CORPUS LUTEUM FUNCTION AND REGULATION IN THE SKATE, *RAJA ERINACEA*.

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In elasmobranchs the homology of the post-ovulatory follicle with the corpus luteum of other vertebrate classes is on firm ground, based on structural and functional correlates (Dodd and Sumpter, in Marshall's Physiology of Reproduction, Vol. 1, pp. 1-126, 1984). Most recently, progesterone has been definitively identified as a product of the corpus luteum of *Squalus acanthias* (Tsang and Callard, J. Exp. Zool. *241*:377-382, 1987). However, little functional information is available for the ovarian compartments of oviparous species such as the little skate, *Raja erinacea* although Koob, Tsang and Callard (Biol. Reprod. *35*:267-275, 1986) have correlated plasma gonadal steroid levels with the ovulatory cycle. Of particular interest is the ovarian source of the peak of plasma progesterone co-incident with the immediate preovulatory period. Since corpora lutea from previous ovulations persist through several cycles (Hisaw and Hisaw, Anat. Rec. *135*:269-278, 1959), these structures and/or the preovulatory follicles could contribute to this peak. In this study we have focused on progesterone synthesis and control by corpora lutea of different ages in order to determine their potential contribution to the preovulatory peak. Female skates were routinely palpated for ovulation (Koob, Tsang and Callard, Biol. Reprod. *35*:267-275, 1986) and maintained in flow-through saltwater tanks at $\simeq 12^{o}C$ for the duration of the experiment. Animals were weighed weekly and force-fed ground shrimp to maintain general health. Hypophysectomy (hypox) and ventral lobectomy (VLX) were performed by a ventral buccal approach much as described by Chevins and Dodd for the genus *Raja* (Gen. Comp. Endocrinol. *15*:232-241, 1970). Recovery and subsequent longevity were excellent. Skates were sacrificed by spinal transection, the ovaries transferred to cold elasmobranch buffer and corpora lutea (C.L.) dissected out. Three major categories were identified based on stage of the ovulatory cycle, weight and diameter: (I) Newly formed, $\simeq$ 9.0 mm diameter, 90 mgs weight, found only in animals with oviducal eggs; (II) present in both pre- and postovulatory animals;
$\simeq$ 8.0 mm and 80 mgs, possibly preceding cycle in origin; and (III) present in both pre- and postovulatory animals, probably at least 2 cycles old, $\simeq$ 4.0 mm and 30 mgs. C.L. were scissor-minced, and cells dispersed in 0.1% collagenase. Cells were recovered, re-suspended in buffer and dispensed in aliquots of 250,000 cells as described by Klosterman and Callard (The Bulletin, MDIBL *26*:119-121, 1987). Aliquots of cells were incubated for 16-18 hours at ambient temperature ($\simeq 20^{o}C$) in the presence or absence of pituitary extract and substrate (25 hydroxycholesterol, 25 OH CH. Progesterone (P) was determined by radioimmunoassay (RIA, Tsang and Callard, Gen. Comp. Endocrinol. *66*:182-189, 1987).

Basal progesterone synthesis in the absence of substrate increased from Stage I (early) to II (mature) C.L. and decreased as C.L. aged (Stage III; Fig. 1a). Addition of substrate markedly increased P synthesis in a dose-dependent manner (Fig. 1b). Both early and mature C.L. had a lower basal P production after hypox (Fig. 2c, mature C.L.); nevertheless, after addition of substrate, P production was markedly increased although to a lesser extent than in C.L. from sham-operated animals (Fig. 2c). Addition of pituitary extract alone stimulated P synthesis by cells from hypox animals to a level similar to that in the presence of substrate alone. There was no synergistic effect of substrate plus pituitary extract (Fig. 2c). After VLX, basal synthesis of P by C.L. appeared unimpaired (Fig. 2b) and substrate addition was more effective in promoting P synthesis than in C.L. from hypox animals. Further, substrate plus pituitary extract were markedly synergistic in this group, P accumulation in the medium being threefold the highest values obtained in cells from sham-operated animals (Figs. 2 and 2b).

These results demonstrate progesterone production by isolated luteal cells of an oviparous elasmobranch for the first time and demonstrate substrate and pituitary dependency as we have shown previously for *Squalus* C.L. (Klosterman and Callard, The Bulletin, MDIBL *26*:119-121, 1987). Further, endogenous P production by C.L. cells suggests an ordered chronological sequence of C.L. differentiation and involution. Both basal and substrate-dependent P production is decreased after hypox but not VLX. Indeed, cells from VLX animals in the presence of substrate and pituitary extract produced more P than control cells similarly treated.

These data raise interesting questions about C.L. function and regulation in this species and possibly other elasmobranchs. It appears that factors from the neurointermediate lobe, as well as the gonadotropic ventral lobe of the pituitary may have an important impact on C.L. function. In order to evaluate more completely the potential interactions of pituitary regulatory factors it will be necessary to determine the full steroid biosynthetic capacity of the C.L. However, from these data it can be deduced that past cycle C.L. may contribute significantly to the preovulatory progesterone peak.

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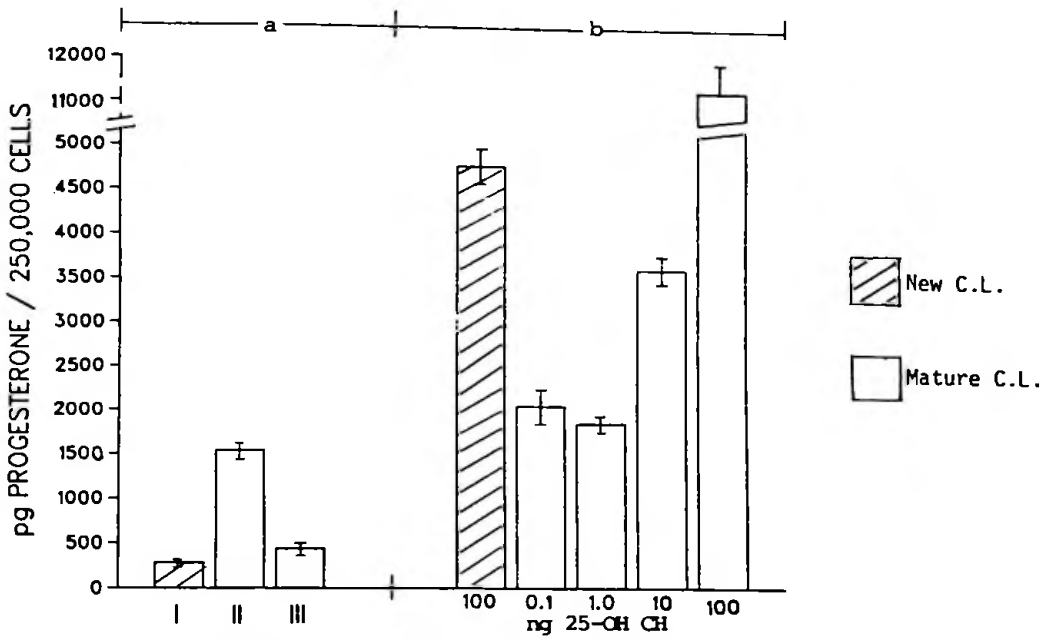

Figure 1:Basal and substrate stimulated P synthesis by C.L. from Raja erinacea.

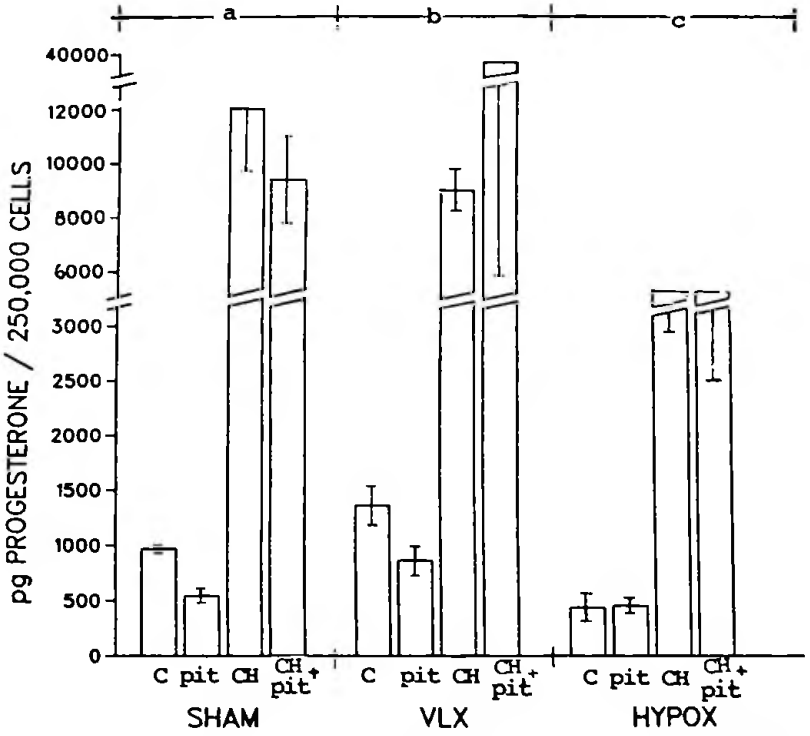

Figure 2:The effects of VLX and hypox on luteal P synthesis in the presence of substrate ± pituitary extract. C, no substrate; pit, 1/150th pituitary extract; CH, 25-OH cholesterol, 100ng/ml.