Mechanosensitive Pelvic Nerve Afferent Fibers Innervating the Colon of the Rat Are Polymodal in Character

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Su, X. and G. F. Gebhart. Mechanosensitive pelvic nerve afferent fibers innervating the colon of the rat are polymodal in character. J. Neurophysiol. 80: 2632–2644, 1998. This report describes the chemical and thermal sensitivity of mechanosensitive pelvic nerve afferent fibers innervating the colon of the rat. A total of 51 fibers in the S1 dorsal root, identified by electrical stimulation of the pelvic nerve, were studied. An ~7 cm length of descending colon was isolated in situ to permit intracolonic perfusion and distension with Krebs solution. Reproducibility of responses to repetitive colorectal distension (CRD, 40 mmHg, 30 s, every 4 min) was documented. All fibers gave monotonic, incrementing responses to graded CRD (5–60 mmHg). Increases (n = 6) or decreases (n = 6) in pH of the perfusate failed to produce any change in resting activity or responses to CRD. Infusion of bile salts increased the resting activity of six of six fibers in a concentration-dependent manner but did not affect the magnitude of responses to CRD. After intracolonic distension of an inflammatory soup (10⁻³ M bradykinin, 10⁻⁵ M PGE₂, 10⁻³ M serotonin, 10⁻⁷ M histamine, and 10⁻³ M KCl), 13/22 fibers exhibited sensitization of responses to CRD. Seventy-three percent of 45 fibers tested responded to intracolonic perfusion of heated Krebs solution. The estimated threshold for response was 45°C and response magnitude increased with the temperature. A smaller proportion (30%) of 37 fibers tested responded to intracolonic perfusion of cold Krebs solution. The estimated threshold for response was 28°C. Of 36 fibers tested, 8 were activated by both heat and cold; typically, fibers activated by heat did not respond to cold. In a sample of 26 fibers tested for response to all three modalities of stimulation, 11 responded to mechanical, chemical, and thermal stimuli; the remaining 15 responded to mechanical and either chemical or thermal stimulation. Changes in intracolonic pressure in response to chemical and thermal stimuli also were evaluated. Inflammatory soup and bile salts did not change intracolonic pressure; heat and cold produced a modest decrease and increase in muscle tension, respectively. These results document that mechanosensitive pelvic nerve afferent fibers are also chemosensitive and/or thermosensitive, supporting the notion that visceral mechanoreceptors in general are likely polymodal in character.

INTRODUCTION

Sherrington (1906) was the first to note that the cutaneous nociceptors he proposed as detectors of stimuli that threaten or damage tissue were polymodal (“anelective”), although the term polymodal was not used in this context until much later. Bessou and Perl (1969) termed polymodal those C-fiber afferents in cutaneous nerves that responded to mechanical, thermal, and particularly noxious chemical (e.g., acid) stimuli. In cutaneous nerves, several groups of nociceptors with diverse receptive properties exist (for recent overview, see Campbell and Meyer 1996). Many cutaneous C-fiber afferents belong to the class of polymodal nociceptive neurons that respond to mechanical, thermal, and chemical stimuli (for recent overview, see Perl 1996). Polymodal receptors are also widely distributed in other tissues, including joints (Schmidt 1996), skeletal muscle (Kumazawa and Mizumura 1977; Mense 1996), dura (Bove and Moskowitz 1997), cornea (Gallar et al. 1993), splanchnic nerve afferents innervating the mesentery (Adelson et al. 1996, 1997), and superior spermatic nerve innervation of the testis and epididymis (Kumazawa and Mizumura 1980a,b; Kumazawa et al. 1987).

In the gastrointestinal tract, afferent nerve terminals are present in the mucosa, muscularis, and serosa. Visceral afferent units typically have been classified according to whether their principal activity is associated with mechanical, thermal, or chemical stimuli, although many respond to more than one stimulus (for review, see Sengupta and Gebhart 1994b). For example, it long has been known that mechanosensitive mucosal afferent fibers are often also sensitive to chemical stimuli (Iggo 1957). Others have since documented that mechanosensitive afferent fibers innervating colon or urinary bladder muscle also respond to chemical stimuli (e.g., bradykinin, acetic acid, mustard oil, xylenes) (Floyd et al. 1977; Häbler et al. 1990; Haupt et al. 1983; Jänic and Koltzenburg 1990; Sengupta and Gebhart 1994a; Sengupta et al. 1996; Su et al. 1997a,b). Longhurst (1995) has extensively studied the chemosensitivity of afferent fibers innervating the stomach, proximal small intestine, and mesentery, many of which are also mechanosensitive.

Thermosensitive afferent fibers have been reported in the vagus and splanchnic nerves (Riedel 1976; Von Euler 1947), and it has been documented that thermoreceptors exist in the esophagus (El Ouazzani and Mei 1982), stomach and duodenum (Gupta et al. 1979), and intestine (Rawson and Quick 1972; Rawson et al. 1969). In humans, warm and cold receptors are distributed along the gastrointestinal tract (Villanova et al. 1997) and anal canal and rectum (Miller et al. 1987). El Ouazzanni and Mei (1982) considered warm, cold, and mixed receptors in the digestive tract to be specific thermoreceptors sensitive only to warm or cold stimuli and not to mechanical and chemical stimuli. Receptors that respond to both mechanical and thermal stimuli have been described in duodenum (Cottrell 1984) and colon and rectum (Clifton et al. 1976).

Much of the recent literature has focused on the mechanosensitivity of gastrointestinal receptors (e.g., Blumberg et
al. 1983; Haupt et al. 1983; Jänig and Koltzenburg 1991; Sengupta and Gebhart 1994a,b) and their potential role in visceral nociception. Virtually all such studies have used balloons to distend hollow organs, making it experimentally difficult to also test the application of chemical or thermal stimuli. We have argued that the adequate stimuli for such receptors are unknown and that they are likely polymodal (Gebhart and Sengupta 1994; Sengupta and Gebhart 1994b).

Accordingly, the objective of the present study was to examine the chemical and thermal sensitivity of mechanosensitive pelvic nerve afferent fibers innervating the rat colon. We adapted the control device used previously for distension of the colon (Gebhart and Sengupta 1995) to provide constant pressure colonic distension with Krebs solution to which we could add chemicals or change temperature of the solution. Preliminary reports of some of these data have been presented previously (Gebhart 1996; Su et al. 1996).

METHODS

General procedures

Male Sprague-Dawley rats weighing 410–530 g (Harlan, Indianapolis, IN) were used. Food, but not water, was withheld for 24 h before an experiment. Rats were anesthetized initially with 40–45 mg/kg ip pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, IL); anesthesia was maintained by infusion of pentobarbital (5–10 mg·kg⁻¹·h⁻¹ iv). A femoral artery and vein were catheterized for measurement of arterial pressure and administration of drugs, respectively; the trachea also was cannulated. Rats were paralyzed with pancuronium bromide (initial 0.3 mg/kg iv; supplemental 0.2–0.3 mg·kg⁻¹·h⁻¹ iv) and subsequently ventilated with room air (55–60 strokes/min, 3–4 ml stroke volume). Mean arterial blood pressure was monitored continuously and was maintained at >80 mmHg by intravenous injection of 5% dextrose in saline given in a bolus of 1–1.5 ml as required. Core body temperature was maintained at 36°C by a hot-water-circulating heating pad placed under the rat and an overhead feedback-controlled heat lamp (thermoprobe inserted into the thoracic esophagus). At the end of experiments, rats were killed by an overdose of intravenous pentobarbital. The experimental protocol was approved by the Institutional Animal Care and Use Committee of The University of Iowa.

Surgical procedures

The lower abdomen was exposed by a 4- to 5-cm-long incision laterally at the left flank. The urinary bladder was emptied and catheterized (PE-100) through the fundus, and urine was evacuated constantly via the fundic catheter. An ~7 cm length of descending colon was exposed and isolated in situ. The blood supply and nerves innervating the colon remained intact. A catheter (5 mm diam) was inserted into and ligated in the proximal end of the descending colon. Another catheter (5 mm diam) was inserted via the anus and similarly ligated, thus permitting intracolonic perfusion or distension of the colon with Krebs-Henseleit (Krebs) solution.

The left testis, vas deferens, and seminal vesicle were tied and with which the isolated colon was perfused. To monitor the temperature of the perfusate, a thermoprobe (Physitemp, type IT-1E) was introduced into the colon via the anal catheter. A laser beam (polyethylene tubing, PE-60) placed in the colon from the proximal end. The pressure reservoir was connected to a pressurized fluid reservoir through the proximal catheter, and intracolonic pressure was measured through a fine catheter (polyethylene tubing, PE-60) placed in the colon from the proximal end. The pressure reservoir was connected to a distension control device via a low-volume pressure transducer (see Gebhart and Sengupta 1995). At rest, 37°C Krebs solution (0 mmHg) remained in the colon. For phasic, constant pressure distension (5–60 mmHg, 30 s), 37°C Krebs solution was introduced via the proximal catheter and the distal catheter was clamped. The experimental arrangement is illustrated in Fig. 1.

If a fiber responded to brief phasic colorectal distension (CRD; 40 mmHg, 2–3 s), a stimulus-response function (SRF) to phasic distending pressures of 5, 10, 20, 30, and 40, and 60 mmHg, 30 s at 4-min intervals was determined. Responses to repeated CRD (10 trials of 40 mmHg, 30 s at 4-min intervals) were examined in six fibers. Thermal and chemical stimulation of the colon was produced by changing the temperature or composition of the Krebs solution with which the isolated colon was perfused. To monitor the temperature of the perfusate, a thermoprobe (Physitemp, type IT-1E) was introduced into the colon via the anal catheter. A total of 51 pelvic nerve afferent fibers were studied, 9 of which were studied after partial characterization of another fiber in the same experiment. After characterizing responses to CRD, responses to thermal (heat and/or cold) stimulation generally were tested before examining responses to chemical stimulation (pH, inflammatory soup, bile...
Thermal stimulation of the colon was produced by ramp increases or decreases in temperature (37°C to 50–60°C or 37°C to 20°C, ~480 s) without changing intracolonic pressure while outflow was open. Responses to CRD (40 mmHg, 30 s) during the peak temperature were tested in some experiments by clamping the distal catheter. The responses of some fibers to repeated heat or cold stimuli (10-min intertrial interval) also were tested.

Chemical stimulation of the colon was produced by changing the pH of the perfusate, by perfusion with an inflammatory soup mixture of sodium cholate and sodium deoxycholate, histamine, and 10^{-5} M KCl, pH 7.35 or pH 5.50 (Handwerker and Reeh 1991) or by adding bile salts (BS) to the perfusate. The pH of the perfusate was adjusted by adding HCl or NaOH to the Krebs solution. The effects of these chemical stimuli on spontaneous activity and responses of fibers to CRD were determined with the chemical-containing fluid in the colon; the colon was flushed with 37°C Krebs solution after chemical stimulation. Testing of other stimuli followed a recovery interval of 40–60 min (at which time responses to CRD returned to control).

**Data analysis**

The resting activity of a fiber was counted for 60 s before CRD, and response was determined as the increase in discharge during distension above its resting activity. In some cases where chemical stimulation of the colon variably increased resting activity, the overall mean change in resting activity was taken into account to determine the response to CRD. SRFs to graded CRD were plotted for each individual fiber, and a least-squares regression line was obtained from the linear part of the SRF. The regression line then was extrapolated to the ordinate (representing distension pressure) to estimate response threshold.

To estimate the response threshold to thermal stimulation, the mean and standard deviation (SD) of the resting activity was determined. Threshold was defined as the temperature at which unit activity increased >2 SD above resting activity. For fibers with no or low background activity, the response threshold was considered that temperature at which the fiber began and continued to discharge. Unit activity during thermal stimulation was counted in 10-s bins, and the maximum response during thermal stimulation was defined as that bin with the greatest number of counts.

All data are expressed as means ± SE. Results were analyzed using Student's t-test or an analysis of variance (ANOVA) for repeated measures; P < 0.05 was considered statistically significant.

**Chemicals and drugs**

Krebs solution of the following composition (in mM): 118.0 NaCl, 0.7 KCl, 24.0 NaHCO₃, 1.2 MgSO₄, 2.5 CaCl₂, 1.1 KH₂PO₄, and 10.0 glucose, pH 7.3–7.4, was prepared from chemicals purchased from Sigma Chemical (St. Louis, MO). Bile salts (BS; a mixture of sodium cholate and sodium deoxycholate), histamine hydrochloride (MW: 184.1), serotonin hydrochloride (MW: 212.7), PGE₂ (MW: 352.5), and bradykinin (MW: 1060.2) were purchased from Sigma and dissolved in distilled water.

**RESULTS**

**Fiber sample**

Thirty-one of the 51 distension-sensitive pelvic nerve afferent fibers studied (61%) were unmyelinated C fibers with a mean CV of 2.0 ± 0.1 m/s (range, 1–2.5 m/s) and 20 (39%) were thinly myelinated Aδ fibers with a mean CV of 5.2 ± 0.9 m/s (range, 2.8–14 m/s). Forty-nine fibers were spontaneously active (mean, 1.3 ± 0.3 imp/s; range, 0.01–7.1 imp/s); two C-fibers had no resting activity. The characteristics of this sample of S₄ pelvic nerve afferent fibers are similar to what we found in earlier studies in which the colon was distended with air using a 7-cm-long latex balloon (Sengupta and Gebhart 1994a; Su and Gebhart 1998; Su et al. 1997a) (Table 1). All fibers gave sustained, phasic responses to fluid distension that were linked temporally to the distending stimulus (e.g., see Fig. 3). None gave on/off type, rapidly adapting responses suggestive of sensitivity to movement of fluid across the mucosa, but the tissue location of these mechanosensitive fibers cannot be determined from these experiments.

**Mechanosensitivity**

All 51 fibers gave monotonically increasing responses to graded CRD. As in previous studies in which the colon was

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**FIG. 1.** Experimental arrangement.
TABLE 1. Summary of characteristics of pelvic nerve mechanosensitive afferent fibers innervating the colon of the rat

<table>
<thead>
<tr>
<th>Characteristics of Fibers</th>
<th>Fluid CRD</th>
<th>Balloon CRD with Air*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conduction velocity, m/s</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>C fibers</td>
<td>2.0 ± 0.1 (31)</td>
<td>1.5 ± 0.1 (43)</td>
</tr>
<tr>
<td>Aδ fibers</td>
<td>5.2 ± 0.9 (20)</td>
<td>4.7 ± 0.6 (10)</td>
</tr>
<tr>
<td>Resting activity, imp/s</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>C fibers</td>
<td>1.7 ± 0.4 (29)</td>
<td>2.9 ± 0.6 (31)</td>
</tr>
<tr>
<td>Aδ fibers</td>
<td>0.8 ± 0.3 (20)</td>
<td>2.2 ± 0.6 (7)</td>
</tr>
<tr>
<td>Response pattern to CRD, n</td>
<td>Adapting</td>
<td>34 (13Aδ; 21 C)</td>
</tr>
<tr>
<td></td>
<td>Nonadapting</td>
<td>17 (7 Aδ; 10 C)</td>
</tr>
<tr>
<td>Response threshold, mmHg</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Low threshold</td>
<td>2.4 ± 0.4 (46)</td>
<td>2.9 ± 1.0 (34)</td>
</tr>
<tr>
<td>High threshold</td>
<td>28.5 ± 0.8 (5)</td>
<td>32.6 ± 1.7 (10)</td>
</tr>
<tr>
<td>Maximum response to 60 mmHg CRD, imp/s</td>
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<td></td>
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<tr>
<td>Low threshold</td>
<td>25.8 ± 1.5</td>
<td>16.0 ± 1.3</td>
</tr>
<tr>
<td>High threshold</td>
<td>13.7 ± 5.6</td>
<td>9.1 ± 1.5</td>
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</table>

Values are means ± SE; parentheses enclose n values. All recordings were made from the decentralized S1 dorsal rootlet. * From Sengupta and Gebhart 1994a (1); Su et al. 1997a (2); and Su and Gebhart 1998 (3).

distended with air and a balloon, the majority of pelvic nerve fibers had low thresholds (LT) for response to fluid distension of the colon (<10 mmHg; n = 46). Five fibers had high thresholds (HT) for response (>25 mmHg). Individual SRFs of LT and HT afferent fibers are shown in Fig. 2, and the corresponding insets show the mean SRFs for each group of fibers. The mean extrapolated thresholds for response of LT and HT pelvic nerve afferent fibers to graded CRD was 2.4 ± 0.4 and 28.5 ± 0.8 mmHg, respectively.

RESPONSE PATTERN. Similar to balloon CRD (Sengupta and Gebhart 1994a), two different response patterns to fluid CRD were noted. One group of fibers (n = 34) gave an initial, dynamic response to CRD that adapted slowly during maintained distension. A second group of fibers (n = 17) gave incrementing responses to CRD during fluid distension. Examples are given in Fig. 3A: Figure 3B summarizes responses over the pressure range studied. In a post hoc analysis, we examined whether the pattern of response to CRD was associated with sensitivity to high thresholds (HT) for response (>25 mmHg). Individual SRFs of 5 fibers that responded at high threshold to CRD were noted. Of the 17 nonadapting fibers identified in Table 2. Aside from the observation that four of the five HT fibers were nonadapting, there are no obvious correlations.

REPRODUCIBILITY OF RESPONSES. Six fibers were tested for responses to repetitive CRD at 40 mmHg (30 s). None of the fibers exhibited any change in response magnitude or pattern to repeated distension at a 4-min interval between distensions. Figure 4 shows the response a C fiber to 10 successive colonic distensions, and the responses of each of six fibers to repeated CRD.
**FIG. 3.** Response patterns of pelvic nerve afferent fibers to colorectal distension (CRD, 30 s). A: examples illustrated as peristimulus time histograms (1-s bin-width) of responses of an adapting and nonadapting fiber to 40 mmHg CRD. B: comparison of responses of adapting (n = 34) and nonadapting (n = 17) fibers, plotted as mean impulses/second during the 1st 10 s of CRD or during the last 10 s of CRD (5–60 mmHg).

**Chemosensitivity**

RESPONSES TO PH. The effect of transiently changing the pH of the perfusate on spontaneous activity and responses to 40 mmHg CRD was tested on eight LT fibers (3 Aδ and 5 C fibers). Four fibers were exposed to both low (3.0) and high (11.0) pH (e.g., Fig. 5A); two fibers each were exposed for ~8 min to only low pH or only high pH. Neither the spontaneous activity nor mechanosensitivity of any of the fibers tested were affected; an example is given in Fig. 5A, and the data are summarized in Fig. 5, B and C; see also Table 2.

RESPONSE TO BS. The mechanosensitive properties of six fibers [5 LT (3 Aδ and 2 C fibers) and 1 HT C fiber] were tested before and after sequential instillation of 0.1, 0.5, and 1% BS into the colon. The mean resting activity of the six fibers increased from a mean 0.2 ± 0.1 imp/s to 0.5 ± 0.3, 3.2 ± 1.2 (P < 0.05), and 4.2 ± 1.6 (P < 0.05) imp/s, respectively (Fig. 6B). No fibers exhibited sensitization of responses to CRD after instillation of BS in the colon (Fig. 6C). Figure 6A illustrates an example of responses to CRD of an unmyelinated LT fiber in the presence of BS.

**TABLE 2.** Summary of responses of pelvic nerve mechanosensitive afferent fibers to chemical and thermal stimuli

| Aδ, LT | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| OH⁻    | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| H⁺     | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| BS     | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| IS     | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| heat   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| cold   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

| C, LT  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
| OH⁻    | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| H⁺     | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| BS     | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| IS     | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| heat   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| cold   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

<table>
<thead>
<tr>
<th>C, HT</th>
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<th>3</th>
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<th>5</th>
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<tr>
<td>BS</td>
<td>+</td>
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<td>cold</td>
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BS, bile salts; IS, inflammatory soup; +, response; −, tested, no response; LT, low threshold; HT, high threshold; underlined numbers identify nonadapting responses to CRD (see text for details). * Tested, but did not record activity until 60 min after IS. † Did not respond to heat stimulation but exhibited an afterdischarge when heat stimulation was terminated.
FIG. 4. Reproducibility of responses to repeated colorectal distension (CRD, 40 mmHg for 30 s). A: example of responses of an unmyelinated fiber (1.71 m/s) to 10 repeated distensions applied every 4 min. Responses of the fiber are illustrated as peristimulus time histograms (1-s binwidth); phasic distending pressure is presented below. B: summary responses of 6 fibers to repeated CRD plotted as the mean increase in impulses/second over the resting activity against the number of trials. A indicates the example illustrated in A.

FIG. 5. Effects of pH. A: responses of a pelvic nerve afferent fiber, illustrated as peristimulus time histograms (1-s binwidth), to colorectal distension (CRD, 40 mmHg, 30 s) before (control) and during intracolonic instillation of pH 3 or pH 11 Krebs solution. Neither resting nerve activity of the 6 fibers tested (B) nor responses of the same fibers to 40 mmHg CRD (C) were affected by changing intracolonic pH.
in resting activity, but its response to CRD after pH 5.5 IS was increased. Figure 7C illustrates responses of these 13 sensitized fibers 60 min after intracolonic instillation of either pH 7.35 ($F = 11.56, P < 0.001$) or pH 5.5 ($F = 6.97, P < 0.01$) IS. Although pH 5.5 IS produced an apparent greater sensitization than did pH 7.35 IS, the difference is not statistically significant ($F = 1.09, P = 0.30$).

The mean threshold for response, another indication of sensitization, decreased in 7/13 fibers with response thresholds $>3$ mmHg before IS treatment (range: 3–30 mmHg). Response threshold decreased from a mean $11.8 \pm 4.1$ to $3.8 \pm 1.5$ ($P < 0.05$) and $2.6 \pm 1.6$ ($P < 0.05$) mmHg before and 30 and 60 min, respectively, after pH 7.35 or 5.5 IS treatment. Pretreatment response thresholds of the
remaining six sensitized fibers (increased responses to CRD) were near 0 mmHg and could not have decreased.

**Thermosensitivity**

The responses of mechano-sensitive afferent fibers to intracolonic perfusion of hot and/or cold Krebs solution also were tested. The method of colonic perfusion did not allow us to determine precisely the temperature at the location of the endings, but three types of receptors were distinguished according to the apparent temperature at which they were activated. Heat receptors responded to temperatures ≥42°C, cold receptors responded to temperatures ≤30°C, and mixed receptors responded to both heat and cold thermal stimuli.

**RESTING ACTIVITY.** Forty-five fibers were tested for response to heat stimulation and 33 (73%) responded (an example is given in Fig. 8A). Mean resting activity increased from 1.5 ± 0.4 to a mean maximum 17.3 ± 2.3 imp/s (n = 33) during intracolonic perfusion with 50°C Krebs solution; the mean estimated response threshold was 44.9 ± 1.2°C. Three of 45 fibers failed to respond during the increase in colonic temperature but exhibited an afterdischarge when heat stimulation was terminated. Eighteen fibers also were tested for possible sensitization of response to a second heat stimulus 10 min after the first stimulus. All responded to both trials (Fig. 8B); the response threshold for one fiber increased from a mean 0.3 ± 0.1 to 14.2 ± 4.5 imp/s during intracolonic perfusion with 20°C Krebs solution. The mean estimated response threshold was 27.7 ± 1.4°C. No fiber exhibited an afterdischarge when cold stimulation was terminated.

**RESPONSE TO CRD.** The responses of 30 fibers (25 heat sensitive and 5 heat insensitive; 18 adapting and 12 nonadapting) to CRD also were tested during the peak temperature (50°C) by clamping colonic outflow. Twenty-two fibers (17 heat sensitive and 5 heat insensitive; 14 adapting and 8 nonadapting) exhibited a significant increase in the initial 10-s dynamic response to CRD (from 26.8 ± 2.2 to 41.4 ± 2.8 imp/s, P < 0.01) during heat stimulation and adapted quickly during maintained 40 mmHg distension. In eight heat-sensitive fibers (4 adapting and 4 nonadapting), the magnitude of responses to CRD during the second heat stimulation was significantly reduced (P < 0.05). The data are summarized in Fig. 9A.

The responses of 29 fibers (8 cold-sensitive and 21 cold-insensitive) to CRD also were tested at 20°C. All fibers gave a decreased response to 40 mmHg CRD (from 28.8 ± 2.4 to 12.2 ± 1.8 imp/s; P < 0.01); data are summarized in Fig. 9B.
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response to CRD at 50 °C

control
heat

15 Hz
5s
n=22

response to CRD at 20 °C

control
cold

40 mmHg
n=29

FIG. 9. Summary of effects of intracolonic instillation of hot or cold Krebs solution on response patterns of pelvic nerve afferent fibers to colorectal distension (CRD, 40 mmHg, 30 s). Responses at peak temperature of heat (A) or cold (B) are illustrated as mean peristimulus time histograms (1-s binwidth).

Conduction velocity (CV)

There were no differences in the distribution of CVs of heat or cold-sensitive and -insensitive fibers (Fig. 10). CV was also measured in some fibers after heat (n = 15), cold (n = 22), BS (n = 3), and IS (n = 7) stimuli; no changes in CV were noted.

Responses of colonic muscles

To determine whether chemical or thermal stimuli affected muscle tone of the colon, intracolonic pressure was recorded during some trials. Heat produced a mean decrease in intracolonic pressure from 7.3 ± 2.4 to 3.9 ± 1.9 mmHg

(n = 8; P > 0.05) and cold produced a slight increase in intracolonic pressure (from 6.4 ± 2.8 to 7.4 ± 2.2 mmHg, n = 7; P > 0.05). BS and IS did not change intracolonic pressure.

Polymodal responses

Twenty-six mechanosensitive fibers were exposed to chemical (BS or IS) and thermal stimuli. Eleven fibers responded to all three modalities of stimulation: mechanical, chemical, and thermal stimuli (7 sensitive only to heat, 3 sensitive to both heat and cold, and 1 sensitive only to cold); 15 fibers responded to either chemical or thermal stimulation (in addition to distension). There were no differences in the thermo- and/or chemosensitivity between Aδ and C fibers (Fig. 11).

All but 4 of the remaining 25/51 mechanosensitive fibers in which only either chemical or thermal stimuli were tested responded to the second stimulus. Thus among the 51 fibers studied, 47 (92%) responded to another modality of stimulation (either chemical and/or thermal) in addition to the distending mechanical stimulus used to initially characterize the fiber. Table 2 summarizes the response of all fibers tested.

DISCUSSION

In previous studies, using constant-pressure air distension of a balloon inserted in the colon, we documented the presence of low- and high-threshold pelvic nerve afferent fiber populations innervating the colon and also documented that responses to repetitive colonic distension were stable (Sengupta and Gebhart 1994a; Su and Gebhart 1998; Su et al. 1997a). Similarly, in the present study, using Krebs solution to distend the colon in situ, we found populations of low- and high-threshold pelvic nerve afferent fibers in the S, dor-

FIG. 10. Frequency histograms of conduction velocity of temperature-sensitive and -insensitive pelvic nerve afferent fibers. n, number of fibers.
We also documented that responses to repeated fluid distension of the colon are stable, thus permitting quantitative evaluation of alterations in mechanosensitivity produced by chemical stimuli.

Chemosensitivity of mechanosensitive afferents

A number of studies have reported that mechanosensitive visceral afferent fibers are also sensitive to chemicals, principally algesic or irritant chemical stimuli (see INTRODUCTION). We previously reported that intracolonic instillation of acetic acid or mustard oil can sensitize mechanosensitive afferent fibers innervating the colon (Sengupta et al. 1996; Su et al. 1997a). In complementary studies, urinary bladder instillation of irritant xylenes or mustard oil was shown to sensitize mechanosensitive pelvic nerve afferent fibers innervating the bladder (Su et al. 1997b). In the present study, intracolonic instillation of IS increased resting activity and the magnitude of response of 13/22 fibers to CRD throughout the range of distending pressures tested. IS also reduced the threshold for response of fibers that had pre-IS response thresholds >3 mmHg. Numerous potential mediators are released and synthesized during tissue injury and inflammation, and one chemical does not likely represent the adequate stimulus. That multiple putative mediators constitute an adequate stimulus was suggested by Handwerker and Reeh (1991). In an in vitro skin-nerve preparation, applying an IS induced greater excitation (80%) of nociceptive C fibers (Kessler et al. 1992) than did application of serotonin (20%), bradykinin (50%) (Lang et al. 1990), or histamine (14%) (Koppert et al. 1993). Bove and Moskowitz (1997) reported that 79% of nasociliary nerve fibers innervating the dura are sensitive to a combination of inflammatory mediators similar to the composition of IS used here. Handwerker and Reeh (1991) further documented that 40% of cutaneous polymodal receptors were excited by increasing the H+ concentration to a pH of 5.2. Similarly, we noted that the effect of IS on responses to CRD was enhanced by increasing the H+ concentration to pH 5.5.

We did not find that transient exposure of the colon to low or high pH Krebs perfusate changed either resting activity or responses to CRD of afferent fibers. In support, colonic mucosal afferent fibers in the ventral root of the cat colon were also not sensitive to 0.1 N HCl (Clifton et al. 1976). We previously reported that pH 3.0 acetic acid (2 ml, 2.5%) retained in the rat colon for 15 min sensitized about one-half of a sample of 32 mechanosensitive pelvic nerve fibers to 60–100 mmHg balloon CRD when tested 30–60 min after acetic acid instillation (Sengupta et al. 1996). Acetic acid can cause mucosal barrier dysfunction (Gardiner et al. 1995) and can lead to neutrophil infiltration, hemorrhage, and necrosis (Lowe and Noronha-Blob 1993). Exposure of the colonic mucosa to low pH in the present experiments was of relatively short duration, and fluid CRD was limited to 40 mmHg; changes in resting activity or responses to CRD were not seen in the sample tested. BS did not increase the magnitude of response to noxious CRD, but BS did significantly increase the resting activity of afferent fibers. BS normally are present in the colon (Rodrigues et al. 1995; Varityam 1992) where they reportedly stimulate synthesis of LTB4, an inflammatory mediator in inflammatory bowel disease (Dias et al. 1994). Rather than sensitizing visceral afferent fibers, LTB4 has been reported to decrease responses of abdominal visceral afferent fibers (Pan et al. 1995). In the present study, all 4 HT fibers tested with BS or IS were affected, whereas 9/25 LT fibers were unresponsive. This is supported by Pan and Longhurst’s (1996) report that ischemia-sensitive abdominal afferent fibers had higher thresholds to mechanical distension, whereas ischemia-insensitive receptors had lower thresholds to mechanical distension.

Thermosensitivity of mechanosensitive afferents

Thermal stimuli are adequate for some visceral sensory fibers, producing both reflex responses (e.g., bladder cooling reflex) (Fall et al. 1990) and conscious sensation (e.g., drinking a cold beverage on an empty stomach) (Webber et al. 1980). We found that 33/45 fibers tested were heat sensitive, 11/37 fibers were cold sensitive, and 8/36 fibers responded to both hot and cold Krebs solution. Thermosensitive fibers were estimated to have response thresholds of 28°C for cold-sensitive fibers and 45°C for heat-sensitive fibers. Apparent sensitization or desensitization was observed in a few fibers by repeat stimulation with heat or...
cold, but generally response thresholds and response magnitudes to thermal stimulation were similar on the second presentation of the thermal stimulus 10 min after the first stimulus. Sensitization, particularly to repeated heat stimulation, might have been expected to develop, but the number of trials (two) and interval between trials (10 min) was limited in the present experiments. These thermosensitive pelvic nerve fibers resemble thermoreceptors in skin and the testis and epididymis (Iggo 1959; Kumazawa and Mizumura 1980a). Compared with other visceral thermoreceptors, pelvic nerve thermoreceptors in the colon have similar response thresholds to those in the vagus nerve in the gastroesophageal region (EL Ouazzani and Mei 1982) and in the gastrointestinal vagal territory (EL Ouazzani and Mei 1979). Splanchnic cold receptors also have been described in the gastrointestinal tract (Gupta et al. 1979), and receptors that respond to both mechanical and thermal stimuli have been described in the duodenum (Cottrell 1984). Mechanosensitive mucosal afferent fibers in sacral ventral roots innervating the colon and rectum of the cat also responded to hot and cold temperatures (Clifton et al. 1976).

Kumazawa et al. (1987) reported that chemical responses of polymodal receptors can be modulated by temperature. Similarly, we noted that responses to mechanical stimulation decreased during cold stimulation. It could be that the decrease in temperature altered stimulus transduction or CV (most likely the former). CV of the fibers, tested by electrical stimulation at a site distant from the receptor, did not change. Heat stimulation produced mixed effects. In 22 fibers, there was an increase in the dynamic component of the response to CRD; in 8 fibers, attenuated responses to CRD were observed at the peak temperature. As in earlier work (Sengupta and Gebhart 1994a), we noted two patterns of response to CRD. Adapting and nonadapting responses of colonic afferents also have been noted by others (Blumberg et al. 1983; Jänic and Koltzenburg 1991). As suggested previously (Sengupta and Gebhart 1994a), the initial dynamic response likely represents an increase in muscle tension that develops during active resistance to distension offered by the muscle and of intrinsic excitatory reflexes produced by the myenteric plexus. The firing rate then slowly declines as muscle tension decreases during the ‘‘receptive’’ reflex; with termination of distension, the firing often ceases because of the sudden loss of muscle tension. Thermal stimulation appeared to have its greatest effect on the dynamic component of the response. The mechanisms and possible functional role of altered mechanosensitivity by thermal stimuli remains to be studied. It is conceivable that changes in adaptation by heat can be caused by changes in the excitability of the spike generator membrane during prolonged distension (e.g., heat may increase the activity of an electrogenic Na-K⁺ pump or membrane inactivation of Na⁺; cold may have an opposite effect).

Polymodal receptors

The effects of chemical and thermal stimuli reported here could be due to a change in conduction of nerve action potentials or compliance of the colon and not on neuron sensory endings (receptors). In the present study, CVs of the fibers remained unaffected after intracolonic application of IS, BS, and hot or cold perfusate. It seems unlikely, however, that changes in CV of the axon, measured distant from the colon, would be produced by intracolonic treatments. Recording of intracolonic pressure revealed that cold and heat stimulation can produce a slight increase or decrease in intracolonic pressure, respectively; IS and BS did not change the tension of the smooth muscle. In experiments in rats pretreated with loperamide to paralyze the smooth muscle, we observed similar effects of IS, BS, heat, and cold (unpublished data). Accordingly, the chemical and thermal effects reported here likely occur at receptors associated with afferent nerves innervating the colon; that is, visceral afferent fibers innervating colon are polymodal in character. Like the high proportion (90%) of polymodal receptors in testis (Kumazawa and Mizumura 1980a, b; Kumazawa et al. 1987), we found that 92% of mechanosensitive receptors in the colon are polymodal (i.e., respond to at least 2 stimulus modalities).

We found no differences between polymodal Aδ and C fibers in the pelvic nerve in response to thermal and/or chemical stimuli. It has been noted in cutaneous nerve that units with faster CVs generally have lower mechanical thresholds and also are less responsive to heat stimulation and algic substances. It was proposed by Kumazawa (1996), however, that the criterion most important in classifying a sensory receptor as transmitting nociceptive information is not CV, but whether it can encode changes in the tissue surrounding the receptor terminals that are produced by noxious events.

Detailed information about the response-properties of polymodal receptors is still lacking. Szolcsányi (1993) reported that polymodal nociceptive axons terminate in a ‘‘chain of beads,’’ which were considered to be multiple sensor sites. Some of these sensor beads can be activated by mechanical stimuli directly or indirectly by releasing chemical mediators from the tissue (Hamill et al. 1992). Other sites are depolarized preferentially by noxious heat or chemical stimuli (Szolcsányi 1993). Most studies of polymodal receptors, including this one, have focused on responses to applied stimuli and do not address either the precise location of the receptors or transduction mechanisms. We acknowledge that some responses reported here could be indirect (e.g., through release of an endogenous chemical from specialized cells in the tissue that activates the nerve terminal) and cannot document that the afferent fibers recorded here and characterized as polymodal contain on their peripheral terminal receptors for all three stimulus energies. Functionally, however, these fibers are polymodal in character and transmit information from the tissue about mechanical, thermal, and chemical stimuli.

Functional significance

Visceral afferent fibers, in the presence of tissue inflammation, can become sensitized, contribute to central hyperexcitability, and lead to visceral hyperalgesia. Most mechanosensitive colon afferent fibers have a wide dynamic range of response and can be sensitized to nonnoxious intensities of distension. There are a number of clinical conditions, categorized as functional bowel disorders, including nonulcer dyspepsia, noncardiac chest pain, and irritable bowel
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syndrome, that are characterized by discomfort and pain in the absence of tissue inflammation or apparent pathology (Mayer and Gebhart 1994). These disorders are complex and involve both peripheral and central contributions. It seems apparent now that a significant component of the discomfort and pain associated with the functional bowel disorders is associated with altered sensory input or altered integration in the CNS. The knowledge of adequate stimuli for receptors in the gastrointestinal tract and improved knowledge of their basic physiology will help understand the extent to which peripheral contributions from the organ itself or visceral receptors contribute to the altered sensations and pain that characterize functional bowel disorders.

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