Abstract—This study examined whether the immunocyte recruitment associated with a mild inflammatory state induced by acetic acid would produce detectable sulfidopeptide leukotriene (LT) levels from colonic tissues or in dialysates. Histological examination and measurements of peroxidase activities of inflamed tissues indicated edema, hyperplasia and neutrophil infiltration. Significant elevated LTB4 and prostaglandin E2 (PGE2) levels were found but only slight elevations in sulfidopeptide LTs occurred. A slight elevation in eosinophil peroxidase indicated that eosinophil infiltration also occurred. The increase in sulfidopeptide LT levels appeared insufficient by itself to alter secretory responses in the distal colon. However, combined with other immunocyte products such as PGs, the sulfidopeptide LTs may influence the symptomology of inflammatory bowel disease.

INTRODUCTION

Immunocyte infiltration, primarily of polymorphonuclear leukocytes (PMN), is associated with inflammatory conditions of the gut such as human inflammatory bowel disease (IBD) and colitis as well as experimental models of these inflammatory states in animals (1–13). As inflammatory mediators can be produced by these recruited cells, it is possible that augmented responses may occur when these cells are activated. Numerous studies have shown that inflammatory mediators can alter physiological functions of the intestinal tract, such as net water and electrolyte fluxes across the mucosa possibly leading to diarrhea (14–24). Specifically, the sulfidopeptide leukotrienes (SPLT) have been found to induce active anion secretion in the distal colon (18, 25, 26). A host defense response, increased colonic secretion has been associated with systemic immu-
nity and intestinal inflammation (27, 28). In previous work, we demonstrated that histamine-independent changes in short-circuit current (Isc), a measure of active transepithelial ion transport, were greater in inflamed than non-inflamed tissues following antigen challenge (28). We also reported that zileuton, a selective 5-lipoxygenase inhibitor (5-LO) and R-840, a dual inhibitor of 5-LO and cyclooxygenase (CO), reduced this antigen-induced increase in transepithelial ion transport in non-inflamed and inflamed guinea pig distal colonic segments. However, indomethacin was less effective in inflamed tissue than in non-inflamed tissue indicating that the augmented response may be partially due to increased SPLT production. As selective leukotriene antagonists and 5-lipoxygenase inhibitors (5-LO) have been suggested as possible new drugs for the treatment of IBD, we wished to determine the extent to which SPLTs were involved in this colonic hyperresponsiveness. The purpose of this study was to determine whether the enhanced response to inflamed tissues could be due to immunocyte products such as arachidonic acid metabolites and the sulfidopeptide LTs in particular. Therefore, we tested the hypothesis that increased transepithelial ion transport, under conditions of mild inflammation, is associated with increased amounts of SPLTs produced in colonic segments after stimulation with antigen or calcium ionophore.

METHODS AND MATERIALS

Materials. All drugs and biochemicals were purchased from Sigma Chemical Company (St. Louis, Missouri). Reagent grade chemicals were purchased from diverse sources.

Animals and Tissue Preparation. Colonic tissues were obtained from antigen sensitized male Hartley guinea pigs (Charles River) that weighed between 400–700 g and were 4 to 9 weeks in age. All animals were sensitized by a single intraperitoneal injection of 50 mg/kg chicken ovalbumin (OVA, grade V, Sigma Chemical, St. Louis, Missouri), at least 14 days prior to usage. The animals were housed for 2 to 7 weeks in groups of five animals. The holding rooms were maintained at 25°C with constant humidity control and were under a 12 hour light-dark cycle with lights on at 0700 hr.

The animals had continuous access to water and standard, non-medicated guinea pig chow (Ralston-Purina, St. Louis, Missouri). Each animal was pretreated with pentobarbital at a dose sufficient to produce surgical anesthesia (approximately 35 mg/kg intraperitoneally). Induction of colonic inflammation was achieved by the rectal instillation of a solution of 0.5% or 0.75% acetic acid in a volume of 2 ml. The enema was followed by a 2 ml saline rinse. The enema was infused through a 8 cm feeding tube with the tip inserted 5–7 cm from the anus. Guinea pigs providing control (non-inflamed) tissues received a 3 ml saline infusion. Experiments were initiated between 18–24 hrs after saline or acetic acid treatment. Each animal was pretreated with pentobarbital at a dose sufficient to produce surgical anesthesia (approximately 35 mg/kg intraperitoneally).

In experiments where isolated tissue samples were required, a midline laparotomy was performed and a segment of distal colon 10–12 cm in length was excised and opened along the mesenteric border. Animals were sacrificed with an intracardiac administration of a saturated potassium chloride solution. Tissues were bathed in ice-cold oxygenated Kreb's-Henseleit buffer solution