Supplementary Materials

Cytoprotective Mechanism of the Novel Gastric Peptide BPC 157 in Gastrointestinal Tract and Cultured Enteric Neurons and Glial Cells

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Animal and Human Intestinal Preparations

Male or female albino Wistar rats (160-200 g) were used in intestinal motility, neural culture, and ELISA experiments. Fresh biopsies of human small and large intestine were obtained from segments of intestines discarded during Roux-en-Y gastric bypass and other GI surgery. All animal protocols were approved by Research Risk and Safety Office and Human Ethics Committee in Jingmen First Hospital (protocol 013R0507 and protocol 013H0688).

Intestinal Motility Recording

Intestinal strips from rat or human were connected to isometric tension transducers (FORT 10g, World Precision Instruments (WPI), Sarasota, FL) and a fixer with a stimulating electrode in the glass chambers (10 ml, WPI) of organ baths. These intestinal preparations were bathed in Krebs solution bubbled with 95% O_2 and 5% CO_2 at 37 ± 0.5°C. Regular recording was performed after the preparations were equilibrated for 30 min and the bath solution was replaced by warm (37°C) solution twice/30 min. The composition of the Krebs solution was (in mM): 120 NaCl, 6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.35 NaH_2PO_4, 14.4 $NaHCO_3$, and 11.5 glucose. Contractions of the intestinal preparations were recorded *via* tension transducers, transducer amplifier, personal PC, and software (Lab Chart 8, AD-Instruments, Colorado Springs, CO). Electrical field stimulation

was output by a stimulus isolation unit connected to a square-wave stimulator (Grass S88, Quincy, MA). Studies were done simultaneously in four tissue baths. Analog changes in muscle tension were digitized and stored for analysis (Fig. 1).

ELISA

Segments of ileum (10–20 cm) proximal to the ileocecal junction and distal colon were removed from rats. The lumen was flushed with ice-cold Krebs solution and then divided into 1-cm segments. The full-thickness segments were placed separately into 1.5-ml microfuge tubes and incubated in 1–2 ml of Dulbecco's modified Eagle's medium (DMEM), which was changed at 10-min intervals for the first 40 min. Full-thickness human intestinal segments (0.5–1 cm long, 0.5 cm wide) were placed separately into 5-ml microfuge tubes (because of the larger size than rat preparations) and handled in the same manner. The protocols for 5-HT measurement after 40 min of equilibration was as follows: 1) after allowing 10 min for basal release, samples were withdrawn for analysis of 5-HT content; and 2) segments were exposed to 100 μM BPC157 for 30 min, and then withdrawn for analysis of 5-HT content. The samples were centrifuged at 200 rpm for 5 min, and the supernatants were stored at −20°C until assay. 5-HT levels were determined with ELISA kits (KA1894; Novus Biologicals, Littleton, CO) according to protocol provided by the supplier. The intestinal segments were blotted on filter paper and their weights recorded. Data are expressed as nanograms or picograms per gram of tissue wet weight.

Enteric Neural and Glial Cell Cultures

Myenteric ganglia were obtained from rat distal ileum and colon by microdissection of the longitudinal muscle layer with the attached myenteric plexus. The ganglia were transferred into a tube for digestion with enzyme cocktail solution (125 mg collagenase type IA, 100 mg protease type IX, and 2.5 mg deoxyribonuclease in 100 ml DMEM) at 37°C with continuous shaking for 35 min. Then the tube was centrifuged at 2000 rpm for 10 min. After the supernatant was discarded, the remaining pellets were washed and centrifuged twice with ice-cold DMEM. The pellets were transferred to Petri dishes containing DMEM and visualized under a stereomicroscope to harvest the ganglia. Five to twenty ganglia were then transferred in 10-μl pipettes onto 22 mm × 22 mm uncoated glass coverslips on the bottom of 35-mm plastic Petri dishes. The dishes were placed in a humidified incubator at 37°C with 5% CO_2 for 1-2 h, then the growth culture medium (DMEM containing 10% fetal bovine serum, 100 μg/ml streptomycin, 100 units/ml penicillin, and 0.05 μg/ml amphotericin B) was replaced with fresh medium. The

medium was replaced twice per week. In order to remove non-neuronal cells, 5-fluorodeoxyuridine (FdU, F0503; Sigma, St Louis, MO) was added to the culture dishes (2 μM FdU/dish) of the enteric neural group on day 4, and after 24 h the medium was replaced by fresh growth medium without FdU. L-glutamate (100 μM) and 5-HT (20 μM) were added to the dishes of the enteric glial group twice per week.

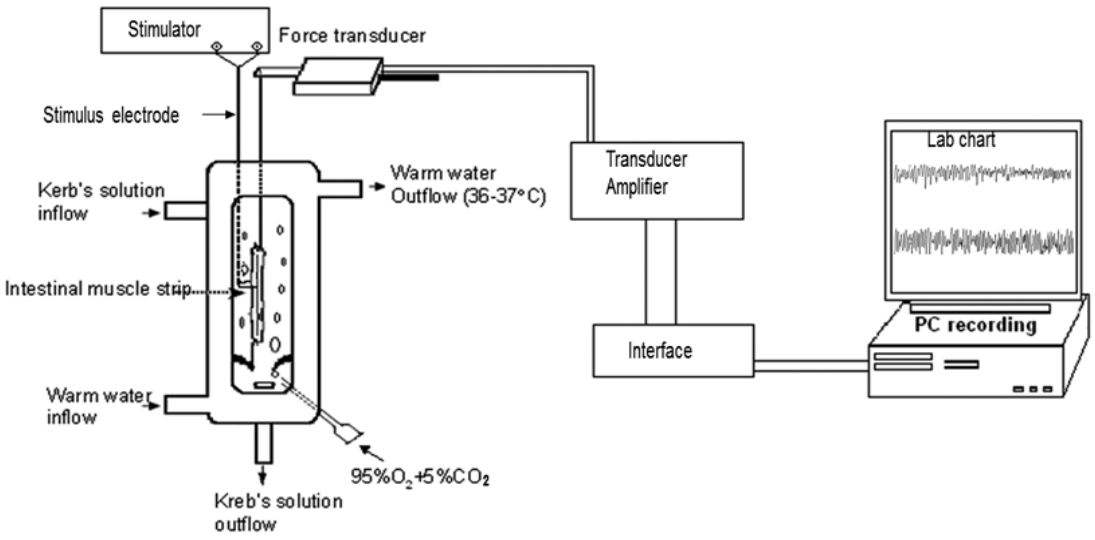

Fig. S1 Modified 4-channel organ bath system consisting of bath chamber, chamber support stand, circulation reservoir, air/gas fitting kit, stimulating electrode, and force transducer. Muscle contraction was measured by the transducer and input into a 4-channel amplifier (TBM4M; WPI). All contractile responses were recorded on a PC *via* the Lab-Trax-4/24T processing system (WPI) or Powerlab 4/30 (AD Instruments). Circular and longitudinal muscle strips were each isolated from human and rat intestinal preparations.

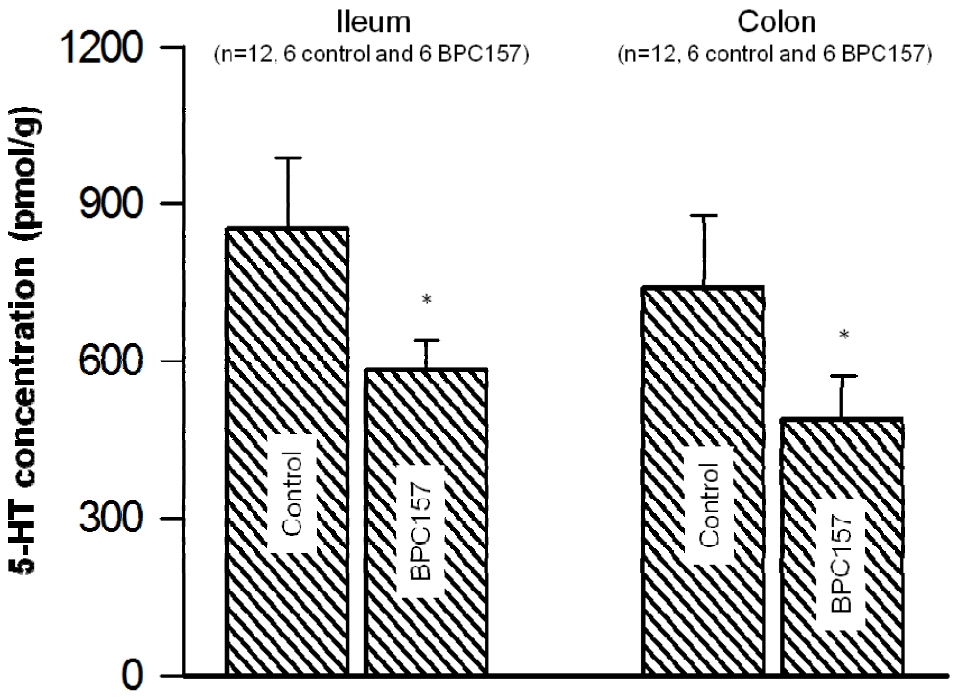

Fig. S2 Application of BPC157 decreases 5-HT concentration in ileal and colonic preparations. All preparations were incubated in Krebs solution with or without BPC157 (100 μmol/L). The 5-HT concentration in the supernatants of tissue homogenates were measured using 5-HT ELISA kits. The data are from 12 ileal and 12 colonic rat preparations. **P* <0.05.

Table S1. Numbers of cultured enteric neurons and EGCs

	Ileum				**Colon**			
	Control		BPS157		Control		BPS157	
	neurons	glia	neurons	glia	neurons	glia	neurons	glia
3rd day	182	248	306	372	237	329	251	323
4th week	35	367	97	595	51	445	79	502
Percentage (%)	19.23	147.98	31.7	159.95	21.51	135.47	31.47	155.42