Ovarian sympathectomy in the guinea pig

I. Effects on follicular development during the estrous cycle

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Summary. The influence of ovarian adrenergic nerves on follicular growth during the estrous cycle in the adult guinea pig was ascertained by comparing follicular development in control and chemically sympathectomized ovaries from the same animal. Selective ovarian sympathectomy was achieved by injecting 6-hydroxydopamine into a surgically closed periovarian membranous sac (bursa) on day 2 of the cycle (day 1 = day of estrus). The contralateral surgically closed ovarian bursa was injected with solvent used for 6-hydroxydopamine. Animals were laparotomized on days 5, 10 and 14 of the cycle. Blood from the utero-ovarian vein was collected bilaterally for measurement of progesterone and androstenedione. The ovaries were processed for histologic examination, and the number of follicles in each ovary was analyzed morphometrically. Sympathectomy on day 2 caused a decrease in healthy preovulatory follicles (>700 μm diameter) on day 10 of the cycle. There were no differences in ovarian weights or the total number of follicles per ovary at this time. On days 5 and 14 of the cycle, there were no differences in ovarian weights, total number of follicles per ovary or follicles in any size classification. Sympathectomy did not alter progesterone levels in the utero-ovarian vein as compared to contralateral control levels. From control ovaries, there was a significant increase in progesterone in the blood of the utero-ovarian vein on day 10 but venous levels of progesterone from sympathectomized ovaries were not significantly different at any day of the cycle. In the venous effluent from sympathectomized ovaries, androstenedione was elevated at day 5 compared to days 10 and 14. These observations suggest that ovarian adrenergic nerves may modulate growth of large antral follicles and ovarian steroidogenesis during the estrous cycle.

Key words: Ovary – Adrenergic nerves – Follicle growth – Guinea pig

The morphology of ovarian nerves has been extensively studied for over a century (see reviews by Neilson et al. 1970; Burden 1978). In contrast, the functional significance of these nerves has received limited attention. It has been suggested that local ovarian innervation may participate with the well established hypothalamo-adenohypophysial axis in several aspects of ovarian function. Because they lie adjacent to developing follicles, one aspect of ovarian function in which nerves may play a role is follicular development. Previous workers have indicated that these nerves may exert a trophic influence on follicle growth (Fink and Schofield 1971; Burden 1972). Removal of these nerves decreased follicular development in mice (Grob 1974) and increased follicular atresia in hamsters (Curry et al. 1982). Furthermore, exogenous norepinephrine (NE) has been reported to stimulate follicle growth and decrease atresia in hypophysectomized mice (Grob and Brink 1973).

The present study examined the role of ovarian adrenergic nerves in follicular development during the estrous cycle in the guinea pig, a species with a dense adrenergic innervation (Burden 1972; Stefenson et al. 1981). The influence of adrenergic nerves on follicle growth was ascertained by comparing follicular development in control and chemically sympathectomized ovaries from the same animal. To accomplish a local selective ovarian chemical sympathectomy, a surgical procedure for constructing a closed periovarian membranous bursal sac was developed. The sympatholytic agent 6-hydroxydopamine (6-OHDA) was injected into the bursal sac.

Materials and methods

Animals. Adult female Dunkin Hartley guinea pigs obtained from Dutchland Laboratories (Denver, Pennsylvania) were maintained on a 12:12 light-dark cycle with lights on at 0700 h. The animals were provided laboratory chow and water ad libitum. The mean length of the estrous cycle is 16 days (Phoenix 1970). The stage of the cycle was monitored by examining the vaginal membrane daily between 0800 and 1000 h and when patent a vaginal smear was taken. The day of maximal vaginal cornification was designated as the day of estrus (day of ovulation, day 1 of the cycle). The preovulatory LH surge occurs on day 16 of the cycle (Croix and Franchimont 1975).

General surgical techniques. Under anesthesia with Meto- fane (Pittman-Moore, Inc., Washington Crossing, New Jersey) and Innovar-Vet (1 cc/10 kg i.m., Pittman-Moore, Inc.) the ovaries were exposed by a dorsolateral flank incision. In the guinea pig, both the mesotubarium superius (superior
mesosalpinx) and the infundibular extremity of the oviductal mesenteries remain free from attachment forming an open periovarial sac (Beck 1972). To provide a closed bursal cavity for local application of drugs, the free margin of the mesotubarium superius and infundibular oviductal mesentry was folded dorsolaterally to enclose the ovary and sutured to the mesometrial fat pad with 6-0 silk (Fig. 1). Injections into the bursal cavity were accomplished with the aid of a surgical microscope with a 30-gauge needle fitted to a 50-μl Hamilton syringe.

**Effect of surgical closure of the bursal cavity on the estrous cycle.** On day 3 of the estrous cycle, the ovaries of six adult guinea pigs were exposed and each ovarian bursa was sutured to form a closed bursal sac. The effect of this surgical procedure on the estrous cycle was examined daily by examination of the vaginal membrane.

**Injection of dye or 6-hydroxydopamine into the bursal cavity.** The ovaries in six adult guinea pigs were exposed and the bursae sutured to verify the effectiveness of a closed bursal cavity for local drug application. One animal received 50 μl of 1% toluidine blue solution in each bursal cavity. The bursal cavities and injection sites were observed for leakage for 15 min with a surgical microscope.

The remaining five guinea pigs were used to verify the effect of injection of 6-OHDA (Regis Chemical Co., Morton Grove, Illinois) into a surgically closed bursal sac on ovarian adrenergic nerves. Fifty microliters of 6-OHDA solution (15 mg of 6-OHDA/50 μl) were injected into one bursal cavity (experimental) and the contralateral cavity (control) was injected with 50 μl of solvent (0.2% ascorbic acid in 0.9% NaCl). Animals were killed 1, 6, 8, 10 and 12 days after injection. The ovaries were removed and processed by a modification of the Falck-Hillarp method (Falck and Owman 1965) for demonstration of adrenergic nerves. The tissue was treated with formaldehyde vapor for 1 h at 80°C and vacuum infiltrated with paraffin. Sections were cut at 10 μm and mounted in Entellan (EM Laboratories, Elmsford, New York). Fluorescence was visualized with a Zeiss Photomicroscope III equipped with a vertical fluorescence illuminator.

**Effect of ovarian sympathectomy on follicular development during the estrous cycle.** Both ovarian bursae in 24 adult guinea pigs (490–790 g) were closed on day 2 of the estrous cycle (day 1 = day of estrus). The bursal cavity on one side (experimental) was injected with 50 μl of 6-OHDA solution (15 mg of 6-OHDA/50 μl). The contralateral bursal cavity (control) received 50 μl of solvent (0.2% ascorbic acid in 0.9% NaCl). Selection of ovaries for experimental and control treatments was alternated between left and right sides in different animals to avoid any innate bias. Animals were laparotomized on days 5 (n=8), 10 (n=8) and 14 (n=8) of the estrous cycle. Blood from each utero-ovarian vein (UOV) was collected with a 30-g needle fitted to a 1 cc syringe for radioimmunoassay of progesterone and androstenedione. Ovaries were removed, cleaned of adnexa, weighed and fixed in Bouin’s solution.

**Histology and morphometrics.** Ovaries were embedded in paraffin, serially sectioned at 8 μm and processed for hematoxylin and eosin (H & E) staining. Corpora lutea (CL) and follicles were counted on every fifth section. Follicles were classified as preantral (characterized by two or more layers of granulosa cells without cavitation) or antral and as healthy or atretic (defined as follicles with at least five granulosa cells with pyknotic nuclei). Two right-angle mea-