Inflammation enhances reflex and spinal neuron responses to noxious visceral stimulation in rats

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Ness, T. J., and G. F. Gebhart. Inflammation enhances reflex and spinal neuron responses to noxious visceral stimulation in rats. Am J Physiol Gastrointest Liver Physiol 280: G649–G657, 2001.—To improve understanding of sensory processes related to visceral inflammation, the effect of turpentine-induced inflammation on reflex (cardiovascular/visceromotor) and extracellularly recorded lumbosacral dorsal horn neuron responses to colorectal distension (CRD) was investigated. A 25% solution of turpentine, applied to the colorectal mucosa, produced inflammation, decreased compliance of the colonic wall, and enhanced reflex responses in unanesthetized rats within 2–6 h. At 24 h posttreatment, pressor responses to CRD (80 mmHg, 20 s) were 20% greater, and intraluminal pressures needed to evoke visceromotor reflexes were 30% lower than controls. Parallel electrophysiological experiments in spinal cord-transected, decerebrate rats demonstrated that two neuronal subgroups excited by CRD were differentially affected by turpentine administered 24 h before testing. During CRD, abrupt neurons were 70% less active and sustained neurons were 25% more active than similar neurons in controls. In summary, reflex and neuronal subgroup (sustained neurons) responses to CRD were both potentiated by chemical inflammation. This suggests that the neurophysiological basis for inflammation-induced increases in reflex responses to CRD is increased activity of this neuronal subgroup.

VISCERAL PAIN IS POORLY understood compared with cutaneous pain. Few human psychophysical studies of visceral pain have been performed, and few validated models of visceral nociception exist (for review, see Ref. 21). As a consequence, much of our understanding of visceral pain is limited to anecdotal clinical descriptions of pain related to visceral pathology. Abdominal pain associated with stimulation of the gastrointestinal tract has been related to sustained or repetitive distension of the gut (15), as occurs pathologically with intestinal obstruction. Psychophysical studies (17, 31) have demonstrated that experimental balloon distension of the sigmoid colon also leads to the sensation of pain, but in some cases only after multiple presentations of the distending stimulus. This stimulus in humans also produces sensitization of neighboring structures that are not being directly stimulated (20, 31), suggesting the activation of central mechanisms. Visceral pain also occurs in association with inflammation of the gut and adjacent structures (38). Inflammation of the gut in and of itself may or may not be an adequate stimulus for pain production, because inflammatory bowel diseases such as ulcerative colitis may be nonpainful until peritonitis or obstruction occurs (3, 8).

Inflammation does, however, reliably lead to abnormal reflexes in the gut, such as those leading to diarrhea, hypermotility, or dysmotility, and makes the abdomen “tender” with increased sensitivity to mechanical stimuli such as palpation (38).

Recently, studies (21) of visceral nociception have employed the stimulus of colorectal distension (CRD) to evoke vigorous physiological, neuronal, and behavioral responses in rats, rabbits, horses, cats, dogs, and primates (including humans) that have been interpreted as painful or nociceptive. Quantitative neurophysiological studies (24, 26) in rats have demonstrated the existence of at least two spinal neuron populations that encode for CRD in an excitatory, graded fashion throughout the noxious range. These neuron groups can be distinguished from each other in several ways. One group, sustained neurons, is characterized by the presence of a sustained afterdischarge for 4–240 s after the termination of a phasic distending stimulus. The other group, abrupt neurons, demonstrates an abrupt cessation of activity immediately after the termination of the distending stimulus. Both abrupt and sustained neurons are excited by noxious cutaneous stimuli presented to same-segmental, receptive fields (e.g., the perineum), but only abrupt neurons are reliably inhibited by the presentation of noxious stimuli to nonsegmental sites (i.e., subject to counter-irritation; Ref. 29). Classifying neurons as abrupt or sustained has proven to be predictive of the differential analgesic efficacy of intravenous morphine, kappa opioid receptor agonists, and lidocaine on neuron responses to CRD (22, 23, 27). The role of these different neuronal subpopulations in the evocation of CRD-related sensations and reflexes is not yet determined.
Inflammation is known to be a potent modifier of visceral sensations and reflexes. Inflammation of the colorectal mucosa by the intracolonic application of turpentine (11), zymosan (5), acetic acid (4, 14), or trinitrobenzene sulfonic acid (12) has been demonstrated to increase behavioral and reflex responses to CRD, and mustard oil (1) has been demonstrated to increase the activity of dorsal horn neurons excited by CRD. In a preliminary study, we (30) examined the effect of intracolonic turpentine on neuronal responses to CRD before, and for 2 h after, turpentine administration. Using a repeated-measures design, we (30) found that sustained neurons had increased spontaneous activity after intracolonic turpentine pretreatment, but the activity of abrupt neurons was unaffected. There was no statistically significant alteration in the way in which abrupt or sustained neurons encoded for CRD, although there was a trend for increased activity at lower CRD intensities in the sustained neurons. In those preliminary experiments (30), there was a potentially “confounding” component of the experimental design in that CRD stimuli were presented every 4 min for over 2 h in both the vehicle- and turpentine-treated rats. This repeated stimulation produced an expansion of the cutaneous receptive fields in some neurons (30) and is known to produce sensory changes in psychophysical studies (20, 31). It has unknown consequences on other physiological processes.

To minimize the methodological problem of repeated measures and to examine, in a more comprehensive fashion, the physiological consequences of colonic inflammation, we assessed in this study multiple physiological responses to CRD at single time points 1–48 h after the induction of inflammation using intracolonic turpentine. Reflex cardiovascular and visceromotor (abdominal-hindlimb contractions) responses to CRD, as well as colorectal compliance, were measured in awake, unanesthetized rats after the induction of inflammation. In addition, spinal dorsal horn neuronal responses to CRD were characterized in cervical spinal cord-transsected, decerebrate rats at the time point demonstrated to correlate with peak changes in reflex responses and vascular permeability (a measure of local inflammation). This characterization, performed 24 h after turpentine treatment, allowed for a determination of quantitative alterations in sensory processing. This measurement of multiple reflex and neuronal responses to CRD were allowed for the gathering of complementary and comparable data. To the best of our knowledge, this is the first report of a quantitative examination of both reflex and neuronal responses to a visceral stimulus in the presence and absence of local inflammation. This parallel measurement of reflex and neuronal responses has allowed for a more comprehensive understanding of sensory changes in the presence of developing and sustained colorectal inflammation.

METHODS

Reflex Responses

General. To evaluate the effect of turpentine pretreatment on reflex responses to CRD, two groups of male Sprague-Dawley rats (380–650 g) were utilized. All reflex responses were obtained in unanesthetized, awake rats, some of which had undergone previous surgery for chronic instrumentation. Rats in group 1 underwent surgery at least 3 days before further testing and had femoral arterial and venous catheters placed and exteriorized at the nape of the neck while deeply anesthetized with pentobarbital sodium (45–50 mg/kg ip). These rats were used in studies of cardiovascular responses to CRD and subsequently used in studies of Evans blue extravasation, a measure of vascular permeability that correlates with local inflammation (e.g., see Ref 19). Rats in group 2 had no previous surgery performed before testing and were employed in colonometric and visceromotor reflex studies. The specific protocols for groups 1 and 2 are given below. At a preset time before testing, all experimental rats had 1 ml of turpentine (25% in oil vehicle) applied to an 8-cm length of colorectal mucosa by a 1-ml syringe inserted via the anus. Rats were briefly anesthetized with diethyl ether during this procedure and allowed to recover in individually marked cages with free access to food and water. Control rats were similarly injected with vehicle alone (1 ml oil) immediately before testing with coincident distension balloon placement or received no pretreatment.

The specific methodology for the cardiovascular and visceromotor responses to CRD have been described and validated in previous studies (4, 5, 11, 23, 25). The different reflex responses to CRD are quantified in different ways. In particular, cardiovascular responses to CRD are evoked by a constant pressure, phasic (on-off) stimulus that leads to a reproducible and reliable pressor response to CRD (25). The visceromotor response to CRD is reliably evoked by intensities of CRD that also produce pressor responses, but the vigor of the visceromotor response has proved difficult to quantify. Therefore the visceromotor response has most commonly been quantified as the visceromotor threshold (VMT). That is, the minimal intraluminal pressure necessary to evoke a visibly apparent or electromyogram-verified abdominal-hindlimb contraction is defined as the VMₚ, which has been demonstrated to be reproducible (e.g., Refs. 11 and 25). A “ramped” CRD stimulus in which the intraluminal pressure is slowly increased until a response is evoked is therefore utilized in studies using the visceromotor response as an end point. Use of a ramped rather than phasic CRD stimulus to evoke threshold rather than vigor responses makes the quantitative comparison of visceromotor and cardiovascular reflex responses less meaningful. Complementary rather than precisely comparable data are generated using these two measures. Additional description of the CRD stimulus employed in the different experimental groups is presented below.

Group 1 protocol. Experimental rats were treated with turpentine 1, 2, 6, 24, or 48 h before testing. Immediately before testing, all rats were briefly anesthetized with diethyl ether, and a 7- to 8-cm distending balloon was inserted into the descending colon and rectum via the anus and a connecting catheter taped to the tail. Chronic femoral venous and arterial catheters were accessed at that time. Rats were allowed to recover for ~10 min until engaging in normal locomotion in their cages. They were then connected to a distension control device described previously (2), and air was used to distend the balloon phasically to a preset pressure. Heart rate and blood pressure were continuously monitored via the femoral arterial catheter using a low-volume pressure transducer and pressure processor. At 4-min intervals, 6 80-mmHg, 20-s CRDs were administered. Then graded, phasic distension stimuli of 20-, 40-, 60-, 80-, and
100-mmHg pressure for 20 s were administered in a randomized order. This was followed by a final 80-mmHg, 20-s CRD. Cardiovascular responses were quantified as the peak change in mean arterial pressure (ΔMAP) from baseline during CRD; resting, baseline MAP was determined during the 30-s period before CRD. After cardiovascular testing, four to six rats in each group were then anesthetized with 20–30 mg/kg intravenous pentobarbital sodium and injected intravenously with 50 mg/kg Evans blue. This was allowed to circulate for 15 min, at which time rats were overdosed with 50 mg/kg pentobarbital sodium. Rats were then perfused with 500 ml normal saline to which 1,000 U heparin were added. The descending colon and rectum were dissected out, blotted dry with tissue paper, immersed in a closed vial containing 10 ml DMSO, and placed on a rocker table over night. The tissue was then removed and dried on a histological warming plate for 48–72 h, after which time the tissue was weighed. The amount of Evans blue in the DMSO extraction solution was determined colorometrically using a light spectrophotometer (Beckman; 620 nm). The amount of Evans blue was normalized to the measured dry tissue weight of the excised colon and rectum. Use of Evans blue extravasation to assess visceral inflammation has been previously described (e.g., Ref. 19).

Group 2 protocol. Rats were treated with vehicle immediately before testing or with turpentine 1, 2, 6, 24, or 48 h before testing in the manner described above. While rats were under brief diethyl ether anesthesia, a 5-cm-long distending balloon was inserted into the descending colon and rectum via the anus, and a connecting catheter was taped to the tail. Rats were allowed to recover from anesthesia for ~10 min until engaging in normal locomotion in their cages. The distending balloon utilized in group 2 experiments was smaller than that used in group 1 experiments, and both the balloon and connecting catheter were filled with water rather than air. These alterations allowed accurate colonometric measures of the compliance of the colon and rectum. Volumes of water were added in 1-ml increments to the distending balloon to a total of 4 ml, and compliance was determined by measuring the intraluminal pressure for a given volume of distension using a low-volume pressure transducer attached to the connecting catheter. A “steady-state” intraluminal pressure could be determined 30 s after infusion of each milliliter of water. After colonometric determinations, the water in the balloon was evacuated and air distension of the colon and rectum was employed to determine a pressure threshold for evocation of a visceromotor response (a reflex contraction of abdominal and hindlimb musculature). A hand-driven, air-filled syringe was used to infuse air into the colorectal distending balloon, providing an accelerating ramped increase in intraluminal pressure until a visceromotor response was noted visually. An inline pressure transducer allowed for determination of the intraluminal pressure, at which time the visceromotor response was evoked, and this was defined as VMF. Five to ten measures of the VMF were determined at 2-min intervals until a stable (±20%) value was determined on three consecutive measures. The mean of these three values was then used for subsequent statistical analysis.

Neuron Responses

General. Neuron studies examined populations of neurons in two groups of male Sprague-Dawley rats (375–520 g). One group (n = 8) was pretreated with intracolonic turpentine (1 ml, 25%; method similar to reflex studies) 24 h (±2 h) before study. Control rats received intracolonic vehicle coupled with subcutaneous administration of the turpentine solution (n = 5) or sham treatment (n = 28). The electrophysiological preparation was identical to that of our previous studies (22–24, 26, 27, 29, 30). Neurons were stratified into two groups based on published criteria (24, 29) that define neurons based on the temporal characteristics of their responses to CRD as well as their responses to a nonsegmental noxious stimulus (pinch of skin in cervical dermatomes). Abrupt neurons were defined as neurons that demonstrated increased activity during CRD, an abrupt return to baseline or subbaseline levels of activity after the termination of the phasic distending stimulus, and >20% inhibition of their spontaneous activity by a nonsegmental noxious stimulus. Sustained neurons were defined as neurons that demonstrated a sustained afterdischarge for >4 s after termination of the phasic distending stimulus and no inhibition by a nonsegmental noxious stimulus. If there was ambiguity regarding the temporal characteristics of the neuron’s responses, the response to a nonsegmental noxious stimulus was considered to be the determining factor because a previous characterization of these neurons (29) demonstrated this to be a reliable discriminating factor between abrupt and sustained neurons.

Electrophysiological preparation. For surgery, rats were deeply anesthetized with inhaled halothane (2–5%). Tracheal, carotid arterial, and jugular venous cannulas were inserted. The upper cervical spinal cord was exposed, and 50 μl of 1% lidocaine hydrochloride were injected bilaterally. The spinal cord was transected at C1, and the brain was mechanically pithed with a forceps. All anesthesia was then discontinued, and rats were ventilated with air-oxygen and allowed to recover for ~4 h. At that time, rats demonstrated vigorous flexion withdrawal reflex responses to tail pinch. Paralysis was then established with pancuronium bromide (0.2 mg/kg iv) Blood pressure was continuously monitored, and rats were kept at physiological temperatures using overhead lamps. Normal saline was administered as needed to prevent hypovolemia. The thoracolumbar or lumbosacral spinal cord was exposed by laminectomy, the rats were suspended from thoracic and lumbar vertebral clamps, and the dura mater was cut.

Unit characterization. Phasic, constant-pressure CRD was produced by inflating with air a 7- to 8-cm-long flexible latex balloon inserted transanally into the descending colon and rectum. Tungsten microelectrodes (0.9–1.2 MΩ; Micro Probe, Potomac, MD) were used for single-unit recordings 0- to 1-mm lateral to midline and 0.1- to 1-mm ventral to the spinal cord dorsum. Brief, phasic CRDs (80 mmHg) were used as the primary search stimuli. All isolated units that were reliably excited by CRD on three trials were characterized further. Responses (excitatory/inhibitory) were determined to cutaneous inputs (nonnoxious brush and noxious pinch). The effect of a 5-s application of a clamp to the skin of the upper body (a nonsegmental somatic noxious stimulus) on spontaneous activity was also determined and quantified. To quantify neuron responses, units were displayed on an oscilloscope for continuous monitoring, discriminated conventionally from background, converted into uniform pulses, and saved by computer. For some units, responses to graded CRD (20, 40, 60, 80, and 100 mmHg for 20 s) were obtained.

Statistics

Descriptive statistics are reported as means ± SE. Statistical comparisons were made using a one or two-way
RESULTS

General Observations

Due to ethical concerns, awake unanesthetized rats were closely observed for any signs of distress after treatment with intracolonic turpentine. Subjectively, before insertion of the distending balloon, rats pretreated with turpentine demonstrated normal motor activity, continued to eat food pellets and drink from water bottles, did not self mutilate, gave no obvious signs of increased sympathetic nervous system activation such as piloerection, and did not vocalize to any greater extent than did control rats when handled. Rats in all groups often defecated before balloon insertion. Rats pretreated with turpentine 1–6 h before testing were noted to have loose stools but no signs of blood. However, rats treated 24–48 h before testing had occasional flecks of blood in harder stools. For studies of reflex measures, there were no statistically significant differences between rats receiving intracolonic vehicle injections and no pretreatment; data from these two groups of rats were combined to form data for control rats.

Cardiovascular Measures

Pressor responses to CRD were enhanced at all times tested after intracolonic turpentine, although this difference was generally apparent only during the initial distensions (Fig. 1A). A “wind-up” phenomenon, whereby repeated CRDs lead to increased responses over the first 5–10 distensions, is apparent in this data and has been noted previously (25). Although there appears to be little effect of intracolonic turpentine on the final, stable, peak pressor responses to CRD in groups treated 1, 2, 6, and 48 h before testing, there was a statistically significant greater pressor effect during the first distension when pretreatment with turpentine occurred 2, 6, or 24 h before testing (P < 0.05; Fig. 1A). Pressor responses in rats treated with turpentine 24 h before testing were significantly greater than pressor responses in control rats over all trials even after multiple CRDs (initial distensions, P < 0.01; last distensions, P < 0.05; Fig. 1A). This difference was also observed in pressor responses to graded CRD (Fig. 1B), which were measured after multiple CRDs. Significantly greater responses were apparent at intensities of 20–80 mmHg in rats treated with turpentine 24 h before testing (P < 0.05 for 20, 40, 60, and 80 mmHg; not significantly different for 100-mmHg measurement). Graded responses to CRD did not differ from controls in rats treated with turpentine 1, 2, 6, and 48 h before testing (Fig. 1B). It is notable that these graded responses were obtained after six or more previous CRDs.

Evidence of Inflammation

Rats treated with turpentine 1–48 h before subsequent distention of the descending colon and rectum had significantly greater amounts of Evans blue extravasated than control rats that received only distension (P < 0.05 at all time points; Fig. 1C). Discrete areas of mucosal and submucosal irritation were apparent in many of the turpentine-treated colons.

Colonometric Measures

As depicted by Fig. 2A, the compliance of the descending colon and rectum as measured colonometrically was decreased by the intracolonic application of turpentine. Distension with a given volume of intraluminal water produced a greater intraluminal pressure in turpentine-treated colons than was produced by the same volume of distending water in uninflamed control colons. That is, turpentine-treated colons are less compliant. Using two-way ANOVA, we found that rats treated with intracolonic turpentine 6, 24, and 48 h
before measurement had colons that were significantly less compliant ($P < 0.05$) than those from control rats. This change in compliance may represent local tissue swelling or a change in tone produced by neural and/or humoral inputs. Post hoc data analysis failed to show statistically significant differences at any specific distending volume. A: colonometric measures of compliance demonstrated significant decreases in compliance (measured as a greater increase in pressure with identical volumes of distension) in rats treated 6, 24, and 48 h before testing (2-way ANOVA analysis with $P < 0.05$). Post hoc analysis did not demonstrate statistically significant differences at any specific distending volume. B: the visceromotor threshold (minimal intraluminal distending pressure necessary to evoke a reflex contraction of abdominal and hindlimb musculature) was lower in rats treated with turpentine 2, 6, 24, and 48 h before testing than in control rats ($n = 5–6/group$). **$P < 0.01$, significantly different from control rats. Values are means $\pm$ SE.

**Visceromotor Responses**

The pressure threshold for evocation of a visceromotor response (contraction of abdominal and hindlimb musculature), $V_{MT}$, was significantly lower in rats pretreated with turpentine 2, 6, 24, and 48 h before testing when compared with measures in control rats ($P < 0.01$ in those groups; Fig. 2B). Changes in $V_{MT}$ were noted at 1 h after treatment, but there was great variability between subjects. Subjectively, there did not appear to be any difference in the vigor of the visceromotor responses in rats pretreated with turpentine when compared with control rats. Because $V_{MT}$ was significantly decreased at a time when colonic compliance had not yet changed (2-h time point) and at intraluminal pressures (25–35 mmHg) associated with virtually identical intraluminal volumes in the different treatment groups (see Fig. 2A), it is unlikely that the reduced $V_{MT}$ was due to a decrease in compliance.

**Neuron Responses**

The characteristics of the studied neurons are given in Table 1. Fifty neurons were characterized in eight rats treated with intracolic turpentine 24 h before testing, and ninety-six neurons were characterized in control rats. The 24-h time point for turpentine pretreatment was selected because the reflex and Evans blue extravasation studies indicated a peak effect of inflammation at that time. All neurons were excited by noxious cutaneous stimuli, and some neurons were also excited by the addition of nonnoxious stimuli. In general, in turpentine-treated rats stimulation of the convergent cutaneous receptive fields of neurons excited by CRD evoked less vigorous responses than similar stimulation of cutaneous receptive fields in control rats. However, this difference was not quantitatively examined. Neurons were classified as abrupt or sustained neurons (as described in METHODS). Abrupt neurons characterized in turpentine-pretreated rats had significantly less spontaneous and CRD-evoked activity than abrupt neurons characterized in control rats ($P < 0.01$ for both measures). This was opposite to the effect noted in sustained neurons: those characterized in turpentine-pretreated rats had significantly more spontaneous ($P < 0.01$) and CRD-evoked activity

Table 1. Characteristics of lumbosacral spinal dorsal horn neurons excited by 80-mmHg, 20-s CRD

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>Treatment</th>
<th>$n$</th>
<th>Spontaneous Activity, Hz</th>
<th>Evoked Activity, counts</th>
<th>Receptive Field</th>
<th>Depth, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrupt</td>
<td>Control</td>
<td>53</td>
<td>18.0±1.9</td>
<td>641±54</td>
<td>44:9</td>
<td>0.51±0.04</td>
</tr>
<tr>
<td></td>
<td>Turp</td>
<td>21</td>
<td>8.6±1.9*</td>
<td>185±47*</td>
<td>15:6</td>
<td>0.48±0.08</td>
</tr>
<tr>
<td>Sustained</td>
<td>Control</td>
<td>43</td>
<td>6.2±1.1</td>
<td>426±46</td>
<td>29:14</td>
<td>0.52±0.05</td>
</tr>
<tr>
<td></td>
<td>Turp</td>
<td>29</td>
<td>20.9±2.9*</td>
<td>527±79**</td>
<td>12:17</td>
<td>0.53±0.05</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE. The Abrupt vs. Sustained classification scheme is described in METHODS and is similar to that found in other studies (22–24, 26–30). Control rats received pretreatment with intracolic vehicle and subcutaneous turpentine ($n = 5$) 24 h before study or received no pretreatment ($n = 28$). Turp, rats ($n = 8$) that received 1 ml of a 25% solution of turpentine applied topically to the colon 24 h before study. Evoked activity represents the no. of neuron depolarizations discriminated during the period of colorectal distension (CRD) minus the prestimulus spontaneous activity. Receptive field indicates the no. of neurons excited by noxious and nonnoxious (class 2) or only noxious cutaneous stimuli (class 3). Depth indicates distance of electrode penetration below the cord dorsum. *$P < 0.01$ and **$P < 0.05$, significantly different from control rats.
(P < 0.05) than sustained neurons characterized in control rats. Other characteristics of abrupt and sustained neurons were similar in the two groups (Table 1).

Both abrupt and sustained neurons encode for CRD in a monotonic, accelerating fashion, but the effect of turpentine pretreatment on the normally linear mean stimulus-response functions differed (Fig. 3). In abrupt neurons, turpentine pretreatment resulted in a reduction in the response magnitude to CRD at all intensities of distension (Fig. 3A). Even when the data are normalized to the response to a 100-mmHg, 20-s CRD (to account for possible sampling bias between treatment groups; Fig. 3A, inset), it is clear that the stimulus-encoding properties of abrupt neurons for CRD are less linear when given the turpentine pretreatment. In contrast to the abrupt neurons, responses of sustained neurons were enhanced by turpentine pretreatment, particularly at low intensities. In the turpentine-pretreated rats, the stimulus response function of the sustained neurons is relatively linear at intensities of 20–80 mmHg but appears to peak with no additional activity evoked by the greater intensity of 100 mmHg (Fig. 3B). As evidenced by the normalized data, there is a leftward shift in the stimulus-response function revealing a two- to threefold greater response to the lower intensities of CRD (20 and 40 mmHg) when compared with neurons in control rats (Fig. 3B, inset).

DISCUSSION

The present study demonstrated that chemical inflammation of the colon-rectum leads to increases in reflex responses to CRD and to increased CRD-evoked activity in a subset of spinal dorsal horn neurons encoding for CRD, the sustained neurons. The magnitude of the alterations in the reflex responses correlated in time with an index of local inflammation, the extravasation of Evans blue into colorectal tissues. The mechanisms of these increased responses may be due to both processes at the level of the primary afferent transducer as well as central processes (spinal cord or brain).

Products of inflammation such as prostaglandins, leukotrienes, histamine, and kinins have been demonstrated to sensitize primary afferent neurons that are normally activated by mechanical or other chemical stimuli. These afferent neurons then become activated by lower intensities of stimulation and/or respond with greater activity to equal intensities of stimulation (13). A subset of primary afferent neurons described in visceral systems (e.g., the urinary bladder; Refs. 9 and 10) are so-called “silent” nociceptors that only become mechanosensitive after chemical irritation. Evidence for such sensitized and silent nociceptors has been presented (37) for primary afferents excited by CRD using mustard oil and acetic acid as proinflammatory agents. In addition to direct neuronal effects, it is also possible that products of inflammation affect the muscle and connective tissue strata to which the nerve endings attach and so secondarily produce increased primary afferent neuron activity. For example, histamine, PGE2, and PGF2α all produce contractions of colonic smooth muscle (34) that might serve to modify the activity of primary afferents adjacent to smooth muscle.

There are numerous studies demonstrating neurochemical and neurophysiological changes in the spinal cord that occur secondary to a peripheral inflammatory process in both somatic (6, 33, 35, 40) and visceral systems (1, 7, 18, 19). As a consequence, it would be
expected that changes in spinal cord processing may occur secondary to the local inflammatory effects of turpentine. The neurophysiological portion of the present study gave clear evidence that such an alteration in the response of spinal dorsal horn neurons to visceral stimuli did occur. However, it is notable that peripheral inflammation did not lead to a global increase in excitability within the spinal dorsal horn but rather to a selective increase in neuronal activity of one neuronal subpopulation that was coupled with a selective decrease in the activity of a different neuronal subpopulation. A comparison of control and turpentine-pretreatment neuron populations indicates that there is an increase in the activity of sustained neurons and a decrease in the activity of abrupt neurons. Because both of these neuronal groups encode for CRD, it would be expected that a generalized increase in primary afferent activity would produce increased activity in both groups. However, this was not the case. As a consequence, it must be hypothesized that either peripheral inflammatory changes selectively decrease the primary afferent activity of inputs selective for abrupt neurons or central inhibitory mechanisms selective for abrupt neurons have been increased. Selective desensitization of primary afferents has not been noted in this system, and as defined in this study, abrupt neurons have a central inhibitory system that is selective for them. Abrupt neurons are inhibited by nonsegmental noxious stimuli, and sustained neurons are not similarly inhibited. Hence, the latter hypothesis that an alteration in central inhibitory mechanisms occurs secondary to peripheral inflammatory changes becomes more tenable.

The present study differs quantitatively from our (30) preliminary repeated-measures study in several ways. In our (30) previous study, neuronal responses were examined for the first 2 h after turpentine application. No statistically significant effect was noted in the activity of abrupt neurons, but their mean activity demonstrated a slight decline. This is in contrast to the present study in which the abrupt neurons were markedly less responsive in turpentine-treated rats than in control rats. In the previous study (30), sustained neurons had increased spontaneous activity and demonstrated a trend toward increased CRD-evoked activity, whereas in the present study both the spontaneous activity and CRD-evoked activity of sustained neurons in turpentine-treated rats was greater than the corresponding activities of sustained neurons in control rats. In all, these findings should not be surprising because the present study demonstrated that the vigor of reflex responses to CRD was only mildly increased 2 h after turpentine treatment but was robustly increased 24 h after turpentine treatment. The neuronal “trends” noted at 2 h after turpentine treatment were similarly more robust at 24 h after turpentine treatment.

The findings of the present and previous studies (30) suggest that pain from the gastrointestinal tract may be related to the altered balance between abrupt and sustained neurons. The precise role of these different neuron subtypes is still, as yet, undefined. Both abrupt and sustained neurons have been demonstrated (24, 26) to have long ascending axonal projections to the brain and are inhibited by analgesics (22, 23, 27), which suggests a role in nociception, but the methodology of the present study does not rule out effects on local circuitry. The abrupt neurons characterized in this study effectively have an “inhibitory surround” to their convergent cutaneous fields because noxious stimulation outside of their excitatory receptive field produces inhibition. For this reason, it is reasonable to speculate that the abrupt neurons are more likely to be involved in the localization of painful events than sustained neurons, which do not have such an inhibitory surround. A natural extension of this speculation is that the poor localization that is a hallmark of clinical visceral pain may be due to the phenomenon noted in this study: increased sustained neuron activity and decreased abrupt neuron activity. We propose that when the gastrointestinal tract has normal physiological functioning there are only transient, localized intraluminal pressure alterations. These lead to afferent neural input from the gut to the spinal cord that is intermittent and low intensity, and, as a consequence, central alterations in processing do not occur. However, with repeated distensions or the sensitization of primary afferents by inflammation, alterations in dorsal horn processing occur and pain ensues.

Clinical medicine suggests that pain processing from the gastrointestinal tract is not unique. That is, other visceral structures are affected similarly. For example, acute and chronic inflammation of the gallbladder leads to tenderness to palpation and pain when obstructed or physiologically activated (e.g., by a fatty meal; Ref. 38), and inflammation of the urinary bladder produced by a bacterial infection leads initially to urgency and tenderness to palpation and pain with urination (39). It is easy to postulate that similar alterations in central processing occur in pain-processing sites corresponding to these organs, resulting in a state of visceral hypersensitivity or hyperalgesia. The “irritable focus” theory of MacKenzie (16) long ago explained visceral pain in such a fashion. Neurophysiological data do not support specifics of MacKenzie’s (16) theory. However, a modification of the “convergence-projection” theory of Ruch (36) as previously proposed (28) to allow for plasticity of responses due to prolonged noxious inputs, does give one modeling of visceral pain that explains both clinical and experimental findings. In humans, colonic inflammation alone does not reliably lead to the sensation of pain. It is interesting therefore that, with the exception of rats treated with turpentine 24 h before testing, the effects of turpentine on reflex responses to CRD could not be distinguished from the effects of repeated distension. A sensitization process occurs both in rats and humans in response to repeated distensions of the distal gastrointestinal tract (17, 25, 26, 31). That is, repeated presentations of the same stimulus lead to progressively greater responses
(or lower thresholds for response) until eventually a stable response or threshold occurs. We (25, 26) have previously postulated that this sensitization process may be due to a combination of local tissue effects and alterations within the spinal cord that occur secondary to prolonged noiceptive input. The present study demonstrated that pretreatment with turpentine augmented this sensitization process.

In summary, application of the chemical irritant turpentine to the colorectal mucosa led to inflammation, alterations in compliance, and augmentation of reflex responses to CRD. The presumed neurophysiological basis for these changes is an increase in the activity of one of the neuron subtypes encoding for the stimulus of CRD, sustained neurons, which argues for the importance of that neuron subgroup in inflammation-related visceral pain. The decrease in the CRD-related activity of abrupt neurons is similarly important and may explain other phenomena of inflammatory visceral pain, such as its poor localization.

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