PRELIMINARY REPORT

Repetitive Deformation and Pressure Activate Small Bowel and Colonic Mucosal Tyrosine Kinase Activity In Vivo

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Physical forces like deformation and pressure modulate signaling and phenotype in cultured cells. However, it is more difficult to establish that such phenomena occur in vivo. We studied the effects of 0 to 10 minutes of rhythmic distension with an isotonic electrolyte and polyethylene glycol solution to 30 cm H_2O pressure on defunctionalized small and large bowel segments in adult male Sprague Dawley rats. Mucosa was harvested at 0, 1, and 10 minutes and assayed for tyrosine kinase activity. Rhythmic distension caused a time-dependent increase in colonic mucosal tyrosine kinase activity, which was statistically significant at 10 minutes (140% ± 41% increase, n = 5, $P < .05$). Small bowel tyrosine kinase activity was markedly lower than that observed in the colon, but achieved a statistically significant increase at 5 minutes after initiation of rhythmic distension. (115% ± 44% increase, n = 5, $P < .05$).

CELL CULTURE studies suggest that repetitive strain and pressure may stimulate proliferation and alter cell phenotype and signaling in cell types as diverse as vascular endothelium and smooth muscle, the intestinal mucosa, pulmonary epithelial cells, and osteoblasts.[1-3] Although the hypothesis is attractive and might explain some of the pathophysiology of disease states as diverse as hypertensive atherosclerosis, pulmonary barotrauma, and mucosal atrophy during starvation or ileus, scant data exist to substantiate such physical force signaling in vivo. In the gastrointestinal tract, ligating the rat common bile duct is associated with increased ductal epithelial proliferation,[4] but chronic biliary obstruction might cause widespread alterations in the physiology of the animal.

Cyclic strain alters Caco-2 intestinal epithelial proliferation and differentiation in association with activation of intracellular tyrosine kinase activity, and tyrosine kinase blockade prevents this effect.[5] We therefore sought to test the hypothesis that repetitive strain and pressure might activate mucosal tyrosine kinase activity in vivo in anesthetized rats. We chose a peak pressure of 30 cm H_2O to be similar to pressures observed in vivo.

MATERIALS AND METHODS

Adult male Sprague Dawley rats were anesthetized with intraperitoneal pentobarbital (40 mg/kg), and 10 cm segments of midjejunal small intestine and 5 cm segments of noncecal right colon were transected proximally and distally and then cannulated proximally with polyethylene catheters, which were secured with silk ties. The bowel segments were rinsed with isotonic electrolyte and polyethylene glycol solution (Go-Litely, Braintree Laboratories, Braintree, MA), the distal ends were ligated, and the bowel segments were then subjected to repetitive pressure at 60 cycles per minute using a hand-held bulb attached to a pressure gauge and a 30-cm fluid column open at the top to permit overflow and regulate maximal pressure to 30 cm H_2O.

At 0 to 10 minutes after initiation of pressure, the jejunal and colonic mucosa was harvested into a 40°C lysing buffer (Dulbecco's phosphate-buffered saline [D-PBS] containing 0.5% Triton X-100 and 0.35 mol/L NaCl). Aliquots of the mucosal lysates were assayed for total protein by bicinchonic acid (BCA) assay (Pierce, Rockford, IL) and then assayed for tyrosine kinase activity using a synthetic substrate and an enzyme-linked immunosorbent assay (ELISA)-based commercial assay kit using the universal tyrosine kinase substrate poly-Glu-Tyr (PGT) (Sigma, St Louis, MO). All BCA and tyrosine kinase activity assays were within the linear range of the assay based on simultaneously assayed standards.

Five animals were studied for each data point. Data were expressed as moles phosphorylated protein/minute/mg of lysate protein and analyzed by Student's *t* test.

RESULTS AND DISCUSSION

Basal mucosal tyrosine kinase specific activity was approximately 2.5-fold higher in the colon than in the small bowel. However, in each case, a time-dependent trend toward increased tyrosine kinase specific activity was observed of similar magnitude. (Fig 1) This stimulation achieved statistical significance at 5 minutes for the small bowel samples (115% ± 44% increase, n = 5, $P < .05$ and at 10 minutes for the colonic samples. (140% ± 41% increase, n = 5, $P < .05$).

Many investigators have described the induction of intracellular signals and alterations in cell biology in culture by physical forces such as deformation, pressure, and shear stress.[1,2] Indeed, cyclic deformation initiates a rapid time-dependent peak of tyrosine kinase activity in human Caco-2 intestinal epithelial cells,[5] and 15 mm Hg pressures may induce tyrosine phosphorylation of proteins in human colon cancer cell lines.[6] We have previously reported that focal adhesion kinase is rapidly activated by cyclic deformation in Caco-2 intestinal epithelial cells in vitro,[3] and src has been reported activated in vitro by deformation in other cell types,[2] so these might certainly be among the tyrosine kinases involved. However, this must await further study.

In vivo assessment of the cellular consequences of applica-

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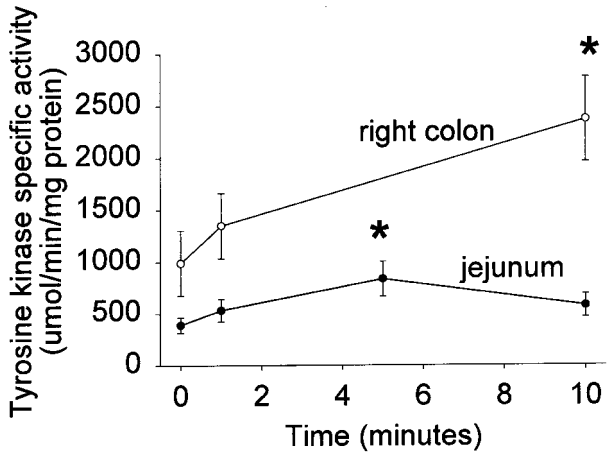

Fig 1. Effect of rhythmic distension on mucosal tyrosine kinase specific activity. Segments of jejunal (●) and noncecal right colonic (○) bowel were repetitively distended to a pressure of 30 cm H_2O for 0, 1, 5, and 10 minutes for the jejunum and 0, 1, and 10 minutes for the colon in anesthetized rats prior to sacrifice, mucosal harvest, and tyrosine kinase specific activity assay. Time-dependent increases in mucosal tyrosine kinase specific activity occurred, which achieved statistical significance at 5 minutes for the jejunal samples and 10 minutes for the colonic samples. Results are expressed as micromole synthetic substrate hydrolyzed per minute per milligram of sample protein ($n = 5$, $P < .05$).

tion of an isolated physical force is more challenging. Hypertension or barotrauma or constipation may be associated with alterations in the vascular endothelium or smooth muscle, the pulmonary epithelium, or the bowel mucosa in vivo. In particular, in the small bowel, a standardized surgical pressure decreases diamine oxidase (a brush border enzyme) and increases mucosal mass.[7] Lipids or bulk agents, which strain mucosa by remodeling villi,[8] may also alter proliferation, differentiation, and barrier function.[9-13] Many actions of fiber are independent of digestion,[14] and mucosal atrophy in defunctionalized Thiry-Vella loops is reversed by luminal water,[15] so it is possible that distention may support the mucosa. Conversely, fecal diversion causes colonic atrophy, although butyrate deficiency could contribute to this.[16,17] However, it is difficult to discern whether such observations represent consequences of the physical forces involved or more complex neuroendocrine or metabolic phenomena.

This study suggests that acute application of a physical force engendering repetitive pressure and distension has cellular consequences in vivo, at least in anesthetized animals. The parameters chosen for force application were admittedly arbitrary. Luminal pressures fluctuate constantly in an irregular fashion in response to the changing patterns of bowel motility and passage of luminal contents. Trying to reproduce such pressure patterns, which vary irregularly in amplitude and frequency, would be technically challenging and difficult to analyze, so we attempted to standardize our stimulus. A 30-cm H_2O pressure is, however, similar in magnitude to the pressures observed in vivo. It seems unlikely that the perfusion resulted in significant fluid shifts across the mucosa and/or the neuroendocrine consequences of such fluid shifts because the solution chosen for perfusion was isotonic and recovered at the end of the perfusion.

If, indeed, physical forces do stimulate rapid tyrosine kinase activity peaks, then the consequences of such signals await exploration. The rapid biphasic stimulation of tyrosine kinase activity by cyclic deformation in cultured Caco-2 intestinal epithelial cells appears to mediate increased proliferation and altered differentiation,[5] consistent with similar observations in other cultured cell types.[1,2] The data presented here validate such concepts in vivo and suggest that isolated physical forces may trigger cellular signals in intact animals.

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