The effect of homologous relaxin and neurointermediate lobe extracts on *in vivo* and *in vitro* myometrial activity in the viviparous dogfish, *Squalus acanthias*.

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Regulation of the mammalian reproductive tract by ovarian steroids and the peptides relaxin and oxytocin is quite well understood, but the antiquity of the endocrine regulatory system for egg, embryo and fetal maintenance *in utero* is unclear. Further, the relative importance of the different endocrine components in oviparous and viviparous non-mammalian species is not understood. The structure of *Squalus acanthias* ovarian relaxin has recently been determined (Bullesbach *et al.*, Eur. J. Biochem., 1986 in press), and the degree of its structural homology with mammalian relaxins leads to questions of functional homology. We have previously demonstrated that porcine relaxin (pRLX) significantly increased cervical cross-sectional area in pregnant *S. acanthias* (Koob, Tsang and Callard, Biol. Reprod. 31, 231-238, 1984) and suggested a role for homologous *Squalus* relaxin (sRLX) in normal parturition in this species. In this study we report on the effect of sRLX on myometrial activity *in vivo* and *in vitro* in pregnant *Squalus*. In addition, we demonstrate an effect of homologous neurointermediate lobe (NIL) extracts, known to contain elasmobranch homologs of oxytocin and vasotocin, on the myometrium.

Stage C (mid- to late-pregnancy) *Squalus acanthias* were gill-netted in Frenchman's Bay, ME and kept in circulating seawater tanks until used at Mount Desert Island Biological Laboratory. For *in vitro* studies, sharks were pithed and the uterus removed to aerated elasmobranch Ringer (Forster, Goldstein and Rosen, Comp. Biochem. Physiol. 42A, 312, 1972) at 16° C. Tissue strips (approximately 5 x 15mm) were suspended in a saline bath at 16 + 1° C and attached to either a force or pressure transducer. For *in vivo* studies, pithed animals were maintained in a saline trough with continuous flow of water over the gills. An intrauterine balloon catheter was introduced into the reproductive tract. Spontaneous rhythmic activity was recorded using a Grass Model 79 Physiograph. Recordings were made until the tissue exhibited a spontaneous repeatable rhythm, and sRLX or NIL extract in elasmobranch buffer were added directly to the bath or through an intravenous catheter and recording continued. Rate, amplitude and duration of contractions were monitored.

sRLX was added to the bath in increasing amounts (10,000 and 1000 ng/ml) and recordings made (Figure 1). Before addition of sRLX, the interval between contractions was 2.98 ± 0.13 minutes. After sRLX this value was sequentially increased to 5.76 ± 0.88 (10 ng/ml sRLX), 15.15 ± 1.71 (100 ng/ml sRLX, $p < .01$) and 18.8 ± 2.53 (1000 ng/ml sRLX, $p < .005$). No significant changes in amplitude or duration of contractions were observed. A similar decrease in spontaneous rhythmic oviduct activity was observed *in vivo* after intravenous administration of both sRLX and porcine (p) RLX (Figure 2). In contrast to the effect of RLX, NIL extracts (0.5 NIL equivalents) administered i.v. provoked a marked contractile response of the oviduct (Figure 3).

These studies demonstrated that the purified naturally occurring homologous ovarian peptide relaxin and native extracts of the pituitary neuro-intermediate lobe have effects on the reproductive tract which are of potential physiologic

importance to the progress of gestation and parturition in this species. These studies complement earlier work in which we showed (Koob, Tsang and Callard, 1984) that pRLX influences the connective tissue properties of the 'cervical' region of Squalus reproductive tract. The data indicate that the actions of RLX and NIL peptides on the vertebrate reproductive tract have been conserved since the earliest era of gnathostome evolution. (3. Supported by NSF Grants PCM 81-041446 and DCB 85-18296 to IPC.)

FIGURE 1

SQUALUS oviduct:
In vitro effects of RLX

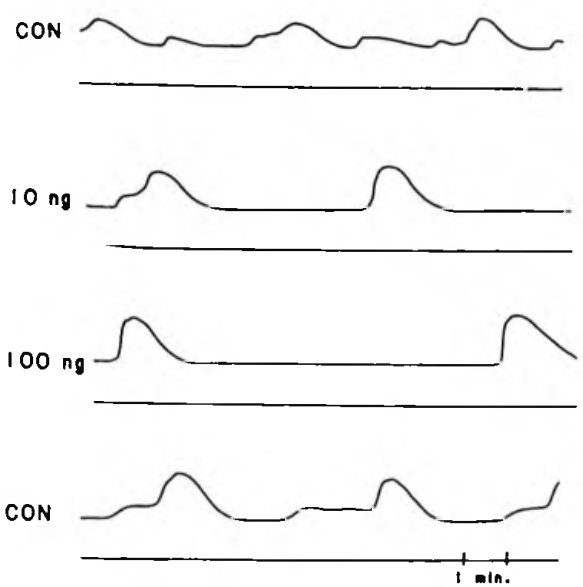

FIGURE 2

SQUALUS oviduct:

in vivo effects of RLX

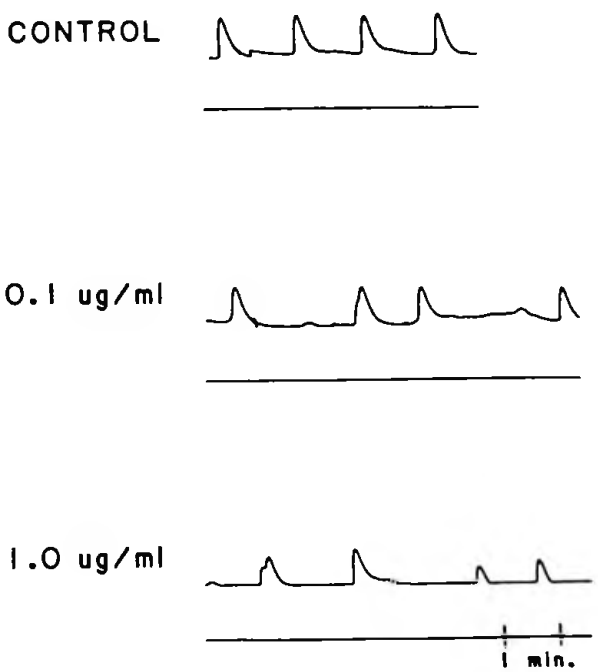

FIGURE 3

SQUALUS oviduct: in vivo effects of NIL