Effects of Somatostatin Analog SMS 201-995 on Enterotoxigenic Diarrhea

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Somatostatin analog SMS 201-995 causes a dose-related suppression of the release and/or action of several gastrointestinal hormones and impairs several anterior pituitary functions. Some patients with illness involving abnormal hormonal activity have responded to treatment with SMS 201-995, including resolution of severe secretory diarrhea. This study examined SMS 201-995 inhibition of Escherichia coli heat-stable enterotoxin STa (STa) effects and effects of the analog in the rabbit RITARD model with enterotoxigenic Escherichia coli. SMS 201-995 did not alter STa binding to its receptor on piglet brush border membranes. The analog, at concentrations of 0.1 μg/ml (0.1 μM) and 1 μg/ml (1 μM) did not significantly alter STa activation of intestinal epithelial cell particulate guanylate cyclase. At maximal dosing the analog significantly reduced intestinal fluid secretion in suckling mice that was induced by either 8-bromo cyclic GMP or STa. In piglets, the analog reduced by 37–44% the amount of diarrhea induced by STa. However, even with maximal dosing, the piglets still had significant diarrhea, although of a lesser amount. In the rabbit RITARD model the drug failed to alter the severe diarrheal response seen when dosing the animals with enterotoxigenic Escherichia coli. Overall, SMS 201-995 had a significant but incomplete effect in reducing the STa effects seen in the various assays. Additionally, in the RITARD model the analog did not alter the clinical responses to various enterotoxigenic bacteria. SMS 201-995 should be useful as a probe into the mechanisms involved in intestinal fluid secretion, but a clinical role in enterotoxigenic gastrointestinal disease was not supported by this study.

KEY WORDS: octreotide; somatostatin; SMS 201-995; Escherichia coli heat-stable enterotoxin.
cell carcinomas have responded to treatment with the somatostatin analog SMS. Several of these conditions have included symptoms of chronic and severe secretory diarrhea; the diarrhea resolved while the patients received the analog (2–8).

Diarrheal disease due to enterotoxins could have an important neurohormonal mechanism. Furthermore, it could be possible that SMS might block a step in the pathway of enterotoxin-induced secretory diarrhea. Escherichia coli heat-stable enterotoxin STa (STa), which activates particulate guanylate cyclase in the intestinal brush border membranes of enterocytes and induces secretory diarrhea, was examined in depth with SMS to evaluate for an inhibitory effect by the analog. Additionally, some experiments tested SMS effects against bacteria secreting other Escherichia coli enterotoxins [heat-stable enterotoxins STa and STb and heat-labile enterotoxin LT (9)].

MATERIALS AND METHODS

Brush Border Membranes. Small intestine epithelial cells and their brush border membranes (BBM) were prepared as previously described (10). Briefly, the cells were isolated from 5- to 10-day-old piglets following the method of Weisss (11). BBM were prepared following the method of Hauser et al (12). The modifications included the addition of 1 mM dithiothreitol and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) in the isolation buffers. The final pellets of BBM were suspended in a modified Dulbecco's buffer at 8 mg protein/ml and stored at −65 °C. There was a 12-fold enrichment of BBM sucrose activity with our isolation method (13).

Purified Escherichia coli STa. Purification of STa followed a modification of the method of Greenberg and Saeed (14). The steps included 15-liter fermentation, 0.2 μm cassette ultrafiltration (Pellicon Systems, Millipore Corp., Bedford, Massachusetts) Amberlite XAD-2 batch absorption chromatography, Sep rake chromatography (Analytium International, Harbor City, California), and C-18 reverse-phase HPLC chromatography. The HPLC column was an Aquapor RP-300 (7.1 mm ID × 25 cm long; Brownnlee Laboratories, Santa Clara, California). The resulting STa had a specific activity of 4 ng/mouse unit (see methods for STa sucking mouse assay).

The purified STa was iodinated by the lactoperoxidase method using a radioiodination kit and following the instructions of the manufacturer (New England Nuclear; Boston, Massachusetts). Specific activity of the labeled STa was 1,600,000 dpm/pM. The labeled STa was biologically active in the sucking mouse assay. Free iodine and unlabeled STa were separated from iodinated STa by HPLC reverse-phase C-18 chromatography.

STa Binding Assay. Increasing amounts of SMS, 0–50 μg, were added to test tubes along with [125I]STa (in the presence or absence of a 1000-fold excess unlabelled STa), 350 μg of piglet BBM, and the buffer solution. Between 40,000 and 50,000 cpm of [125I]STa were in each assay tube. The buffer was a modified Dulbecco's buffer containing 0.25% bovine serum albumin. The mixture was incubated at 37 °C for 45 min and terminated by filtration through polyethyleneimine (0.3%) treated Whatman GF/B glass fiber filters under suction and followed by copious washing with phosphate-buffered saline (PBS). The positive control was the incubation with both iodinated and noniodinated STa. Specific binding, demonstrated by the positive control, was the difference between the binding obtained with BBM with iodinated STa only and the binding obtained with incubating BBM with both iodinated STa and 1000-fold excess noniodinated STa. This binding assay has been standardized for membrane protein per tube (manuscript submitted).

Guanylate Cyclase Assay. Male piglets less than 21 days old were sacrificed. The small intestines were removed and rinsed with a cold solution of 0.25 M sucrose, 10 mM Tris buffer (pH 8), 1 mM EDTA, and 1 mM dithiothreitol. Mucosal scrapings were obtained with a glass slide and the scrapings were homogenized in the same solution. Homogenates were centrifuged at 105,000 g for 1 hr. The pellet that contained the particulate fraction was either used immediately or stored at −65 °C for later use in the enzyme assay. All guanylate cyclase assays in this study used the same membrane preparation.

Guanylate cyclase activity was determined in 100 μl of suspension reactions. The mixtures contained the particulate membrane fraction (10–70 μg protein), 50 mM Tris HCl (pH 7.6), 10 mM theophylline, 7.5 mM creatine phosphate, 20 μg creatine phosphokinase, and 1 mM GTP, 4 mM MgCl2, with or without STa and/or SMS (15). The reaction was initiated with the addition of the GTP and MgCl2 and incubated for 10 min at 37 °C. The reaction was terminated by the addition of ice-cold sodium acetate buffer pH 4.0 followed by heating for 3 min at 95 °C. The cyclic GMP formed was measured by radioimmun assay with acetylation of samples (16) (New England Nuclear). The protein was measured by the Pierce Micro BCA Protein assay (Application note 23235, Pierce Chemical Co., Rockford, Illinois).

Suckling Mouse Assay. Two- to 4-day-old sucking mice (from a colony of DUR/ICR mice) were given 100 μl of test solution per oral–gastric tube. The oral test solution contained STa or saline with or without SMS. Additionally, the mice received, as indicated, 100 μl of SMS or saline subcutaneously at the indicated time prior to oral dosing of STa. After a 3-hr incubation at room temperature the mice were sacrificed and the intestines and remaining body weight were recorded. One mouse unit (4 ng of purified STa) gave a ratio of 0.087 (14, 15, 17).

Adult Rabbit RITARD (Removable Intestinal Tie-Adult Rabbit Diarrhea) Model. The method of Spira et al was followed (18). The adult rabbits were male New Zealand white rabbits weighing between 1.6 and 2.7 kg. The animals were healthy prior to the procedure and had been in the facility for at least two weeks. The bacteria used in this model were Escherichia coli, strain 1108 [STa+, STb+, and LT+ (heat-labile enterotoxin)], and a nontoxicogenic Escherichia coli, strain 10405. The Escherichia coli were grown in brain–heart infusion broth. A concentration of 106 viable cells/ml in PBS were prepared for the positive control.