Histochemical Studies on the Dependence of Secretory Function of the Major Vestibular Gland (Bartholin's Gland) on Ovarian Steroid Hormones in the Cat
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ABSTRACT. The dependency of the secretory function of the feline major vestibular gland (Bartholin's gland) on the ovarian steroid hormones was studied histochemically. After estrogen (estradiol-17β or diethylstilbestrol), progesterone or a combination of these was administered to cats which were previously ovariohysterectomized, the major vestibular glands were removed, embedded in paraffin, sectioned, and stained by alcian blue, periodic acid-Schiff or peroxidase-labeled lectin (peanut agglutinin and wheat germ agglutinin). The vividly positive reactions to the stainings applied were observed in the secretory epithelial cells of the major vestibular glands in the estrogen treated animals. The dependency of the secretory function of the major vestibular glands on the estrogen was demonstrated in this study.—KEY WORDS: Bartholin’s gland, cat, estrogen.


The major vestibular gland has been found in many species of mammals including the human, cow, horse, monkey, cat, bat and opossum [5, 8]. Our previous study showed that in the gland of the cat, glycoconjugates with vicinal diol, acidic and sulfate groupings were present in its secretory cells and in its luminal secretion particularly at the estrous stage [2]. Our lectin histochemistry also showed that glycoconjugates with D-galactose and N-acetyl-D-glucosamine were obviously present in acinar cells of the gland [2]. The relationship between the secretory function of the gland and the reproductive stage, i.e. the condition of ovarian function, has not yet been clearly shown. In the calf, however, the fact that the secretory activity of the major vestibular gland depends on synthetic estrogens has no far been established and led to a speculation that the estrogenic condition would enhance the secretory activity [3, 6]. Therefore, the present study was designed to evaluate the effect of ovarian steroid hormones on the functional and morphological pattern of this gland in the cat.

Twenty-four Japanese female domestic cats, which had been clinically checked as normal, were used. First of all, the estrous and the diestrous groups were prepared, each consisting of 3 cats. The estrus was induced by giving the animals pregnant mare serum gonadotrophin as described previously [2]. The remaining 18 cats were ovariohysterectomized under ketamine hydrochloride anesthesia. One month later, they were divided into 6 groups according to the kind of treatments, each group consisting of 3 cats. Hormonal substances given to the animals were estradiol-17β (E2; Teikoku Zoki), diethylstilbestrol (DES; Sigma) and progesterone (P; Teikoku Zoki). Each substance was embedded in a silastic tube (10 mm in length) and the tube was implanted subcutaneously into the neck of each cat. Empty tubes were implanted into control animals. One week after the implantation, all the animals were subjected to euthanasia with ketamine hydrochloride. During euthanasia, the vestibule of the vagina of each cat was perfused with Bouin's solution. The specimen was dissected out and cut into small pieces and immersed in the same fixative for 24 hrs. Each piece was then dehydrated through increasing concentrations of ethanol and embedded in paraffin. Sections were made at 6 μm in thickness, deparaffinized in xylene, hydrated through decreasing concentrations of ethanol and then subjected to histological or histochemical staining. Hema-toxylin and eosin staining was used for histological observation. The vicinal diol group of glycoconjugates was visualized by periodic acid-Schiff (PAS) method [9]. Sulphated glycoconjugates were demonstrated by alcain blue (AB) at pH 1.0 [4], and acidic glycoconjugates at pH 2.5 [7]. Using peroxidase (PO)-labeled lectins, peanut agglutinin (PNA)- and wheat germ agglutinin (WGA)-diaminobenzidine (DAB) procedure [1, 7] were performed to assess particular saccharide residues of glycoconjugates (PNA for D-galactose and WGA for N-acetyl-D-glucosamine). PO-labeled lectins were obtained from EY Laboratory (San Mateo, Calif., U.S.A.). In order to detect the activity of endogenous PO in tissues, some control sections were incubated with DAB alone. Specimens were observed under an interference contrast microscope.

The height of epithelial cells in the major vestibular gland changed drastically after treatments. It was extremely lowered after ovariohysterectomy as compared with that of the estrus or the diestru. However, it was intensely recovered by E2 or DES administration, but not recovered by P. Histochemical reactions in the epithelium of the major vestibular gland after various treatments are summarized in Table 1. Strong positive reactions to PAS, AB at pH 1.0 and AB at pH 2.5 were observed only in those animals which were at the estrous stage or were ovariohysterectomized and given E2 (Figs. 1, 2; pictures of PAS and AB at pH 1.0 are not shown), DES or E2 plus P. This indicates that the secretory epithelial cells are abundant in acidic and neutral glycoconjugates under the estrogenic condition. When the sections were treated with PO-WGA-DAB, the secretory glandular cells exhibited a strong positive reaction in those cats at the estrus and the diestru and in those cats ovariohysterectomized and given E2 (Fig. 3), DES or E2 plus P. PO-PNA-DAB also showed a result similar to that of PO-WGA-DAB (Fig. 5).
Fig. 1. Glandular cells of the major vestibular gland of an ovariohysterectomized cat. The cells exhibit a negative reaction to AB at pH 2.5. × 400.

Fig. 2. Glandular cells of the major vestibular gland of an ovariohysterectomized cat given estradiol-17β. The cells show a strong positive reaction to AB at pH 2.5. × 400.

Fig. 3. Glandular cells of the major vestibular gland of an ovariohysterectomized cat given estradiol-17β. The cells show a positive reaction to WGA. × 400.

Fig. 4. Glandular cells of the major vestibular gland of an ovariohysterectomized cat given progesterone. Only a few cells show a positive reaction to PNA. × 400.

Fig. 5. Glandular cells of the major vestibular gland of an ovariohysterectomized cat given estradiol-17β. The cells show a positive reaction to PNA. × 400.

Fig. 6. Glandular cells of the major vestibular gland of an ovariohysterectomized cat given progesterone and estradiol-17β. The cells show a mosaic-like positive reaction to PNA. × 400.
When the ovariohysterectomy cats were given both E2 and P, the glandular cells showed a mosaic-like positive reaction to PO-PNA-DAB (Fig. 6). On the other hand, no vivid positive reaction to PO-PNA-DAB was observed in the ovariohysterectomized cat given P alone (Fig. 4). Such drastic changes of lectin-binding affinity has not been reported in the calf [6]. The foregoing results in the present study imply that, in the major vestibular gland of the cat, terminal saccharide residues such as N-acetyl-D-glucosamine and D-galactose accumulate in the secretory epithelial cells, and that this accumulation is activated under the estrogenic condition. Thus, it is proven in the present study that the secretory function of the major vestibular gland of the cat depends on estrogen. Therefore, it can be speculated that there exist estrogen receptors in the major vestibular gland of the cat. This speculation is now under our investigation by means of immunohistochemical techniques.

REFERENCES