Adaptation of stress-induced mucosal pathophysiology in rat colon involves opioid pathways

DERRICK A. YATES, JAVIER SANTOS, JOHAN D. SÖDERHOLM, AND MARY H. PERDUE
Intestinal Disease Research Program, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Received 12 December 2000; accepted in final form 5 February 2001.

Yates, Derrick A., Javier Santos, Johan D. Söderholm, and Mary H. Perdue. Adaptation of stress-induced mucosal pathophysiology in rat colon involves opioid pathways. Am J Physiol Gastrointest Liver Physiol 281: G124–G128, 2001.—Acute stress increases ion secretion and permeability of rat colonic epithelium. However, it is not known if stress-induced mucosal changes are subject to adaptation. Wistar-Kyoto rats were exposed to either continuous water-avoidance stress (CS) for 60 min or intermittent stress (IS) for three 20-min periods. Distal colonic segments were mounted in Ussing Chambers, and ion-transport (short-circuit current (Isc)) and permeability (conductance and flux of horseradish peroxidase (HRP)) parameters were measured. CS significantly increased Isc, conductance, and HRP flux compared with control values. In contrast, in IS rats these variables were similar to those in nonstressed controls. To study the pathways involved in IS-induced adaptation, rats were pretreated intraperitoneally with the opioid antagonists naloxone or methylnaloxone. Opioid antagonists had no effect on values in control or CS rats. However, in the IS group, naloxone and methylnaloxone reversed the adaptive responses, and all variables increased to CS values. We conclude that stress-induced colonic mucosal pathophysiology is subject to rapid adaptation, which involves opioid pathways.

Clinical data implicate psychological stress as an important determinant in the reactivation of inflammatory bowel disease (1, 20), as well as a contributing factor in irritable bowel syndrome, gastric ulcers, and gastric hemorrhage (4, 13). Experimental studies (2, 9, 21, 23, 24, 32) indicate that the gastrointestinal tract is susceptible to stress-induced pathophysiology. Previous studies (25–27) in our laboratory demonstrated that exposure of rats to acute stress stimulated intestinal ion secretion and enhanced epithelial permeability. Such alterations may result in a loss of electrolytes and water, leading to diarrhea, and enhanced uptake of bacterial products and/or antigens across the epithelium (18), possibly leading to the development of intestinal mucosal inflammation (31). Although all humans encounter life stress to various degrees, only a portion of the population develops overt gastrointestinal disorders. However, little is known about defense mechanisms to counteract stress-induced dysfunction in the gastrointestinal tract.

Adaptive responses in the body are responsible for maintaining internal homeostasis and represent a mechanism for dealing with stress (16). Endogenous opioids, small peptides released in an active form from neurons in both the brain and the periphery, are one class of mediators associated with adaptation (7). Centrally, opioids act on a number of targets, including the hypothalamic-pituitary-adrenal (HPA) axis where they downregulate the release of corticotropin-releasing hormone (CRH), a key component in the central stress response (8). However, the role of opioids in mediating adaptation of stress-induced abnormalities in peripheral systems has not been thoroughly explored.

Opioids, such as codeine and loperamide, are used to treat diarrhea by inhibiting electrolyte secretion and decreasing intestinal motility (11). The presence of opioid receptors has been demonstrated on enteric neurons, as well as on epithelial cells in porcine and guinea pig intestine (6, 19, 22). On the basis of our (25–27) previous findings demonstrating stress-induced functional changes in rat colonic mucosa, we hypothesized that opioid-dependent adaptive mechanisms may serve to limit intestinal pathophysiology. To study the adaptive phenomenon, we developed a model of acute intermittent exposure to stress. We found that stress-induced increases in colonic ion secretion and permeability were subject to adaptation and that endogenous opioids were involved in this response. This information may lead to new approaches for treating intestinal disorders influenced by stress.

**METHODS**

**Animals**

Male Wistar-Kyoto rats (300–350 g, Charles River Laboratories, St. Constant, QC, Canada), housed two per cage, were maintained on a normal 12:12-h light-dark cycle and provided with food and water ad libitum. Rats were handled daily by one investigator for 2 wk before the study. All the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: M. H. Perdue, Intestinal Disease Research Program, HSC-3N5C, McMaster Univ., 1200 Main St. West, Hamilton, Ontario, Canada L8N 3Z5 (E-mail: perdue@mcmaster.ca).
experimental stress procedures were performed between 9:30 and 11:30 AM to minimize the effect of circadian rhythm. Rats were euthanized by decapitation. All procedures were approved by the Animal Care Committee at McMaster University.

**Stress Protocols**

Rats were randomly exposed to either continuous stress (CS) for 60 min or intermittent stress (IS) for three 20-min periods separated by 5 min of rest in the home cage. The stress protocol consisted of placing rats individually in a tank (56 × 50 cm) on a small platform (8 × 6 cm) surrounded by room temperature water −1–2 cm below the platform. Controls, sham stressed (SS) rats, were placed on the same platform in the tank without water.

**Drug Treatments**

Naloxone (Sigma Chemical, St. Louis, MO) was dissolved according to the manufacturer’s instructions, aliquoted, and stored at −80°C. Naloxone was used at a dose of 5 mg/kg ip, which was previously shown to be effective for nonspecific blocking of opioid receptors in various models in the rat (5, 15). Naloxone methyl bromide (methylnaloxone) (Sigma-RBI, Natick, MA), a quaternary derivative of naloxone that does not cross the blood-brain barrier, was used to distinguish peripheral from central effects. Methylnaloxone was administered at 50 mg/kg, because it is less potent than naloxone (12).

**Colonic Epithelial Physiology**

**Ussing chamber studies.** The distal colon was removed, placed in 37°C oxygenated Krebs buffer, stripped of longitudinal muscle and myenteric plexus, and opened along the mesenteric border. Four adjacent pieces from each rat were mounted in Ussing chambers (W-P Instruments, Narco Scientific, Mississauga, ON, Canada). The chamber opening exposed 0.6 cm² of tissue surface area to 8 ml of circulating oxygenated Krebs buffer at 37°C. The buffer contained (in mM) 115 NaCl, 1.25 CaCl₂, 1.2 MgCl₂, 2 K₂HPO₄, and 25 NaHCO₃, pH 7.35 ± 0.02. In addition, the serosal buffer also contained 10 mM glucose as an energy source, which was osmotically balanced by 10 mM manitol in the mucosal buffer. The chambers contained agar-salt bridges to monitor the potential difference across the tissue and to inject the required short-circuit current (Iₛₑ) to maintain a zero potential difference as registered via an automated voltage clamp (W-P Instruments). Iₛₑ (in μA/cm²) was recorded continuously by a computer connected to the voltage clamp system. Tissue conductance (in mS/cm²) was calculated according to Ohm’s law. Baseline values for Iₛₑ as an indicator of ion secretion, and conductance, an indicator of ion permeability, were calculated at equilibrium, 15 min after the tissues were mounted.

Horseradish peroxidase (HRP) (Sigma Chemical) was used as a model protein probe to examine macromolecular permeability. Fifteen minutes after mounting the tissues, type VI HRP (10⁻⁵ M) was added to the luminal buffer and allowed to equilibrate for 30 min. Serosal samples (0.5 ml) were obtained at 30-min intervals for 2 h and replaced with buffer to maintain a constant volume in the chambers. HRP activity was determined by a modified Worthington method, as previously described (18). The mucosal-to-serosal flux of HRP was the average value of two consecutive stable flux periods.

**Colonic Motility**

Colonic motility in each rat was estimated by the fecal pellet output. The number of pellets expelled during the stress period was recorded.

**Serum Corticosterone**

Blood samples were collected from rats immediately after decapitation, and serum was obtained. Corticosterone concentrations were measured by RIA (Immuchem, ICN Biomedicals, Costa Mesa, CA).

**Experimental Design**

In the first part of the study, we determined the effect of exposing rats to CS on colonic Iₛₑ, conductance, and flux of HRP and compared the values with those of rats exposed to IS and SS. In addition, to determine if 20 min of water-avoidance stress was sufficient to induce abnormalities, we conducted some studies at this time point.

In the second part of the study, we evaluated whether naloxone or methyl-naloxone, administered intraperitoneally, was able to reverse the adaptive inhibition of the stress-induced pathophysiology. Rats were injected intraperitoneally with either saline, naloxone, or methyl-naloxone, 20 min before being subjected to either CS, IS, or SS. Ussing chamber studies were conducted, and colonic Iₛₑ, conductance, and HRP flux were examined.

**Statistical Analysis**

Results are expressed as means ± SE. Data were analyzed by ANOVA for multiple group comparisons. Fisher’s post hoc test or Student’s t-test was used where appropriate. Statistical significance was designated as P < 0.05.

**RESULTS**

**CS Altered Mucosal Physiology**

CS for 60 min resulted in a significant increase in baseline Iₛₑ, tissue conductance, and flux of the macromolecule HRP compared with SS control rats (Fig. 1). In contrast, IS for three 20-min periods had no effect on either baseline Iₛₑ or conductance values (Fig. 1, A and B). In addition, the HRP flux in the IS group was not different from that of SS controls (Fig. 1C).

Serum corticosterone levels of 161 ± 3.5, 260 ± 23.1, and 193 ± 6.7 ng/ml in SS, CS (P < 0.05 vs. SS), and IS rats (P < 0.05 vs. SS and CS), respectively, suggested that the IS rats had experienced stress. To further examine this issue, we compared rats exposed to one 20-min period of stress with CS and SS rats. The rats exposed to a single 20-min period of stress demonstrated significantly increased (P < 0.05) values for Iₛₑ, as well as conductance and flux of HRP, relative to SS controls (Table 1). Increases in conductance and HRP flux at 20 min were equal to or greater than those observed after 60 min CS, whereas the increase in Iₛₑ was approximately one-half of the 60-min CS value, but still significantly elevated above SS control values. These results contrasted with those of the IS group, in which there were no differences compared with SS rats (Fig. 1). This suggests that adaptive responses were activated by the IS protocol.
Fig. 1. Adaptation to stress-induced changes in colonic mucosal function. Results are indicated for short-circuit current ($I_{sc}$, A), tissue conductance ($G$, B), and horseradish peroxidase (HRP) flux (C). Rats were exposed to sham stress (SS), continuous water-avoidance stress (CS) for 60 min, or intermittent water-avoidance stress (IS) for 3 20-min periods with 5-min rest periods. Bars represent means ± SE; $n = 5–6$ rats/group, with 3–4 tissues averaged per rat. *$P < 0.05$ vs. SS; **$P < 0.01$ vs. SS.

**Opioid Receptor Antagonists Reversed Adaptation to Stress-Induced Mucosal Physiology**

To study the possible involvement of opioids in the adaptive response to stress, we used the nonspecific opioid antagonists naloxone and methylnaloxone (which does not cross the blood-brain barrier). Naloxone (ip) did not alter epithelial parameters in SS rats nor did it have effects on CS-induced epithelial pathophysiology (Fig. 2). However, rats injected with naloxone before IS demonstrated a significant increase ($P < 0.01$) in $I_{sc}$, conductance, and flux of HRP compared with SS and saline-injected IS rats. In fact, values for ion secretion, conductance, and macromolecular permeability were similar to those observed after CS. Injection with methylnaloxone resulted in similar effects to those observed with naloxone. In IS rats pretreated with methylnaloxone, a significant increase ($P < 0.02$) in $I_{sc}$, conductance, and HRP flux occurred compared with SS and nontreated IS rats (Fig. 2), whereas methylnaloxone pretreatment had no effect on values in SS or CS rats.

**Stress Increased Fecal Output**

Colonic propulsive motor activity was increased during exposure to stress, as shown by the greater number of fecal pellets expelled by stressed vs. SS rats. In both the IS and CS rat groups, all pellets were expelled during the first 20 min, i.e., before the first rest period in the IS group. The effect was significant for both CS (10.5 ± 0.9 pellets/h) and IS rats (8.6 ± 2.2 pellets/h) compared with SS rats (2 ± 0.8 pellets/h). Pretreatment with naloxone (9 ± 0.9 and 9.2 ± 1.5, pellets/h, for CS and IS, respectively) and methylnaloxone (7.2 ± 2.5 and 7.5 ± 1.9, pellets/h, for CS and IS, respectively) did not affect pellet expulsion in any of the stress protocols.

**DISCUSSION**

In this study, we have shown that rat colonic mucosa demonstrates adaptation to acute stress when experienced intermittently, whereas CS stimulates an increase in ion secretion, as well as permeability to ions and macromolecules. The homeostatic response observed in IS, i.e., secretory and permeability parameters maintained at control levels, was inhibited by intraperitoneal injection of either naloxone or methylnaloxone, suggesting that the adaptive effects were mediated by peripheral opioid receptors.

The effects of stress on the intestine have been well documented in recent years (31). Studies in rodents have demonstrated stress-induced changes in gastrointestinal motility (32), mucus production (9), epithelial ion secretion (27), and macromolecule permeability (18), as well as reactivation of experimental colitis (21). Peripherally released CRH and mucosal mast cells have proven to be key players in this stress-induced mucosal pathophysiology (9, 10, 23, 25). The results from animal studies agree with observations of increased jejunal ion and water secretion after acute stress in humans (2, 24).

Adaptation maintains internal homeostasis under stressful conditions. Although the central adaptive response to stress has been characterized with respect to brain structure and function (16), mechanisms involved in adaptation of stress-induced pathophysiology in peripheral organs have not been described. In the present study, we have developed a model of IS, in which rats are maintained two 5-min periods of rest during 60 min of water-avoidance stress. As previously shown (25), 60 min of CS significantly increased ion secretion and tissue conductance as well as macromolecular permeability. In contrast, in our model of IS, no change was observed in any of these parameters compared with SS rats. To determine if this result represented an inhibitory mechanism or was simply a lack of sufficient stress to elicit a response, we examined the effect of 20 min of CS, equivalent to the first IS stress period. We found that 20 min of CS were sufficient to elicit a

Table 1. Effects of 60- and 20-min CS on colonic mucosal function

<table>
<thead>
<tr>
<th>Stress Protocol</th>
<th>$I_{sc}$, $\mu$A/cm²</th>
<th>$G$, mS/cm²</th>
<th>HRP, pmol·h⁻¹·cm⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-min SS</td>
<td>32.4 ± 5.7</td>
<td>15.7 ± 1.1</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>60-min CS</td>
<td>79.3 ± 15*</td>
<td>25.9 ± 3.8*</td>
<td>13.3 ± 3.6*</td>
</tr>
<tr>
<td>20-min CS</td>
<td>52.9 ± 4.4*</td>
<td>32.4 ± 9*</td>
<td>18.9 ± 3.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 5–6$ rats/group, with 3–4 tissues averaged per rat. Rats were submitted to either sham stress (60-min SS), continuous water avoidance stress for 60 min (60-min CS), or a single, continuous stress 20-min period (20-min CS). Immediately after stress, colonic segments were mounted in Ussing chambers and assessed for short-circuit current ($I_{sc}$), tissue conductance ($G$), and transmucosal flux of horseradish peroxidase (HRP). *$P < 0.05$ vs. SS.
significant increase in $I_{sc}$, conductance, and flux of HRP. In addition, levels of circulating corticosterone were elevated above control values in IS rats. Therefore, we concluded that an adaptive response, promoted by the IS protocol, was responsible for reversing the epithelial pathophysiology.

The involvement of opioid receptors in the modulation of adaptive responses to stress-induced colonic pathophysiology is also a novel finding of this study. Centrally, endogenous opioids mediate adaptive responses by inhibiting the release of CRH in the HPA axis, thereby down-regulating the stress response (7, 28). Similarly, opioids have been shown (14, 33) to be of importance for the regulation of stress-induced behavioral changes. In the periphery, opioid receptors have been localized in the myenteric and submucosal plexi (6) and have also been shown (19) on enterocytes of intestinal villi and crypts. In the porcine jejunum, activation of peripheral opioid receptors on submucosal neurons inhibited the release of neurotransmitters that stimulate chloride secretion (22). Moreover, several in vitro studies (17, 19, 29) have demonstrated a proabsorptive role for $\mu$- and $\delta$-opioid receptor agonists in the submucosal plexus and enterocytes. However, to our knowledge, the role of peripheral opioids in mediating stress-induced mucosal pathophysiology has not been explored. Our present results show that pretreatment of IS rats with the nonspecific opioid antagonist naloxone abolished all adaptive responses in the mucosa, restoring stress-induced increases in $I_{sc}$, conductance, and HRP flux to values similar to those in rats exposed to CS. Furthermore, intraperitoneal injection with methylnaloxone, which does not cross the blood-brain barrier, rendered similar blocking effects on IS-induced inhibition. This strongly suggests the adaptive response to be a peripherally mediated event. However, there is also evidence that stress may induce a change in the permeability of the blood-brain barrier (3). Therefore, we cannot exclude the possibility that methylnaloxone was acting on central receptors (30). Previously, we have shown (25) that stress-induced changes in colonic epithelial function are mediated by CRH, by a mechanism that is not altered by blocking corticosterone synthesis. Although our present study does not indicate the precise site of action for opioids, it is possible that, analogous to effects in the CNS, peripheral opioid-containing nerves inhibit CRH release to counteract the mucosal pathophysiology. Further study is required to determine the location of the opioid receptors modulating the adaptive response in this model.

Morphine has been used to treat diarrhea for centuries, and it is well known that opioids have inhibitory effects on colonic motility, as well as ion secretion (11), but the IS protocol did not affect the stress-induced increase in fecal output. This is not surprising, because the pellets were expelled during the first 20 min of exposure to stress, i.e., before the first rest period in the IS group. This suggests that all pellets in the colon were expelled before activation of the adaptive response, and the opioid release, probably induced during the first period of rest, was too late to reverse the motility effect. In line with this, naloxone had no effect on pellet expulsion, because there was no adaptive response demonstrated.

In summary, we have developed a model of acute IS that promotes rapid adaptive responses, restoring normal mucosal physiology. We have shown that this brief relief from water-avoidance stress reverses the stress-
induced barrier and transport defects in the colon. Our results also show that endogenous opioid pathways are involved in this protective response, possibly acting on peripheral receptors in the gastrointestinal tract. Although the clinical relevance of these findings remains to be determined, we have identified a mechanism in rodents for coping with intestinal physiological changes in response to acute stress. Ion and water secretion may be of some short-term benefit in washing away noxious substances, but excessive secretion results in diarrhea. An increased intestinal permeability to macromolecules may initiate and/or promote mucosal inflammation. Impaired opioid-mediated control of secretion and permeability could result in an inability to maintain mucosal integrity, which may be of pathophysiological importance. In conclusion, it is possible that promotion of opioid-mediated adaptive responses in the intestine can be beneficial in stressed-related intestinal disorders.

This study was supported by a grant from the Canadian Institutes for Health Research. D. A. Yates received graduate student support from RX and D. Canada. J. Santos was a recipient of a Canadian Association of Gastroenterology and Medical Research Council (Astra Research Initiative) Fellowship. J. D. Söderholm was supported by the Swedish Society for Medical Research, the Swedish Institute, the Swedish Association of Gastroenterology and Medical Research Council (Association de recherche sur les maladies de l’intestin et du tube digestif).J. D. Söderholm was supported by a grant from the Canadian Institutes for Health Research for Health Research.

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