Distributions of Steroid 5 α -Reductase and 17 α -Hydroxylase/C17,20-lyase (P450c17) Immunoreactivities in Rat Gastric Mucosa

Hiromichi Yokoi^{1,2}, Yoshihiro Tsuruo³, Shiro Kominami⁴, Takeshi Yamazaki⁴ and Kazunori Ishimura¹

¹Department of Anatomy and Cell Biology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770–8503, Japan, ²Kagawa National Children's Hospital, Zentsuji 765–8501, Japan, ³Department of Anatomy and Cell Biology, Wakayama Medical University School of Medicine, Wakayama 641–8509, Japan and ⁴Faculty of Integrated Arts and Sciences, Material and Life Sciences, Hiroshima University, Higashihiroshima 739–8521, Japan

Received January 31, 2005; accepted February 14, 2005

We examined the immunohistochemical localization of steroid 5 α -reductase type 1 and 17 α -hydroxylase/C17,20-lyase (P450c17) in rat gastric mucosa. Immunoreactivity of 5 α -reductase was localized in the chief cells and the mucous neck cells. P450c17 immunoreactivity was localized in the parietal cells. Sub-cellular localizations of both 5 α -reduc-

tase and P450c17 were on the membrane of endoplasmic reticulum. P450c17 is a key enzyme of androgen biosynthesis, and 5 α -reductase has a high affinity for testosterone and catalyzes the conversion of testosterone to its 5 α -reduced metabolite. Thus, it is suggested that there is an androgenic metabolic pathway in rat gastric mucosa.

Key words: steroid, 5 α -reductase, P450c17, gastric mucosa, immunohistochemistry

I. Introduction

Steroid 5 α -reductase catalyzes the conversion of several steroids with a C-4,5 double bond and C-3 ketone (i.e. progesterone, androstenedione, testosterone, glucocorticoids and mineralocorticoids) into 5 α -reduced metabolites. Although the biochemical activity and mRNA of 5 α -reductase have been demonstrated in the rat stomach [2, 8, 11], no immunohistochemical analysis on 5 α -reductase has been shown in the gastric mucosa. In the present study, we examined the immunocytochemical localization of 5 α -reductase in rat gastric mucosa using a polyclonal antibody against 5 α -reductase rat type 1 [7, 12]. It has been reported that 5 α -reductase has a higher affinity for androgens, such as androstenedione and testosterone, than glucocorticoids [8]. Hence, we also carried out an immunocytochemical analysis of 17 α -hydroxylase/C17,20-lyase (P450c17), a key enzyme of androgen biosynthesis, which has been reported to be localized in rat gastric mucosa [5].

II. Materials and Methods

Animals

Adult Wistar rats (8–12 weeks old, Japan SLC, Shizuoka, Japan) of both sexes were maintained on a 12-hr light-dark cycle at 20°C and fed lab chow and water *ad libitum*. Study protocols were approved by the Committee on Animal Research, the University of Tokushima.

Tissue preparation

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The animals were perfused through the left ventricle with 50 ml of 0.1 M phosphate buffer (PB), pH 7.4, heparinized and warmed at 37°C, followed with 100 ml of fixative (4% paraformaldehyde and 0.2% picric acid in 0.1 M PB, pH 7.4) at 4°C. Stomachs were removed and further immersed in the same fixative for 2 hr at 4°C. The tissues were soaked in 10, 15, and 20% sucrose in 0.01 M phosphate-buffered saline (PBS), pH 7.4, successively until they sank to the bottom of the container. The specimens were then frozen and cut into 8- μ m-thick sections using a cryostat. The frozen sections were thaw-mounted on glass slides coated with chrome alum gelatin and air-dried for about 1 hr.

Immunocytochemical procedure

The sections were rinsed with 0.01 M PBS for 10 min

Correspondence to: Hiromichi Yokoi, Kagawa National Children's Hospital, Zentsuji 765–8501, Japan.
E-mail: hyokoi@kagawasyoni.hosp.go.jp

and soaked in 0.2% Triton X-100 in 0.01 M PBS for 20 min. Endogenous peroxidase was blocked by incubation with 3% hydrogen peroxide in distilled water for 20 min at room temperature, and endogenous avidin/biotin binding was blocked with avidin D, followed by biotin (Vectastain, Vector Laboratories, Burlingame, CA, USA). The sections were then incubated with antiserum against 5α -reductase rat type 1 [7, 12] diluted 1:10,000 in 0.01 M PBS containing 1% normal goat serum (NGS) overnight at 4°C. Some sections were incubated with an antiserum against P450c17 [3, 10] diluted 1:4,000 in 0.01 M PBS containing 1% NGS overnight at 4°C. This was followed by biotinylated anti-rabbit IgG diluted 1:100 in 0.01 M PBS containing 1% NGS for 1 hr at 32°C, and then with Elite ABC solution (50 μ l of avidin and biotin-peroxidase in 5 ml of 0.01 M PBS containing 1% NGS) (Vectastain, Vector, USA) for 1 hr at 32°C. The immunoreaction was visualized in 50 mM TRIS-HCl buffer, pH 7.6, containing 0.01% 3,3'-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide, and examined by light microscopy.

The sections were then treated with 1% osmium tetroxide for 1 hr at room temperature, rinsed with 10% sucrose in distilled water, stained with a saturated solution of uranyl acetate for 30 min at room temperature, rinsed with 10% sucrose in distilled water, dehydrated in a graded series of ethanol, and embedded in Epon. Thin sections were examined under an electron microscope without uranyl

acetate or lead staining.

The sections with antigenic signals were confirmed by antigen-peptide competition experiments (absorption with 1 mM of the antigen).

III. Results

Light microscopic observations

In the rat stomach, both 5α -reductase immunoreactivity and P450c17 immunoreactivity were localized in the gastric gland, but not in other layers such as the surface mucous cell layer, lamina propria, submucosa, muscle layer and serous membrane (Figs. 1, 2). In the glandular neck, 5α -reductase immunopositive cells were scattered and slender, triangular, or irregular in shape (Fig. 2a). In the glandular base, they were continuously located and granular-looking (Fig. 2b). When using antigen-absorbed antiserum against 5α -reductase, no immunoreaction was observed (Fig. 1b). Immunopositive cells for P450c17 were round or spherical in shape, and their shape and distribution in the gland were different from those of 5α -reductase immunopositive cells.

There was no significant difference between male and female rats (data not shown).

Electron microscopic observations

The cellular and subcellular localizations of 5α -reduc-

Fig. 1. **a.** Immunostaining of 5α -reductase type 1 in the rat stomach. Immunoreactivity is localized in the gastric mucosa (arrows). mm, muscularis mucosae; sm, submucosal layer; ml, muscle layer. **b.** No immunoreactivity is seen after preabsorption of the antiserum against 5α -reductase type 1 with the antigen. **c.** Immunostaining of P450c17 in the rat stomach. Immunoreactivity is also localized in the gastric mucosa (arrows), but its staining-pattern is different from the 5α -reductase immunostaining-pattern (**a**). Bar=100 μ m.

Fig. 2. a, b. Immunoreaction of 5 α -reductase type 1 in the gastric mucosa. Immunoreactive cells are present in the gastric glands. No immunoreactivity is observed in the surface mucous cells (smc). In the glandular neck (gn), immunoreactive cells are scattered and have slender, triangular, or irregular shapes (a). In the glandular base (gb), immunoreactive cells are continuously located and granular-looking (b). c, d. Immunoreaction of P450c17 in the gastric mucosa. Immunoreactive cells are present in the gastric gland. They have round or spherical shape. Immunoreaction is localized in the cytoplasm. Bar=20 μ m.

Fig. 3. a, b. Electron micrograph of the gastric mucosa. a. Immunocytochemistry of 5 α -reductase type 1. The immunoreactive cell (asterisk) has abundant rough endoplasmic reticulum. Immunoreaction products are observed on the rough endoplasmic reticulum. No immunoreaction is observed in the other cells (stars). b. Immunocytochemistry of P450c17. The immunoreactive cell (asterisk) has intracellular canaliculi (c) and many mitochondria (m). Immunoreaction products (arrowheads) are observed in the cytoplasm. The other cells (stars) show no immunoreaction. Bars=1 μ m.

Fig. 4. **a, b.** Subcellular localization of immunoreaction products. **a.** 5 α -reductase positive cell. Immunoreaction products (arrowheads) are present on the membrane of rough endoplasmic reticulum, but not in the mitochondria (m) or the nucleus (n). **b.** P450c17 positive cell. Immunoreaction products (arrowheads) are present on the endoplasmic reticulum, but not in the mitochondria (m) or the intracellular canaliculi (c). Bars=0.5 μ m.

tase and P450c17 immunoreactivities were determined by electron microscopy using the pre-embedding method. The 5 α -reductase immunostained cell showing positive reaction products in the cytoplasm, was columnar-shaped and had abundant rough endoplasmic reticulum (Fig. 3a). These ultrastructural features were the characteristics of the chief cell. The immunoreaction products were located mainly on

the membrane of rough endoplasmic reticulum (Fig. 4a). The P450c17-positive cells had many mitochondria and intracellular canaliculi (Fig. 3b). The immunoreaction products were present mainly on the endoplasmic reticulum, but not in the mitochondria or intracellular canaliculi (Fig. 4b). The characteristics of the P450c17-positive cell were those of the parietal cell.

IV. Discussion

The present immunocytochemical analysis has revealed the occurrence of steroid 5α -reductase-containing cells in the rat stomach. The immunoreactivity was localized in the chief cells and the mucous neck cells in the gastric gland, but not in the parietal cells. Previously, we reported that 5α -reductase immunoreactivity was localized in the glial cells of myenteric plexus in rat gastrointestinal tract [14]. In the rat stomach, therefore, 5α -reductase was immunocytochemically localized in the chief cells, the mucous neck cells and the glial cells of myenteric plexus.

Two isozymes of 5α -reductase, type 1 and 2, have been identified in humans and rats [9]. The type 1 isozyme is widely distributed throughout the whole body, and it has a broad pH optimum in the neutral range and a low sensitivity to finasteride, a 5α -reductase inhibitor. The type 2 isozyme is preferentially located in male reproductive organs, and it has a narrow acidic pH optimum and a high sensitivity to the inhibitor. In the rat stomach, type 1 mRNA predominates [8]. This study demonstrated that 5α -reductase type 1 is immunocytochemically localized in the rat stomach.

P450c17 immunoreactivity was localized in the parietal cells, and not in the chief cells or mucous neck cells. These results are compatible with the report of Le Goascogne *et al.*, in which P450c17 protein and mRNA were localized in the parietal cells [5]. It was also shown that when a purified preparation of parietal cells was incubated with pregnenolone or progesterone, pregnenolone was mainly converted to androstenediol and progesterone was converted to testosterone [5]. Because androstenediol has the C-5,6 double bond and 5α -reductase catalyzes the conversion of steroids with the C-4,5 double bond, androstenediol cannot be converted by 5α -reductase. This enzyme has a high affinity for testosterone, and catalyzes the conversion of testosterone to its 5α -reduced metabolite, dihydrotestosterone (DHT) [8]. Testosterone produced in the parietal cells, therefore, may be converted to DHT by 5α -reductase localized in the chief cells and mucous neck cells. DHT binds to the same intracellular receptor as testosterone, and DHT-receptor complexes are more stable than those of testosterone in target cells. Thus, DHT formation is a way of amplifying the action of testosterone in target tissues [13]. In the small intestine, it was demonstrated that DHT formation exerts stimulatory effects on epithelial proliferation and on some mucosal enzyme activities [2]. DHT converted in the gastric mucosa may have the same function as in the intestine.

This study has immunocytochemically demonstrated that the subcellular localizations of both 5α -reductase and P450c17 are on the membranes of endoplasmic reticulum. Previous biochemical studies have also reported that both enzymes are membrane-bound [6]. Although 5α -reductase was reported to be occasionally localized in the nucleus, especially on the nuclear membrane [13], no immunoreaction has been observed in the nucleus or on the nuclear membrane of gastric mucosal cells.

It has been suggested that the sexual dimorphism of

gastroduodenal ulceration relates to the action of endogenous sexual steroids. Laszlo *et al.* reported an aggressive role of testosterone in the generation of cysteamine-induced gastroduodenal ulceration [4]. This mucosal injury can be prevented by orchidectomy and also pretreatment with the anti-androgen, cyproterone acetate. Pretreatment with testosterone dose-dependently aggravated the cysteamine-induced gastroduodenal mucosal injury. 5α -reduction of testosterone may modulate the role of testosterone on the mucosal injury. In female rats, pregnancy and lactation markedly reduce steroid- and cysteamine-induced gastroduodenal lesions [1]. 5α -reductase can catalyze the conversion of progesterone to dihydroprogesterone, but any role of dihydroprogesterone in steroid-induced gastroduodenal ulcer has not been demonstrated. In our study, there was no sexual difference in the immunoreaction of 5α -reductase or P450c17 in rat gastric mucosa. Further investigation, such as gonadectomy or hormone-replacement study, is needed to elucidate the relationship of gastroduodenal ulceration and steroid synthesizing enzymes in the gastric mucosa.

In summary, this study immunocytochemically localized 5α -reductase type 1 in the rat gastric gland. The immunoreaction was located mainly in the chief cells, and not in the parietal cells. We also immunohistochemically demonstrated that P450c17 was localized in the parietal cells. These results suggest that there is an androgenic metabolic pathway in rat gastric mucosa.

V. References

1. Drago, F., Montoneri, C., Varga, C. and Laszlo, F. (1999) Dual effect of female sex steroids on drug-induced gastroduodenal ulcers in the rat. *Life Sci.* 64; 2341–2350.
2. Farthing, M. J., Vinson, G. P., Edwards, C. R. and Dawson, A. M. (1982) Testosterone metabolism by the rat gastrointestinal tract, in vitro and in vivo. *Gut* 23; 226–234.
3. Kominami, S., Shinzawa, K. and Takemori, S. (1982) Purification and some properties of cytochrome P-450 specific for steroid 17 α -hydroxylation and C17-C20 bond cleavage from guinea pig adrenal microsomes. *Biochem. Biophys. Res. Commun.* 109; 916–921.
4. Laszlo, F., Varga, C., Montoneri, C. and Drago, F. (1997) Damaging actions of testosterone on cysteamine-induced gastroduodenal ulceration and vascular leakage in the rat. *Eur. J. Pharmacol.* 337; 275–278.
5. Le Goascogne, C., Sananes, N., Eychenne, B., Guezou, M., Baulieu, E. E. and Robel, P. (1995) Androgen biosynthesis in the stomach: expression of cytochrome P450 17 α -hydroxylase/17,20-lyase messenger ribonucleic acid and protein, and metabolism of pregnenolone and progesterone by parietal cells of the rat gastric mucosa. *Endocrinology* 136; 1744–1752.
6. Miller, W. L. (1988) Molecular biology of steroid hormone synthesis. *Endocr. Rev.* 9; 295–318.
7. Miyamoto, T., Kagawa, S., Kitagawa, K., Futaki, S., Yokoi, H., Tsuruo, Y. and Ishimura, K. (1996) Immunocytochemical localization of 5α -reductase type 1 in the prostate of normal and castrated rats. *Histochem. Cell Biol.* 105; 101–109.
8. Normington, K. and Russell, D. W. (1992) Tissue distribution and kinetic characteristics of rat steroid 5α -reductase isozyme. Evidence for distinct physiological functions. *J. Biol. Chem.* 267; 19548–19554.
9. Russell, D. W. and Wilson, J. D. (1994) Steroid 5α -reductase:

- two genes/two enzymes. *Ann. Rev. Biochem.* 63; 25–61.
10. Shinzawa, K., Ishibashi, S., Murakoshi, M., Watanabe, K., Kominami, S., Kawahara, A. and Takemori, S. (1988) Relationship between zonal distribution of microsomal cytochrome P-450s (P-450_{17 α} lyase and P-450C21) and steroidogenic activities in guinea-pig adrenal cortex. *J. Endocrinol.* 119; 191–200.
 11. Stenstad, P. and Eik-Nes, K. B. (1982) In vitro metabolism of testosterone to 17 β -hydroxy-5 α -androstane-3-one and 5 α -androstane-3 α , 17 β -diol by the rat intestine. *J. Steroid Biochem.* 17; 401–406.
 12. Tsuruo, Y., Miyamoto, T., Yokoi, H., Kitagawa, K., Futaki, S. and Ishimura, K. (1996) Immunohistochemical presence of 5 α -reductase rat type 1-containing cells in the rat brain. *Brain Res.* 722; 207–211.
 13. Yokoi, H., Tsuruo, Y., Miyamoto, T., Kitagawa, K., Futaki, S. and Ishimura, K. (1996) Steroid 5 α -reductase type 1 immunolocalized in the anterior pituitary of intact and castrated male rats. *Histochem. Cell Biol.* 106; 359–366.
 14. Yokoi, H., Tsuruo, Y. and Ishimura, K. (1998) Steroid 5 α -reductase type 1 immunolocalized in the rat peripheral nervous system and paraganglia. *Histochem. J.* 30; 731–739.