NIDAMENTAL GLAND CATECHOL OXIDASE ACTIVITY IN THE LITTLE SKATE, *RAJA ERINACEA*

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Previous work has suggested that the elasmobranch nidamental gland is under endocrine regulation by the ovary (Koob et al., *Biol. Repro.* 35:267-275, 1986). Thus, glands are small in immature animals and increase in size in a manner which is directly correlated with plasma estradiol levels and ovarian follicular size. Since the female skate reproductive tract expresses both estrogen and progesterone receptors (Reese and Callard. *Gen. Comp. Endo.* 84:170-181, 1991; Paolucci and Callard. *Gen. Comp. Endo.* In press, 1997), it is likely that this complex synthetic/secretory process is coordinated by the ovarian steroids (see Koob et al, *Biol. Repro.* 35:267-275, 1986). The protein products of the gland are combined to produce the tertiary membranes of the egg, which become tanned by a quinone oxidation process after the oocyte has become encapsulated (Koob and Cox. *Environ. Biol. Fishes.* 38:151-157, 1993). Both histological (Rusaouën et al., *Comp. Biochem. Physiol.* 53B:539-543, 1976; Rusaouën. *Arch. Anat. Microsc.* 67:107-119, 1978) and biochemical studies (Koob and Cox, *Biol. Bull.* 175:202-211, 1988) have demonstrated a diversity of secretory products, one of which is catechol oxidase. This enzyme appears to play an integral role in the capsular tanning process. It is believed that catechol oxidase is stored within nidamental gland cytoplasmic granules as a zymogen and is secreted upon receipt of an unknown stimulus. The enzyme becomes active after a latent phase and is then incorporated into the capsular matrix where it retains oxidative potential *in situ* for an extended period. The initiator(s) of synthesis and secretion have not been identified.

Female skates were captured by trawling in Frenchman Bay, Mount Desert Island, Maine from May to July and were maintained in 2400 L circulating seawater tanks at ambient temperatures. They were fed a diet of squid. Under these conditions animals undergo successive ovulatory cycles each followed by twin ovipositions for several months. Females were determined to be reproductively active by the presence of large follicles easily visualized through the ventral body wall. The reproductive cycle was tracked by palpating daily for the presence of capsules.

Nidamental gland samples were taken from five groups: non-reproductively active; first day capsules detected *in utero*; 2 days post-capsule detection; 2 days post-laying and 4 days post-laying (n=3). Tissue was minced over ice and homogenized in a ground glass homogenizer in the presence of 20 ml/g extraction buffer (0.5M NaCl, 0.05M NaH_2PO_4, pH 7.0). Three sections per animal were processed to control for regional variations in catechol oxidase expression. The milky-white homogenate was centrifuged at 37,000 g for 30 minutes at 4°C. The resulting pellet was rehomogenized in extraction buffer and centrifuged again. The supernatant was frozen at -70°C.

Catechol oxidase activity was determined spectrophotometrically by monitoring the oxidation of 4-methylcatechol to its quinone derivative at 400 nm. The 1 ml reaction mixture contained 0.25 ml extract, 0.4 ml extraction buffer, 0.25 mL dH_2O, and 0.1 ml 10mM 4-methylcatechol, 10mM HCl

(Koob and Cox, *Biol. Bull.* 175:202-211, 1988). The resulting activity of the extracts was standardized for protein content by the Bradford method.

As demonstrated in Figure 1., catechol oxidase activity did not vary significantly from the first day of capsular detection to seven days later (four days after laying). However, enzyme activity levels remained 15-fold higher than that found in non-reproducing controls. Given that plasma estradiol, progesterone and testosterone levels fluctuate during the reproductive cycle (Koob et al. *Biol. Reprod.* 35:267-275, 1986), it appears that once induced, possibly by rising plasma steroid hormone levels in the first functional ovarian cycle, synthesis and secretion of the enzyme is constitutively maintained.

In conclusion, although catechol oxidase is not a good marker for endocrine regulation of the nidamental gland, it will provide a valuable control marker against which fluctuating levels of structural components of the gland may be standardized in future studies of hormonal regulation. The initial hypothesis, that catechol oxidase activity levels decrease after capsular formation and increase again as the female prepares to form another pair of capsules, appears to be refuted by the data reported.

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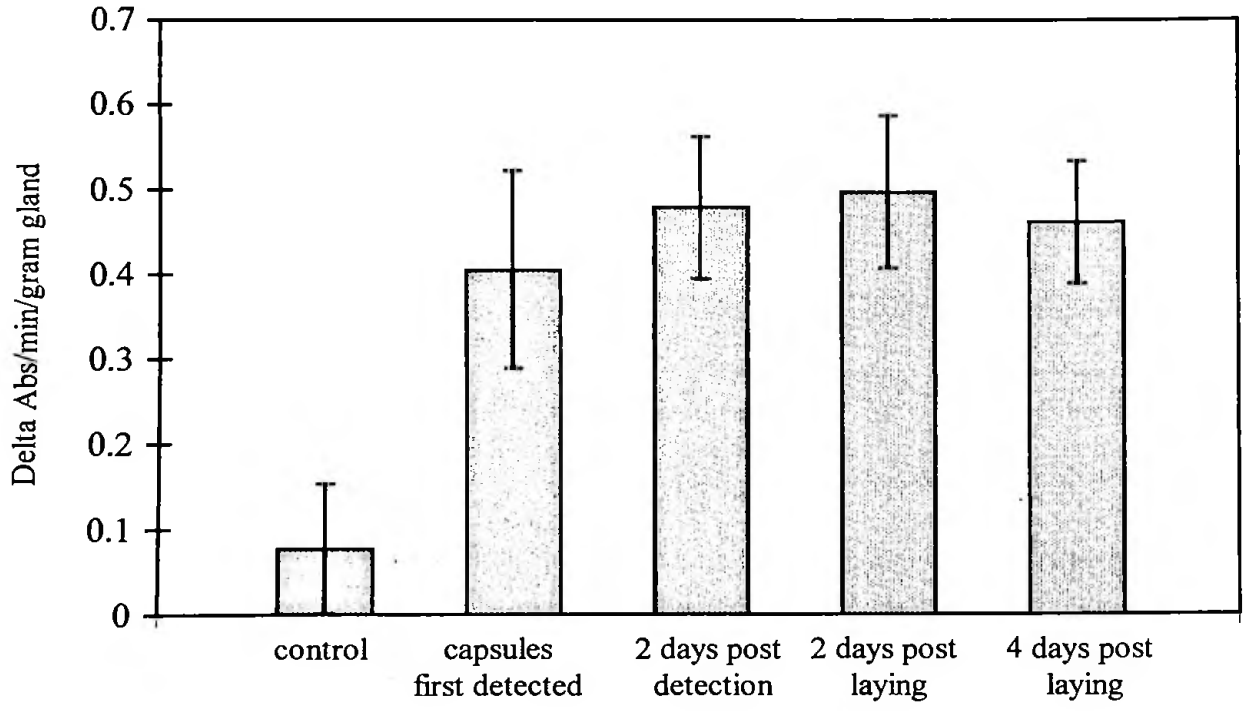

Figure 1. Activity of catechol oxidase from *Raja erinacea* nidamental glands. Once capsule formation begins, catechol oxidase activity remains relatively constant when standardized to gram of gland tissue (n=3).