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# Localization of 11β-Hydroxysteroid Dehydrogenase Type II in Human Epithelial Tissues

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**ABSTRACT**

The enzyme 11β-hydroxysteroid dehydrogenase type II (11βHSD2) confers specificity on the renal mineralocorticoid receptor by inactivating glucocorticoids. Mutations in this gene give rise to the syndrome of apparent mineralocorticoid excess, a congenital condition characterized by sodium retention, severe hypertension, and often by growth retardation. It is not known whether 11βHSD2 or another enzyme confers specificity in nonrenal sodium transporting epithelia, such as those in the sweat gland, salivary gland, and gastrointestinal tract. We previously have used the HUH23 antibody to localize 11βHSD2 in the human kidney, vascular smooth muscle cells, and placenta. In the present study, we have examined a range of human epithelia for the presence of 11βHSD2. In the skin, staining was seen in eccrine sweat glands and arterioles, whereas weak HUH23 immunostaining was observed in the epidermis. Staining was absent from sebaceous glands and hair follicles. In the parotid gland, the 11βHSD2 enzyme was present in striated and excretory ducts, whereas in the submandibular gland, it was found in striated and interlobular ducts. Acini, adipocytes, and associated tumor tissue did not stain with the HUH23 antibody. In the gastrointestinal tract, HUH23 stained ileal enterocytes, colonic absorptive cells, and epithelial goblet cells, whereas the rectum contained areas of staining and nonstaining absorptive cells. Gastrointestinal structures, such as the lamina propria, Peyer's patch, and goblet cells within the crypts of Lieberkuhn did not stain with the antibody. This study demonstrates the presence of 11βHSD2 in nonrenal sodium-transporting epithelia and describes a range of tissues affected in the syndrome of apparent mineralocorticoid excess. (*J Clin Endocrinol Metab* **81:** 3244–3248, 1996)

THE ENZYME 11β-hydroxysteroid dehydrogenase type II (11βHSD2) is thought to confer specificity on the nonselective mineralocorticoid receptor (1) by inactivating glucocorticoids (2, 3). Inhibition of enzyme activity, as occurs in licorice intoxication or as a side effect of the administration of the antiulcer drug Carbenoxolone, leads to overactivation of mineralocorticoid activity by cortisol and results in elevated blood pressure. A congenital defect in 11βHSD2 gives rise to the syndrome of apparent mineralocorticoid excess (AME), in which patients show marked sodium retention, hypokalemia, and severe hypertension in the face of low plasma renin activity (4). Recently, we and others have identified mutations in the gene coding for 11βHSD2 in AME patients (5, 6, 7). Immunohistochemical studies suggest that it is the renal distal tubule and vascular smooth muscle cell that play a pivotal role in the development of this form of hypertension (8). High amounts of 11βHSD2 enzyme also have been found in placental syncytiotrophoblasts (8), where it is thought to protect the fetus from high circulating levels of maternal glucocorticoids and may be important in the maintenance of normal pregnancy (7).

The 11βHSD2 enzyme is assumed to confer mineralocorticoid specificity in all other sodium transporting epithelia. There is abundant evidence for the presence of mineralocorticoid receptors in the sweat glands and salivary glands, where sodium is reabsorbed during the formation of serous secretions (9, 10). The gastrointestinal tract also is a well characterized target for aldosterone, with the descending colon possessing the highest concentration of mineralocorticoid receptors of all tissues (11). In the present study, we have used a monospecific polyclonal antibody directed against the carboxy-terminus of the human enzyme to show that it is the 11βHSD2 isoform that endows mineralocorticoid specificity in a wide range of epithelia.

## Materials and Methods

### Antibody production and characterization

The generation and characterization of the HUH23 antibody have been described previously (8). Antibodies were raised in rabbits against a synthetic peptide corresponding to the last 16 amino acid residues of human 11βHSD2, and the antiserum immunopurified on the peptide coupled to an Affigel-10 column.

### Tissue collection and pathology

Human skin was obtained fresh from buttock biopsies, frozen in OCT embedding medium (Miles Inc., Elkhart, IN) and cut into 5-micron sections. All other samples were normal tissue dissected from pathological specimens obtained from the Department of Pathology, Tohoku University School of Medicine, Sendai, Japan. Pathology specimens were fixed in 10% formalin, embedded in paraffin, and cut into 3-micron sections.

Skin was obtained from the buttock biopsy of a 22-yr-old male Cau-

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casian volunteer with no obvious pathology. A parotid gland was obtained from a 34-yr-old female Japanese who presented with a mass in the parotid gland 3 yr previously and was diagnosed with pleomorphic adenoma. A submandibular gland was from a 16-yr-old Japanese with mucocele. The ileal specimen was from a 51-yr-old Japanese female who was treated for perforation of diverticulum and localized peritonitis; the normal ileum specimen was obtained 10 cm from the ileocecal junction. Descending colon was from a 60-yr-old Japanese who had a moderately differentiated adenocarcinoma of the colon with infiltration into the serosa; a specimen of normal colonic mucosa was obtained 30 cm distal to the carcinoma. Normal rectum was obtained from a 73-yr-old male Japanese with moderately differentiated adenocarcinoma of the rectum.

### *Immunostaining*

Paraffin sections were dewaxed, rehydrated, and stained by a 3-layer immunoperoxidase technique as previously described (8). The immunopurified HUH23 antibody was used at a concentration of 7.5 μg/mL for paraffin sections and at 10 μg/mL for frozen sections. The anti-von Willebrand factor antibody was raised in rabbits against the human antigen (DAKO Laboratories, Carpintaria, CA) and used at 5 μg/mL. The control antiserum was a solid-phase absorbed rabbit Ig fraction from healthy nonimmunized animals (DAKO Laboratories). It was used at a concentration of 5 μg/mL.

Photography was performed with a Weild Leitz microphotography system (MPS 46). A violet filter was used to reduce the hematoxylin signal.

## Results

When sections of human buttock skin were incubated with the HUH23 antibody, marked staining was observed in arterioles and much lighter staining in the epidermis, where reactivity seemed to be localized in the stratum germinativum and absent from the stratum corneum (Fig. 1A). Nonimmune rabbit serum did not stain a similar section (Fig. 1B), confirming the specificity of staining with HUH23. Furthermore, use of an anti-von Willebrand factor antibody (Fig. 1C), which stains endothelial cells in the vasculature, confirmed the assignment of the structures in Fig. 1A as arterioles. Eccrine sweat glands also stained with the HUH23 antibody (Fig. 2A), and the specificity of this reaction was again confirmed by the use of a nonimmune rabbit serum (Fig. 2B). However, neither sebaceous glands nor hair follicles showed evidence of the 11βHSD2 enzyme (Fig. 2C).

Salivary glands also showed evidence of 11βHSD2. When parotid gland was stained with the HUH23 antibody, strong staining was seen in the striated ducts (Fig. 3A), while adjacent tumor cells were negative (not shown). Specificity was confirmed by the use of nonimmune rabbit serum (Fig. 3B). Weaker HUH23 staining also was observed in parotid excretory ducts (Fig. 3C). Striated ducts in the submandibular gland stained positively with HUH23, with mucous acini devoid of immunoreactivity (Fig. 4A). Submandibular gland interlobular ducts also were found to contain the 11βHSD2 enzyme (Fig. 4B). The lack of staining of this structure with a nonimmune rabbit serum again confirmed the specificity of staining by HUH23 (Fig. 4C).

We next localized the 11βHSD2 enzyme in the human intestinal tract. The HUH23 antibody strongly stained enterocytes of ileal villi, and the cytoplasm of ileal goblet cells also seemed to stain, whereas the lamina propria and mucous vacuoles of goblet cells did not react (Fig. 5A). Ileal villi did not stain with nonimmune rabbit serum (Fig. 5B). Incubation of a section of ileum containing a Peyer's patch with HUH23

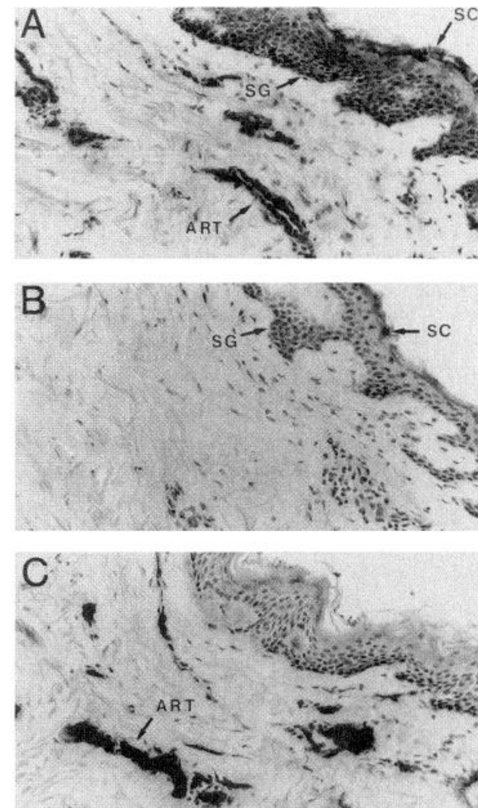


FIG. 1. Localization of 11βHSD2 in human skin. A, HUH23 immunostaining of the epidermis and arterioles (ART) of the dermis; SG, stratum germinativum; SC, stratum corneum. B, Section of human skin stained with nonimmune rabbit serum. C, Human skin stained with an antibody against von Willebrand factor, showing endothelial cell staining of an arteriole (ART). Magnification ×150.

showed that intestinal lymph nodes do not contain immunoreactive 11βHSD2 (Fig. 5C). Absorptive cells of the distal colon were strongly positive for the presence of 11βHSD2, while the goblet cells in the crypts of Lieberkuhn and the lamina propria were negative (Fig. 6A). However, mature goblet cells located amongst the absorptive cells of the epithelium invariably exhibited cytoplasmic staining. The HUH23 antibody seems to stain absorptive cells throughout the colon, with identical results for transverse colon and cecum (results not shown). In contrast, in the rectum, a high proportion of absorptive cells does not stain with the HUH23 antibody; Figs. 6B and 6C show two fields of a section of rectum in which the majority of absorptive cells are positive (Fig. 6B) or negative (Fig. 6C) for HUH23 immunostaining.

For each tissue, additional patients were examined, and there was no discernable difference in the patterns of staining, though modest differences in intensity were sometimes observed.

## Discussion

There is considerable evidence that the 11βHSD2 enzyme confers specificity on the mineralocorticoid receptor in the kidney (8, 12, 13), and it is a commonly held assumption that this isozyme also confers specificity in other mineralocorticoid target tissues, such as those in sweat glands, salivary

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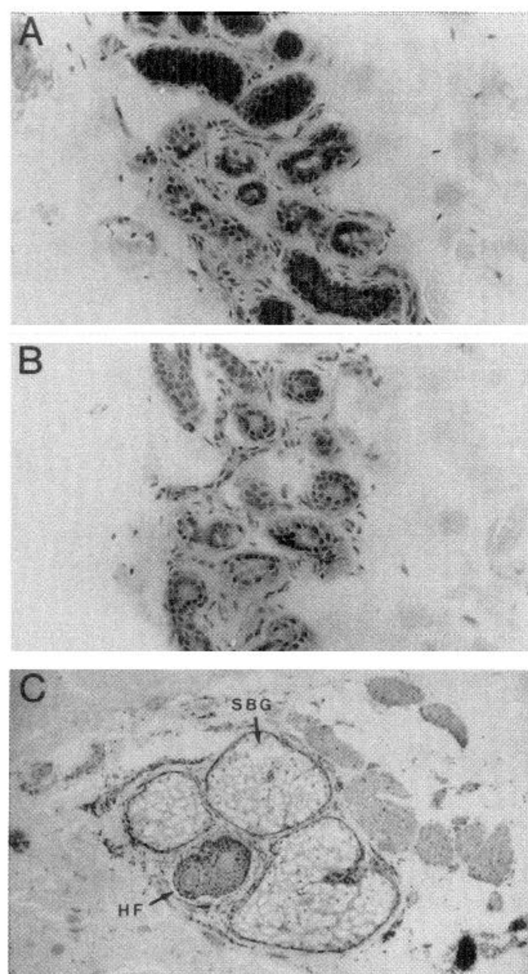


FIG. 2. Staining of exocrine structures in the skin. A, HUH23 staining of an eccrine sweat gland; B, staining of eccrine sweat gland with nonimmune rabbit serum; C, HUH23 staining of sebaceous glands (SBG) and hair follicle (HF). Magnification ×60.

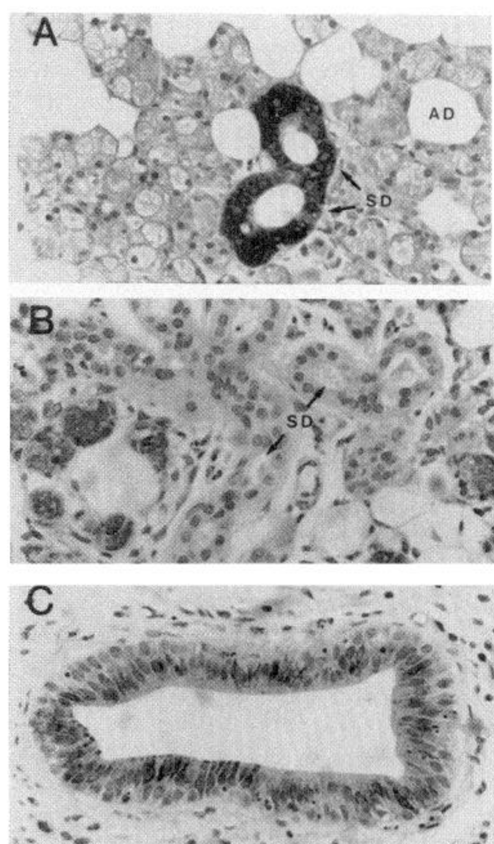


FIG. 3. Localization of 11βHSD2 in the parotid gland. A, HUH23 staining of striated ducts (SD); adipocytes (AD) did not stain. B, Parotid gland incubated with nonimmune rabbit serum. C, HUH23 staining of an excretory duct in the parotid gland. Magnification ×240.

glands, and the gastrointestinal tract. An alternative explanation is that there are other tissue-specific mechanisms, including perhaps other 11βHSD isoenzymes, that perform the same function. The present study has thus examined the localization of the 11βHSD2 isoform in a range of nonrenal epithelia.

In the skin, 11βHSD2 immunoreactivity was found to be present in the vasculature, and at lower levels, in the epidermis. We previously have localized the 11βHSD2 enzyme in vascular smooth muscle cells of renal interlobular arteries (8), but a recent study, using an antibody raised against a fusion protein that does not contain the C-terminal peptide, has reported the absence of staining in the vasculature (14). These differences may be explained by sensitivity of the vascular C-terminal epitope to experimental conditions, such as the use of fresh-frozen *vs.* formalin-fixed, paraffin-embedded sections. In addition, we have observed vascular staining of frozen sections of human foreskin when using an antibody directed against residues $^{267}$T-$^{281}$Q of the 11βHSD2 protein (unpublished observations). Another structure in the skin that stained with the HUH23 antibody was the epidermis. Here the 11βHSD2 enzyme may serve to mitigate the atrophic effects of glucocorticoids and could play a role in the etiology of psoriasis and eczema.

The HUH23 antibody also stained eccrine sweat glands, consistent with the detection of NAD-dependent 11βHSD activity in isolated structures (10). Mineralocorticoid receptor immunoreactivity recently has been demonstrated in sweat glands, sebaceous glands, and hair follicles (10), but in the present study, the HUH23 antibody stained only sweat glands, consistent with demonstrable sodium transport in this tissue alone. A previous comparison of enzymatic activities showed that sweat glands have over two orders of magnitude more 11βHSD2 than the epidermis (10). Sebaceous constituents are not known to be modulated by mineralocorticoid hormones, suggesting the presence of a nonselective receptor in these structures.

The parotid gland frequently has been used to study changes in sodium flux in response to mineralocorticoid administration (15). There is evidence for mineralocorticoid receptor gene expression in the rat parotid, and the aldosterone selective receptor has been localized to ductal elements by binding studies and immunohistochemistry (9). In the present study, HUH23 immunostaining was observed in the cuboidal cells comprising the striated ducts in both the parotid and submandibular gland. These ducts play an important role in the modification of serous products by secretion

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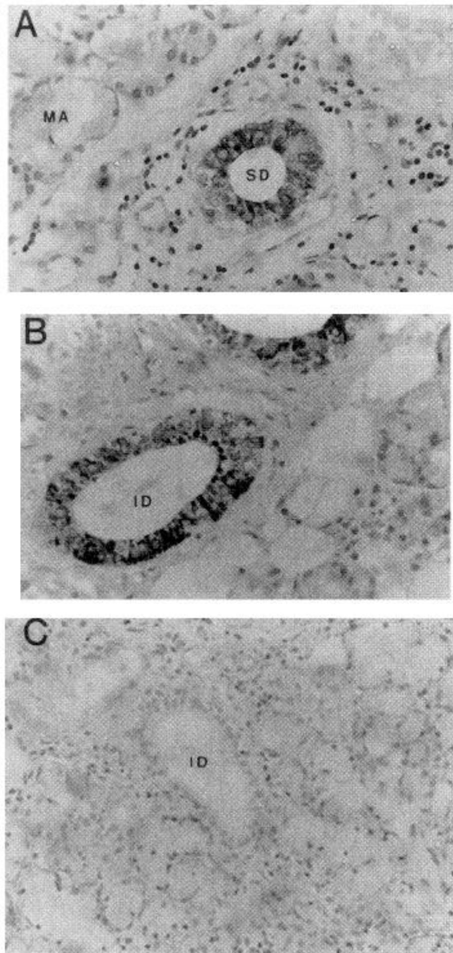


FIG. 4. Staining for the presence of 11βHSD2 in a submandibular gland. A, HUH23 immunostaining of a striated duct (SD). Mucous acini (MA) were devoid of immunoreactivity. B, HUH23 staining of a submandibular interlobular duct (ID). C, Lack of submandibular interlobular duct staining with nonimmune rabbit serum. Magnification ×240.

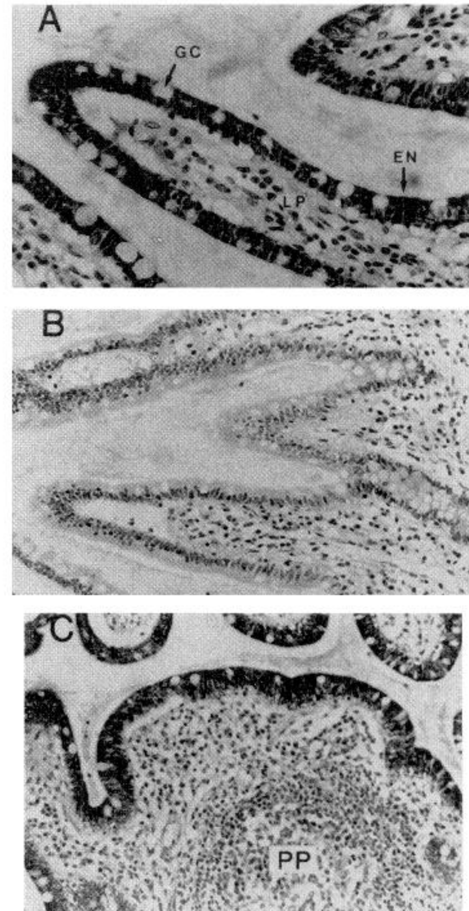


FIG. 5. Localization of 11βHSD2 in the lower gastrointestinal tract. A, HUH23 staining in the ileum; EN, enterocytes; GC, goblet cell mucous vacuole; LP, lamina propria (Magnification ×240). B, Staining of ileum with nonimmune rabbit serum (Magnification ×150). C, HUH23 staining of the ileum showing the absence of immunoreactivity in the Peyer's patch (PP). Magnification ×150.

and reabsorption of electrolytes and secretion of glycoproteins; in the rat parotid gland, these cells also have been shown to internalize exogenous proteins (16). Interestingly, the sodium concentration in parotid saliva is decreased in a number of hypertensive models, and it would be informative to measure salivary cortisol to cortisone ratios in these individuals to determine whether 11βHSD2 plays any role in these forms of hypertension (17).

The gastrointestinal tract also is a well-studied mineralocorticoid target, consisting of aldosterone sensitive and insensitive segments (18). Our demonstration of HUH23 immunoreactivity in the ileum is consistent with observations of mineralocorticoid selectivity in this tissue, in that aldosterone, but not corticosterone, activates magnesium-bicarbonate ATPase and carbonic anhydrase (19). The absence of detectable HUH23 staining in the ileal Peyer's patch is noteworthy, given the demonstration of mineralocorticoid receptors in lymphocytes (20).

The distal colon is the major site of sodium transport in the gut, and immunohistochemical studies have localized the mineralocorticoid receptor to absorptive cells in this tissue (9). Our observation of HUH23 staining in distal colonic absorptive cells is consistent with a recent independent study on the sigmoid colon (14). The 11βHSD2 enzyme seems to play a second role in these cells by modulating the glucocorticoid induced increase in Na/K ATPase activity (21). Chemical shear and microdissection techniques have provided evidence for both isoforms of the enzyme in rat colonic mucosa, with the 11βHSD1 isoform localized to the lamina propria, and 11βHSD2 to surface and crypt epithelial cells (22). In a previous study on inflammatory bowel disease, cortisol was shown to be rapidly inactivated by 11βHSD1 in scrapings of human intestinal mucosa (23); in retrospect, 11βHSD2 also may have contributed to this activity. Given the important role played by glucocorticoids in the inflammatory process, changes in 11βHSD isozyme levels thus may contribute to the etiology of inflammatory bowel disease.

The rectum also responds to aldosterone with electrogenic sodium reabsorption (24). In the present study, we observed HUH23 staining in some absorptive cells in the rectum but not in others from the same section. The degree to which age and disease contributed to the heterogeneity is unknown, but

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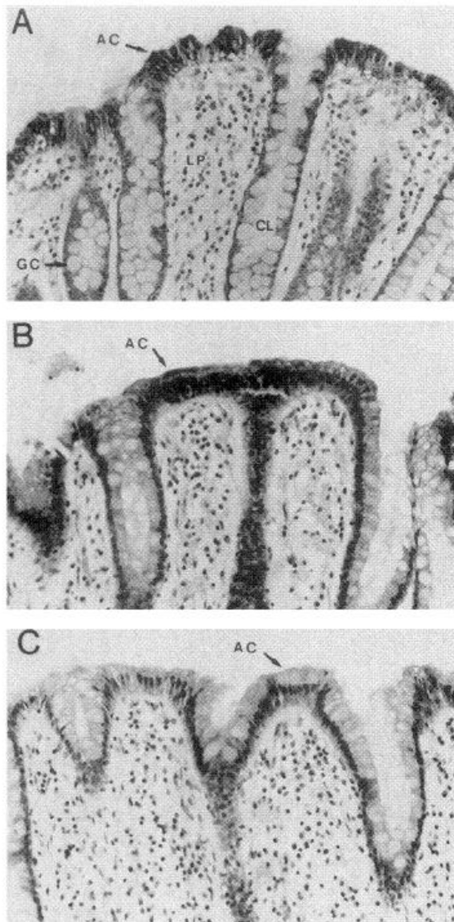


FIG. 6. HUH23 staining of lower intestinal tract. A, Descending colon; AC, absorptive cells; CL, crypts of Lieberkuhn; LP, lamina propria; GC, goblet cell mucous vacuole. B and C, Rectum; AC, absorptive cells. (Magnification ×150).

the degree of cellular maturity may also determine the ability of epithelial cells to express 11βHSD2. Clinical studies previously have demonstrated the presence of variable amounts of 11βHSD activity between patients and have suggested a dependence of enzyme activity on cofactor availability and nutritional factors (23). The present work suggests that this heterogeneity also may result from variable expression of the enzyme.

The present study suggests that the 11βHSD2 enzyme is a common determinant of mineralocorticoid and glucocorticoid receptor occupancy in all sodium transporting epithelia and adds to the list of tissues that are directly affected in the syndrome of AME.

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