a depressant of the initiation of lactation. High levels of relaxin which are maintained through the latter part of pregnancy are abruptly decreased at parturition only to be followed by the onset of lactation. The removal of relaxin's deterrent upon RNA levels could feasibly be as essential as progesterone levels.

The data obtained from this study indicate that relaxin alone has the capacity to elicit cell proliferation of the mammary glands of rats while at the same time damping the cellular activity of the same tissue. It is implied that relaxin will cause additional mammary gland growth when in combination with either estrogen alone or estrogen and progesterone together although in the latter case dose appears to be an important factor. In terms of RNA levels relaxin alone depresses tissue levels only about 13% whereas in conjunction with estrogen or estrogen and progesterone reduction of these values can be as much as 24%. The conclusion which may be drawn from this is that relaxin is acting synergistically with estrogen and progesterone to develop the mammary apparatus during pregnancy and at the same time is a suppressant on the initiation of lactation. Removing the inhibitory effects of relaxin at parturition could possibly be an important prelude to lactogenesis.

Summary. Mammary DNA, RNA and RNA/DNA were measured in groups of 10 ovariectomized rats treated for 20 days with relaxin (R) in doses of 20 and 90 guinea pig units (GPU) alone or in combination with 1 μg estradiol benzoate (EB) or EB plus 3 mg progesterone (P). No treatment, EB only, and EB plus P served as control groups. Twenty GPU R alone significantly increased DNA over the no treatment group while 90 GPU did not increase DNA any higher. Although DNA values for the EB group were higher than no treatment and EB plus P higher than EB, R was not effective in increasing DNA in combination with either EB or EB plus P. R alone at either level did not change RNA from the no treatment group, but 90 GPU R in association with EB and both levels of R in association with EB plus P significantly reduced RNA in relation to the appropriate control groups. Relaxin acts synergistically with estrogen and progesterone to develop the mammary apparatus and at the same time to suppress lactation. Removing the inhibitory effects of relaxin at parturition may be an important prelude to lactogenesis.

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