

## Plasma Estradiol and Progesterone after Hypophysectomy and Substitution with Pituitary in Female Sea Lampreys (*Petromyzon marinus*)

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This study investigated plasma steroid concentrations 1 week after total hypophysectomy in adult female sea lampreys. Plasma estradiol significantly decreased (to  $1.19 \pm 0.17$  SE ng/ml) following hypophysectomy and was elevated (to  $1.91 \pm 0.11$  ng/ml) to a value similar to controls by a single injection of two pituitary equivalents in adult female sea lampreys in the stage of final maturation. Plasma progesterone also significantly decreased (to  $69 \pm 10$  pg/ml) following hypophysectomy and was elevated (to  $183 \pm 71$  pg/ml) to a value not different from uninjected controls by a single injection of two pituitary equivalents in females. Injection of two pituitary equivalents did not affect plasma estradiol in intact lampreys but surprisingly decreased plasma progesterone (to  $67 \pm 14$  pg/ml) compared to saline-injected intact lampreys ( $318 \pm 142$  pg/ml). The decrease in estradiol and progesterone after hypophysectomy and their normalization after injection of pituitaries support the concept that the secretion of estradiol and progesterone is stimulated by gonadotropin or other pituitary hormones. © 1991 Academic Press, Inc.

In river lampreys (*Lampetra fluviatilis*) hypophysectomy and substitution therapy with pituitary extracts or mammalian gonadotropins have been extensively studied. The pro- and mesoadenohypophyses have gonadotropic activity, but there is little or no information about the chemical identity or cell type responsible for synthesis and release of gonadotropin(s) (for review see Larsen and Rothwell, 1972). Gonadectomy and substitution therapy with estradiol and testosterone have also been performed in *L. fluviatilis* (Larsen, 1974, 1987). According to these studies one would expect estradiol to be the female sex hormone and testosterone the male sex hormone. However, other studies on *L. fluviatilis* (Kime and Rafter, 1981; Kime and Larsen, 1987) have indicated that the biologically active sex steroids may not be estradiol or testosterone

one but rather unidentified derivatives of estradiol or testosterone.

In sea lampreys, *Petromyzon marinus*, there are no published reports on the effects of hypophysectomy or gonadectomy but immunocytochemistry has shown an immunoreactive mammalian-like luteinizing hormone in the mesoadenohypophysis (Wright, 1983). Additionally, injections of salmon gonadotropin preparation into adult sea lamprey advanced ovulation by several weeks (Sower *et al.*, 1983) and elevated estradiol levels (Sower *et al.*, 1983, 1985a; Sower, 1989). Estradiol in sea lampreys (Katz *et al.*, 1982; Sower *et al.*, 1985b, 1987) and Japanese river lamprey, *L. japonica* (Fukayama and Takahashi, 1985), and progesterone in sea lampreys (Linville *et al.*, 1987; Sower *et al.*, 1987) are two steroids that have been demonstrated to be associated with reproductive activity.

The purpose of this study was to develop a method for hypophysectomy in sea lam-

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preys based on the technique developed in *L. fluviatilis*. Plasma estradiol and progesterone were measured in female sea lampreys following hypophysectomy and substitution therapy with pituitary preparations. Estradiol and progesterone were measured because of the results obtained by Sower and her co-workers on time course of plasma concentrations of estradiol and progesterone during the final stage of maturation and their response to salmon gonadotropin, GnRH, or GnRH analogs (for review see Sower, 1990).

## METHODS AND MATERIALS

Landlocked adult female sea lampreys were captured by trap in the Cheboygan River, Michigan, during their spawning migration following completion of their parasitic lake phase. The animals were retained in raceways at Hammond Bay Biological Station supplied with flowing lake water ranging in ambient temperature between 16 and 18° under natural photoperiod. The lampreys averaged 200 g in body weight. The females used were approximately 2 to 3 weeks prior to ovulation.

Six groups of 8 to 10 lampreys each were separated into groups in the raceways which were divided into sections by screens. From July 7 to 10, two groups were completely hypophysectomized (pro-, meso-, and metaadenohypophysis), two groups were sham-operated and two groups were not operated (controls). One hypophysectomized lamprey died. Hypophysectomy procedures followed those described by Larsen (1965, 1969). Briefly, the lampreys were anesthetized with 1 g/liter ethyl *m*-aminobenzoate methanesulfonate (MS 222) for 15 min prior to surgery in ice-cold water supplied with aeration. During the operation, the lampreys were wrapped in a wet cloth surrounded by pieces of ice. The operations were performed under a Dansanto surgical microscope. A longitudinal cut of about 3 cm was made through the skin on the ventral side, a few millimeters behind the mouth. Cuts were made until the nasohypophysial duct was visible. Removal of the pituitary involved first cutting the connective tissue surrounding the pituitary with a specialized cutting tool and then gently removing the pituitary by mouth suction through a fine glass pipe. The area around the brain was visually inspected with the microscope following the completion of the experiment, and no remnants of hypophysial tissue were noted. Sham operations were performed in the same manner up to the point of cutting and removing the pituitary.

One week after the operations, lampreys were in-

jected intraperitoneally with saline (control) or pituitary preparation (equivalent to two pituitaries per lamprey) in saline. Forty pituitaries were collected and prepared the same day as the injections were given. The fresh pituitaries were immediately placed in 0.6% saline at 4° following removal and homogenized using a Dounce homogenizer, and 0.2 ml (equivalent to two pituitaries per injection) was injected. Blood samples were collected in heparinized syringes by cardiac puncture 19 hr following the injection. Plasma was drawn off and stored frozen at -20° until assayed for estradiol and progesterone. Plasma estradiol and progesterone were measured by radioimmunoassay as previously described (Sower and Schreck, 1982; Sower *et al.*, 1983, 1987). The lower limits of detection were 7 and 7.8 pg/tube for progesterone and estradiol, respectively.

Data were analyzed by a Student-Newman-Keuls test after preliminary analysis of variance. The level of significance for differing groups was  $P < 0.05$ .

## RESULTS AND DISCUSSION

These data demonstrate that plasma estradiol significantly decreased ( $1.19 \pm 0.17$  SE ng/ml;  $P < 0.05$ ) following hypophysectomy and returned to levels ( $1.91 \pm 0.11$  ng/ml), similar to those of controls after a single injection of two pituitary equivalents in adult female sea lampreys undergoing final maturation (Fig. 1). Plasma progesterone also significantly decreased from  $69 \pm 10$  pg/ml ( $P < 0.05$ ) following hypophysectomy and significantly increased ( $P < 0.05$ ) to  $183 \pm 71$  after a single injection of two pituitary equivalents in the females (Fig. 2). Injection of pituitaries did not affect plasma

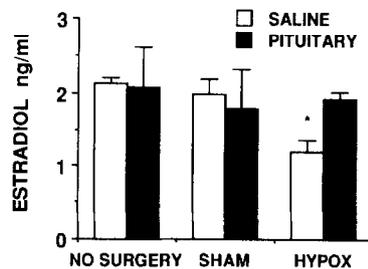


FIG. 1. Plasma estradiol in female sea lampreys injected with saline (control) or two pituitary equivalents that were not operated, sham-operated (SHAM), or hypophysectomized (HYPOX). Bars represent means  $\pm$  SE. \*Significant difference ( $P < 0.05$ ) between HYPOX and SHAM or No Surgery.

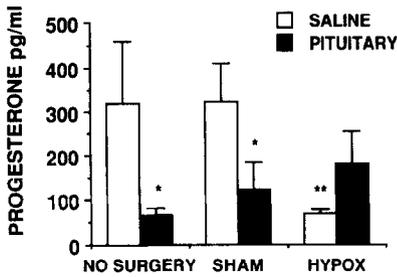


FIG. 2. Plasma progesterone in female sea lampreys injected with saline (control) or two pituitary equivalents that were not operated, sham-operated (SHAM), or hypophysectomized (HYPOX). Bars represent means  $\pm$  SE. \*Significant difference ( $P < 0.05$ ) between saline- and pituitary-injected lampreys, \*\*Significant difference ( $P < 0.05$ ) between HYPOX and SHAM or No Surgery.

estradiol in intact lampreys but decreased plasma progesterone ( $67 \pm 14$  pg/ml) compared to saline-injected intact lampreys ( $318 \pm 142$ ); a similar decrease was noted in sham-operated lampreys.

The decrease in estradiol and progesterone after hypophysectomy and their normalization after injection of pituitaries support the concept that the secretion of estradiol and progesterone is stimulated by gonadotropin or other pituitary hormones. One possible explanation of estradiol secretion not being stimulated beyond the value of control lampreys may be that maximal pituitary stimulation already occurred in controls. The decrease in progesterone after injection of pituitaries was unexpected. However, since the pituitaries probably contain a whole series of hormones (see Larsen and Rothwell, 1972), an inhibitory factor could well be present. Or the pituitary extract may cause rapid conversion of progesterone to another steroid.

The only comparable investigation on hypophysectomy in lampreys was performed on *L. fluviatilis* (Kime and Larsen, 1987). In this experiment, the pro- and mesoadenohypophyses or the gonad was extirpated in males and females during winter months before sexual maturation (appearance of secondary sex characters). Blood samples

were taken 1 to 4 weeks after the surgery and plasma estradiol, testosterone, progesterone, and dehydroepiandrosterone were measured by radioimmunoassay. Plasma progesterone was not detected in any of the lampreys from the different groups, but the sensitivity of these assays was 500 pg/ml compared to 50 pg/ml in the present study. Estradiol showed no decrease; on the contrary, the values were somewhat increased in gonadectomized males and in hypophysectomized females. Possible reasons for these differences between the two studies may be due to species differences, different stage of reproduction, different time interval between surgery and blood sampling, and differences between the type of surgery. In the present study, total hypophysectomy was performed, whereas in the study by Kime and Larsen (1987), only the pro- and mesoadenohypophyses were removed.

As mentioned in the introduction, there is uncertainty about the identity of the biologically active sex steroids. There is no doubt that the lamprey ovary and testis are capable of producing and secreting estradiol and progesterone (see review Sower, 1990). It is also well documented that implants of estradiol and testosterone are able to elicit sex-specific secondary sex characters in river lampreys (Larsen, 1974). However, there is evidence that estradiol and testosterone may also be secreted from a nongonadal source which was suggested by Kime and Larsen (1987) from their gonadectomy experiment.

In summary, the present study provides support for the pituitary regulation of the ovary and confirms earlier work on a possible relationship between estradiol and final reproduction in female sea lampreys. However, these results do emphasize the need for further research with regard to effects of partial hypophysectomy versus total hypophysectomy, pituitary control of gonadal hormone secretion, gonadal steroid synthesis, identification of other sites (e.g.,

interrenal) of steroid secretion, and identification of the biologically active sex hormones.

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