PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 558-561 (1977)

The Response to Endogenous Relaxin of Guinea Pigs Refractory to Porcine Relaxin (39850)

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Guinea pigs, used repeatedly for the bioassay of porcine relaxin during 6 to 8 weeks, show a marked (15- to 20-fold) decline in sensitivity to relaxin from this source (1, 2). The reduction in sensitivity to porcine relaxin is accompanied by a much smaller (two- to four-fold) diminution in sensitivity to pregnant rabbit serum relaxin (1). This loss of sensitivity is not due to aging or to repeated exposure to estrogen (3). It was suggested that the development of a refractory state was due to the formation of antibodies to relaxin in guinea pigs, although such antibodies have not been unequivocally demonstrated in this species. However, in response to adjuvant-treated porcine relaxin, rabbits develop antisera which are capable of inhibiting the elongation of the pubic ligament in mice injected with relaxin obtained from pigs, rabbits, rats, roosters, and whales (4, 5).

The public symphyses of guinea pigs relax spontaneously during pregnancy, apparently in response to a relaxin of placental origin (6, 7). Palpable mobility of the symphysis can be detected about 25 to 30 days before parturition (31-36 days after mating). The degree of relaxation increases steadily until just prior to parturition, when the two halves of the symphysis are widely separated and can be moved independently of each other. This response can also be induced in nonpregnant (intact or ovariectomized) guinea pigs pretreated with estrogen (10 μg of estradiol/day) for 10 days followed by estrogen plus progesterone (1 mg/day) beginning on Day 11. Pelvic relaxation which can be detected by Day 13 or 14 is attributable to the formation of relaxin by reproductive tract tissue, primarily the uterus and vagina (6).

The maximum concentration of relaxin in guinea pig serum during the latter half of pregnancy was estimated to be 0.5 GPU/ml using a guinea pig bioassay (8) but only 0.0013 GPU/ml (0.5 ng/ml)[1] on the basis of radioimmunoassay using rabbit antisera to porcine relaxin (7). The discrepancy between these two measures indicates minimal cross-reactivity between guinea pig relaxin and rabbit anti-porcine relaxin antisera.

The experiments described in this paper were performed to determine whether resistance induced in guinea pigs by repeated injections of porcine relaxin alters the response to relaxin produced endogenously during pregnancy or in response to the injection of steroid hormones.

Materials and methods. Partially purified porcine relaxin (NIH, 440 GPU/mg) was injected sc dissolved in 0.15 *M* NaCl. Estradiol benzoate and progesterone (Nutritional Biochemicals) were dissolved in sesame oil and administered im.

The guinea pigs used for these experiments included two groups of animals which had been used previously for assay of porcine relaxin. The animals had been introduced into the assay colony when they reached 275-325 g. The assay protocol consisted of a priming injection of 5 μg of estradiol benzoate each Monday followed by relaxin injected each Friday; the animals were palpated 6 hr later. Two weeks after the first estrogen injection, all animals responded to 2 GPU of (0.005 mg) NIH relaxin. After approximately 2 months of weekly injections, more than half the animals failed to respond to 8-10 GPU.

In the first experiment, 11 females resistant to porcine relaxin and six untreated females (controls) of the same weight were placed in groups of two to three with males for 1 month. The pelvises of the females were palpated every other day until parturition appeared imminent, after which they

[1] Purified porcine relaxin contains approximately 2500 GPU/mg (9).

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ISSN 0037-9727

were examined daily until 10 days postpartum. The degree of pelvic relaxation was scored on a scale of 0–3. One month after weaning (60 days postpartum) all animals were injected sc with estradiol benzoate (15 μg/kg body weight) and four days later with 0.25 mg/kg of porcine relaxin. Six hours after the relaxin injection the animals were palpated and scored as above.

In the second experiment nine controls and 48 assay colony discards (porcine relaxin-resistant) were used. Each animal received estradiol benzoate (20 μg/kg) every other day for 16 days; daily progesterone injections (2 mg/kg) were begun on Day 11 and continued for 6 days. All animals were palpated daily, and responses were scored as indicated above; the animals were coded and their identity remained unknown to the operator (EHF).

Three weeks after the last progesterone injection, some of the resistant guinea pigs were selected to form a group of eight animals exhibiting maximum response to estrogen and progesterone and another group of eight animals which had responded minimally or not at all to steroid hormones. These animals as well as the seven remaining controls were placed with males and palpated every other day until shortly before parturition and then daily until parturition at which time the pups were weighed and discarded.

Results. Experiment I. All controls and 9 of the 11 resistant colony discards became pregnant. Detectable relaxation (mean score >1) was evident in control animals by 25 to 28 days before parturition, while the resistant animals showed no detectable relaxation until about 20 days prior to giving birth (Fig. 1). At comparable stages of gestation, pelvic relaxation of control animals was nearly 1 response unit greater than in the resistant ones until 4 days before parturition, by which time the two groups were equivalent. Four days after parturition the degree of pelvic relaxation of the resistant animals was only one-half that of the controls, while both groups returned to the normal unrelaxed state by 8 days. Sixty days postpartum, all the control animals responded with marked relaxation (mean response = 2.2) to a priming dose of estradiol benzoate followed 4 days later by 25 μg/kg of NIH porcine relaxin; however, the resistant group remained unresponsive (mean response = 0.4).

Experiment II. Progesterone treatment following 10 days of estrogen priming produced detectable pelvic relaxation after 3 to 4 days (Fig. 2). The response continued to increase to its maximum 2 days after the last progesterone injection (Day 18) and then decayed to zero over the next 4 days (Day

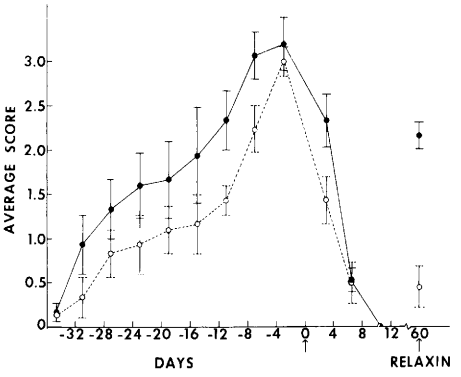

FIG. 1. Pelvic relaxation scores averaged for 4-day periods during the latter half of gestation in control and porcine relaxin-resistant guinea pigs (Experiment I). Parturition is indicated by the arrow at time 0. Scoring was continued for 2 weeks postpartum. Sixty days postpartum, the response to 25 μg/kg of porcine relaxin was measured. ⏚ : controls (n = 6); ⍜ : relaxin-resistant (n = 9); mean scores ± SE.

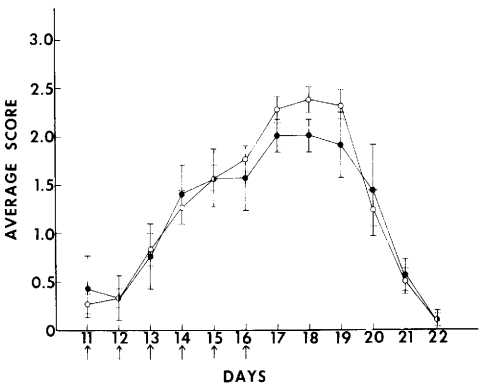

FIG. 2. The effect of progesterone (2 mg/kg/day) on pelvic relaxation in control and porcine relaxin-resistant guinea pigs. Each animal received 20 μg/kg of estradiol benzoate every other day from Day 0–16. Progesterone was injected daily from day 11–16 (arrows). ⏚ : controls (n = 9); ⍜ : relaxin-resistant (n = 48); mean scores ± SE.

22). The average response was the same in the control and resistant animals, although the response within each group was highly variable (four resistant and one control did not respond at all).

Experiment III. Experiment III was a repeat of Experiment I in which resistant animals were selected and grouped according to their response to progesterone. Pelvic relaxation during gestation replicated Experiment I in that all the porcine relaxin-resistant animals lagged about 1 unit behind the controls until a few days before parturition at which time sufficient enlargement of the birth canal was achieved to permit passage of fetuses of normal size (Fig. 3, Table I). However, in the progesterone-responsive group the number of pups and total litter weight were significantly ($p < .05$) lower than the controls.

Discussion. Relaxation of the symphysis pubis during gestation in the guinea pig (Figs. 1 and 3) follows a course parallel to plasma relaxin concentration as measured by radioimmunoassay (7). Animals resistant to porcine relaxin lag behind control animals with respect to pelvic relaxation until a few days prior to parturition. These differences could be the result of a reduction in relaxin secretion, neutralization of circulating relaxin by antibodies, or a loss of responsiveness of the target tissue. The finding here that resistant animals respond to estrogen and progesterone to the same extent as controls but are deficient in relaxin activity during gestation suggests the secretion of a relaxin by the reproductive tract which is less homologous to porcine relaxin than is placental relaxin. In support of the possibility of structural heterogeneity of guinea pig relaxin, it has been established that three different molecular species with relaxin activity can be isolated from porcine relaxin concentrates (9).

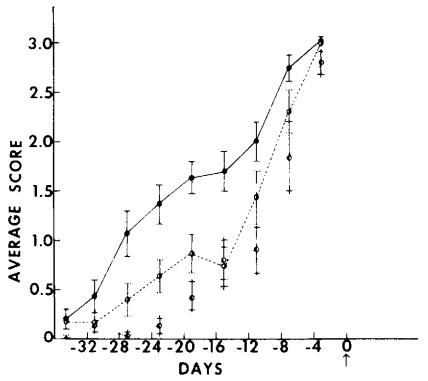

FIG. 3. Pelvic relaxation scores during the latter half of gestation in control and porcine relaxin-resistant guinea pigs (Experiment II). Parturition is indicated by the arrow. ● : controls (group I, $n = 7$); ◐ : relaxin-resistant, progesterone-responsive (group II, $n = 8$); ◑ : relaxin-resistant, progesterone-insensitive (group III, $n = 8$); mean scores (averaged over 4-day periods) ± SE.

The modest cross-reactivity between porcine relaxin and guinea pig plasma relaxin (7) is consistent with the hypothesis that porcine relaxin is highly antigenic in guinea pigs. Ouchterlony tests of our guinea pig sera against porcine relaxin were negative, but this does not exclude all possible antibody types. Following parturition reformation of the pelvis occurs somewhat more rapidly in resistant animals (Fig. 1); this further supports the existence of circulating antibodies.

As indicated in Table I and Fig. 3 the animals in group II of experiment II (relaxin-resistant and progesterone-sensitive) were slowest to achieve maximum pelvic relaxation. These animals also produced the smallest number of offspring. If the main source of relaxin during pregnancy is the placenta, part of the delay in achieving maximum relaxation may be due to the smaller amount of placental tissue that would be

TABLE I. SIZE AND WEIGHT OF LITTERS DERIVED FROM RELAXIN-RESISTANT GUINEA PIGS.[a]

Treatment	Litter size	Pup weight (g)	Litter weight (g)
Group I: Control (7)	3.8 ± 0.3	97.0 ± 2.8	372 ± 33
Group II: Relaxin-resistant; progesterone-responsive (8)	2.7 ± 0.4	106.5 ± 6.3	278 ± 31
Group III: Relaxin-resistant; progesterone-unresponsive (8)	3.3 ± 0.4	108.3 ± 8.1	332 ± 33

[a] Values are means ± SE.

expected in this group of animals. However, comparison of litter size with pelvic relaxation at several stages prior to parturition in all three groups indicated no correlation, although we do not have data on actual placental weights.

Summary. Guinea pigs made resistant to porcine relaxin by repeated injections were able to complete gestation and give birth to pups weighing the same as control animals. The progression of pelvic relaxation as gestation proceeded was retarded in the resistant animals although the optimum degree of relaxation was achieved just prior to parturition. Estrogen-primed, resistant animals responded to progesterone to the same extent as did controls, suggesting that the delay in pelvic relaxation during gestation is unlikely to be due to a reduction in secretion of relaxin or to a loss of tissue sensitivity to relaxin but rather to neutralization of placental relaxin by circulating antibodies.

Note added in proof. Dr. B. G. Steinetz, of Ciba-Geigy Corporation, Ardsley, N.Y., has demonstrated the existence of anti-porcine relaxin antibodies in the sera of the relaxin-resistant animals used in these experiments. The antibody content was measured by the ability of the serum specifically to bind ^{125}I-polytyrosyl relaxin. Of the labeled relaxin added, 14 and 12%, respectively, was bound by 25 μl of serum from animals in groups II and III (Table I); the fraction bound by the same quantity of serum from control animals averaged less than 2%.

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Received March 12, 1977. P.S.E.B.M. 1977, Vol. 155.