Inhibition of type IV phosphodiesterase (PDE IV) activity reduces the production of various proinflammatory cytokine and suppresses neutrophil activation. Nonsteroidal anti-inflammatory drugs such as aspirin induce gastric mucosal lesions. In the pathogenesis of aspirin-induced gastric mucosal lesion, the contributions of activated inflammatory cells and proinflammatory cytokine production are critical. The specific PDE IV inhibitor rolipram is known to be a potent inhibitor of inflammation by increasing intracellular cyclic AMP in leukocytes. The aim of the present study was to determine whether rolipram can ameliorate aspirin-induced gastric mucosal lesions in rats and whether the agent can inhibit the increase in neutrophil accumulation and the production of proinflammatory cytokines. Gastric lesions were produced by administration of aspirin (200 mg/kg) and HCl (0.15 N; 8.0 ml/kg). Rolipram was injected 30 min before aspirin administration. The tissue myeloperoxidase concentration in gastric mucosa was measured as an indicator of neutrophil infiltration. The gastric mucosal concentrations of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were determined by ELISA. The intragastric administration of aspirin induced multiple hemorrhagic erosions in rat gastric mucosa. Gastric mucosal lesions induced by aspirin were significantly inhibited by treatment with rolipram. The mucosal myeloperoxidase concentration was also suppressed by rolipram. Increases in the gastric content of TNF-α and IL-1β after aspirin administration were inhibited by pretreatment with rolipram. We demonstrated that the specific type IV PDE inhibitor, rolipram, could have a potent antiulcer effect, presumably mediated by its anti-inflammatory properties.

KEY WORDS: phosphodiesterase; cytokine; aspirin; cAMP.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin are widely used as anti-inflammatory, analgesic agents (1–3). However, NSAIDs are known to have adverse effects on the gastrointestinal tract, which frequently can be serious and life-threatening. Therefore effective strategies to protect the gastrointestinal mucosa from NSAIDs are required. Although, as the mechanisms of gastric lesions induced by NSAIDs, suppression of endogenous prostaglandin (PG) production by cyclooxygenase (COX) inhibition is considered to be important, the exact pathogenesis remains to be elucidated (4–6). Several investigators have reported that intraperitoneal injection of antineutrophil serum and immunoneutralization of adhesion molecules expressed on neutrophils and endothelial cells significantly attenuate the gastric mucosal injury induced by NSAIDs (8, 9).
Also, recent studies have reported that tumor necrosis factor-α (TNF-α) accumulates in the gastric mucosa after aspirin administration, and pretreatment with TNF-α inhibitors suppresses the gastric mucosal injury caused by aspirin (10, 11). Therefore, activation and infiltration of neutrophils and proinflammatory cytokines are critical factors in the development of gastric mucosal lesions induced by NSAIDs. On the other hand, recently the potential usefulness of the specific type IV phosphodiesterase inhibitor rolipram as a novel anti-inflammatory agent has been reported (12, 13). This specific type IV phosphodiesterase inhibitor increases intracellular cAMP in neutrophils by suppression of breakdown of cAMP to 5AMP and decreases TNF-α and N-formyl-methionyl-leucyl-phenylalnine (FMLP)-stimulated neutrophil adherence, production of reactive oxygen species, and degranulation (14). In addition, rolipram has been reported to ameliorate experimentally induced colonic mucosal lesions and ischemia reperfusion injury to the kidney in animal models (15, 16). Also, rolipram suppressed indomethacin-induced gastric mucosal injury (17). However, the effect of rolipram or the significance of type IV phosphodiesterase in the pathogenesis of gastric mucosal lesions has not been reported. In this study, we examined the effect of a specific type IV phosphodiesterase inhibitor, rolipram, on aspirin-induced gastric mucosal lesions and myeloperoxidase concentration in the gastric mucosa to understand the pathogenesis of aspirin-induced gastric mucosal lesions. Also, the effect of rolipram on proinflammatory cytokine production in gastric mucosa was studied.

MATERIALS AND METHODS

Animals. Experiments were approved by the Akita University Animal Care Committee. Male Sprague–Dawley rats (250–300 g) were fed on standard laboratory diet and water ad libitum and kept in cages at a temperature (22 ± 2°C) and humidity (55 ± 5%)-controlled room with a 12-hr dark–light cycle before and during experiments.

Chemicals. The specific phosphodiesterase IV inhibitor, rolipram, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rolipram was dissolved in a small volume of dimethylsulfoxide and then diluted with physiological saline just before injection.

Effect of the Specific Type IV Phosphodiesterase Inhibitor in an Aspirin-Induced Gastric Mucosal Injury Model. Aspirin-induced gastric injury was produced by intragastric administration of aspirin (200 mg/kg) and HCl (0.15 N; 8 ml/kg). Rolipram (2.5 or 5 mg/kg; n = 5 at each dose) or vehicle was injected intraperitoneally 30 min prior to aspirin administration. Control rats were given only physiological saline. The animals were sacrificed by stunning and cervical dislocation 6 hr after aspirin administration and the stomach was removed. Gastric mucosal lesions were measured by two independent observers blind to the treatment. The ulcer index was calculated as the sum of the lengths of all lesions (18).

Effect of the Specific Type IV Phosphodiesterase Inhibitor on the Myeloperoxidase (MPO) Concentration in the Gastric Mucosa. An assay of gastric mucosal MPO concentration was used to quantify the degree of neutrophil infiltration. Three hundred milligrams of scraped mucosa was homogenized for 30 sec with a polytron homogenizer (PT 1200; Kinematica AG, Littau, Switzerland) in 1.0 ml of ice-cold 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer (pH 6.0). Hexadecyltrimethylammonium bromide was used to negate the pseudoperoxidase activity of hemoglobin and to solubilize membrane-bound MPO. The homogenate was sonicated (US0 IKA Werke GrmlH and Co. KG, Staufen, Germany) for 10 sec, freeze–thawed three times, and centrifuged for 20 min at 18,000g. The supernatant was taken for determination of the enzyme activity utilizing an ELISA kit (Bioxytech, Oxis International, Inc., Portland, OR, USA). The change in absorbance at 405 nm was measured with a spectrophotometer (Microplate Reader Model 3550; Bio-Rad, Hercules, CA, USA). The concentration of MPO is expressed as nanograms per milligram of protein using Bradford’s method (19).

Effect of the Specific Type IV Phosphodiesterase Inhibitor on the Gastric Concentration of TNF-α and IL-1β. One hundred milligrams of scraped mucosa was homogenized for 30 sec with a polytron homogenizer (PT 1200; Kinematica AG) in 1.0 ml of ice-cold potassium phosphate buffer (pH 7.4). Aliquots of homogenate supernatant, in PBS were obtained by centrifugation (10,000 g for 10 min). Total protein was measured by Bradford’s method. The concentration of TNF-α and IL-1β in the supernatant of mucosal homogenates was determined with an ELISA kit specific for rat TNF-α and IL-1β (R&D Systems Inc., Minneapolis, MN, USA). The assay was performed according to the manufacturer’s instructions. After color development, optimal density was measured with a microplate reader. The concentration of TNF-α and IL-1β is expressed as picograms per milligram of protein.

Statistical Analysis. All data are expressed as mean ± SE. Statistical significance was determined by Mann–Whitney U test using the Statview-J 4.11 statistical program (Abacus Concepts, Berkeley, CA, USA) for Macintosh computer. P values < 0.05 were considered statistically significant.

RESULTS

Effect of the Specific Type IV Phosphodiesterase Inhibitor on Aspirin-Induced Gastric Mucosal Injury. Administration of aspirin–HCl induced multiple hemorrhagic erosions in the rat stomach. In contrast, pretreatment with rolipram inhibited the gastric lesions. The ulcer index in vehicle-treated rats was 30.5 ± 2.9 mm. The ulcer index in rats pretreated with rolipram was significantly suppressed, to 5.6 ± 1.1 mm (2.5 mg/kg; P < 0.01) or 4.3 ± 1.8 mm (5 mg/kg; P < 0.01) (Figure 1). The protective effect of rolipram was also confirmed also histologically. Aspirin administration resulted in large areas of epithelial crypt loss, predominantly neutrophilic infiltration, erosions, and mucosal bleeding. In contrast, pretreatment with rolipram resulted in smaller erosions with few neutrophils (Figure 2).