The Vanilloid Receptor Initiates and Maintains Colonic Hypersensitivity Induced by Neonatal Colon Irritation in Rats

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Background & Aims: **Robust chemical or mechanical irritation of the colon of neonatal rats leads to chronic visceral hypersensitivity. The clinical and physiologic relevance of such noxious stimulation in the context of human irritable bowel syndrome is questionable. The aims of this study were to determine whether mild chemical irritation of the colon of neonatal rats produced persistent changes in visceral sensitivity and to evaluate the role of transient receptor potential vanilloid 1 (TRPV1) in the initiation and maintenance of visceral hypersensitivity.** ***Methods:*** **Ten-day-old rat pups received an intracolonic infusion of 0.5% acetic acid in saline. TRPV1 inhibitors were administered 30 minutes before acetic acid sensitization. Sensitivity of the colon to balloon distention (CRD) in adults was measured by grading their abdominal withdrawal reflex and electromyographic responses. In adult rats, TRPV1 antagonist was injected intraperitoneally 30 minutes before CRD.** ***Results:*** **Neonatal acetic acid treatment resulted in higher sensitivity to CRD in adult rats compared with controls in the absence of histopathologic signs of inflammation. Treatment of colons of adult rats with acetic acid did not produce persistent sensitization. Antagonism of the TRPV1 before neonatal administration of acetic acid and after established visceral hypersensitivity attenuated sensitivity to CRD. TRPV1 expression was increased in dorsal root ganglia–containing colon afferent neurons.** ***Conclusions:*** **We have described a new model for persistent colonic sensory dysfunction following a transient noxious stimulus in the neonatal period and a potentially important role for TRPV1 in initiation and maintenance of persistent visceral hypersensitivity.**

Irritable bowel syndrome (IBS) is defined by the occurrence of intermittent periods of abdominal pain and altered bowel habits in the absence of observable biological abnormalities.[1–3] Although no pathogenic mechanisms have been defined, human studies show that IBS is associated with a state of chronic visceral hypersensitivity, but the mechanisms responsible for the generation and maintenance of visceral hypersensitivity in patients with IBS are not known.[4–7] A popular theory is that IBS has its roots early in life with various factors being implicated, including psychological stress, parental influence, physical/social abuse, dietary and/or chemical intolerance, and infections.[8–10] The diversity of these factors suggests that the early life period may be associated with an inherent predisposition of the gastrointestinal tract and associated nervous elements to develop long-term changes in response to transient stressors. This concept is partly supported by animal models that have utilized either maternal deprivation or inflammation of the colon as initiating events in neonatal animals.[11–14] Our laboratory has previously described an animal model of chronic visceral hypersensitivity based on mechanical and chemical irritation of the colon of neonatal rats.[11] However, the magnitude of the insults used in that study (balloon distention or mustard oil) were capable of producing robust inflammation and/or injury. The clinical and physiologic relevance of such noxious stimulation in the context of human IBS is questionable.

The aims of this study were to further characterize the phenomenon of neonatal vulnerability to colonic sensitization by using a much milder chemical irritation of the colon (dilute acetic acid) in neonatal rats and to examine the long-lasting changes, if any, in visceral sensitivity. Further, we wished to determine the molecular basis for the induction as well as maintenance of this sensitization, focusing our initial studies on the vanilloid receptor, transient receptor potential vanilloid 1 (TRPV1), based on recent studies supporting a role for it in mechanosensation and in mediating mechanical hypersensitivity of visceral afferents. TRPV1 knockout mice exhibit decreased mechanical and acid sensitivity of jejunal afferents[15] and decreased mechanical sensitivity and impaired chemical sensitization of colonic afferents.[16] The

Abbreviations used in this paper: ANOVA, analysis of variance; AWR, abdominal withdrawal reflex; CRD, colorectal distention; DII, 1,1′-dioleyl-3,3,3′,3-tetramethylindocarbocyanine methanesulfonate; DRG, dorsal root ganglia; EMG, electromyographic; IBS, irritable bowel syndrome; IFN, interferon; IL, interleukin; I-RTX, 5′-iodoresiniferatoxin; LS, lumbar sacral; MPO, myeloperoxidase; PFA, paraformaldehyde; P10, 10-day-old rat pups; TL, thoracolumbar; TRPV1, transient receptor potential vanilloid 1.

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TRPV1 antagonist capsazepine inhibited colon afferent fiber responses to stretch[16] and jejunal afferent responses to acid.[15] In the rat, TRPV1 immunoreactivity is detected on 82% of thoracolumbar (TL) and 50% of lumbar sacral (LS) colonic dorsal root ganglia (DRG) neurons[17] and is observed in nerve fibers within the myenteric ganglia, the muscle layers, and the mucosa of the colon.[18] However, the role of this receptor in persistent visceral hypersensitivity in vivo is not known. We therefore wished to determine whether antagonism of TRPV1 before neonatal administration of acetic acid attenuates or prevents the development of sensitivity to colorectal distention (CRD) Our results suggest that the neonatal colon is uniquely capable of being sensitized even by stimuli that do not cause overt inflammation or injury; further, TRPV1 plays a significant role in both the initiation and the maintenance of this sensitization. These findings have implications for the pathogenesis of IBS in humans.

Materials and Methods

Male Sprague–Dawley rats were used in all experiments. The Institutional Animal Care and Use Committee of the University of Texas Medical Branch approved procedures performed on animals.

Colonic Sensitization

For neonatal sensitization, rats were obtained as litters of 4-day-old pups. Ten-day-old rat pups (P10) received an infusion of 0.2 mL of 0.5% acetic acid solution in saline into the colon 2 cm from the anus, and controls received an equal volume of saline. To treat neonatal rats with capsaicin, 10-day-old rat pups received an infusion of 0.2 mL of 50 μg/mL capsaicin or 0.2 mL vehicle (10% ethanol, 10% Tween 20, 80% saline) or were left unmanipulated. Visceral sensitivity and inflammatory parameters were measured in these rats between 8 and 12 weeks of age. For adult sensitization, 8-week-old rats were administered 1 mL each of a 5% solution of acetic acid in saline into the descending colon; controls received saline. After 4 weeks, rats were tested for sensitivity to CRD.

Visceral Sensitivity

In rats, high-intensity CRD represents a noxious visceral stimulus that produces both aversive behaviors and visceromotor responses (contraction of the abdominal and hind limb muscles) that are quantifiable and reproducible measures of acute visceral pain.[19] These effects are also observed in humans where colorectal pain is accompanied by cardiovascular and respiratory reflexes and by increases in the tension of abdominal wall muscles.[20] In initial studies, we used a grading system based on the abdominal withdrawal reflex (AWR) as well as a measure of the electromyographic (EMG) activity of the external oblique muscle. The AWR represents a characteristic set of behaviors produced by involuntary motor reflexes that are activated by CRD. Visceral hypersensitivity was measured by grading the response of rats to CRD as previously described.[11] Briefly, under mild sedation with 1% methohexital sodium (Brevital; Eli Lily & Co, Indianapolis, IN) 25 mg/kg intraperitoneally, a flexible balloon (5 cm) constructed from a surgical glove finger attached to a Tygon tubing was inserted 8 cm into the descending colon and rectum via the anus and held in place by taping the tubing to the tail. Rats were placed in small Lucite cubicles (20 $\times$ 8 $\times$ 8 cm) (Bioengineering Department, University of Texas Medical Branch, Galveston, TX) and allowed to adapt for 30 minutes. CRD was performed by rapidly inflating the balloon to constant pressure. Pressure was measured using a sphygmomanometer connected to a pressure transducer. The balloon was inflated to various pressures (10, 20, 30, 40, 50, 60, 70, and 80 mm Hg) for a 20-second stimulation period followed by a 2-minute rest. Behavioral responses to CRD were measured by visual observation of the AWR by blinded observer and the assignment of an AWR score as follows: 1, normal behavior without response; 2, contraction of abdominal muscles; 3, lifting of abdominal wall; 4, body arching and lifting of pelvic structures.

To obtain EMG measurements of visceromotor responses, under anesthesia with pentobarbital sodium 50 mg/kg intraperitoneally (Nembutal; Abbott Laboratories, North Chicago, IL), 2 electrodes were implanted in the external oblique muscle and externalized behind the head. Rats were allowed 1 week to recover from the surgery. CRD was performed as described previously with 20 seconds of distention followed by 2-minute rest between distentions of 20, 40, 60, and 80 mm Hg. EMG was recorded continuously during the experiment on a Biopac Systems EMG 100 C, MP100A-CE (Biopac Systems, Inc, Santa Barbara, CA). The EMG signal was amplified, filtered at 300 Hz, and digitized using Acknowledge (Biopac Systems, Inc). The area under the curve for the EMG signal (during each 20 seconds of distention plus 10-second postdistention period for a total of 30 seconds) was calculated using an in-house written computer program. The net value for each distention was calculated by subtracting the baseline value derived from the average area under the curve (30-second interval) for the 2-minute predistention period.

Evaluation of the Colon for Inflammation/Damage

Colons were divided into proximal, middle, and distal segments; portions of each segment were placed in 10% buffered formalin, and portions were frozen in liquid nitrogen. H&E-stained paraffin sections were scored for inflammation by a pathologist as described.[21] Myeloperoxidase (MPO) assays were performed as described.[22] Protein was measured by the bicinchoninic acid method (Pierce, Rockford, IL). Activity was expressed as the change in absorbance $\cdot$ min^{-1} $\cdot$ mg protein^{-1}. Cytokine levels were measured in colon extracts using the BioRad