STRESS induces chemical changes in the central nervous system which alters the biochemistry and physiology of the digestive tract. The present study determines arachidonic acid oxidation and damage in the colon following stress. Ten rats were stressed by the cold-restraint method; ten were controls. Stress induced 0.5 ± 0.7 (S.D.) mucosal erosions whereas controls had none. Subepithelial hemorrhage and erosions occurred only in the proximal two-thirds of the colon. Prostaglandin E_2 synthesis was increased after stress compared to the control (381 ± 130 vs. 1610 ± 372 ng/g/min). Leukotriene C_4 synthesis also increased after stress (4217 ± 994 vs. 11300 ± 1662 ng/g/min). Synthesis of prostaglandin E_2 increased ($r = 0.9381$) with leukotriene C_4 . The response of the colon to stress is less severe than that in the stomach and may be related to regional regulation of prostaglandin and leukotriene synthesis.

Key words: Colitis, Stress, Prostaglandin, Leukotriene

Stress induced experimental colitis

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Introduction

The alimentary tract is very sensitive to stress. The upper gastrointestinal system appears to be more easily damaged by stressful stimuli than the large bowel. The occurrence of peptic ulcers has been linked to stress-induced acid secretion.¹ Dysfunction of intestinal motility and movement is also stress-related.² In patients with inflammatory bowel disease, emotional stress may slow small bowel movement but increase motility in the colon.^{3–5} Psychological stress may also cause abdominal pain, constipation or diarrhoea, symptoms of irritable bowel syndrome.^{6,7} Dysfunction of the gastrointestinal system is probably caused by alteration in the neurochemical control of motor activity and blood flow. Synthesis and release of neuropeptides and catecholamines in the brain mediate these functions through sympathetic and parasympathetic nerves. The different responses of the proximal and distal gastrointestinal tract to stress may be related in part to the neural control of the synthesis of vasoactive prostaglandins and leukotrienes in these tissues. The effect of stress on these arachidonic acid oxidation products and damage to the colonic mucosa was determined in the present study.

Methods

Animals: Rats were purchased from Holtzman Co., Madison, WI, and weighed 125–150 g. The rats were housed in an American Association for Laboratory Animal Services approved facility in which humidity and temperature were controlled,

and fed Rat Chow (Ralston Purina Co., St Louis, MO). *The Guide for the Care and Use of Laboratory Animals* was followed as recommended by the Institute of Laboratory Animal Resources National Research Council (Division of Research Resources, Bethesda, MD). Animals adapted to the environment for at least 1 week before being used experimentally. Ten were stressed by the fasting-cold-restraint method.⁸ Food was removed for 24 h before immobilizing the animal under a tight wire screen at 4°C for 4 h. Ten control animals remained in their cages undisturbed.

Tissue sampling: Following termination by cervical dislocation, the colon was excised, and the proximal and distal ends were marked. Then the colon was opened along the antimesenteric border. After washing the colons in saline at 4°C, the serosa was positioned on a glass plate to position the mucosa outward and lesions were identified by one investigator (L.K.) who was blinded to the treatment group. The colon was divided into thirds: proximal, midportion and distal. Lesions with a 2–3 mm adjacent area and normal appearing tissues from each section were excised for histology. The remaining mucosa was then scraped with the edge of a glass slide, weighed and used for enzyme assay. The stomach was also removed, opened along the lesser curvature and examined for the presence of blood, indicating that stress caused gastric hemorrhage. There was clotted blood in the stomachs from rats which had been stressed, but not in the control group.

Assays: As a measure of the activity of prostaglandin G/H synthase and 5-lipoxygenase, PGE_2 and LTC_4

were determined. Approximately 150 mg of tissue was suspended in 1 ml of 0.1 M NaCl, 4.8 mM KCl, 1.2 mM KPO_4 , 5 mM glutathione, 1 mM hemin, 3 mM $MgSO_4$, 8.8 mM HEPES, and 5 mM poloxamer 188 (a nonionic detergent) at pH 8 to permeabilize the cell membrane for 1 min. The tissue suspension was centrifuged for 1 min at $1500 \times g$. The supernatant was decanted and discarded. The tissue was resuspended in 1 ml of the identical buffer containing 132 mM sodium arachidonate, and incubated at $23^\circ C$ for 10 min. The synthesis of prostaglandins and leukotrienes by oxidation of arachidonate was terminated by the addition of 1.5 ml of acetone at $0^\circ C$. Arachidonic acid oxidation products were extracted as previously described.⁹ Prostaglandin E_2 and LTC_4 were derivatized with panacyl bromide, separated, identified and quantitated by reversed-phase HPLC.¹⁰

Calculations: The rate of synthesis was calculated in units of ng/min/g wet weight, and the means and standard errors of the mean were determined. Statistical significance between groups was determined by the ANOVA and the relationship between variables was determined by the product-moment correlation.

Histology: Samples of mucosa containing lesions and normal appearing tissues were obtained from stressed rats, and were fixed in a buffered 10% formaldehyde solution for histology. Mucosa from controls were taken from similar areas of the colon. Specimens were then embedded in paraffin, and 4 μm thick sections were cut for placement on a microscope slide. Sections were made at several different levels and stained with hematoxylin and eosin. The occurrence of subepithelial hemorrhage (petechiae), erosions (visible break in the mucosa) and ulcers (penetration of the muscularis mucosae) was determined.¹¹ A break in the mucosal surface, such as an erosion or ulcer, was more easily quantifiable in the colon than comparing degrees of inflammation, and only the number of breaks were counted.

Results

Gross and microscopic appearance: The mucosa from control rats was pinkish and had only few erythemic areas. Microscopically, subepithelial hemorrhages occurred only rarely and in two colons, but no erosions were identified (Fig. 1). The surface epithelium was intact, and the crypts of Lieberkühn contained goblet cells. A few lymphocytes were present in the interglandular lamina propria, and lymphatic nodules were well defined.

After cold-restraint stress the gross appearance of the mucosa became paler with an increase in areas

FIG. 1. Colon from control rats which was sampled from the middle third of the bowel. Note the intact surface epithelium and lymph nodule. (Magnification $\times 200$, H & E stain)

of erythema. Four colons had well-defined erosions. The mean number of erosions in the stress group was 0.5 ± 0.7 (S.E.M.) and occurred only in the proximal two-thirds of the colon (Fig. 2). Subepithelial hemorrhages were occasionally present in the colons and were located near the surface epithelium. The columnar epithelium was lost and erosions extended almost to the base of the crypts but not into the muscularis mucosa (Fig. 3).

FIG. 2. Inflammation of the mid-colon from a rat stressed for 4 h. Note denuded epithelium, inflammatory cells and hemorrhage in the lamina propria. (Magnification $\times 200$, H & E stain)

FIG. 3. Ulceration and inflammation of the mid-colon in the rat 4 h after stress. (Magnification $\times 50$, H & E stain)

Table 1. Effect of stress on arachidonate products

Group	Prostaglandin E ₂	Leukotriene C ₄
Control	381 ± 130	4 217 ± 994
Stress	1 610 ± 372*	11 300 ± 1 662*

Values are in ng/g/min and the mean ± S.E.M. are shown.

**p* < 0.01, compared to control.

Inflammatory cells were present in the exudate. Lymphatic nodules appeared larger and more lymphocytes were in the interglandular lamina propria than in controls.

Effect of stress on PGE₂ and LTC₄ synthesis: Prostaglandin E₂ synthesis increased three-fold after stress compared to the control mucosa (Table 1), but there was no relationship between ulceration and prostaglandin E₂ synthesis (*r* = -0.35667). Leukotriene C₄ synthesis increased by two-fold in the mucosa after stress, but also had no association with ulceration. A significant correlation (*r* = 0.93814, *p* < 0.001) occurred for the synthesis of prostaglandin E₂ and leukotriene C₄.

Discussion

After inducing stress for 4 h, all stomachs were hemorrhagic and had many lesions, but only a few colons had erosions. Although erythema and subepithelial hemorrhage was not scored or given a subjective number, the gross appearance of the mucosa suggested that cold-restraint stress induces colitis in many rats. Psychological stress could be responsible for the mild symptoms of gastrointestinal dysfunction experienced by healthy individuals.

In the normal colon, arachidonate appears to be primarily oxidized via the lipoxygenase rather than by the prostaglandin G/H synthase pathway. In the stomach, prostaglandin synthase is the major pathway, since leukotriene synthesis is approximately half of prostaglandin synthesis.¹² Stress induces an increase in leukotriene synthesis in the stomach which, compared to PGE₂, indicates that arachidonate oxidation changed toward leukotriene production. The normal colon appears to have a greater capacity for leukotriene synthesis than the stomach. While stress induces a significant increase in leukotriene synthesis in the stomach, there is only a two-fold increase in the colon. Comparing the relative synthesis of leukotriene and prostaglandin, there is no change in the direction of arachidonate oxidation after stress in the colon. The positive correlation of prostaglandin and leukotriene synthesis indicates that a common substrate to both enzymes must be limiting synthesis. Since

specimens are incubated in an excess of arachidonate for assay, the amount of oxygen available in tissue for synthesis is probably limiting oxidation. The lack of a significant correlation of erosions and synthesis of prostaglandins and leukotrienes is due to the small number of lesions.

The stress-related decrease in PGE₂ synthesis which occurs in gastric mucosa appears to be related to inadequate levels of arachidonic acid and oxygen.¹²⁻¹⁴ Corticosteroids inhibit phospholipase A₂ activity and the release of arachidonic acid from membrane phospholipids.^{15,16} Decreased blood flow would also diminish the delivery of extracolonic fatty acids to the mucosa for prostaglandin synthesis. The activity of prostaglandin synthase activity in the gastric mucosa is not impaired during stress if adequate levels of arachidonic acid are present.¹² Lipoxygenase activity, however, increases significantly with stress, and the shunting of arachidonic acid from prostaglandin synthesis to leukotriene synthesis is correlated with gastric erosions.¹² The colon sustains less injury after stress but both enzyme activities are increased. No shunting of arachidonic acid from PGE₂ to leukotrienes is apparent in the colon. The greater capacity of the colon for PGE₂ and LTC₄ synthesis suggests that blood flow and mucosal oxygenation was adequate during stress.

Intracellular arachidonate levels, however, may be limited in the colonic mucosa, affecting the synthesis of PGE₂ and LTC₄, decreasing blood flow and inducing ulceration in some animals.

Some evidence supporting a role for prostaglandin as mediators of cytoprotection in the colonic mucosa exists. Exogenous PGE₂ administration reduces damage to the mucosa of the colon induced by bile salts or ethanol.^{17,18} Prostaglandin E₂ prevents also gastric mucosal ulceration in a variety of stress models and its synthesis is important in cytoprotection against injurious agents.^{19,20} The increased synthesis of PGE₂ seen in inflammatory bowel disease may be related to a protective feedback mechanism once an insult to the mucosa occurs.

Subepithelial hemorrhage and lesions in the proximal colon and increased synthesis of PGE₂ and LTC₄ after stress may be related to its innervation. The distal colon, which had no visual damage after stress, receives parasympathetic neural fibers via pelvic nerves rather than the vagus nerve. Neurochemicals act with specificity and selectivity at different sites in the brain stem. Corticotropin-releasing factor (CRF) inhibits gastric acid secretion, gastric emptying and small bowel movement but stimulates colonic movement and faecal excretion.^{21,22} Subdiaphragmatic vagotomy prevents the delay in gastric emptying but has no effect on colonic movement.²² The central nucleus of the

amygdala may be particularly sensitive to neurotensin, β -endorphins and thyrotropin-releasing hormone and probably integrates stress-related inputs to connect the cortex with the hypothalamus and lower brain stem.²³ Neurotensin increases PGE₂ synthesis through an α -adrenergic mechanism which decreases acid secretion and increases mucosal blood flow.²⁴ Differences in response of the colon and stomach to stressful stimuli are probably related, in part, to the neural control of prostaglandin and leukotriene synthesis in these tissues.

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