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Combined administration of secretin and oxytocin inhibits chronic colitis and associated activation of forebrain neurons

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Abstract

Background—The pathogenesis of inflammatory bowel disease is unknown; however, the disorder is aggravated by psychological stress and is itself psychologically stressful. Chronic intestinal inflammation, moreover, has been reported to activate forebrain neurons. We tested the hypotheses that the chronically inflamed bowel signals to the brain through the vagi and that administration of a combination of secretin (S) and oxytocin (OT) inhibits this signaling.

Methods—Three daily enemas containing 2,4,6-trinitrobenzene sulfonic acid (TNBS), which were given to rats produced chronic colitis and ongoing activation of Fos in brain neurons.

Key Results—Fos was induced in neurons in the paraventricular nucleus of the hypothalamus, basolateral amygdala, central amygdala, and piriform cortex. Subdiaphragmatic vagotomy failed to inhibit this activation of Fos, suggesting that colitis activates forebrain neurons independently of the vagi. When administered intravenously, but not when given intracerebroventricularly, in doses that were individually ineffective, combined S/OT prevented colitis-associated activation of central neurons. Strikingly, S/OT decreased inflammatory infiltrates into the colon and colonic expression of tumor necrosis factor-α and interferon-γ.

Conclusions & Inferences—These observations suggest that chronic colonic inflammation is ameliorated by the systemic administration of S/OT, which probably explains the parallel ability of systemic S/OT to inhibit the colitis-associated activation of forebrain neurons.

It is possible that S and OT, which are endogenous to the colon, might normally combine to restrict the severity of colonic inflammatory responses and that advantage might be taken of this system to develop novel means of treating inflammation-associated intestinal disorders.

Keywords

autism; gut-brain signaling; inflammatory bowel disease; interferon- γ (IFNγ); neuropeptides; tumor necrosis factor-α (TNFα)

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Introduction

Bidirectional signaling occurs between the brain and the gut and can disturb function in both organs. Anxiety, for example, often leads to gastrointestinal (GI) discomfort, and GI disorders, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) share a high degree of co-morbidity with psychiatric illness.[1,2] Intestinal discomfort, furthermore, occurs frequently in individuals with an autistic spectrum disorder (ASD).[3–5] Gastrointestinal symptoms occurring in a series of autistic children and adopted orphans have successfully been treated with intensive family nurture. [6,7] Nurture is known to be associated with the release of secretin (S) and oxytocin (OT).[8,9]

Secretin is best known for its role as a duodenal hormone released in response to acidification of the intestinal lumen. [10] Secretin, however, can also activate vagal sensory nerves; [11,12] moreover, S is synthesized within the brain and can activate hypothalamic neurons.[13–16] Mice lacking S receptors, furthermore, exhibit defects in social and cognitive behaviors, suggesting that the central actions of S are significant.[17] Although S treatment was reported to be beneficial in autism and associated GI abnormalities,[18] its efficacy was not confirmed in subsequent clinical trials;[19] moreover, studies have cast doubt on the existence of a specific relationship between autism and IBD. [19] Conceivably, S may have to act in concert with another regulatory hormone.

Oxytocin is best known for its ability to stimulate milk let down and uterine contraction. Oxytocin, however, also promotes adaptive affiliative behaviors that oppose stress. [20–23] Oxytocin also delays gastric emptying and slows intestinal transit. [24–27] Enteric neurons produce OT, and OT receptors have been identified on enteric neurons and the intestinal epithelium. [28] The gastrointestinal effects of OT may be relevant to its behavioral actions; breastfeeding, which is associated with the delivery of significant quantities of OT to neonates,[29] confers long-term protection against GI inflammation. [30]

Because of the ability of S to stimulate the vagi and that of OT to oppose stress, we tested the hypothesis that S and OT in combination modulate transmission of signals from the inflamed bowel to the brain. To do so, chronic intestinal inflammation was induced by rectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS)[31–33] and we determined the effects of subdiaphragmatic vagotomy and S and OT combinations on the inflammation-associated activation of neurons in the paraventricular nucleus of the hypothalamus (PVH), central amygdala (CeA), basolateral amygdala (BLA), and piriform cortex (PIR). Observations suggest that signals from the TNBS-inflamed gut are transmitted to PVH, CeA, BLA, and PIR independently of the vagi; this signaling is antagonized by peripheral, but not central, administration of S/OT, which also decreases the intensity of TNBS-induced colitis.

Methods

Animals

Male Sprague–Dawley rats (250–300 g) were obtained from Hilltop Lab Animals, Inc. (Scottdale, PA, USA) and housed at the New York State Psychiatric Institute Housing Facility. All procedures were approved both by the Institutional Animal Care and Utilization Committees of Columbia University and that of the New York State Psychiatric Institute.

Haptene-induced colitis

After fasting overnight, animals were anesthetized with ketamine [60 mg kg^{-1} intraperitoneally (i.p.)] and xylazine (7 mg kg^{-1} i.p.). A glass microsyringe equipped with a gastric intubation needle was used to administer a 1, 0.5, or 0.5 mL enema of TNBS in

ethanol/PBS (1: 1: 2), respectively, on three consecutive days. The final concentration of TNBS was 1.25%. An equal volume of phosphate buffered saline (PBS) was administered identically to controls.

Systemic S/OT

Four days after the final TNBS/control enemas, animals were anesthetized with ketamine and xylazine. An Alzet™ (DURECT Corp., Cupertino, CA, USA) osmotic pump (delivery rate = 0.25 μL h^{-1}) was implanted intraperitoneally (i.p.) in control and experimental animals to infuse combinations of S, OT, and S/OT (100 μg each; Phoenix Pharmaceuticals, Burlingame, CA, USA) in 250 μL of saline or equivolume saline into the femoral vein for 21 days. Wounds were sutured, anesthesia was discontinued, bacitracin was applied as a topical antiseptic jelly, and animals were returned to individual cages for postoperative care and observation.

Intracerebroventricular S/OT

Four days after the final TNBS administration, animals were anesthetized with ketamine/ xylazine. An Alzet™ osmotic pump was surgically implanted under the neck to infuse equivolume saline or S/OT (50 μg of each peptide in 250 μL of saline; Phoenix Pharmaceuticals) intracerebro-ventricularly (ICV) 0.5 μL h^{-1} for 21 days.

Brain preparation

Animals were euthanized at the treatment endpoint by rapid i.p. injection of ketamine/ xylazine. The animals were then transcardially perfused, sequentially with heparinized physiological saline and a 4% solution of formaldehyde (freshly prepared from paraformaldehyde) in sodium phosphate buffer (pH 7.4). The brains were removed, post-fixed for 2–3 h in the same solution and cryoprotected overnight at 4 °C in a solution of 20% sucrose in 0.1 mol L^{-1} PBS. Frozen sections were cut on a sliding microtome at 30 μm in the transverse plane. Every fourth section was processed immunocytochemically to demonstrate Fos. Control and experimental tissues were processed simultaneously in the same solutions in order to control for potential variability in immunocytochemistry. Incubations were carried out in separate test wells on a rotator table. Tissues were collected in 0.1 mol L^{-1} PBS (pH 7.4) in spot test wells and washed in Tris-buffered saline (TBS) between each step. Non-specific binding sites were blocked by pre-incubating with 1% bovine serum albumin (BSA) in TBS for 30 min. Sections were incubated overnight at room temperature with rabbit primary antibodies to Fos (diluted 1: 10 000) (Oncogene, Cambridge, MA, USA) in TBS containing 0.1% BSA, to which 0.25% Triton X-100 was added to facilitate antibody penetration. Sections were washed with TBS for 10 × 3 min and exposed for 1 h to biotinylated goat antibodies to rabbit IgG (1: 200). Sections were again washed for 10 × 3 min and incubated for 45 min with a preformed complex of avidin-biotin peroxidase (1: 100) (ABC Elite Kit, Vector Labs, Burlingame, CA, USA). Sites of primary antibody binding were visualized by demonstrating peroxidase activity with H_2O_2 and 3, 3′-diamonobenzidine (DAB).

Assessment of Fos activation

Digital images of tissue sections were obtained with a SPOT-RT Slider camera (Diagnostic Instruments, Sterling Heights, MI, USA) mounted on a Nikon Microphot microscope (Nikon Instruments, Melville, NY, USA) interfaced with a Dell Pentium III computer (Dell, Round Rock, TX, USA). Images were not altered except to adjust brightness and contrast. Plates were arranged using Adobe Photoshop CS3 software (Adobe, New York, NY, USA). Topographic distribution and density patterns of immunocytochemically labeled neurons were mapped microscopically. Fos-ir nuclei counts were obtained in the PIR, CeA, BLA,

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and PVH, the boundaries of which corresponded to those of Swanson's Atlas. [34] Quantitative data were collected from images of three sections, located 1.08, 1.53, and 2.00 mm behind the Bregma, using a two-dimensional counting paradigm. The fields through the CeA that were counted measured 0.5 × 0.5 mm, while those through the PVH measured 0.5 × 0.625 mm. The PIR was considered to include three samples, each 0.3 × 0.3 mm, enclosed within an area defined by a line running from the rhinal fissure to the midpoint on the ventral surface of the cortex and which were located within 0.5 mm of the outer edge of the section.

Colon damage and histopathology scores

After euthanasia, the distal 8 cm of the colon was opened longitudinally along the mesenteric border, cleaned of luminal content, and gently rinsed in saline. Segments were blocked for histological assessment. Inflammatory cells infiltrating the colonic mucosa and submucosa were identified morphologically in paraffin sections stained with hematoxylin and eosin (H&E). Two animal histopathologists, who were blinded to the treatment of the animals, scored severity of inflammatory lesions. Submucosal edema, tissue damage/ necrosis, inflammatory cell infiltration, and vasculitis were rated on a scale of 0–3 (0 = none; 3 = severe); perforation was scored as 0 (absent) or 1 (present). [35]

Statistics

Data for counts of Fos-ir nuclei in brain regions, macroscopic, and histological colon damage scores were reported as means ± standard error (SEM). One-way analysis of variance (ANOVA) or Student's *t*-test were used to compare means. Values of $P < 0.05$ were regarded as significant. Calculations were carried out with the assistance of SPSS (Version 15.0; SPSS Inc., Chicago, IL, USA) or GRAPHPAD PRISM software (La Jolla, CA, USA).

Vagotomy

Subdiaphragmatic vagotomy was carried out on rats anesthetized with ketamine/xylazine. A 2 cm incision was made extending from the midline immediately below and parallel to the ribcage. The liver was gently retracted with a Q-tip. Using a stereomicroscope, the right and then left vagus nerves were visualized along the lower esophagus and a length (0.5 cm) of the nerve was excised. In control animals, the incision was made and the vagi were visualized but left intact. Following the vagotomy in experimental animals or the sham procedure in controls, wounds were sutured, anesthesia was discontinued, bacitracin was applied topically to the surgical site and animals were returned to individual cages for postoperative care and observation. Both vagotomized and sham-operated control rats were placed on a liquid diet following vagotomy. The animals were found to lose little weight on the diet, which enabled them to tolerate the procedure without recourse to pyloroplasty. The effectiveness of vagotomy was monitored by demonstrating the accumulation of NADPH diaphorase activity in the neurons of the dorsal motor nucleus of the vagus (Fig. S1). This activity has been demonstrated to increase in the cell bodies of the vagal motor neurons following axotomy.[36]

Reverse-transcriptase polymerase chain reaction (RT-PCR)

Total RNA was isolated from segments of rat gut with Trizol (Invitrogen, Carlsbad, CA, USA) and stored for further use at −80 °C. Complementary DNA (cDNA) was prepared from 3 μg of total RNA by reverse transcription in a 30 μL reaction volume with 0.5 μg random hexamer primers, 0.5 mmol L^{-1} dNTPs, 40 units of RNAsin™, and 400 units of Maloney Murine Leukemia Virus reverse transcriptase (MMLV; Promega, Madison, WI, USA). Real-time PCR was used to quantify enteric transcripts encoding glyceraldehyde 3-

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phosphate dehydrogenase (GAPDH), S, interleukin-6 (IL-6), interferon-gamma (IFNγ), and tumor necrosis factor-alpha (TNFα). Transcripts encoding S, IL-6, IFNγ, and TNFα were normalized to those of GAPDH. The real-time PCR reaction mixture contained 5 μL of the cDNA along with the primers for S, IL-6, IFNγ, and TNFα and PCR master mix [Applied Biosystems (ABI), Foster City, CA, USA]. cDNA levels were quantified using a GeneAmp 7500 sequence detection system (ABI). Duplicate reactions of each standard or sample were incubated for 2 min at 50 °C, denatured for 10 min at 95 °C, and subjected to 40 cycles of annealing at 60 °C for 20 s, extension at 60 °C for 1 min, and denaturation at 95 °C for 15 s. Data were analyzed with computer assistance employing the TAQMAN 7500™ software (Applied Biosystems).

Results

Macroscopic and microscopic evaluation of colonic lesions

The colons of rats given enemas containing PBS (control; Fig. 1A,B) or TNBS (Fig. 1C–F) were fixed and stained with H&E in order to evaluate colonic inflammation. Relatively little residual evidence of inflammation was found in the colons of most of the rats, which were examined 21 days after the last treatment with TNBS. Microscopic damage scores averaged 0.08 ± 0.05, which was not significantly different from 0 and there was no evidence of vasculitis or perforation of the bowel wall. In saline-treated rats that had been subjected to enemas containing TNBS (Fig. 1D–F), however, there was significant evidence of edema (score 0.9 ± 0.1; $P < 0.0001$) along with infiltration of neutrophils and macrophages into the mucosa and submucosa (score 0.5 ± 0.1; $P < 0.001$). Cratered ulcers were observed in 2/8 of TNBS-exposed rats that were treated with saline (Fig. 1D–F).

Considerable fibrosis was evident under the denuded surfaces of the ulcers and an extensive ingrowth of new blood vessels were seen in the adjacent submucosa. Similar ulcers were never observed in the colons of rats that received PBS enemas instead of TNBS or in animals that were subjected to TNBS-containing enemas but which were treated with S/OT, although residual edema was observed in S/OT-treated animals (0.8 ± 0.1; $P < 0.0001$). No residual ulcers were detected in any of 11 TNBS-treated colons after infusion of S/OT (score 0; $P = 0.001$; Fisher's exact test) *vs* control rats in which TNBS-induced colitis was treated with saline ($n = 8$). The edema score in S/OT-treated rats was not significantly different from that found in the colons of animals given PBS-containing enemas. Secretin/OT infusion, however, significantly reduced the infiltration of the mucosa and submucosa by neutrophils and macrophages (Fig. 2; $P < 0.01$ *vs* infusion of saline). These observations suggest that inflammatory infiltrates and edema were still present in the colons of rats 21 days after exposure to TNBS and a subset had residual ulcers, although other histological signs of inflammation had resolved. After infusion of S/OT there was a significant reduction in inflammatory infiltrates and residual ulcers.

S/OT combined treatment reduces pro-inflammatory cytokines TNFα and IFNγ

Further studies were carried out to determine whether an indicator that was more sensitive than histological scoring might confirm whether or not residual inflammation was still present in the gut 3 weeks after administration of TNBS enemas. Expression of the pro-inflammatory cytokines TNFα, IFNγ, and IL-6 was therefore quantified 3 weeks after subjection of the animals to enemas containing PBS or TNBS. Infusions of saline (control) or S/OT were then given for 3 weeks to each group of rats. Transcripts encoding TNFα and those encoding IFNγ were each found to be significantly more abundant in the colons of rats exposed to TNBS ($n = 8$) than in those exposed to saline ($n = 7$) (Fig. 3A,B). However, the abundance of transcripts encoding IL-6, which is known to decrease in chronic TNBS-induced colitis, [37] was not significantly different in the two groups of rats (Fig. 3C). These

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data suggest that, despite the relatively normal macroscopic and microscopic damage scores, significant inflammation remains in the colon 3 weeks following administration of TNBS. The infusion of the S/OT combination reduced the abundance of transcripts encoding TNFα and those encoding IFNγ to the level found in the control animals that received saline enemas (Fig. 3A,B), but had no significant effect on the abundance of transcripts encoding IL-6 (Fig. 3C). When infused individually, neither infusion of S ($n = 4$) nor that of OT ($n = 4$) was able to reduce the abundance of transcripts encoding TNFα (Fig. 3D), IFNγ (Fig. 3E), or IL-6 (Fig. 3F) in the colons of rats subjected to enemas containing TNBS.

Colitis-induced excitation of central neurons

The ability of TNBS-induced colitis to activate neurons in the hypothalamus, amygdala, and piriform cortex has previously been reported. [38] 2,4,6-trinitrobenzene sulfonic acid-containing enemas were administered to rats, which were then treated for 3 weeks with saline or the S/OT combination and Fos-IR was demonstrated (Fig. 4A,B). The numbers of neurons with Fos-ir nuclei were then determined in the PVH (Fig. 4C), CeA (Fig. 4A,B,D), BLA (Fig. 4E), and PIR (Fig. 4F). Systemic treatment of rats with the S/OT combination instead of saline reduced the number of Fos-ir neurons in these areas.

The S/OT combination, therefore, reduces both the manifestations of inflammation in the intestine and the accompanying excitation of central neurons 3 weeks after the induction of chronic TNBS-induced colitis. Despite the relatively normal appearance of the colons of the animals 3 weeks after the induction of colitis with TNBS, therefore, the brains still display evidence of neuronal excitation, which is sensitive to reduction by systemic administration of S/OT. In contrast to systemic treatment, ICV administration of S/OT failed to alter the numbers of neurons with Fos-immunoreactivity in any of the brain regions examined (Fig. 5).

Vagotomy

Bilateral subdiaphragmatic vagotomy and sham operations were carried out to determine whether the vagi carry the signals generated by an inflamed colon to the brain. Again, the numbers of neurons with Fos-ir nuclei were determined in the PVH, CeA, BLA, and PIR. Vagotomy did not reduce the number of neurons displaying nuclear Fos immunoreactivity significantly below that found in rats receiving sham operations in any of these regions (Table S1). Interestingly, vagotomy itself increased the excitation of neurons, as evidenced by their Fos-ir nuclei, in the BLA (Fig. 6; $P < 0.04$). These observations suggest that inflammation associated signaling is transmitted from the colon to the brain via a pathway that is not totally vagus-dependent. The data are also consistent with the idea that vagal activity constitutively inhibits activity in the BLA, which then increases when the vagi are cut.

Secretin in the colon

Transcripts encoding S were found in the colon; however, their abundance in the duodenum was >20-fold greater than in the colon (the ratio to GAPDH was 550 ± 63 in the duodenum and 25 ± 5; $P < 0.0001$).

Discussion

Prior studies established that chronic TNBS-induced inflammation in the colon activates neurons in brain regions involved in emotion and autonomic regulation. [38] Although the systemic treatment of animals with S and OT was not found to influence the inflammation-associated activation of central neurons when these agents were administered individually, treatment with the peptides in combination was not evaluated. The current study was carried

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out to determine whether the systemic administration of a S/OT combination would oppose the colitis-associated activation of neurons in PVH, CeA, BLA, and PIR. Systemic, but not ICV, administration of S/OT to rats with TNBS-induced colitis significantly reduced the numbers of Fos-ir neurons in the PVH, CeA, BLA, and the PIR. The efficacy of the S/OT combination in inhibiting the colitis-associated activation of neurons in the brain thus appears to be related to peripheral effects of these hormones. Because vagotomy did not prevent colitis-associated activation of neurons in any region, it seems unlikely that S/OT inhibits the activation of central neurons by interfering with vagal afferents. It seems more likely that S/OT's ability to decrease colitis-associated activation of central neurons is related to its parallel ability to inhibit chronic TNBS-induced colitis. Although neither S nor OT, by itself, modulates the severity of chronic TNBS-induced colitis, the S/OT combination reduces the number of residual ulcers, the degree to which neutrophils and macrophages infiltrate the intestinal mucosa, and the expression of the pro-inflammatory cytokines, TNFα and IFNγ. The ability of systemic S/OT to attenuate TNBS-induced colonic inflammation thus provides an adequate explanation for the ability of systemic S/OT to oppose the colitis-associated activation of neurons in the brain.

The ability of enteric neuropeptides/neurotransmitters to influence intestinal inflammation is well known. [39] Some, such as vasoactive intestinal peptide (VIP), exert anti-inflammatory effects.[40–45] Secretin is a member of the same peptide family as VIP. Receptors, both for S and VIP (VPAC1), are family B class II guanine nucleotide-binding proteins that are coupled to the production of cAMP. [46,47] VPAC1, which binds VIP with high affinity, also binds S, albeit with lower affinity. [48,49] Similarly, the S receptor binds S and VIP, but with equal affinity. [48,50] VPAC1 and S receptors, moreover, form oligomeric complexes when co-expressed [51,52] and both utilize 'a hidden endogenous agonist' within the receptor that is exposed when the receptor binds a ligand. [53] Although the conformation of the VPAC1 ectodomain normally restricts access of S to the binding site, it is possible that conditions of inflammation facilitate an interaction of S with VPAC1.[49] The resultant action of S on VPAC1 could be anti-inflammatory. Alternatively, the activation of shared signal transduction pathways downstream of cAMP may allow S to amplify the anti-inflammatory effects of endogenous VIP. The OT receptor is also positively coupled to the generation of cAMP; [54] therefore, the synergy observed in the combined administration of S/OT may be a phenomenon of cross-talk at the signal transduction level.

Little is currently known about the colonic functions of S or OT; nevertheless, S, [55] OT,[56] the S receptor, [28,46,57] and the OT receptor [28] are all endogenous to the colon. Secretin is produced in the duodenum by the 'S' subtype of enteroendocrine cell, but these cells are rare [58] or absent [57] in adult colon. Colonic S cells are more common during development. [55] Transcripts encoding S were found in the colon; however, their duodenal abundance was >20-fold that of the colon. Lymphocytes, which are abundant in the colon, express both S and its receptor; moreover, transgenic ablation of S-expressing lymphocytes leads to the sudden onset of colitis. [59] This colitis is associated with the specific loss of the S-expressing subset of $CD4^{+}$ lymphocytes, which has been postulated to be the cause of the colitis. Colitis, however, could equally well be caused by the concurrent loss of S that is expressed by the subset of colonic enterochromaffin cells that derive from progenitors common to S cells. [55] The importance of duodenal S in the regulation of pancreatic ductal secretion is very well known, as is its ability to stimulate vagal afferent nerves. [60–62] Neither of these properties would seem to account for the expression of S in the colon. The known functions of OT in milk let-down and uterine contraction also fail to account for its colonic presence. Within the colon, OT is present in neurons, primarily in the myenteric plexus, but OT receptors are epithelial and neuronal. [28] It is conceivable that endogenous S/OT interact to exert an anti-inflammatory effect that restrains inflammation in the colon. This possibility is

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supported by the very small amount of exogenous S and OT that have to be combined to oppose inflammation.

Microbial provocation of innate and acquired immunity is intense in the colon; [63–65] therefore, a multiplicity of regulatory mechanisms, perhaps involving S/OT, might have evolved in that organ to prevent excessive inflammation-induced tissue damage. Oxytocin receptors, moreover, are expressed selectively in junctional complexes of intestinal epithelial cells, [28] a location that is consistent with the possibility that OT receptor signaling is involved in the maintenance of the colonic epithelial barrier. Such an action might impair the translocation of microbes from the lumen to the intestinal wall. [66] The sudden onset of colon inflammation that accompanies ablation of S-expressing cells is consistent with the possibility that S, which promotes growth of the intestinal epithelium in the duodenum and might also do so in the colon, [67] interacts with OT to help maintain mucosal integrity. An interaction between endogenous S and OT in the colon might contribute to the prevention of IBD.

Both OT[68] and S receptors[59] are expressed by T lymphocytes. Their effects on lymphocytes have not been extensively studied; nevertheless, the lymphocytic expression of these receptors is consistent with the possibility that immune or inflammatory responses could be affected directly by an action of S/OT on lymphocytes. Whether or not endogenous S and OT combine to regulate mucosal integrity or inflammatory responses, it is of considerable interest that the anti-inflammatory effect of the combination of these agents might be useful in the treatment of IBS or IBD.

Because vagotomy failed to decrease the colitis-associated activation of central neurons, it is apparent that the vagi are not solely responsible for this signaling. The vagus nerves have previously been shown to modulate inflammation and vagotomy to aggravate colitis. [69] Vagal afferents protect against TNBS-induced colitis [70] and vagal efferents modulate immune effectors. [71] In fact, we observed a significant increase in neurons displaying activated Fos in the BLA after vagotomy. This increase in activity may well be due to the loss of vagal attenuation of the colitis. Whatever effect the vagi exert on the colon, however, it remains clear that the persistence of central signaling from the colon following vagotomy rules the vagus nerves out as the sole conduit of this information to the brain. Although the S/OT combination reduced the colitis-associated activation of neurons within the PVH, CeA, BLA, and PIR, this action cannot be attributed to an effect of these molecules on the vagi. The S/OT combination was ineffective after ICV administration; moreover, the ability of the S/OT combination to inhibit the activation of the brain in response to chronic TNBS-induced colitis would seem logically to follow from the parallel S/OT amelioration of the colitis. Cytokines, such as TNFα, the expression of which is diminished by S/OT administration, cross the blood–brain barrier[72–74] especially in the region of the hypothalamus. [75] Transport of colitis-liberated cytokines from the blood to the brain might thus be responsible for the colitis-associated activation of neurons in the PVH, CeA, BLA, and PIR. Because these regions are concerned with stress adaptation and visceral-emotional responses,[76–80] their activation during chronic colitis could contribute to adverse central manifestations of intestinal inflammation. The anti-inflammatory effects of S/OT would thus seem to be centrally beneficial even if they are the result of a peripheral action.

In conclusion, our experimental data shows that administration of S and OT in combination inhibits chronic colitis. Anti-inflammatory efficacy, manifested as significant reductions in the colon of leukocyte invasion and expression of the inflammatory cytokines, TNFα, and IFNγ, requires that S/OT be combined because neither hormone individually reduces leukocytic infiltration or cytokine expression. Because intracerebroventricular administration of S/OT did not affect colitis or the activation of central neurons consequent

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to it, the anti-inflammatory actions of S/OT are peripheral, not central. Both S and OT, and their receptors, are endogenous to the colon. These observations are thus consistent with the ideas that colonic S and OT might normally combine to restrict the severity of inflammation and that advantage could be taken of this system to develop novel means of treating IBD or other inflammation-associated intestinal disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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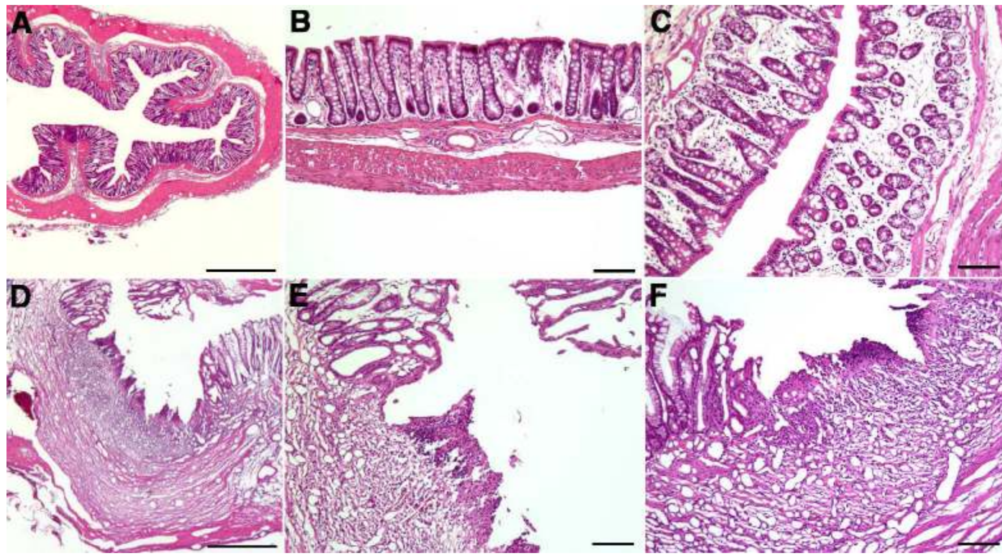

Figure 1.

Moderate inflammation is morphologically detectable in the colons of rats 3 weeks following intra-colonic adminstration of 2,4,6-trinitrobenzene sulfonic acid (TNBS). Animals received enemas containing phosphate buffered saline (PBS, control) or TNBS and were treated for 3 weeks with saline administered via an osmotic minipump. (A) Control colon. No evidence of inflammation is apparent. The marker = 500 μm. (B) Control colon. There is no evidence of mucosal disruption, edema, or infiltration of leukocytes. The marker = 100 μm. (C) Colon subjected to TNBS enemas. There is mucosal edema but the epithelium is intact. The marker = 100 μm. (D, E) Colon subjected to TNBS enemas. A large ulcer disrupts the mucosal epithelium. Fibrosis and infiltration of leukocytes are evident in the ulcer crater. The markers D = 500 μm; E = 100 μm. (F) Colon subjected to TNBS enemas. Another ulcer is illustrated. Again the epithelium is disrupted and the presence of many new blood vessels (empty of blood because the preparation was fixed by perfusion) indicates that the ulcer is a chronic one. The marker = 100 μm.

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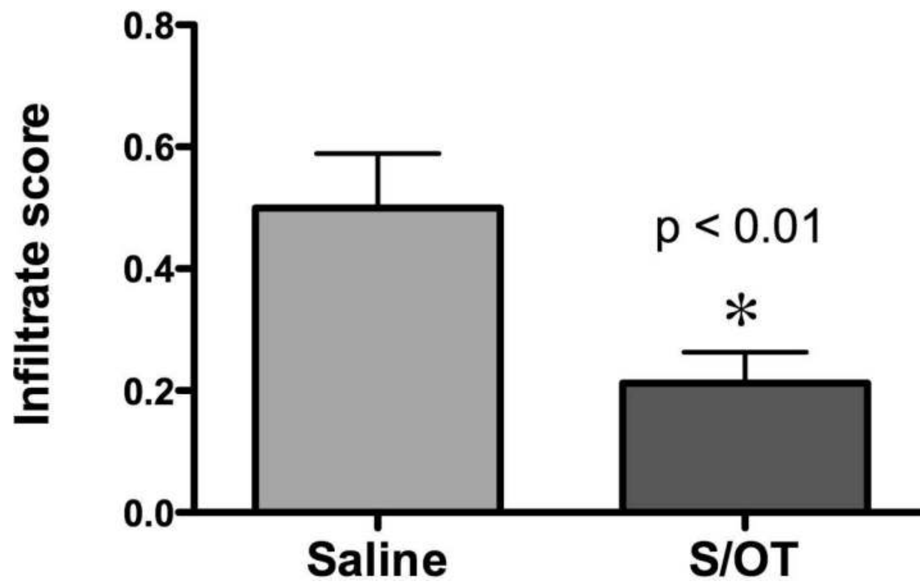

Figure 2.

Treatment of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis with the secretin/ oxytocin (S/OT) combination reduces the extent of mucosal infiltration with leukocytes. Two pathologists who were blinded as to the treatment scored the degree to which the colonic wall was infiltrated with neutrophils and macrophages. Animals that were subjected to TNBS-induced colitis were treated either with saline ($n = 8$) or with the S/OT combination ($n = 11$) and the resulting infiltration scores were compared. Treatment with S/ OT significantly reduced the inflammation scores (*).

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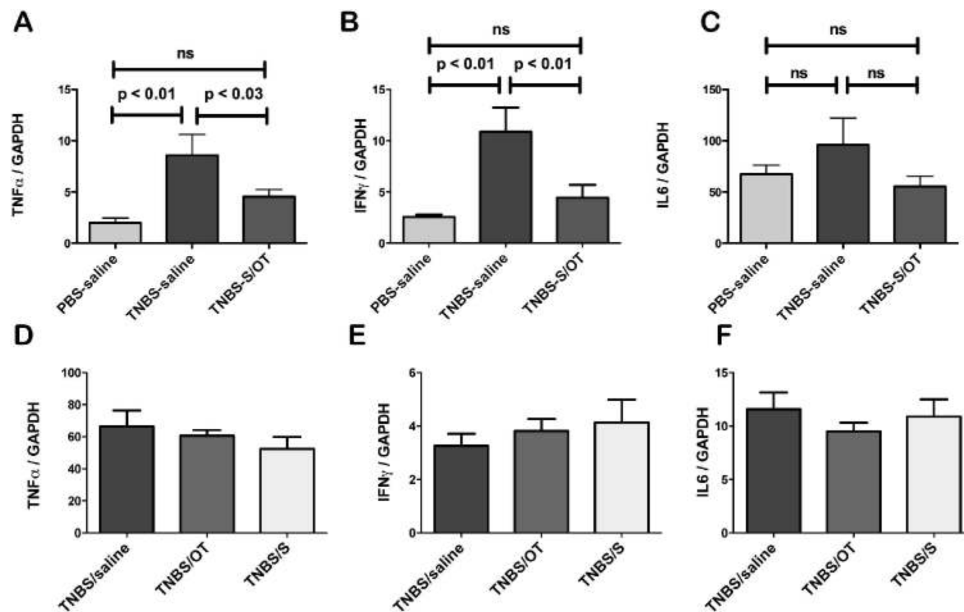

Figure 3.

Combined, but not individual, treatment with secretin (S) and oxytocin (OT) inhibits the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-colitis evoked increase in expression of pro-inflammatory cytokines. (A–C) Animals were given enemas containing phosphate buffered saline (PBS, control) or TNBS and treated with saline or S/OT in combination. (A) TNBS-induced colitis is associated with a significant increase in the abundance of transcripts encoding TNFα; treatment with S/OT prevents this increase. (B) TNBS-induced colitis is associated with a significant increase in the abundance of transcripts encoding IFNγ; treatment with S/OT prevents this increase. (C) TNBS-induced colitis is not associated with a significant change in the abundance of transcripts encoding IL-6; treatment with S/OT does not alter this abundance. (D–F) All animals were subjected to enemas containing TNBS. Treatment with saline was compared with that with OT or S, which were administered individually. No significant differences were found in the abundance of transcripts encoding TNFα (D), IFNγ (E) or IL-6 (F) between animals treated with saline, OT, or S, when these hormones were administered individually.

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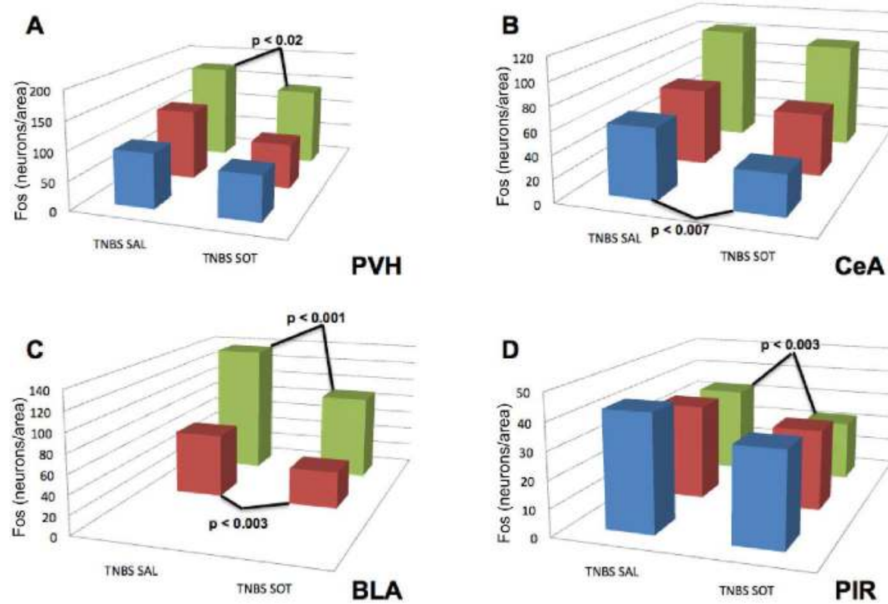

Figure 4.

Treatment with the systemic secretin/oxytocin (S/OT) combination inhibits the colitis-evoked activation of neurons in regions of the brain concerned with arousal (PVH), visceral nociception (CeA), reward (BLA), and odor aversion (PIR). Animals were subjected to 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis and treated with saline or the S/OT combination. Fos immunoreactivity was demonstrated and the numbers of Fos-immunoreactive neurons/unit area was determined at multiple levels (blue bar = level 23/anterior, red bar = level 25, green bar = level 27/posterior) in four regions of the brain. (A) TNBS-induced colitis activates Fos in neurons of the CeA. (B) Systemic treatment with S/OT decreases the number of neurons in the CeA in which Fos is activated in response to TNBS-induced colitis. The density of activated neurons in saline-treated animals was quantified and compared with that of S/OT-treated rats at each of the four levels of the brain that were analyzed. Significantly fewer neurons with Fos-ir neurons were found in at least one level in each of the four brain regions examined. (C) PVH; (D) CeA; (E) BLA; (F) PIR.

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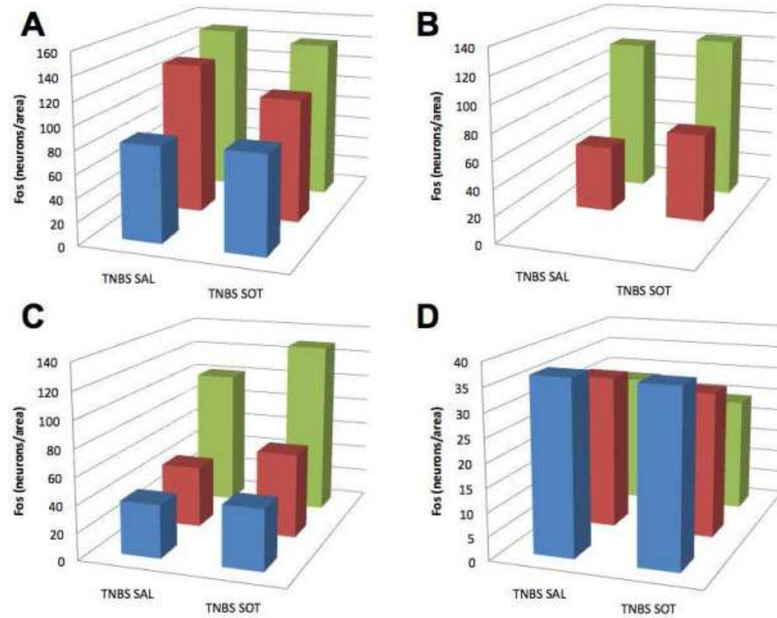

Figure 5.

Intracerebro-ventricular (ICV) administration of the secretin/oxytocin (S/OT) combination does not inhibit the colitis-evoked activation of neurons in any of the regions of the brain where activation was opposed by the systemic administration of S/OT. Animals were subjected to 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis and treated with ICV saline or S/OT. Fos immunoreactivity was again demonstrated and the numbers of neurons/unit area was determined at multiple levels. The density of activated neurons in ICV saline-treated animals was compared with that of ICV S/OT-treated rats at each level. No significant differences were found in any of the brain regions that were examined. (A) PVH; (B) CeA; (C) BLA; (D) PIR.

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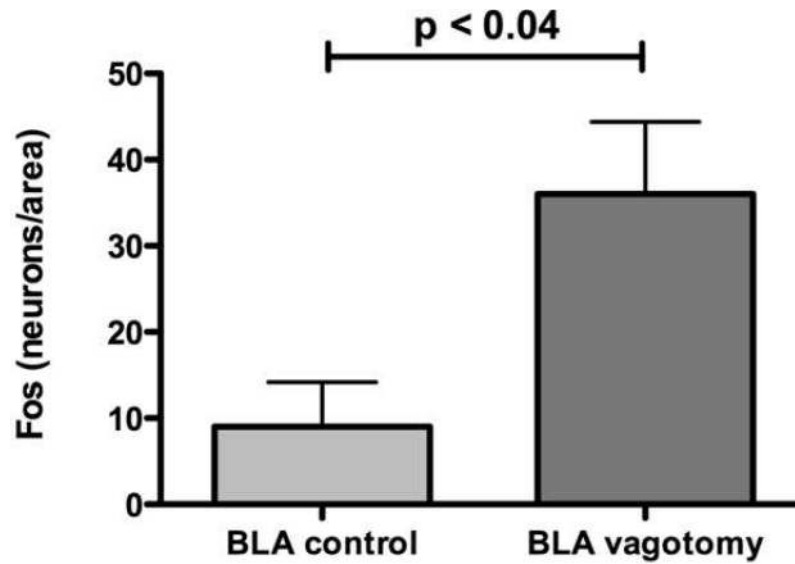

Figure 6.

Animals were subjected to 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis and to vagotomy (n = 4) or sham (n = 4) operation. Fos immunoreactivity was again demonstrated and the numbers of neurons/unit area was determined at multiple levels. Except in the anterior basolateral amygdala (BLA), no significant differences were found in the brain regions that were examined. Unlike the paraventricular nucleus of the hypothalamus (PVH), central amygdala (CeA), and piriform cortex (PIR), the density (number/unit area) of Fos-ir neurons at this level of the basolateral amygdala (BLA) was significantly increased, not decreased by subdiaphragmatic vagotomy, suggesting that tonic vagal inhibition of the activity of neurons occurs in the BLA.