# THE MYOMETRIUM OF THE SPINY DOGFISH, SQUALUS ACANTHIAS: A MODEL FOR STEROID AND PEPTIDE REGULATION

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During late pregnancy in mammals, estrogen and progesterone levels are relatively high. Estrogen acts primarily to prepare the uterus for labor and parturition. Progesterone predominantly counteracts the effects of estrogen therefore maintaining pregnancy. The withdrawal of progesterone is thought to initiate labor.

Mammalian relaxin has been shown to inhibit myometrial activity of the pregnant uterus. Rudzik and Miller (J. Pharmacol. Exp. Ther. 138:88, 1962) demonstrated that the response of the uterus to purified relaxin is eliminated following ovariectomy, hypophysectomy, or adrenalectomy. Estrogen administration reverses this inhibition while progesterone antagonizes the effects of estrogen. Mercado-Simmen et al. (Endocrinol. 110:220, 1982) have shown that relaxin receptor levels are high in mammals pretreated with estrogen or at proestrus and estrus when estrogen levels are naturally high and progesterone levels are low. The primary known effects of relaxin are thought to be an involvement by the hormone in uterine and cervical metabolism which results in a change in the physical properties of these tissues and the inhibition and coordination of myometrial contractions (Sanborn, B.M. In: The Physiology and Biochemistry of the Uterus in Pregnancy and Labor, Huszar G., Ed. CRC Press, Fla., pp. 225-238, 1986).

In the viviparous elasmobranch, Squalus acanthias, the uterus is spontaneously active throughout pregnancy and circulating estrogen levels are low (100-200 pg/ml plasma) during the first stages of pregnancy and then increase (10ng/ml plasma) in the late stages (Tsang and Callard, Gen. Comp. Endocrinol. 66:182-189, 1987). Basal progesterone production is low in Stage C animals (Tsang and Callard, J. Exp. Zool. 241:377-382, 1987). Previous studies from this laboratory have demonstrated that porcine relaxin significantly increases cervical cross area in pregnant Stage C dogfish (Koob, Laffan, and Callard, Biol. Reprod. 31:231-238, 1984). It has also been shown that homologous Squalus relaxin (sRLX) increases the interval between contractions in the pregnant Stage C uterus in vitro and in vivo in a dose-dependent manner; sRLX coordinates myometrial contractions in the dogfish. This study demonstrates that the effects of homologous relaxin on an estrogen primed uterus can be blocked by progesterone. This evidence suggests that the elasmobranch uterus is an excellent model for studying the reproductive endocrinology of the uterus.

Pregnant Stage C Dogfish (5-14 kg) were gill netted in Frenchman's Bay, Maine. Animals were maintained in tanks equipped with running sea water. Control animals were injected on Days 1, 4, and 5 intramuscularly with the vehicle (vegetable oil). Estrogen treated animals were injected i.m. with a 10 mg/kg bolus of 17B-estradiol on day 1 and progesterone treated animals were injected i.m. with 20 mg/kg progesterone vehicle on days 4 and 5. Estrogen and progesterone treated animals were injected i.m. with 10 mg/kg 17B-estradiol on Day 1 and 20 mg/kg progesterone on Days 4 and 5.

All pressure recordings were obtained on Day 8 of the steroid treatment schedule. Animals were anesthetized with 10 mg/kg sodium pentobarbital (Abbott). During surgery and throughout in vivo testing, the animal was

maintained in a trough equipped with continuous flowing fresh sea water. Intra-uterine pressure cycles were measured via balloon catheter filled with distilled water surgically implanted in the pregnant uterus. The balloon catheter was connected to a Grass pressure transducer which was in turn connected to a Grass model 79 physiograph. A standard pressure curve was generated and initial pressure in the balloon catheter determined. All recordings were obtained at an initial pressure of 15 mm $H_2O$. Evaluation of initial activity was performed 50-60 minutes after surgery to allow for stabilization of activity. Squalus relaxin (sRLX) was provided by Dr. C. Schwabe, the Medical University of S. Carolina. The hormone was injected i.v. into a cannula introduced into the caudal vein of the animal. 10 ug of sRLX was injected into the animals during the time of recording. The pattern of spontaneous contraction was allowed to stabilize before sRLX was administered. Evaluation of the recordings was performed 10 minutes after addition of the hormone.

Control animals and estrogen treated animals exhibited a decrease in the rate of spontaneous activity of $2.29 \pm 0.77$ to $5.46 \pm 2.21$ minutes and $2.50 \pm 0.85$ to $6.94 \pm 2.55$ minutes respectively after injection of 10 ug sRLX. The interval between contractions increased in the control group, $1.34 \pm 0.91$ to $3.47 \pm 1.1$ minutes and in the estrogen treated group, $0.41 \pm 0.26$ to $8.36 \pm 5.4$ minutes. sRLX had no significant effect on intensity (amplitude) or duration of contraction. The progesterone treated group showed no significant difference in rate (1.69 to 1.52 minutes) or interval (0.293 to 0.267 minutes) after injection of sRLX. The estrogen and progesterone treated group also demonstrated no significant effect of sRLX on rate ($3.10 \pm 1.18$ to $3.05 \pm 1.10$ minutes) or interval ($1.78 \pm 0.54$ to $1.49 \pm 0.40$ minutes). It is apparent that sRLX effects the interval in control and estrogen treated animals but does not have any effect on progesterone or estrogen and progesterone treated animals. (Refer to Figure 1 and 2 and Table 1).

These results suggest that homologous sRLX can slow the rate of spontaneous uterine contraction in 3rd trimester sharks (Stage C) in which endogenous progesterone is reduced and the estrogen level is rising (Tsang and Callard, J. Exp. Zool. 241:377-382, 1987). However, injection of progesterone or estrogen and progesterone but not estrogen alone, markedly slowed spontaneous uterine activity. Under these circumstances, no effect of relaxin could be observed. From these and other results, we suggest that progesterone plays an important physiological role in regulation of spontaneous uterine activity during gestation in this elasmobranch; further, relaxin, a peptide found in shark ovarian tissue, may also have important myometrial slowing activity in the absence of progesterone and when plasma estrogen levels are high. In future work, we propose to investigate the cellular mechanism(s) of action of steroids and peptides on isolated elasmobranch myometrial cells.

It is evident from this study that the steroidal and ovarian peptide regulation of the elasmobranch myometrium is very similar to the regulation observed in mammals, suggesting that regulation of the reproductive tract has been conserved for 400 million years since the origin of elasmobranchs.
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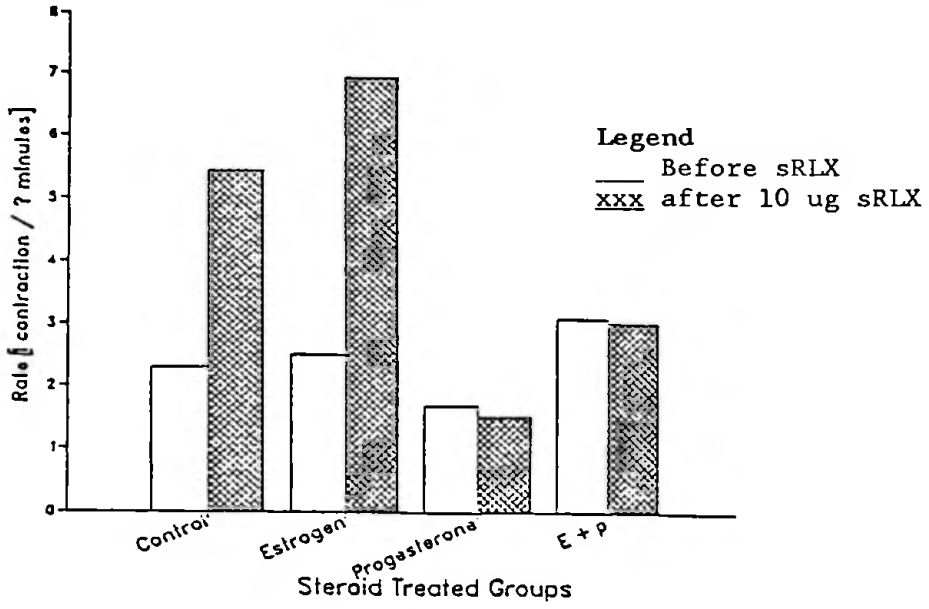


Figure 1: The graph indicates that in control and estrogen treated dogfish, there was a marked decrease in the rate (1 contraction / ? minute) of spontaneous myometrial contractions after the injection of 10 ug sRLX i.v. No significant effect was observed in animals receiving progesterone.

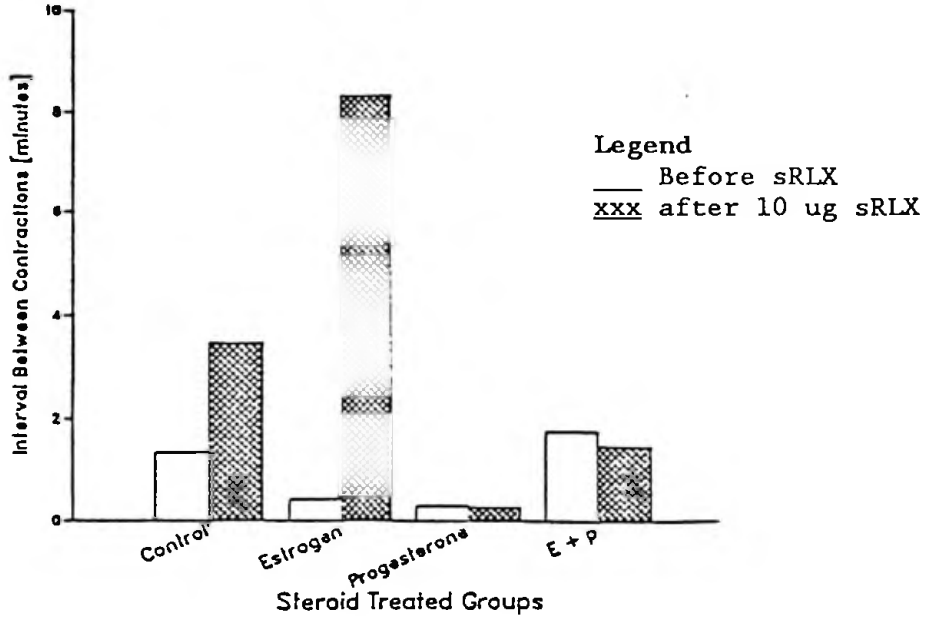


Figure 2: The graph indicates that in control and estrogen treated dogfish, there was an increase in the interval between contractions (minutes) after administration of 10 ug sRLX. No significant effect was observed in animals receiving progesterone.

Table 1: The effect of 10 ug sRLX on rate ( 1 contraction / ? minutes) and interval between contraction (minutes) of *in vivo* recordings of spontaneous myometrial activity in control, estrogen, progesterone, and estrogen and progesterone treated pregnant (Stage C) dogfish. (Mean ± Standard Error)

| GROUP | RATE | | INTERVAL | |
|---|---|---|---|---|
| | Control | 10 ug sRLX | Control | 10 ug sRLX |
| CONTROL | 2.29 ± 0.77 | 5.46 ± 2.21 | 1.34 ± 0.91 | 3.47 ± 1.10 |
| ESTROGEN | 2.50 ± 0.85 | 6.94 ± 2.55 | 0.41 ± 0.26 | 8.36 ± 5.41 |
| PROGESTERONE | 1.69 | 1.52 | 0.293 | 0.267 |
| ESTROGEN & PROGESTERONE | 3.10 ± 1.18 | 3.05 ± 1.10 | 1.78 ± 0.54 | 1.49 ± 0.40 |