COLITIS REDUCES SHORT-CIRCUIT CURRENT RESPONSE TO INFLAMMATORY MEDIATORS IN RAT COLONIC MUCOSA


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Abstract—Inflammatory mediators may contribute to the diarrhea associated with colitis. Although the secretory action of such mediators is reported in normal tissue, there is little information regarding their effects on inflamed tissue. We examined the short-circuit current response (Isc) to these mediators, in mitomycin-C (MC)-induced colitis, a model with histological similarities to colitis in man. Rats were injected once with MC (3.25 mg/kg, intraperitoneally) or vehicle. The colons were removed three and seven days later and mounted, devoid of muscularis, in Ussing chambers for measurement of Isc, potential difference (PD), and resistance (Rt). MC-treated rats had diarrhea after three days, and microscopic studies revealed colonic inflammation. There were no significant differences in Rt, PD, and Isc between control and MC-treated tissues at three and seven days. Maximal increases in Isc to bradykinin, prostaglandin E,

INTRODUCTION

Several inflammatory mediators evoke colonic electrolyte secretion when tested on normal, healthy colonic mucosa (1). Whether or not this effect contributes to the secretory diarrhea associated with inflammatory bowel disease (IBD) in
humans remains to be confirmed. The inflamed mucosa may behave differently toward these mediators. There are reports of decreased colonic Na\(^+\)K\(^+\)-ATPase activity (2), defective stimulation of cyclic AMP by prostaglandin E\(_2\) (3), and aberrations in enteric neural physiology (4–7) in the inflamed intestine.

Mitomycin-C (MC) is an antitumor antibiotic that produces colitis in rats, which closely resembles human ulcerative colitis (8). After a single intraperitoneal injection of MC, there is histologic evidence of diffuse colonic inflammation, limited to the mucosal layer. The inflammation is present within three to seven days after the injection and begins to subside after 14 days. This new model of colitis provides convenient means for evaluating the responsiveness of the inflamed colonic mucosa to stimulants of intestinal secretion.

We investigated the pattern of stimulation of short circuit current (I\(_{sc}\)), a measure of anion secretion in the colon, by the secretagogues bradykinin, substance P, prostaglandin E\(_1\), serotonin (5HT), carbachol, and theophylline (9). We reasoned that the response to these inflammatory mediators in the inflamed tissue might be different than in healthy mucosa due to alteration of their receptors or membrane components essential for signal transduction. Carbachol and theophylline were used as secretagogues without inflammatory activity. Theophylline was used as a type of control because its action is believed to be primarily intracellular, through an effect upon cyclic nucleotide phosphodiesterase, rather than through a membrane-associated receptor mechanism.

**MATERIALS AND METHODS**

*Induction of Colonic Inflammation in Rats.* Male Sprague-Dawley rats (200–300 g) were used. Experimental rats were given one intraperitoneal injection (3.25 mg/kg) of MC (a gift from Bristol-Meyers, Evansville, Indiana) and control rats were given one intraperitoneal injection of an identical volume of sterile water. Animals were allowed to eat and drink freely throughout the experiments. The animals were anesthetized using 45 mg/kg pentobarbital (Butler, Alsip, Illinois) at three and seven days after MC injection, and the colon was removed. Tissues were mounted on a paraffin plate, fixed in 10% buffered formalin, serially sectioned, and processed for histological analysis.

*Histological Evaluation of Inflammation in Colon.* Slides were coded to conceal the identity of the treatment groups and independently evaluated in a blind fashion. The inflammatory scoring system used has been described previously (8). In brief, the following eight histological parameters were assessed: vascular, dilatation, edema, epithelial cell loss (necrosis), cellular mucin depletion, neutrophil eosinophil and mononuclear cell infiltration, and fibrosis. Each criterion was scored on an ascending scale of 0 to 4, with a maximum total score of 32 (absence of any abnormality = 0, most severe inflammation = 32).

For assessing small intestinal inflammation, the following histological criteria were graded from 0 = no abnormality to 4 = severe abnormality: reduction in villi number, villi and mucosal thickening, chronic and acute inflammatory cell infiltrates, regenerating epithelial cells, and fibrosis (possible total score from 0 = no abnormality to 28 = severe inflammation).