Decreased Vasoactive Intestinal Peptide Levels and Glutathione Depletion in Acquired Megacolon

TIMOTHY R. KOCH, MD, ARND SCHULTE-BOCKHOLT, MD, MARY F. OTTENSON, MD, GORDON L. TELFORD, MD, STEVEN J. STRYKER, MD, T. BALLARD, MD, and EMMANUEL C. Opara, PhD

We reported decreased vasoactive intestinal peptide levels in acquired megacolon. The origin of altered neuropeptide levels is unknown, but recent work suggested that tissue antioxidants may function as neuroprotectants. Our hypothesis was that altered levels of inhibitory neurotransmitters in human colon are associated with depletion of the tripeptide thiol, glutathione. Normal colon samples (N = 10; from patients 41–80 years old) and acquired megacolon samples (N = 10; from patients 31–98 years old) were obtained at surgery. Vasoactive intestinal peptide levels were decreased in muscularis externa from acquired megacolon (P = 0.01), while there was a modest increase in NADPH diaphorase activity in muscularis externa from megacolon (P = 0.10). Glutathione in acquired megacolon was detectable in muscularis externa from only five specimens (P < 0.05), but was not significantly different (P > 0.05) in the mucosal–submucosal layer. The results supported the presence of vasoactive intestinal peptide and NADPH diaphorase in distinct subpopulations of nerves in human colon. The results also supported the hypothesis that glutathione functions as a neuroprotectant in a subset of patients with acquired megacolon.

KEY WORDS: colon; megacolon; vasoactive intestinal peptide; glutathione; NADPH diaphorase.

Colonic inhibitory nerves contain at least two neurochemical systems: nitric oxide (NO) synthase and vasoactive intestinal peptide (VIP). In colon, inhibition of NO synthase partially reduces nerve-mediated relaxation (1), and NO produces smooth muscle membrane hyperpolarization (3). Enteric NO could be produced by two or more sources: inhibitory nerves containing neuronal NO synthase may produce NO (4), or a membrane NO synthase coupled to a membrane receptor activated by VIP or VIP-like peptide could produce NO (5, 6).

Neuronal nicotinamide adenine dinucleotide phosphate diaphorase (NADPH diaphorase; EC 1.8.1.4) is contained in a class of flavin-bound enzymes that catalyze reduction of dyes using reduced NADPH as hydride donor. Neuronal NADPH diaphorase and neuronal NO synthase are identical in rat brain and small intestine (7). There is a direct correlation between NADPH diaphorase histochemical staining and immunoreactive neuronal NO synthase in human, rat, dog, cat, and guinea pig gut (8–12). NADPH diaphorase activity may serve as a marker.
for neuronal NO synthase, and it may be more useful than enzymatic determination of NO synthase activity, since multiple isoforms of NO synthase are present in the gut.

A second inhibitory neurochemical, VIP, is a 28-amino-acid neuropeptide synthesized as a preproVIP peptide (13). In human intestine, exogenous VIP produces smooth muscle relaxation (14) and VIP antagonist partially abolishes descending relaxation (15).

Constipation is a common chronic disorder in the United States, affecting 2% of the population (16). It has been attributed to inadequate dietary fiber intake, decreased water intake, decreased physical activity, or hormonal changes. These theories have not been consistently supported by previous studies (17-19).

The origin of constipation could be related to an alteration of enteric nerves. An epidemiologic study demonstrated associations between neurological disorders and constipation (20). In addition, we have examined the amplitudes of hyperpolarizations evoked by field stimulation using colonic circular smooth muscle from young and elderly humans (21). Hyperpolarizations were lower in older individuals. This finding mimicked the observation that activation of opiate receptors in human colon diminishes the amplitude of hyperpolarization evoked by field stimulation (22). Opiates appear to induce constipation in man by increasing nonpropagating contractions of distal gut (23). Diminishing inhibitory nerve activity in aging colon could lead to constipation by an opiate-like mechanism (termed “spastic constipation” in older literature).

Constipation is a common symptom in acquired megacolon since up to 77% of patients with megacolon have constipation at the time of evaluation (24). There are multiple causes of megacolon, but neuronal cell dysfunction appears to be a commonly observed factor (25-27). Despite the multiple causes of megacolon, a decrease in VIP levels in muscularis externa from megacolon appears to be a consistent underlying abnormality (28).

The etiology of altered enteric nerves in patients with constipation is not known. In studies of neural tissue, free radicals have been examined as potential neurotoxins in the brain (29, 30). Oxygen-derived free radicals can react with polyunsaturated fatty acids in cell membranes to form lipid peroxides, which contain highly reactive hydroxyl radicals; the extent of cellular damage may be related to a balance between free radicals and tissue levels of antioxidants (31).

The tripeptide thiol, glutathione, is a nonenzymatic tissue antioxidant synthesized by most animal cells (32). Antioxidants such as glutathione reduce peroxides and can prevent damage to DNA and cell membranes. Among the organ damage induced by glutathione deficiency, extensive intestinal damage occurs in a mouse model (33).

Based on these studies, we hypothesized that abnormal levels of inhibitory neurotransmitters in acquired megacolon would be associated with depletion of colonic glutathione. The present study was designed to examine this hypothesis by measuring VIP, NADPH diaphorase activity, and glutathione levels in grossly normal colon and in acquired megacolon obtained at surgery.

MATERIALS AND METHODS

Chemicals. NADPH in reduced form, flavin mononucleotide, L-arginine HCl, N-nitro-L-arginine (NOARG), DL-dithiothreitol, trifluoperazine HCl, p-iodonitrotetrazolium violet, dichloro-5-dihydroxyphenolphthalein, and N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid] (HEPES) were obtained from Sigma Chemical Company (St. Louis, Missouri). Diaphorase (EC 1.6.99.1) was obtained from Worthington Biochemical Corporation. A Bio-Rad protein assay kit was obtained from Bio-Rad Laboratories, Inc. (Hercules, California). All other chemicals were of at least reagent grade.

Human Colonic Tissue. Permission for human studies was granted by the Human Research Review Committee at the Medical College of Wisconsin. As controls, grossly normal descending sigmoid colon from the antimesenteric border was obtained at surgery from patients (N = 10; age range: 41 to 80 years) with nonobstructing colonic carcinoma or polyps. Colon was taken at least 3 cm from the stapled ends and 3 cm from any colonic neoplasia, quick frozen on Dry Ice, and stored at -76°C.

Acquired megacolon was defined by radiological criteria (sigmoid megacolon: >6.5 cm) (34). Surgical intervention to treat acquired megacolon is rare and indications included a history of sigmoid volvulus or symptoms intractable to medical and dietary therapy that markedly compromised the patient’s lifestyle (35, 36). Patients underwent abdominopereineal colectomy and ileorectal anastomosis or total abdominal proctocolectomy and ileostomy. Descending sigmoid colon obtained from six patients (five men and one woman) with a mean age of 70 years (range: 47–98 years) and colon from four previously described patients (28) were used in this present study. The clinical characteristics of these 10 patients are summarized in Table 1. Colon was quick frozen on Dry Ice and then stored at -76°C.

Radioimmunoassay. Muscularis propria was separated from the mucosal–submucosal layer by microdissection. VIP was extracted and measured by radioimmunoassay (37). Rabbit antiserum 4823 shows no cross-reactivity to structurally related peptides, including pituitary adrenylate cyclase activating peptide-27 and peptide histidine-methionine-27. Immunoreactive species measured in hu-