○ Special Article

J.J. Bonica Lecture—2000: Physiology, Pathophysiology, and Pharmacology of Visceral Pain

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Background and Objectives: The principal objective of this report is to review recent experimental advances in our understanding of the physiology and pathophysiology of visceral pain and to describe results of studies of opioid modulation of visceral nociception.

Methods: Results are drawn from electrophysiological studies of single pelvic nerve afferent fibers innervating the descending colon in the rat.

Results: The important findings include the following: identification of a subset of pelvic nerve fibers that likely function to signal acute visceral pain, sensitization of mechanosensitive pelvic nerve fibers, and documentation of the presence of silent nociceptors in the pelvic nerve. With respect to pharmacological modulation of pelvic nerve fiber responses to colonic distension, only kappa-opioid receptor agonists, and not mu- or delta-opioid receptor agonists, were effective.

Conclusions: All pelvic nerve fibers innervating the descending colon can be sensitized and contribute to visceral pain; their responses are modulated by kappa-opioid receptor agonists acting in the periphery. *Reg Anesth Pain Med 2000;25:632-638.*

Key Words: Kappa opioids, Mechanoreceptors, Colon distension, Afferent fibers, Polymodal, Antisense.

Visceral pain, particularly visceral hyperalgesia such as that associated with the functional bowel disorders, is poorly understood. Functional bowel disorders like irritable bowel syndrome (IBS) are characterized by pain and discomfort and enhanced sensitivity to gastrointestinal stimuli in the absence of tissue injury or inflammation. Visceral hyperalgesia thus differs from somatic hyperalgesia, which is commonly associated with tissue injury and inflammation. Visceral hyperalgesia could develop and be maintained entirely by either peripheral or central mechanisms. This overview of research from the author's laboratory will focus on examination and characterization of peripheral contributions to the development and maintenance of visceral hyperalgesia as well as peripheral opioid modulation of visceral nociception.

Functional bowel disorders exhibit multiple characteristics that suggest the presence of visceral hyperalgesia. For example, Ritchie[1] documented in 1973 that IBS patients reported pain at lower volumes of colonic distension than did normal subjects. As illustrated in Fig 1, there is a leftward shift of the psychophysical function in IBS patients. Fewer than 10% of normal subjects reported pain at a distending volume of 60 mL, whereas greater than 50% of IBS patients reported pain at the same distending volume. Qualitatively similar outcomes have been confirmed and extended to other hollow viscera in patients with other functional bowel disorders (noncardiac chest pain and nonulcer dyspepsia).[2-4] Accordingly, the altered sensations (bloating, discomfort, and abdominal pain) reported by greater than 50% of patients with IBS or other functional bowel disorders are considered to represent visceral hyperalgesia.[5,6] If this hypothesis is correct, then visceral afferent fibers that innervate these organs should exhibit sensitization. Further, the spinal neurons on which they terminate should undergo a change in excitability. Indeed, there is ample evidence of referred hyperalgesia and expansion of areas of referred sensation in IBS patients,[7] sensitization of visceral sensory afferent fibers and spinal cord neurons[8,9] (see Results), and recruitment of so-called silent or sleeping afferent fibers.[10] Our own work, described in this report, has contributed to better understanding of mechanisms of visceral pain and of visceral hyperalgesia.

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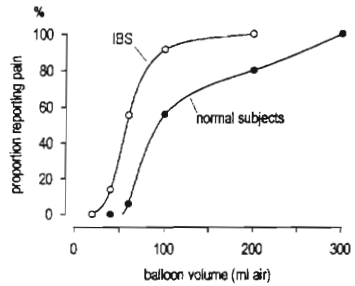

Fig 1. Proportion of normal subjects and of IBS patients reporting pain from balloon distension of the pelvic colon. (Adapted and reprinted from *Gut*, 1973, vol 14, pp 125-132, with permission from the BMJ Publishing Group.[1])

Methods

Reports of experiments described here were obtained in adult male Sprague-Dawley–derived rats (Harlan, Indianapolis, IN). Rats were anesthetized initially with 40 to 45 mg/kg intraperitoneal pentobarbital sodium; anesthesia was maintained during electrophysiological experiments by infusion of pentobarbital (5 to 10 mg/kg/h intravenous [IV]). A femoral artery and vein were catheterized for measurement of arterial pressure and administration of drugs, respectively; the trachea was also cannulated to provide artificial ventilation. Rats were paralyzed with pancuronium bromide. Core body temperature was maintained at 37°C by a hot-water–circulating heating pad placed under the rat and overhead feedback-controlled heat lamps. At the end of experiments, rats were killed by an overdose of IV pentobarbital. The experimental protocols were approved by the Institutional Animal Care and Use Committee of The University of Iowa.

The surgical procedures have been described in detail elsewhere[11] and are only briefly described here. The lower abdomen was exposed by a 3- to 4-cm incision laterally at the left flank. The urinary bladder was emptied and catheterized through the fundus. The urethra was ligated close to its entry to the penis, and urine was constantly evacuated via the fundic catheter. The left testis, vas deferens, and seminal vesicle were tied and removed. The prostate was reflected laterally to access the major pelvic ganglion and pelvic nerve. The pelvic nerve was isolated from surrounding fatty tissue, and a pair of Teflon-coated stainless steel wires stripped at the tips were wrapped around the pelvic nerve and sealed with nonreactive silicone gel. The hypogastric, pudendal, and femoral nerves were isolated and transected. The sciatic nerve was approached through the ischiatic notch and transected. The lateral tail nerve was approached at the root of the tail and transected, and the abdomen was closed with silk sutures.

The lumbosacral spinal cord was exposed by laminectomy and the rat suspended in a stereotaxic frame by thoracic vertebra and ischia clamps. The dura was carefully removed, and the spinal cord was covered with warm (37°C) mineral oil. The S1 and L6 dorsal roots were identified and decentralized close to their entry to the spinal cord. Recordings were made from the distal cut end of the central processes of primary afferent fibers. The dorsal rootlet was split into thin bundles, and a fine filament was isolated from the bundle to obtain a single unit. Electrical activity of the single unit was recorded by placing the teased fiber over one arm of a bipolar silver-silver chloride electrode. Action potentials were monitored continuously by analog delay and displayed on a storage oscilloscope after initial amplification through a low-noise alternating current (AC) differential amplifier. The action potentials were processed through a window discriminator and frequency of impulses was counted online using the spike 2/CED1401 data acquisition program (Cambridge Electronic Design, Cambridge, England). Peristimulus time histograms (1 second binwidth), intracolonic pressure, and blood pressure were displayed on-line continuously and recorded on tape for additional analysis.

For colonic distension, a 6- to 7-cm–long, 2- to 3-cm–diameter flaccid, flexible latex balloon was inserted intra-anally into the descending colon and rectum. When inflated, the diameter of the balloon was greater than the intraluminal diameter of the colon of the rat. Therefore, the pressure measured during distension reflected actual intracolonic pressure. The balloon catheter was connected to a distension control device through a low-volume pressure transducer. Pelvic nerve afferent fibers that responded to colonic distension were studied throughout the dynamic range of distending pres-

sures (5 to 100 mm Hg). Distension was phasic, lasting 30 seconds and repeated at 4-minute intervals. Drugs were administered intravenously or intra-arterially using cumulative-dosing paradigm. Because the preparation was decentralized, drug action was restricted to the periphery.

Results

In studying the mechanosensitive properties of pelvic nerve afferent fibers innervating the rat colon, we found that a large proportion of the sample (~80%) had low thresholds for response, whereas a smaller proportion of the sample (~20%) had high thresholds for response to distension. Figure 2 illustrates the distribution of response thresholds for a sample of pelvic nerve fibers studied in the laboratory. Mean response thresholds for most of the fibers studied are less than 5 mm Hg and, thus, respond well within the physiological range. The smaller proportion of fibers have high response thresholds, responding first at a mean threshold greater than 30 mm Hg. We interpreted this outcome to indicate that there exists in the innervation of the colon (and in subsequent studies of the urinary bladder[12]) a proportion of fibers that respond to acute, noxious intensities of colonic (or urinary bladder) distension.

When the encoding properties of these low-threshold and high-threshold pelvic nerve afferent fibers were studied, it was noted that, as a group, the low-threshold fibers gave greater magnitudes of response at all distending pressures tested and, further, encoded distending pressures well into the noxious range (see Fig 3).

Another interesting finding was that both low-threshold and high-threshold afferent fibers could be sensitized by experimental inflammation of the

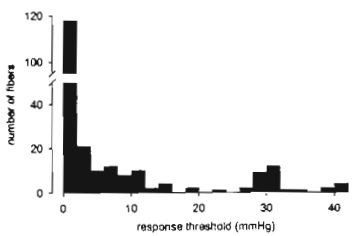

Fig 2. Frequency histogram of extrapolated response thresholds of rat pelvic nerve afferent fibers recorded in the S1 dorsal root to colonic distension. (Data from references 11, 13, and 16.)

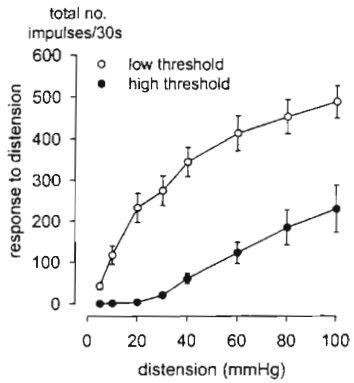

Fig 3. Summary of responses of pelvic nerve afferent fibers to graded intensities of colorectal distension. Two populations of pelvic nerve fibers, low threshold and high threshold, innervate the colon. Low-threshold fibers typically have response thresholds less than 5 mm Hg; high-threshold fibers typically have response thresholds ≥30 mm Hg. Both low- and high-threshold fibers encode distending pressures in the noxious range (>30 mm Hg in the rat). (Data from references 11, 13, and 15.)

colon. That is, as illustrated in Fig 4, response magnitude for both low-threshold and high-threshold pelvic nerve afferent fibers were increased significantly after colonic inflammation and, in some cases, spontaneous activity of these fibers also increased. These outcomes suggested that both low- and high-threshold mechanosensitive pelvic nerve afferent fibers have the ability to contribute to visceral pain. Because over-filling and muscle cramping are the most common acute visceral stimuli, acute pelvic pain likely arises from activation of high-threshold mechanosensitive receptors in organ muscle layers. In functional bowel disorders such as IBS, sensitized low- and high-threshold mechanosensitive muscle endings, as well as mechanically insensitive, so-called silent nociceptors, contribute to the discomfort and pain that characterizes these disorders. An example of a mechanically insensitive pelvic nerve fiber innervating the rat colon is illustrated in Fig 5. These mechanically insensitive fibers are not responsive to the highest intensities of colonic distension (e.g., 100 mm Hg), but acquire spontaneous activity and mechanical sensitivity following colonic inflammation. Accordingly, in the presence of colonic irritation/inflam-

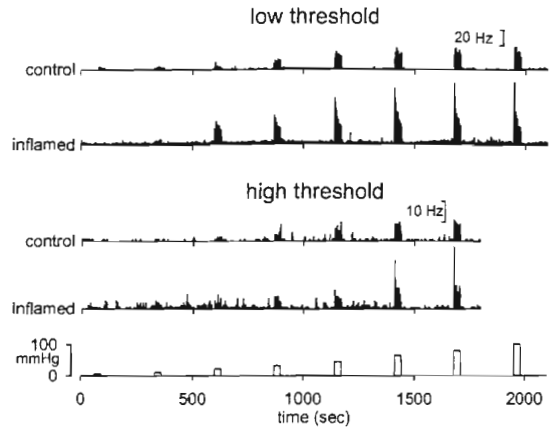

Fig 4. Examples of sensitization of both low-threshold and high-threshold pelvic nerve afferent fibers. Colorectal distending pressures are given below and responses are represented as peristimulus time histograms (1-second binwidth) before (control) and after (inflamed) zymosan instillation into the rat colon. In both examples, spontaneous activity and response magnitude increase after experimental colonic inflammation. (Unpublished data from S. Coutinho and G. Gebhart, 1998.)

mation, there is increased and exaggerated input from the organ to the central nervous system, likely contributing to the altered sensations that characterize the functional bowel disorders.

In other studies,[14] we found that mechanosensitive pelvic nerve fibers innervating the colon were polymodal in character. That is, in addition to mechanosensitivity, many of them were also thermosensitive and/or chemosensitive (Fig 6).

In addition to studying the physiology and pathophysiology of the afferent innervation of the distal gastrointestinal tract, we were also interested in examining means by which the primary afferent input could be modulated, because such a strategy could prove to be useful in the control of discomfort and pain associated with the functional bowel disorders. We studied the effects of receptor-selective opioid receptor agonists (ORAs) on responses of pelvic nerve afferent fibers to colonic distension.[14-16] To our surprise, we found that neither μ-ORAs (e.g., morphine and fentanyl) nor δ-ORAs (DPDPE or SNC-80) had any effect on responses to distension. In contrast, a variety of κ-ORAs tested were capable of dose-dependently attenuating re-

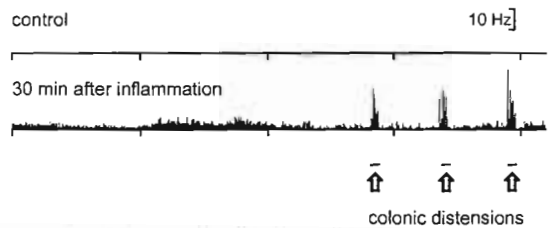

Fig 5. Example of a mechanically insensitive ("silent") pelvic nerve afferent fiber that innervates the colon of the rat. In the absence of tissue insult (control) there is no spontaneous activity or response to colonic distension. After experimental inflammation of the colon with zymosan, there is significant spontaneous activity and also response to distension (60 mm Hg). Data are presented as peristimulus time histograms (1-second binwidth). (Unpublished data from S. Coutinho and G. Gebhart, 1998.)

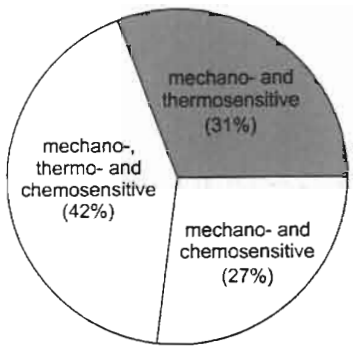

Fig 6. Illustration of the polymodal sensitivity of pelvic nerve afferent fibers innervating the rat colon. Forty-two percent of the fibers studied responded to all 3 modalities of stimulation (mechanical, thermal, and chemical), and 58% of the sample responded to mechanical distension and either thermal or chemical stimuli. (Data from Su and Gebhart.[11])

sponses of pelvic nerve afferent fibers to colonic distension (Fig 7). The evidence provided in Fig 7 and other experimental results suggest that the peripheral receptor at which these κ-ORAs act to modulate visceral nociception differs from the κ-opioid receptor cloned from the central nervous system. For example, Fig 7 shows an unusual clustering of dose-effect functions for the κ-ORAs tested, which was also true for their effects on urinary bladder distension.[17] This receptor in the periphery appears to be opioid-like because naloxone, when administered in a high, nonselective dose, partially antagonizes the effect of κ-ORAs on responses to hollow organ distension. Interestingly, 2 κ-opioid receptor-selective antagonists did not antagonize the effects of any of the κ-ORAs tested. That the effects of the κ-ORAs are peripheral was established in experiments where drugs were administered intracolonically. Again κ- but not μ- or δ-ORAs were effective in attenuating responses to colonic distension when the drug was restricted to the colon.[18]

We subsequently provided evidence that the peripheral site of action of κ-ORAs includes the cell body in the S1 dorsal root ganglion. This site of action was examined by studying opioid effects on high-voltage–activated calcium currents in identified pelvic nerve sensory neurons from the S1 dorsal root ganglion.[19] In these experiments, the dicarbocyanine dye Di-I was injected at multiple sites into the smooth muscle of the colon. Ten to 14 days later, the S1 dorsal root ganglion was removed and Di-I–labeled cells, identified by their red-orange color under Hoffman contrast optics, were recorded in culture using whole-cell patch-clamp methods. Neither morphine, DAMGO, DPDPE, nor SNC-80 (μ- and δ-ORAs) affected high-voltage–activated calcium currents in identified colon sensory neurons. In contrast, κ-ORAs attenuated high-voltage–activated calcium currents in a concentration-dependent manner. These calcium currents were reduced in the presence of N-, P- or Q-, but not L-type calcium channel antagonists. We conclude from these studies that modulation of calcium channel function contributes to the peripheral analgesic action of κ-ORAs in visceral nociception.

Most recently, we have used an antisense oligodeoxynucleotide (ASO) strategy to "knock down" the κ-opioid receptor cloned from the central nervous system.[20] Antisense or mismatch oligodeoxynucleotides were administered into the intrathecal space every 12 hours for 4 days. On the 5th day, the efficacy of ASO treatment was tested by administering μ-, δ-, or κ-ORAs into the rat hindpaw just before the intra-paw injection of 2.5% formalin. After the formalin test, the same rats were anesthetized and prepared for pelvic nerve afferent fiber recording in the S1 dorsal rootlet. Kappa-receptor ASO treatment, but not mismatch oligodeoxynucleotide treatment, blocked the peripheral antinociceptive effects of κ-ORAs; the

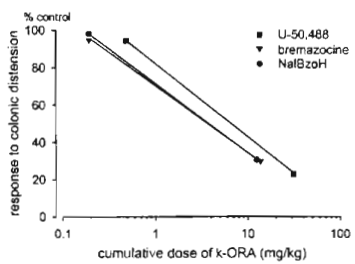

Fig 7. Dose-response functions for δ κ_1-, κ_2-, and κ_3-selective opioid receptor agonists (U-50,488, bremazocine, and NalBzoH, respectively) on responses of pelvic nerve afferent fibers to colonic distension (80 mm Hg). All subtypes of κ-opioid receptor agonists dose-dependently attenuated responses to colonic distension. (Data from Sengupta et al.[13] and Su et al.[18])

peripheral antinociceptive effects of DAMGO (μ-ORA) and deltorphin (δ-ORA) were not affected by either κ-receptor ASO treatment or mismatch oligodeoxynucleotide treatment. In recording experiments in the same rats, the ability of κ-ORAs to attenuate responses of pelvic nerve fibers to colonic distension was not affected by either antisense or mismatch oligodeoxynucleotide treatment. These results indicate that the receptor in the colon at which κ-ORAs act to attenuate visceral pain is not the cloned κ-opioid receptor.

Discussion

The experiments described here, conducted over the past 7 years, have revealed features of visceral sensory neurons that have led to improved understanding of the physiology and pathophysiology of visceral sensory neurons. The key findings include documentation of the presence of both low-threshold and high-threshold mechanosensitive afferent fibers innervating the colon and urinary bladder. Others have reported similar findings for other hollow organs.[21] Interestingly, whereas these low-threshold afferent fibers encode throughout the physiological range, they also appear to encode well into the noxious range and, moreover, give greater magnitude responses than do the high-threshold proportion of mechanosensitive fibers. This finding, in conjunction with the demonstration that all mechanosensitive afferent fibers innervating the colon and bladder can be sensitized after irritation or inflammation, suggests a role for all mechanosensitive afferent fibers in visceral pain. In addition, we documented the presence of so-called silent or sleeping nociceptors in the pelvic nerve innervation of the rat colon, and our estimate is that as much as 30% to 35% of the afferent fiber innervation of the colon could function in this capacity.

Finally, we were able to document what had long been assumed, namely that these mechanosensitive afferent fibers were also thermal and/or chemosensitive.[13] The importance of this latter demonstration relates to the likelihood that normal bowel constituents, in the presence of tissue irritation, could contribute to altered sensations arising from the gut.

With respect to the modulation and control of visceral pain, our work clearly supports an interesting and unique role for κ-ORAs in visceral pain modulation. Because these experiments were conducted in decerebrated preparations, the effects of the drugs administered were restricted to the periphery. We provided further documentation for a peripheral site of action in studies where drugs were administered intracolonically[14] and where drug effects on calcium channels in colon sensory neurons were studied.[19] κ-ORAs have been tested in humans as analgesics. They are not presently available because of undesirable dysphoric effects produced when κ-ORAs gain access to the central nervous system. Accordingly, a peripherally restricted κ-ORA could be a useful drug for modulation of visceral discomfort and pain such as that present in the functional bowel disorders. Unfortunately, we have not been able to identify the receptor at which κ-ORAs act to attenuate responses to colonic, urinary bladder, or gastric afferent fiber responses to distension,[22] but we are confident that it is not the cloned κ-opioid receptor. Additional experiments are required to determine the mechanism by which κ-ORAs act and whether a strategy developed to more specifically interact with that site and mechanism of action would provide an improved analgesic for states of visceral hyperalgesia.

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