Effect of Glucocorticoid on Expression of Rat MUC5AC mRNA in Rat Gastric Mucosa in Vivo and in Vitro

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The gastric mucosa is an important factor in the gastric mucosal protection from acid, pepsin and various reagents (alcohol, aspirin, etc.). MUC5AC is the mucin secreted from surface mucous cells, and belongs to the gel-forming mucin. We examined the regulation of rat MUC5AC (rMUC5AC) mRNA by glucocorticoid in vivo and in vitro, comparing it with that of pepsinogen (Pg) mRNA. By adrenal gland resection, rMUC5AC and Pg mRNA levels and Pg content in rats significantly decreased to 70%, 46% and 42% of those in the sham operated controls, respectively. With the treatment of hydrocortisone (1, 5 and 50 mg/kg), Pg mRNA level and Pg content in adrenalectomized rats was restored. On the other hand, the rMUC5AC mRNA level exceeded the control with 1 or 5 mg/kg injections of hydrocortisone, but drastically decreased to 18% of sham operation levels with it (50 mg/kg). Similar results were obtained in normal rats with the treatment of hydrocortisone (50 mg/kg). Mucin and DNA content of cultured rat gastric epithelial cells were not affected by hydrocortisone, but rMUC5AC mRNA level was significantly decreased in a dose-dependent manner.

From the in vivo and in vitro results, at least a physiological concentration of glucocorticoid was necessary in the expression of rMUC5AC mRNA. However, high dose of hydrocortisone directly suppressed the expression of rMUC5AC mRNA. These results suggested that hydrocortisone might directly cause the suppression and indirectly the enhancement of the mucin biosynthesis.

Key words MUC5AC; pepsinogen; hydrocortisone; mRNA; rat

MATERIALS AND METHODS

Chemicals Hydrocortisone acetate was purchased from Wako Pure Chemical Industries (Osaka, Japan). Biotinylated soybean agglutinin (BSA) and horseradish peroxidase-avidin D (HRP-avidin D) were from Vector Laboratories, Inc. (Burlingame, CA, U.S.A.). Dispase I and bovine serum albumin (BSA) (fraction V) were from Godo Shusei Co. (Tokyo, Japan) and Sigma Chem. Co. (St. Louis, MO, U.S.A.). Immobilon polyvinylidene difluoride (PVDF) transfer membrane was from Japan Millipore, Ltd. (Tokyo, Japan). Anti-sense oligodeoxynucleotides for rMUC5AC and Pg mRNA were customised by Greiner Japan (Tokyo, Japan), as follows: rMUC5AC, 5'-GGTGGTGAGCTGGTGATGTGTTTG-3', which was estimated by Inatomi et al.,6) and for Pg, 5'-TGTGTCTACAATGCCTTGGCAGCCCTG-3', which was estimated by Ichihara, et al.,7) then labeled by 32P with the Oligonucleotide 5'-End Labeling System (Du Pont NEN Res. Pro., Boston, MA, U.S.A.). All other reagents were of the best commercial quality available.

Preparation of Adrenalectomy and Treatment with Hydrocortisone Male Wistar rats weighing about 200 g were used in experiments. Adrenalectomy was performed following the method of Grollman.8) After adrenalectomy, rats were starved for 24 h and were sacrificed under ether anesthesia.

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Culture of Rat Gastric Epithelial Cells and Treatment with Hydrocortisone  
Culture of rat epithelial cells was prepared by our method as previously described.11) Briefly, everted sacs of the stomachs were filled with medium, immersed in incubation medium containing Dispase I (1 kU/ml), and gassed with 95% O₂ and 5% CO₂ at 37 °C for 1 h. Mucosal cells were gently isolated from the surface of the gastric mucosa by pipetting. Cells obtained (2×10⁶ cells/dish) were inoculated on plastic dishes (35 mm diameter) covered with collagen gel, and cultured in a Dulbecco’s modified eagle medium:Ham’s F12 (1 : 1) medium (GIBCO BRL/Life Tech., Eggenstein, Germany) supplemented with 10% fetal calf serum (Biofluids, Inc., Rockville, MD, U.S.A.) and 100 μg/ml gentamicin sulfate at 37 °C for 24 h in a CO₂ incubator. After the 24 h culture, hydrocortisone was added to final concentrations of 0.1, 1 and 10 μg/ml and incubated at 37 °C for 24 h in a CO₂ incubator.

Measurement of Pepsinogen  
The homogenate of the glandular portion of the stomach (10%) with 50 mM phosphate buffer (pH 7.5) was centrifuged at 10000 x g for 10 min after freezing and thawing. The supernatant was used to measure peptic activity by the method of Anson and Mirsky,12) and protein concentrations by the method of Bradford (Bio-Rad Protein Assay; Bio-Rad Lab. Co., Richmond, CA, U.S.A.).

Measurement of Mucin and DNA in Cultured Epithelial Cells  
A dot blot analysis was used for the measurement of mucin. Samples or various concentrations of purified gastric mucin in 4 m guanidine hydrochloride were applied in a microfiltration apparatus (Immuno dot, Atto Co., Ltd., Tokyo) and adsorbed onto a sheet of PVDF-membrane by aspiration. After blocking the blotted sheet with 3% BSA in TBS-T (0.05% tween 20 in 50 mM tris HCl buffered saline [pH 7.3]) at room temperature for 30 min and washing it with TBS-T, the sheet was incubated with 5 μg/ml biotinylated-SBA in 0.1% BSA and 1 mM NaCl in TBS-T for 30 min and washed 6 times with TBS-T at room temperature for 5 min. The same procedure was carried out for HRP-avidin D. Enzyme activity was measured with 0.03% H₂O₂ and 0.02% 3-amino-9-ethylcarbazole in 50 mM acetate buffer (pH 5). DNA concentration of the cultured epithelial cells was measured using a method of fluorescence enhancement of 4’,6-diamidino-2-phenylindole (DAPI).14)}
and Mucus and DNA Content in Cultured Gastric Epithelial Cells

The changes in mucus content, rMUC5AC mRNA level and DNA content in cultured rat gastric epithelial cells for 96 h are shown in Fig. 3; all three factors reached a peak at 72 h. However, the ratios of mucus/DNA were almost the same during the culture.

Hydrocortisone was added to the culture medium 24 h after inoculation and the cells were incubated for 24 h, after which, mucus content, rMUC5AC mRNA level and DNA content were measured. Mucus and DNA contents were not affected by hydrocortisone; however, the expression of rMUC5AC mRNA was significantly decreased to 41% of the control (Fig. 4).

DISCUSSION

It is well known that glucocorticoid is essential for maturation in gastrointestinal development and it has an ulcerogenic side effect on the gastric mucosa. A pharmacological dose of corticosteroids usually inhibits phospholipase A2 via the lipocortin, while there are reports that glucocorticoid does not inhibit prostaglandin synthesis in the gastric mucosa.

We therefore examined the effect of hydrocortisone on the expression of rMUC5AC mRNA in the rat gastric mucosa, and compared it with that on Pg mRNA. After adrenalectomy, rMUC5AC and Pg mRNA levels and Pg content were significantly decreased. With the treatment of hydrocortisone (1 and 5 mg/kg) the rMUC5AC mRNA level was restored to the sham operation level, whereas by the treatment of hydrocortisone (50 mg/kg), it drastically dropped to 18% of sham operation level, much lower than that of the adrenalectomized rats. On the other hand, Pg mRNA level and Pg content increased with the treatment of hydrocortisone (1, 5 and 50 mg/kg). Similar results with hydrocortisone treatment were also obtained in normal rats. There is a report that the gastric mesenchyme secretes humoral factors that are induced by glucocorticoids. The reduction of the rMUC5AC mRNA level by the adrenalectomy showed that a physiological concentration of glucocorticoid was necessary at least for the expression of rMUC5AC mRNA. Therefore, increase in the rMUC5AC mRNA levels might be induced by certain factors, one of which is from gastric mesenchyme with the treatment of an appropriate amount of hydrocortisone. The decrease in the rMUC5AC mRNA level by the treatment with hydrocortisone (50 mg/kg) was thought to be the sum total of enhancement and suppression, different from Pg. This discrepancy shows the existence of two mechanisms in vivo: hydrocortisone increases or decreases mucin mRNA expression both directly and indirectly.

Based on the above results, we examined whether or not hydrocortisone regulates the expression of rMUC5AC mRNA directly, using cultured rat gastric mucosal epithelial cells. We reported earlier the mucus secretion from cultured rat gastric epithelial cells. In this study, we confirmed that rMUC5AC mRNA was expressed in mucosal cells of our established primary monolayer culture. Hydrocortisone suppressed the expression of rMUC5AC mRNA in mucosal cell culture in a dose-dependent manner, although it did not affect mucus or DNA content. These results suggested that hydrocortisone (0.1—10 μg/ml) directly suppressed the expression of rMUC5AC mRNA in gastric epithelial cells without affecting the cellular viability. There is a report that dexamethasone directly suppressed gastric mucin secretion and expression of MUC1 mRNA on the human stomach in vitro. Our study had similar results on this point. In this culture system, epithelial cells continuously expressed rMUC5AC mRNA, although the existence of a trace amount of glucocorticoid in fetal calf serum was not absolutely ruled out.

Taking in vivo and in vitro results into consideration, the expression of rMUC5AC mRNA might be balanced by indirect enhancement by a certain factor that is regulated by hydrocortisone, from other cells like mesenchyme, and by direct suppression by hydrocortisone. Therefore, pharmacological doses of glucocorticoids increase synthesis of Pg, and inhibit mucin biosynthesis, that is, they induce the reinforcement of an aggressive factor and the declination of a protective factor. Suppression of rMUC5AC in the epithelial cells also seems to be involved in the pathogenesis of the steroid therapy-induced gastric mucosal damage.

REFERENCES

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