

Immunohistochemical Evidence for the Presence of Progesterone Receptor in Rat Submandibular Glands

Satoru Ozono¹, Minoru Onozuka*, Kazuyoshi Sato, and Yumi Ito

Department of Pathology, Kanagawa Dental College, Yokosuka 238 and *Department of Anatomy, Gifu University School of Medicine, Gifu 500, Japan

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ABSTRACT. Immunohistochemical analysis of progesterone receptor was carried out in rat submandibular glands. Immunoreactivity to progesterone receptors was found in cell nuclei of the intralobular duct system with in male and female rat submandibular glands. The female glands contained more immunoreactive cells than the male glands. In ovariectomized rats progesterone receptor-containing cells decreased in number while testectomized glands revealed an increase. When estradiol was administered to gonadectomized rats of both sexes, the immunoreactivity in cells of the intralobular duct system markedly increased. These results suggest the possibility that progesterone may modulate various metabolic functions in the rat submandibular glands.

The submandibular glands of rats contain the receptor protein which specially binds to testosterone (2). Recently, Paulo and Szoetys (6) found the other steroid hormone progesterone in the submandibular gland homogenates of both male and female rats. There is, however, as yet no direct evidence for the presence of progesterone receptor protein in the glands.

By immunohistochemical analysis using a monoclonal antibody against progesterone receptor, several workers have shown that this receptor is accumulated in cell nuclei in the progesterone target tissues, such as oviduct, endometrium and myometrium (3, 7, 8). This finding suggests the possibility that the progesterone receptor in the submandibular glands may be demonstrable by use of this antibody. In the present study, this possibility was immunohistochemically tested in male and female rats using a monoclonal antiprogestosterone receptor antibody. We also examined the effect of gonadectomy and estradiol administration on the immunoreactivity of this receptor protein in the submandibular glands.

Male and female Wistar rats were used. At 4 weeks of age, gonadectomy was carried out in both sexes under anesthesia with sodium pentobarbital (30 mg/Kg). Four weeks after surgery, 17 β -estradiol (1 mg/Kg body weight, Sigma; emulsified in sesame oil at a concentration of 1 mg/ml) was administered intraperitoneally once every three days for 4 weeks. Animals were sacrificed about 24 hrs after the last administration. The castrated control animals were given sesame oil of the same

volume and at the same interval. Animals were killed by exsanguination under ether anesthesia, and the submandibular glands were rapidly removed. The glands were fixed in 3.7% neutral-buffered formalin (in 0.1 M sodium phosphate buffer, pH 7.2) for 12 hrs, dehydrated, embedded in paraffin, and sectioned at 5 μ m.

The immunohistochemical determination of progesterone receptor in the submandibular glands was carried out according to the method of Hyde *et al.* (3). Sections were deparaffinized and treated with 0.01 M sodium metaperiodate in 0.05 M Tris-buffered saline (TBS, pH 7.6) for 15 min and 1% sodium borohydride (in TBS) for 10 min, to remove residual aldehydes. Sections were then permeated with 1% Triton X-100 for 5 min, followed by 10 min in 5% dimethylsulfoxide (both in TBS). After washing with TBS, the preparation was incubated for 20 min in 1% hydrogen peroxidase containing 20% normal goat serum and 1% bovine serum albumin (in TBS) to eliminate endogenous peroxidase activity and minimize nonspecific binding. They were then incubated overnight at 4°C with mPRIV (Transbio Sarl), a mouse monoclonal antibody against rat progesterone receptor, at a concentration of 15 μ g/ml in TBS containing 0.5% Triton X-100, 0.1% gelatin and 0.02% sodium azide. The samples were washed in TBS and then incubated for 30 min at room temperature with secondary antiserum (peroxidase-conjugated goat anti-mouse immunoglobulins, Antibodies) diluted 1 : 25 in TBS. Following washing with TBS, sections were incubated for 10 min in diaminobenzidine (0.05% in TBS) in the presence of hydrogen peroxide (0.01%) followed by a wash with distilled water. Then sections were coun-

¹ To whom all correspondence should be sent.

terstained lightly with hematoxylin to help identify immunoreactive cells. After dehydration, the specimen was mounted in permount for light microscopic examination. Control immunohistochemical analysis was carried out by the substitution of preimmune mouse serum for the primary antibody. Some rat uterine sections were also stained for progesterone receptor using the same immunoperoxidase procedure.

In order to evaluate the antiserum against progesterone receptor, we first examined the distribution of immunoreactivity to progesterone receptor in sections of

rat uterus. Immunoreactivity for progesterone receptor was, in agreement with the previous findings obtained from rabbit (8) and guinea pig (3), localized to the nuclei of both stromal and epithelial cells in the uterus (data not shown). Thus, immunohistochemical staining of the sections prepared from the normal female rats was carried out using this antiserum. In the submandibular glands of female rats, immunoreactivity was detected mainly in cells of the intralobular duct system; the striated ducts (long arrows in Fig. 1a) were the most reactive, followed by the granular convoluted ducts

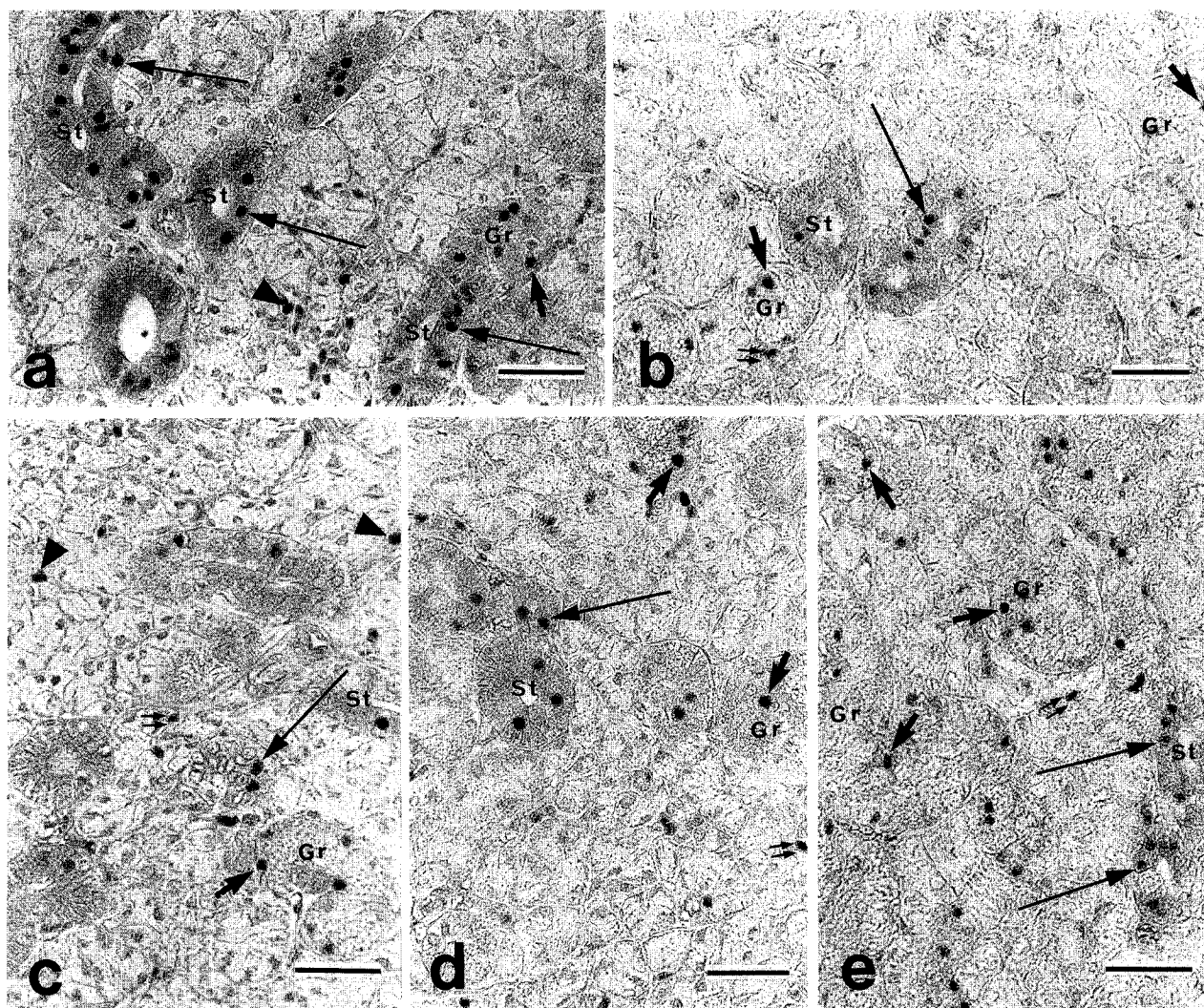


Fig. 1. Photomicrographs of rat submandibular glands immunostained with the monoclonal antibody against progesterone receptor. All sections were lightly counterstained with hematoxylin. St; Striated duct. Gr; granular convoluted duct. Long arrows, short arrows, arrowheads and double arrows indicate the progesterone receptor-positive striated duct cells, granular convoluted duct cells, intercalated duct cells, and acinar cells respectively. **a**, Normal female rat. The immunoreactivity is confined to the cell nuclei, and the most abundant immunoreactive cells appear to be localized in the striated ducts. **b**, Normal male rat. Several immunoreactive cells are recognized. **c**, Overiectomized female rat. The decrease in number of the immunoreactive cells are observed. **d**, Testectomized male rat. The increase in number of the immunoreactive cells is observed. **e**, Estradiol-treated, testectomized male rat. Note a marked increase in the number of immunoreactive cells of the ductal portions of the glands. Each scale bar, 50 μ m.

(short arrows in Fig. 1a) and the intercalated ducts (arrowhead in Fig. 1a). In the acini region, only a few scattered cells were progesterone receptor-positive. The peroxidase conjugate of progesterone receptor was confined within the cell nuclei throughout the submandibular glands, but not in the cytoplasm. In contrast, the reactivity of the submandibular glands of male rats was much less, but its distribution pattern was nearly identical with that of female rats (Fig. 1b). These results suggest that the striated ducts are identified as the most reactive site of progesterone in the glands.

It has been shown that the differentiation of the submandibular glands of the rat is completed at about 10–12 weeks of age (4). Thus, we also examined the effect of either ovariectomy or testectomy at 4 weeks of age on the immunoreactivity of progesterone receptor in the submandibular glands. As shown in Figs. 1a and 1b, the glands of females usually contained more immunoreactive cell nuclei than those of males. In the submandibular glands of testectomized rats the number of immunoreactive cells increased while the ovariectomized rat glands revealed a decrease in the number of reactive cells. However, there was no obvious and consistent sexual difference in the frequency of these reactive cell nuclei in the gonadectomized glands of both sexes (Figs. 1c and 1d).

Since it has been shown that estradiol elevates the concentration of progesterone receptors in the reproductive tract tissue (1, 5), we further examined the effect of estradiol administered to the testectomized and ovariectomized rats. The immunoreactive cell nuclei were significantly increased in number in the intralobular duct system in the gonadectomized rat glands of both sexes (Fig. 1e). This increase may be attributed to estradiol-induced alteration to the immunoreactive cell nuclei which are normally negative to progesterone receptors. In the submandibular glands, when sections were incubated either in the absence of primary antibody or with preimmune serum after endogenous peroxidase activity was blocked, non-specific immunoreactivity was not detectable in either the nucleus or cytoplasm (data not shown).

This study presents the first evidence in rats of the presence of progesterone receptor in the submandibular glands, suggesting the significance of progesterone action on a variety of metabolic events in this gland. In addition, the concentration of the progesterone receptor in the submandibular gland, as in the reproductive tract tissue (9), may be controlled by the quantity of gonadal

steroid hormones in the blood circulation. In the present experiments, the immunoreactive products were observed only within the cell nuclei. This finding agrees with the previous observation obtained in the oviduct, endometrium and myometrium of rabbit and guinea pig (3, 8). However, we can not rule out the possibility either that the cytoplasmic concentration of progesterone receptor was beyond the limit of immunohistochemical detection at a light microscopic level, or that the receptor antigenicity was lost during experimental procedures. Indeed, a recent immunoelectron microscopic observation has revealed extremely small amounts of progesterone receptor molecules present in the cytoplasm of rabbit uterus cells (7).

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