Troglitazone Inhibits Bicarbonate Secretion in Rat and Human Duodenum


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ABSTRACT

Troglitazone is a new, orally effective antidiabetic agent that decreases plasma glucose in obese patients with non-insulin-dependent diabetes mellitus. Unfortunately, troglitazone also has a propensity to cause edema. This study was designed to determine how troglitazone affects intestinal ion transport and water absorption. Short circuit current (Isc) was measured in rat and human duodenal mucosa in Ussing chambers. Five minutes later, the serosal addition of troglitazone caused Isc to decrease gradually, and after 50 min, Isc reached the peak of decrease. EC50 values and maximum response to Isc in rat and human mucosa were 8.4 and 8.7 μM and 8.56 ± 1.0 and 8.00 ± 2.0 μA/cm², respectively. In an HCO₃⁻/CO₂-free system, the decrease in Isc caused by troglitazone was 1.31 ± 0.83 μA/cm². When 10 mM acetazolamide was preadministered, the small decrease in Isc evoked by troglitazone (20 μM) was 4.56 ± 0.22 μA/cm², whereas the preadministration of 100 μM amiloride and 100 nM tetrodotoxin did not influence the decrease in Isc evoked by troglitazone. The serosal preadministration of 100 nM vasoactive intestinal peptide potently enhanced the decrease in Isc evoked by 20 μM troglitazone (21.1 ± 1.63 μA/cm²). The cyclic AMP contents of rat duodenal mucosa incubated with and without troglitazone (20 μM) for 50 min were 3.2 ± 0.25 and 5.8 ± 0.46 pmol/mg protein, respectively (P < .01). These results indicate that the ionic basis for the decrease in Isc that is induced by troglitazone may be inhibition of electrogenic bicarbonate secretion. The alteration of intestinal ion transport by troglitazone could cause edema.

Troglitazone (Fig. 1) is a new, orally effective antidiabetic agent that decreases plasma glucose in insulin-resistant obese and diabetic rodent models in which sulfonylureas are ineffective (Fujita et al., 1983; Fujiwara et al., 1988). The agent decreases the insulin resistance that is such an important initial event in the pathogenesis of type II diabetes. Troglitazone, however, has a propensity to cause edema, although in most instances, it has been reversible with the use of diuretics. Walker et al. (1998) reported that troglitazone has a vasodilative effect on small human arteries in vitro that might be related to the edema and hemodilution.

This study was designed to determine the effect of troglitazone on intestinal ion transport and water absorption. This comparative study was performed in both rat and human duodenum because samples of human duodenum mucosa can be easily and safely taken during gastrointestinal endoscopy. We measured short circuit current (Isc) and cyclic AMP (cAMP) content in muscle-stripped mucosa of rat duodenum after stimulation with troglitazone. We also measured the changes in Isc evoked by troglitazone in human duodenal mucosa. Our results show that troglitazone inhibits electrogenic bicarbonate secretion in duodenal mucosa.

Materials and Methods

Animals. Male Wistar rats weighing 180 to 200 g were housed in a temperature (25.2°C)- and moisture (50%)-controlled room with a 12-h light/dark cycle (lights on at 6:00 AM and off at 6:00 PM). They were fed standard rat food (Oriental Yeast, Osaka, Japan) and tap water ad libitum. Animals were sacrificed by cervical dislocation between 1:00 and 3:00 PM. A 15- to 20-mm segment of the duodenum was quickly obtained. The duodenum was opened longitudinally into a flat sheet, and the mucosa was separated from the underlying connective tissue and musculature.

Human Duodenum Biopsy Specimens. Duodenum specimens were obtained at biopsy by consent from patients who underwent gastrointestinal endoscopy in Kyoto University Hospital. These specimens were used for ion transport studies according to a method.

ABBREVIATIONS: Isc, short circuit current; TTX, tetrodotoxin; cAMP, cyclic AMP; DMSO, dimethyl sulfoxide; VIP, vasoactive intestinal peptide.
described previously (Tominaga et al., 1996). An electronic endoscope (TGF-3000D; Toshiba, Tokyo, Japan) was used to confirm that volunteers did not have any gastrointestinal diseases that might affect the study results. We also included only patients who were not treated with medicines that might affect intestinal ion transport, such as diuretics, cardiovascular drugs, bronchodilators, steroid hormones, and nonsteroidal anti-inflammatory drugs.

**Isc Measurements.** The duodenal mucosa was mounted vertically between temperature (37°C)-controlled Ussing-type chambers (10 ml each) with an exposed area of 0.38 cm² (for rats) or 0.01 cm² (for human biopsy specimens) according to a method described previously (Tominaga et al., 1996). Ringer’s solution that contained 115 mM NaCl, 1.2 mM CaCl₂, 1.2 mM MgSO₄, 25 mM NaHCO₃, 2.4 mM K₂HPO₄, 0.4 mM KH₂PO₄, and 10 mM glucose was used as the standard bathing solution. The bathing solutions were gassed with a mixture of 95% O₂/5% CO₂ resulting in pH 7.4. HCO₃⁻/CO₂-free Ringer solution with Cl⁻ substituted for HCO₃⁻ and was bubbled with pure O₂. The latter solution was titrated to pH 7.4 on the day of use. All drugs were applied to the serosal bathing solution.

Iₑ was measured using an automatic voltage clamping device (CEZ-9100; Nihon Kohden, Tokyo, Japan) that compensates for resistance of the solution between the potential measuring electrodes. The transepithelial potential was recorded through 3 M KCl-agar bridges connected to a pair of calomel half-cells. The transepithelial current was recorded across the tissue via a pair of Ag/AgCl electrodes that were kept in contact with the mucosal and serosal bathing solution using a pair of 3 M KCl-agar bridges. All experiments were done under short-circuit conditions. The Iₑ is referred to as negative when current flows from mucosa to serosa. The electrical properties of the tissues attained a stable value within 20 min after the start of preincubation with various drugs.

In the experiments with human duodenal mucosa, the steady-state values for Iₑ varied over a wide range; therefore, changes in Iₑ per baseline value of Iₑ were calculated for comparison.

**cAMP Measurements.** The duodenal mucosa (~200 mg wet weight) was incubated at 37°C for 50 min with various drugs in 20 ml of Ringer’s solution in the presence of 1 mM 3-isobutyl-1-methylxanthine. Incubation was terminated by rapid aspiration of the incubation medium followed by flash freezing in liquid nitrogen. Intra-cellular cAMP was extracted by homogenization with 0.25 ml of 0.2 M HCl for 10 min at 4°C. The protein was pelleted by centrifugation (2000g for 15 min). After neutralization of the extract with 0.2 M NaOH, the cAMP content was determined with a Correlate-EIA cAMP Enzyme Immunoassay Kit (Assay Designs, Inc., Ann Arbor, MI). Protein concentration was determined by using a protein assay kit (Bio-Rad, Hercules, CA)

**Materials.** Acetazolamide, amiloride, and epinephrine were purchased from Sigma Chemical Co. (St. Louis, MO). Tetrodotoxin (TTX) was purchased from Sankyo Seiyaku (Tokyo, Japan). Vasoactive intestinal peptide (VIP) was obtained from Peptide Institute (Osaka, Japan). Troglitazone was a generous gift from Sankyo Seiyaku; it was diluted in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the Ussing chamber was 0.1%, and vehicle DMSO did not affect Iₑ at the concentration. All other chemicals were of reagent grade.

**Statistical Analysis.** The results are given as mean ± S.E. (n = number of tissue preparations). Statistical significance was evaluated using two-tailed Student’s t tests. Differences among groups were also statistically examined by one-way ANOVA (Fisher’s PLSD test). P < .05 was considered significant.

**Ethical Considerations.** All of our studies were performed in the laboratories of the Department of Metabolism and Clinical Nutrition, Kyoto University, in accordance with the Declaration of Helsinki. The rats were treated in an appropriate manner. The patients who underwent gastroendoscopy at Kyoto University Hospital volunteered and gave their informed consent to participation in this study.

**Results**

**In Rats.** Before the administration of troglitazone, the steady-state value for Iₑ of duodenal mucosa was 28.9 ± 1.8 μA/cm² (n = 6).

Five minutes after the administration of 20 μM troglitazone to the serosal side chamber, Iₑ gradually decreased (Fig. 2). The maximal decrease (8.6 ± 1.0 μA/cm², n = 6) was observed after approximately 50 min. The decrease in Iₑ was observed to occur in a concentration-dependent manner with 5 to 40 μM troglitazone, showing an EC₅₀ value of 8.4 μM (Fig. 3). Troglitazone at a concentration of 5 to 40 μM did not affect Iₑ after mucosal administration (data not shown).

In an HCO₃⁻/CO₂ free system, troglitazone caused only a small decrease in Iₑ (1.31 ± 0.83 μA/cm², n = 6; Table 1). The preadministration of 10 mM acetazolamide to both side chambers caused a rapid decrease in Iₑ (2.89 ± 0.27 μA/cm², n = 6), and the sequential administration of troglitazone (20 μM) caused a small decrease in Iₑ (4.56 ± 0.22 μA/cm², n = 6). However, the mucosal preadministration of 100 μM amiloride did not affect the increase in Iₑ evoked by troglitazone (8.02 ± 0.74 μA/cm², n = 6). Amiloride (100 μM) itself did not alter Iₑ. These results indicate that the ionic basis for the decrease in Iₑ induced by troglitazone is accounted for by the inhibition of electrogenic bicarbonate secretion.

To determine whether the effect of troglitazone is caused by direct action on duodenal epithelia or through a neural intermediary, we examined the effects of the neural blocker TTX. The serosal preadministration of 100 nM TTX caused a rapid decrease in Iₑ (8.68 ± 0.81 μA/cm², n = 6), and the sequential administration of troglitazone (20 μM) caused the decrease in Iₑ (8.46 ± 0.88 μA/cm², n = 6). Thus, the preadministration of TTX did not affect the Iₑ response evoked by troglitazone. The serosal preadministration of 100 nM VIP caused a marked increase in Iₑ (8.59 ± 0.69 μA/cm², n = 6), and the subsequent administration of troglitazone showed a significant large decrease in Iₑ (21.1 ± 1.63 μA/cm², n = 6) compared with that of troglitazone alone. The effects of troglitazone on Iₑ in the various experimental conditions are summarized in Table 1.

The cAMP content of rat duodenal mucosa incubated with...
and without troglitazone (20 μM) was 3.2 ± 0.25 and 5.8 ± 0.46 pmol/mg protein, respectively. Thus, 50-min exposure to troglitazone (20 μM) resulted in a decrease in cAMP content compared with control.

VIP (100 nM), a cAMP-mediated secretagogue, resulted in a 15-fold increase in cAMP content compared with control (97.3 ± 4.42 pmol/mg protein). Troglitazone (20 μM) potently inhibited the cAMP production evoked by 100 nM VIP (62.1 ± 3.00 pmol/mg protein). Thus, troglitazone showed the ability to decrease both basal and agonist-stimulated cAMP production. The effects of troglitazone in suppressing cAMP levels in rat duodenal mucosa are summarized in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Decrease in Isc (µA/cm²)</th>
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<tbody>
<tr>
<td>Troglitazone (20 μM)</td>
<td>8.56 ± 1.01</td>
</tr>
<tr>
<td>+ acetazolamide (10 mM)</td>
<td>4.56 ± 0.22*</td>
</tr>
<tr>
<td>+ TTX (100 nM)</td>
<td>8.46 ± 0.88</td>
</tr>
<tr>
<td>+ amiloride (100 μM)</td>
<td>8.02 ± 0.74</td>
</tr>
<tr>
<td>+ VIP (100 nM)</td>
<td>21.1 ± 1.63*</td>
</tr>
<tr>
<td>Troglitazone (20 μM)</td>
<td>1.31 ± 0.83*</td>
</tr>
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*P < .01 compared with troglitazone-alone group by unpaired Student’s t test. Each value is mean ± S.E. (n = 6).
In Humans. In the human experiments with duodenal biopsy specimens, the steady-state value for Isc varied within a wide range (85.3 ± 17.2 μA/cm², n = 6). Five minutes after the administration of 20 μM troglitazone to the serosal side chamber, Isc gradually decreased (Fig. 4). The maximal decrease (8.0 ± 2.0 μA/cm², n = 6) was observed after approximately 50 min. The decrease in Isc was observed to occur in a concentration-dependent manner from 5 to 40 μM troglitazone, showing an EC₅₀ value of 8.7 μM (Fig. 5). As shown in Fig. 4, the results of the experiments with human duodenal biopsy specimens closely resembled those of the experiments with rats.

Discussion

This is the first report on the effect of troglitazone on the gastrointestinal tract. Troglitazone is a new, orally effective antidiabetic agent that decreases plasma glucose in insulin-resistant obese and/or diabetic rodent models in which sulfonylureas are ineffective. Unfortunately, troglitazone has a propensity to cause edema, which in most instances has been reversible with the use of diuretics. A mild decrease in red blood cell, hemoglobin, and hematocrit levels was more prevalent in the troglitazone group than in the placebo group (Kosaka et al., 1993). We have shown that troglitazone inhibits the electrogenic bicarbonate secretion, probably via a direct action on duodenal epithelia. It is generally believed that electrogenic HCO₃⁻ secretion is accompanied by passive Na⁺ and water movement toward the intestinal lumen via a paracellular pathway (Powell, 1986). Troglitazone therefore may evoke a tendency to retain water in the body of patients.

Walker et al. (1998) reported that troglitazone has a vasodilator effect on small human arteries in vitro, which could also be related to the edema and hemodilution. There seems to be no direct relationship, however, between the vasodilatation of small arteries and the change in intestinal ion transport.

Only a few substances, including epinephrine (Field and McColl, 1973; Laburthe et al., 1982) and somatostatin (Dharmsathaphorn et al., 1980; Warhurst et al., 1996), are known to decrease Isc that accompanies the increment of Na⁺ and Cl⁻ absorption. There was a difference between troglitazone and the other peptides in the time course of Isc decrease. In contrast to the prompt response evoked by epinephrine, the effect of troglitazone developed more slowly, usually taking 5 min to occur. However, it is likely that troglitazone stimulates the basolateral membrane receptor of epithelia, as well as the other peptide, because Isc response to the serosal addition of troglitazone is more sensitive than that to mucosal addition. Lee et al. (1996) reported that the inhibition of ATP-sensitive K⁺ channel activity by troglitazone in insulin-secreting cells took 15 to 20 min to develop, showing a good agreement with our findings. The exact reason for this slow onset of action remains to be elucidated.

Alternatively, there was no significant difference in either EC₅₀ values or the maximum response of Isc to troglitazone between human and rat duodenal mucosa in this study.

Forman et al. (1995) reported that troglitazone binds to the γ isoform of the peroxisome proliferator-activated receptor. This receptor, which is a member of the nuclear receptor superfamily, is a transcription factor that facilitates the differentiation of fibroblasts into adipose cells and the expression of genes involved in intermediary metabolism. Nevertheless, because the effect of troglitazone on intestinal ion transport...
transport began to be observed only 5 min after the administration of troglitazone, it is unlikely that the effect of troglitazone on $I_{sc}$ is mediated by peroxisome proliferator-activated receptor-γ.

They also reported that stimulation of α-adrenergic receptors in the ileal mucosa reduces $I_{sc}$ by inhibiting the $HCO_3^-$ secretion that is associated with a lowering of the cAMP concentration (Field and McColl, 1973; Laburthe et al., 1982). We have demonstrated that troglitazone decreases $I_{sc}$ and suppressed cAMP content in rat duodenal mucosa. Furthermore, in the presence of VIP, a cAMP-mediated secretagogue, troglitazone evoked a large decrease in both $I_{sc}$ and cAMP content. We conclude that the troglitazone-induced inhibition of $HCO_3^-$ secretion has a significant involvement in the cAMP-mediated process.

Another organ directly involved in the regulation of electrolytes and water metabolism is the kidney. Moderate additional water absorption probably would be promptly excreted unless there was a corresponding effect on renal ion transport. The effects of troglitazone on the kidney also are unclear at the present, yet it is likely that troglitazone also would alter the regulation of ion transport in the kidney. However, the edema-inducing effects of troglitazone may be at least partially due to its effect on intestinal ion transport.

Accordingly, the administration of diuretics should safely relieve the fluid retention sometimes induced by troglitazone. Acetazolamide is a widely used diuretic that particularly inhibits the change of intestinal ion transport caused by troglitazone found in this study; accordingly, we demonstrated that it is possible to combine the combined prescription of troglitazone and diuretics for the patients with edema.

Acknowledgment
We thank Sankyou Pharmaceutical Co., Ltd., for kindly providing us with troglitazone.

References

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