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Effects of Relaxin on Early Pregnancy in Rats (39410)

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The polypeptide hormone, relaxin, prepares the reproductive tracts for parturition in several mammalian species. In rats, relaxin is secreted by the ovaries during the last week of a 22-day pregnancy (1), and its most obvious effect is to soften and dilate the uterine cervix (2). When rats are ovariectomized on Day 15 of gestation, pregnancy may be maintained by daily injections of progesterone and estradiol. However, the cervix fails to soften, placental detachment is faulty, and parturition is impaired unless relaxin is administered together with the steroid hormones (2).

In mice, in addition to a gradual softening of the cervix, a long interpubic ligament forms during the last week of a 20–21-day gestation period and the uterus becomes more responsive to oxytocin (3). These changes correlate with increased blood levels of relaxin (1). As in rats, there is a failure of parturition in ovariectomized pregnant mice unless relaxin therapy is instituted (2).

The effects of relaxin on early pregnancy have not been documented. It seemed possible that such early administration might bring about a very premature "parturition." In the present study we have injected relaxin into rats in the peri-implantation period. In addition, we have investigated the effects of relaxin on the response of the pregnant rat to subabortifacient doses of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), another substance involved in the initiation of parturition (4–7).

Materials and methods. Animals. Charles River CD or Marland Breeding Farms MFS adult female rats (both Sprague–Dawley-derived) were used. Pregnancies were timed by the suppliers (midnight ± 6 hr) and the animals were delivered to our laboratory on Day 1 or 2 of pregnancy. Day 1 is the morning on which spermatozoa are found in the vaginal smear.

Hormone and drug treatments. Swine ovarian relaxin preparations (8) ranging in potency from 1000 to 3000 units (9) per mg were suspended in a 5% beeswax in peanut oil vehicle and injected sc at the doses indicated in the text and tables. Groups of control rats were injected sc with equivalent milligram doses of bovine serum albumin (BSA) or a nonrelaxin polypeptide fraction prepared from swine ovaries in the beeswax-oil suspension. Progesterone was injected sc in sesame oil. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was injected sc in 0.1 *M* phosphate buffer (pH 7.4). Dexamethasone and indomethacin were administered by gavage as aqueous suspensions.

Experimental. Relaxin or control preparations were administered sc as single doses on Days 3, 4, or 5 of pregnancy or as multiple doses on Days 3–5 or 4 and 5 in rats. Progesterone was injected sc on Days 3–6 or 2–9 of pregnancy in rats. Dexamethasone was administered orally on Days 4–6. Indomethacin was given by gavage days 3–6. $PGF_{2\alpha}$ was injected sc as a single dose on Day 5 of pregnancy alone or in combination with relaxin. In all experiments, rats were killed on Day 10 and living fetuses, resorptions, and total implantation sites were counted. In one experiment blood was drawn for progesterone determination on Day 6, 24 hr after a single sc injection of relaxin. The rats were then killed on Day 9 and the uterine contents observed as above.

Progesterone radioimmunoassay. Serum progesterone levels were determined by the method of Abraham *et al.* (10) as modified in our laboratory (11).

Results. Injection of 0.5 (but not 0.1) mg of relaxin sc daily on Days 3–5 of pregnancy induced resorption of a large number of fetuses (Table I). This effect was not duplicated by 0.5-mg daily doses of bovine serum albumin (BSA) or a nonrelaxin polypeptide fraction of sow ovaries obtained as a by-product during the extraction of relaxin (Table I).

Single doses of 1 mg of relaxin administered on Day 4 or 5, but *not* on Day 3, likewise terminated pregnancy in rats (Table II). Progesterone, but not indomethacin on Days 3–5, prevented termination of pregnancy by relaxin on Day 5 (Table II). Similarly, resorption of litters induced by 0.5-mg doses of relaxin on Days 4 and 5 was prevented by daily injections of progesterone from the second to the ninth day of pregnancy (Table II). Injections of 10 or 30 μg of dexamethasone on Days 4, 5, and 6 partially or completely reversed the adverse effects of relaxin on litter survival (Table III).

Smaller daily doses of relaxin (0.1 mg) did not induce resorptions (Table IV). A single injection of 1 mg of $PGF_{2\alpha}$ on Day 5 of pregnancy likewise failed to affect the course of pregnancy (Table IV). However, when 1 mg of $PGF_{2\alpha}$ was injected on Day 5 following 0.1-mg doses of relaxin on Days 3, 4, and 5, nearly complete resorption of litters was observed (Table IV).

Serum progesterone levels were found to be similar to those of controls 24 hr after single injections of 3 mg of partially purified or 1 mg of highly purified relaxin (Table V).

Discussion. Two or three injections of relatively large doses of purified porcine relaxin (0.5–1.0 mg) around the expected time of implantation terminated pregnancy in rats. However, this effect appeared to be specific for the relaxin hormone per se as it was not duplicated by injections of nonrelaxin polypeptides obtained from pregnant sow ovaries or injection of a foreign protein such as BSA. Single injections of 1 mg of relaxin on Day 4 or 5, but not on Day 3, likewise terminated pregnancy. In no case did relaxin interfere with implantation. The uterine contents were protected against the effects of relaxin by injections of progesterone or dexamethasone. The latter steroid

TABLE I. EFFECTS OF RELAXIN ON EARLY PREGNANCY IN RATS.

Treatment[a] (Days 3–5)	No. rats	Av no. living fetuses[d]	Av no. resorptions[d]	Av no. implants[d]
Control	6	12.3 ± 1.1	0.4 ± 0.2	13.5 ± 1.2
Relaxin[b] 0.5 mg	5	2.6 ± 2.6[c]	8.0 ± 2.8[c]	10.6 ± 2.1
Relaxin[b] 0.1 mg	5	11.2 ± 0.8	0	11.2 ± 0.8
Control ovarian extract 0.5 mg	6	13.2 ± 0.8	0	13.2 ± 0.8
BSA 0.5 mg	6	10.0 ± 3.0	0	10.0 ± 3.0

[a] In 0.2 ml of 5% beeswax in peanut oil.
[b] 21 AAE relaxin, 3000 units/mg.
[c] $P < 0.02$ versus control.
[d] Determined at autopsy on Day 10 of pregnancy.

TABLE II. EFFECT OF SINGLE DOSES OF RELAXIN ON EARLY PREGNANCY IN RATS AND THE ANTIABORTIFACIENT ACTION OF PROGESTERONE.

Treatment[a]	Injection on Day:	No. rats	Av no. living fetuses	Av no. resorptions[d]	Av no. implants[d]
Control	–	7	10.6 ± 2.7	0	10.6 ± 2.7
Relaxin[b] 1 mg sc	3	6	10.8 ± 1.4	1.8 ± 0.8	12.6 ± 0.7
Relaxin 1 mg sc	4	7	1.8 ± 1.5[c]	8.8 ± 2.7[c]	10.7 ± 2.2
Relaxin 1 mg sc	5	6	0.5 ± 0.5[c]	8.3 ± 2.9[c]	8.8 ± 2.9
Relaxin 1 mg sc + Progesterone 10 mg sc	5 3-6	4	12.5 ± 0.6	0	12.5 ± 0.6
Relaxin 1 mg sc + Indomethacin 0.5 mg po	5 3-6	4	1.5 ± 1.5	10.5 ± 3.4	12.0 ± 3.2
Relaxin 0.5 mg sc	4, 5	6	1.7 ± 1.7[c]	7.2 ± 2.8[c]	8.8 ± 2.5
Relaxin 0.5 mg sc + Progesterone 5 mg sc	4, 5 2-9	6	10.1 ± 1.4	1.6 ± 0.8	11.8 ± 0.8

[a] In 0.2 ml of 5% beeswax in peanut oil.
[b] Relaxin preparation 21 AAE (3000 U/mg).
[c] Significantly different from control, $P < 0.01$.
[d] Determined at autopsy on Day 10 of pregnancy.

TABLE III. REVERSAL OF RELAXIN–INDUCED RESORPTIONS BY DEXAMETHASONE IN RATS.

Treatment	No. rats	Living fetuses[c]	Resorptions[c]	Total implants[c]
Control	10	12.4 ± 0.5	0.1 ± 0.1	12.5 ± 0.5
Relaxin[a] 0.5 mg sc Days 4 and 5	10	1.6 ± 1.0[b]	11.0 ± 1.1[b]	12.6 ± 0.6
Relaxin[a] 0.5 mg sc Days 4 and 5 + Dexamethasone 10 μg po Days 4–6	5	6.8 ± 3.0	3.0 ± 1.5	9.5 ± 2.2
Relaxin[a] 0.5 mg sc Days 4 and 5 + Dexamethasone 30 μg po Days 4–6	5	11.2 ± 0.7	0.2 ± 0.2	11.4 ± 0.4
Dexamethasone 10 μg po Days 4–6	5	12.6 ± 1.2	0	12.6 ± 1.2
Dexamethasone 30 μg sc Days 4–6	5	13.2 ± 0.4	0	13.2 ± 0.4

[a] Relaxin preparation No. *15 AAE* (1000 units/mg) in 0.2 ml of 5% beeswax in peanut oil.
[b] Significantly different from control, $P < 0.01$.
[c] Determined at autopsy on Day 10 of pregnancy.

TABLE IV. EFFECTS OF RELAXIN AND $PGF_{2\alpha}$ ON PREGNANCY IN RATS.

Treatment	Days	No. rats	Fetuses	Resorptions[c]	Total implants[c]
Control (BSA 0.5 mg sc)	3–5	5	12.0 ± 2.8	0	12.0 ± 2.8
Relaxin[a] 0.1 mg sc	3–5	5	11.2 ± 0.8	0	11.2 ± 0.8
$PGF_{2\alpha}$ 1 mg sc	5	5	13.6 ± 1.2	0.8 ± 0.4	14.5 ± 1.3
Relaxin 0.1 mg sc + $PGF_{2\alpha}$ 1 mg sc	3–5 5	5	1.2 ± 1.2[b]	8.4 ± 2.5[b]	9.6 ± 2.6

[a] Relaxin preparation No. *21 AAE* (3000 units/mg) in 0.2 ml of 5% beeswax in peanut oil.
[b] Significantly different from control, $P < 0.01$.
[c] Determined at autopsy on Day 10 of pregnancy.

TABLE V. SERUM PROGESTERONE LEVELS 24 HR AFTER RELAXIN INJECTION IN EARLY PREGNANT RATS.

Treatment[a]	Daily dose (mg)	Days of injection	No. rats	Av no. living fetuses[b]	Av no. resorptions[b]	Serum progesterone (ng/ml)[c]
None	–	–	4	13.5	0	66.7 ± 2.6
Relaxin (15 AAE)	3.0	4, 5	3	0	8.6	75.7 ± 12.8
Relaxin (21 AAE)	1.0	5	5	5.5	7.8	68.5 ± 10.3

[a] Injected in 0.2 ml of 5% beeswax in peanut oil.
[b] Observed on Day 9 of pregnancy.
[c] Blood drawn on Day 6 of pregnancy.

has no progestational activity but corticosteroids may induce secretion of prolactin (12, 13). Prolactin, in turn, would increase progesterone synthesis by the ovaries. It is also possible that dexamethasone, by virtue of its antiinflammatory activity, may somehow directly antagonize the effects of relaxin. (Relaxin is known to induce inflammatorylike changes in permeability and composition of the uterus; 14). However, a nonsteroidal antiinflammatory agent, indomethacin did not prevent the abortifacient effects of relaxin.

Relaxin and $PGF_{2\alpha}$ in doses which separately had no adverse effects on pregnancy, induced resorption of litters when given in combination. We had considered the possibility that relaxin may exert its action by inducing prostaglandin synthesis. However, the failure of a prostaglandin synthetase inhibitor, indomethacin to prevent the abortifacient action of relaxin makes this appear unlikely. It does seem possible that relaxin and PGs may act synergistically at term to bring about parturition.

The mechanism(s) whereby large doses of relaxin terminate pregnancy is not known. The hormone did not appear to alter serum progesterone levels and yet exogenous progesterone antagonized the abortifacient action of relaxin. This finding suggests that the effect of relaxin may be exerted on the uterus inasmuch as this organ is the major site of action of progesterone in maintaining

pregnancy. Relaxin may inhibit decidualization (15). Relaxin also is known to affect the composition (14), structural framework (16, 17), and motility (e.g., 17) of the uterus. Relaxin may be able to reverse the progesterone "block" to the propagation of coordinated uterine contractile activity.

Summary. Administration of relaxin in the peri-implantation period terminated pregnancy in rats. The effect was blocked by progesterone and dexamethasone but not by indomethacin. When relaxin and $PGF_{2\alpha}$ were given concomitantly in doses which singly did not interfere with pregnancy, resorption of fetuses was observed. It is suggested that relaxin may antagonize the progesterone "block" to coordinated uterine contractions.

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