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Octreotide effectively decreases mucosal damage in experimental colitis

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Abstract

The effect of octreotide, a synthetic analogue of somatostatin, on the modulation of the acetic acid model of experimental colitis was examined. Colitis was induced by intracolonic administration of 2 ml of 5% acetic acid. The inflammatory response elicited by the acetic acid resulted in increased colonic synthesis of platelet activating factor, leukotriene B_4 and decreased mucosal somatostatin levels. Subcutaneous administration of octreotide (10 μg/rat) 1 hour before or immediately after damage induction, as well as 1 and 23 hours after acetic acid application, resulted in a significant reduction in mucosal damage. The protective effect was accompanied by a significant reduction in platelet activating factor activity, leukotriene B_4, and vasoactive intestinal peptide concentrations. There were no significant changes in mucosal leukotriene C_4 and calcitonin gene related peptide levels. This study shows that acetic acid induced colitis is pharmacologically manipulated by octreotide. The mechanism of action of octreotide has not yet been fully determined. The potential use of octreotide in treating active inflammatory bowel disease remains to be evaluated.

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Somatostatin, a cyclic tetradecapeptide, is widely distributed throughout the gastrointestinal tract. The highest concentrations of somatostatin secreting cells are found in the antrum, duodenum, and pancreas.[1-3] Minute amounts are found in neural cells along with other neuropeptides, as well as within the mucosal and submucosal layers in the human small intestine and colon.[1-4] Among the biological actions of somatostatin in the gut is its ability to inhibit neuroendocrine secretion of gastrin, vasoactive intestinal peptide (VIP), and motilin – all known for their ability to induce diarrhoea.[1 2] Somatostatin decreases intestinal fluid secretion and stimulates sodium and chloride absorption.[5 6] In animal models, somatostatin inhibits proliferation of gut epithelial and ileal crypt cells.[7 8]

Somatostatin concentrations in the mucosa and submucosa of patients with inflammatory bowel disease were reported to be either reduced or unaltered.[8-11] High concentrations of circulating immunoreactive somatostatin were found in patients with active ulcerative colitis but these returned to normal on remission.[12] We found low colonic mucosal somatostatin values in patients with active ulcerative colitis and normal concentrations on remission.[13] When used for treatment, somatostatin should be administered continuously because of its very short half life. Octreotide, its synthetic analogue, has a longer half life, is administered subcutaneously, and its serum concentration increases in linear fashion with increased doses.[2] Octreotide is currently the most effective drug for various diarrhoeal disorders—carcinoid syndrome, and vipoma, and is beneficial in ileostomy diarrhoea, short bowel syndrome, AIDS, and diabetic diarrhoea.[2 14-19]

In the present study the possible modulation of acetic acid induced experimental colitis[20] by octreotide was evaluated.

Methods

ACETIC ACID INDUCED COLITIS

Hebrew University strain male rats, 200–250 g body weight, were fasted for 24 hours. Under light ether anaesthesia, a midline abdominal incision was made, the colon was isolated, and the junction of caecum and ascending colon was ligated. Two ml of 5% acetic acid were injected into the lumen of the colon at its proximal part through a 25 G needle, followed by 3 ml of air, which cleared most of the acetic acid from the colon.[20] The midline incision was closed. Twenty four hours later the rats were killed. The colon was isolated and a 10 cm segment of the distal colon proximal to the anus was resected, its lumen was rinsed with ice cold saline, and it was weighed. A cross section was obtained for histology and the whole mucosa was scraped, minced, and divided into samples for radioimmunoassay (RIA) and platelet activating factor (PAF) determination. Treated rats received octreotide (10 μg/rat) subcutaneously, 1 hour before and 1 and 23 hours after damage induction. Another group of rats received octreotide (10 μg/rat) immediately after and 8 and 23 hours after damage induction by acetic acid.

DETERMINATION OF MUCOSAL DAMAGE

Mucosal damage was quantitated by the scoring system of Wallace *et al.*[21] In this system: 0=no damage; 1=hyperaemia, no ulcers; 2=linear ulceration without hyperaemia or bowel wall thickening; 3=linear ulcer with inflammation at one site; 4=two or more sites of ulceration and inflammation; 5=two or more sites of major ulceration and inflammation, or one major site of damage extending more than 1 cm along the length of the colon; 6–10=when area of ulceration and inflammation extends more than 2 cm along the length of the colon, the score is increased by one mark for each additional cm of involvement. Mucosal damage was also measured macroscopically and expressed in mm^2/rat. All scoring and measurements of damage were performed by

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two observers. Interobserver variability was less than 1% error. For the purpose of scoring, inflammation was defined as hyperaemia and thickening of the bowel wall. The time taken to assess the extent of mucosal damage was less than a few minutes.

MORPHOLOGICAL STUDIES

Sections of colon were obtained from the same areas of the large intestine, fixed in phosphate buffered formaldehyde, embedded in paraffin and routine 5 μm sections were prepared. Tissues were routinely stained with haematoxylin and eosin and evaluated blindly by light microscopy.

DETERMINATION OF LIPOXYGENASE PRODUCTS

One hundred and fifty mg of mucosa were placed in preweighed tubes containing 1 ml of phosphate buffer (50 mM, pH 7·4). The mucosa was minced with scissors and centrifuged in an Eppendorf centrifuge for 10 seconds. The pellet was resuspended in 1 ml of the above buffer, incubated for 1 minute in a vortex mixer, indomethacin (10 μg) was added, and the tubes were centrifuged for 60 seconds. The supernatants were kept at −20°C until RIAs were performed. The capability of the mucosa to generate leukotriene C_4 (LTC_4) and B4 (LTB_4) was expressed as ng/g wet tissue weight.

MEASUREMENT OF LTB_4

LTB_4 immunoreactivity was determined by a RIA kit (Amersham, TRK 940). The assay combines the use of a high specific activity LTB_4 tracer, an antiserum specific for LTB_4 (cross reactivity 100%), and a leukotriene standard (range 1·6 to 200 pg/tube). The specific binding of tracer is 42·5%, and non-specific binding is 2·4%. Fifty per cent B/Bo displacement is obtained with 15 pg/tube and 90% B/Bo displacement with 2·2 pg/tube of LTB_4. The percentage coefficient of variation (CV) for within assay precision ranges from 8·26 (low) to 8·61 (high) (n=20). The percentage CV for the precision profile of the assay (10 assays of standard curve) ranges from 2·31 to 6·3. The percentage CV for between assay reproducibility ranges from 0·81 to 5·78 (n=10).

MEASUREMENT OF LTC_4

LTC_4 immunoreactivity was determined by RIA (Amersham, TRK 905). The assay combines the use of a high specific activity LTC_4 tritiated tracer with a monoclonal antibody specific for LTC_4 and LTC_4 standard (8–500 pg/tube). The assay uses highly specific LTC_4 antiserum (cross reactivity=100%) and has low cross reactivity with leukotriene D_4 (LTD_4) (<5%). The specific binding of tracer is 40%-45% and non-specific binding is 1%-3%. Fifty per cent B/Bo displacement is obtained with 34 pg/tube, and 80% B/Bo displacement with 9·5 pg/tube of LTC_4. The percentage CV for within assay precision ranges from 7·68 (low) to 3·94 (high). The percentage CV for the precision profile of the assay ranges from 1·64 to 3·49 (n=9). The percentage CV for between assay reproducibility ranges from 1·64 to 3·49 (n=9).

PAF DETERMINATION

Extraction of PAF from colonic mucosa

To extract PAF from the mucosa, 0·5 ml of ethanol 80% was added to preweighed colonic mucosa (20–30 mg) 24 hours before performance of the aggregation assay.

Platelet preparation

Fifty ml of rabbit venous blood were collected into Falcon tubes, mixed with 1 ml 0·2 M ethylenediamine-tetra-acetic acid and centrifuged for 20 minutes at 1600 rpm. Plasma was transferred to another tube and centrifuged for 15 minutes at 3000 rpm. The platelet pellet was reconstituted with a washing buffer (pH 6·5) containing 2·6 mM KCl. 1 mM $MgCl_2$, 137 mM NaCl, 0·2 mM ethyleneglycol-bis (beta-aminoethylether)-N,N'-tetraacetic acid, 5·5 mM glucose, and 0·25% gelatine and centrifuged for 15 minutes at 3000 rpm. The platelet pellet was resuspended in the above buffer and treated with 0·1 mM aspirin for 15 minutes at room temperature, followed by another centrifugation for 15 minutes at 3000 rpm. Platelets were resuspended in washing buffer at appropriate concentrations for the measurement of platelet aggregation.

AGGREGATION ASSAY

PAF activity was measured by platelet aggregation using a Chrono-Log Corporation aggregometer. Platelets were stirred in 400 μl buffer containing 2·6 mM KCl, 1 mM $MgCl_2$ 137 mM NaCl, 12 mM $NaHCO_3$, 1·5 mM $CaCl_2$, 5·5 mM glucose, 0·25% gelatine, 1 mM creatine phosphate and 10 U/ml creatine phosphokinase (pH 7·4). Samples of 10 ul were added and aggregation recorded. WEB 2086 BS inhibited dose dependently (0·17–17 μM) the PAF induced platelet aggregation (r=0·891; y=140–8·8x).

Extraction of neuropeptides

Twenty to 30 mg of colonic mucosa were frozen in liquid nitrogen, weighed, and homogenised in 10 volumes of acetic acid 2 M, containing 10 mM mercaptoethanol and phenylmethylsulphonylfluoride (1 μg/ml). The homogenate was centrifuged for 10 minutes at 3000 rpm (4°C). The supernatant was transferred to another tube, the pellet rehomogenised and centrifuged as above. Supernatants were combined and kept at −20°C until they were lyophilised. After lyophilisation the protein was resuspended in 1 ml phosphate buffer, 50 mM, pH 7·4, containing NaCl 145 mM, gelatin 0·1% and NaN_3 0·02%. Somatostatin, VIP, and calcitonin gene related peptide (cGRP) were determined by RIA.

Somatostatin RIA

Somatostatin RIA employs simultaneous addition of sample or standard (range 0–500

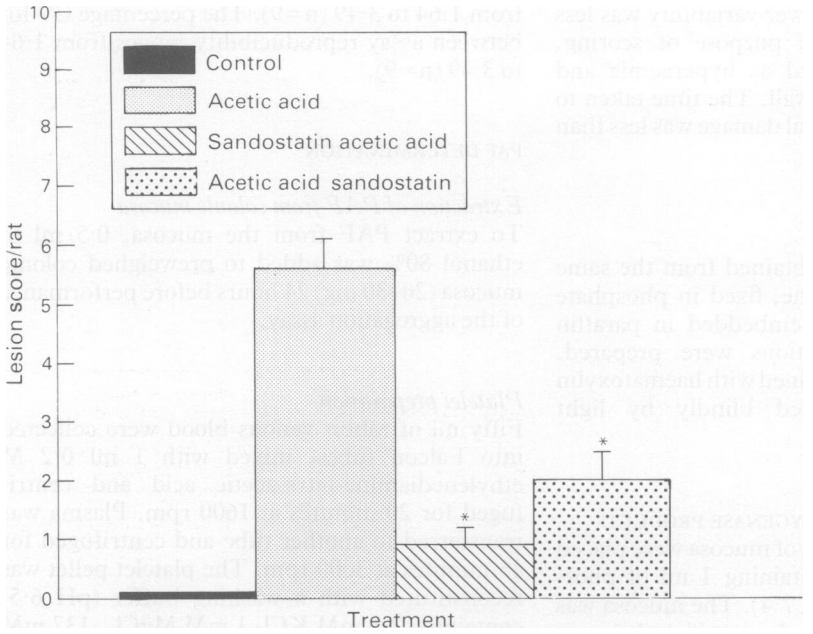

Figure 1: Colonic lesion score 24 hours after intracolonic administration of 5% acetic acid with or without octreotide cotreatment. Results are mean (SEM) of 8 to 11 rats per group.
**Significantly different from acetic acid treated rats; p<0·05, Mann-Whitney non-parametric test and Student's t test.*

pg/ml), rabbit anti-somatostatin antibody (binding capacity 40%–45%), and ^{125}I-somatostatin followed by an overnight incubation at 4°C. Phase separation was accomplished by the addition of a complex goat anti-rabbit serum, carrier rabbit serum, and polyethylene glycol. After 25 minutes incubation the tubes were centrifuged, decanted, and counted in a Packard auto-gamma scintillation spectrometer. The percentage cross reactivity of somatostatin antibody was less than 0·007 for each of the following peptides: beta endorphin, substance P, leucine enkephalin, methionine enkephalin, arginine vasopressin, insulin, thyroid releasing hormone, and VIP.

VIP RIA

VIP was measured by RIA using delayed tracer addition to increase sensitivity. Sample or standard (range 0–290 pg/ml) and rabbit anti-VIP (binding capacity 40%–50%) were added followed by a 24 hour incubation at 4°C. ^{125}I-VIP was then added followed by a second incubation for 24 hours at 4°C. Preprecipitated carrier, second antibody, and polyethylene glycol were added in a single step. The assay was incubated for a further two hours, centrifuged, decanted, and counted in a gamma counter for at least 60 seconds. The percentage cross reactivity of the VIP and antibody was less than 0·1 for each of the following peptides: somatostatin, p-motilin, GIP, glucagon, GRP, gastrin 17, gastrin 34, p-secretin, PPP, p-proinsulin, p-insulin, cholecystokinin (CCK-8), substance P, neurotensin, methionine enkephalin, leucine enkephalin, and bombesin. The percentage CV for within assay variation ranges from ±1·9 (low) to ±3·0 (high) (n=5). The percentage CV for between assay variation ranges from 9·58 (low) to 7·07 (high) (n=73).

cGRP RIA

The cGRP RIA employs simultaneous addition of samples or standard (range 0–1000 fmol), rabbit antiserum to cGRP human (binding capacity 35%-40% and (2-[^{125} I] iodohistidyl^{10}) cGRP followed by five days incubation at 4°C. Phase separation is accomplished by the addition of dextran/charcoal suspension. The tubes are centrifuged for 20 minutes at 2000 *g*, bound (supernatant) and free (pellet) are counted in a Packard auto-gamma scintillation spectrometer.

STATISTICAL ANALYSIS

Data are expressed as median, range, and mean (SEM). Statistical analysis for significant differences was performed according to the Student's *t* test for unpaired data and the Mann-Whitney non-parametric test.

MATERIALS

Acetic acid (Frutarom, Israel); aspirin (Aspegic, Lab Egic, Amilly, France); creatine phosphate, creatine phosphokinase, fatty acid free bovine serum albumin, LTC_4, indomethacin (all from Sigma Laboratories, Israel); PAF (C18) (Bachem, Switzerland); octretotide – sandostatin (Sandoz Ltd, Basle, Switzerland); LTB_4 RIA; LTC_4 RIA (Amersham, England); somatostatin and cGRP RIA (Amersham, England). VIP RIA (Incstar Corp, Stillwater, Minnesota, USA).

Results

ACETIC ACID INDUCED COLITIS

Mucosal Damage

Acetic acid induced extensive colitis. Twenty four hours after administration of 5% acetic acid the mean (SEM) lesion score escalated to 5·6 (0·5) (median 5·0; range 3–9) and lesion area to 268 (71) mm^2 (median 175; range 11–508) (Figs 1 and 2). All but one of the treated rats experienced diarrhoea, defined as loose/liquid stools. The damage induced by acetic acid was accompanied by increase in segmental weight from 0·66 (0·02) g/10 cm (median 0·64; range 0·55–0·79) in control rats to 1·02 (0·08) g/10 cm (median 0·92; range 0·70–1·52) ($p<0·05$) in acetic acid treated rats. The damage induced by acetic acid was accompanied by a significant increase in mucosal LTB_4 generation from 3·2 (0·3) (median 3·3; range 1·56–5·10) in control rats to 5·0 (1·1) ng/g wet weight (median 6·2; range 3·8–11·6) in acetic acid treated rats, as well as PAF activity from 26·4 (3·7) (median 22·1; range 10·2–58·3) to 40·0 (3·8) pg/10 mg wet weight (median 35·7; range 28·2–62·8) respectively (Figs 3 and 4). Somatostatin values decreased significantly, by more than five fold from 66·0 (2·9) (median 66; range 25·1–106·0) to 12·1 (2·9) pg/mg in acetic acid treated rats compared with control rats (median 8·15; range 3·4–26·6) (Table). The mucosal VIP and cGRP content decreased in acetic acid treated rats, although the latter did not reach statistical significance (Table).

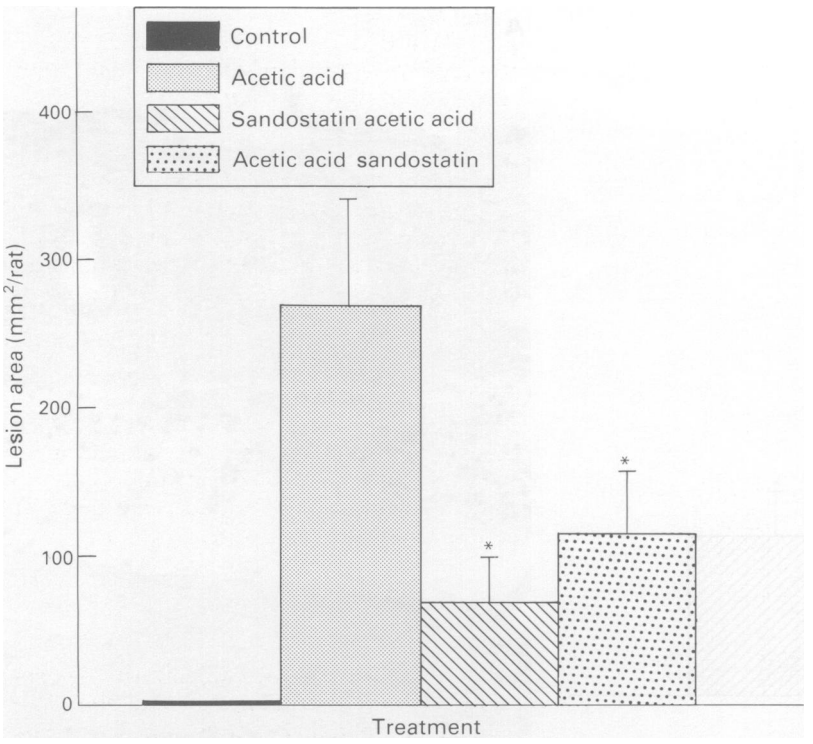

*Figure 2: Colonic lesion area 24 hours after intracolonic administration of 5% acetic acid with or without octreotide cotreatment. Results are mean (SEM) of 8 to 11 rats per group. *Significantly different from acetic acid treated rats, p<0·05, Mann-Whitney non-parametric test and Student's* t *test.*

EFFECTS OF OCTREOTIDE ON MUCOSAL DAMAGE

Treatment with octreotide, 10 μg/rat twice daily immediately after induction of damage by 5% acetic acid, significantly reduced mucosal damage. It reduced the lesion area by 57% (from 268 (71) mm² in the acetic acid treated rats to 114 (42) mm² (median 95; range 0–400) in the acetic acid and octreotide treated rats, p<0·05 Mann-Whitney test) and the lesion score by 64% (from 5·6 (0·5) to 2·0 (0·5) (median 1·5; range 0–5), respectively, p<0·05) (Figs 1 and 2). Pretreatment with octreotide, 1 hour before damage induction by acetic acid had similar and even more impressive effects, decreasing the lesion area by 75% and the lesion score by 84%, although there was no statistical significance between the two treated groups (Figs 1 and 2). Octreotide treatment decreased the segmental weight from 1·02 (0·08) g/10 cm to 0·88 (0·06) g/10 cm (median 0·93; range 0·70–1·15).

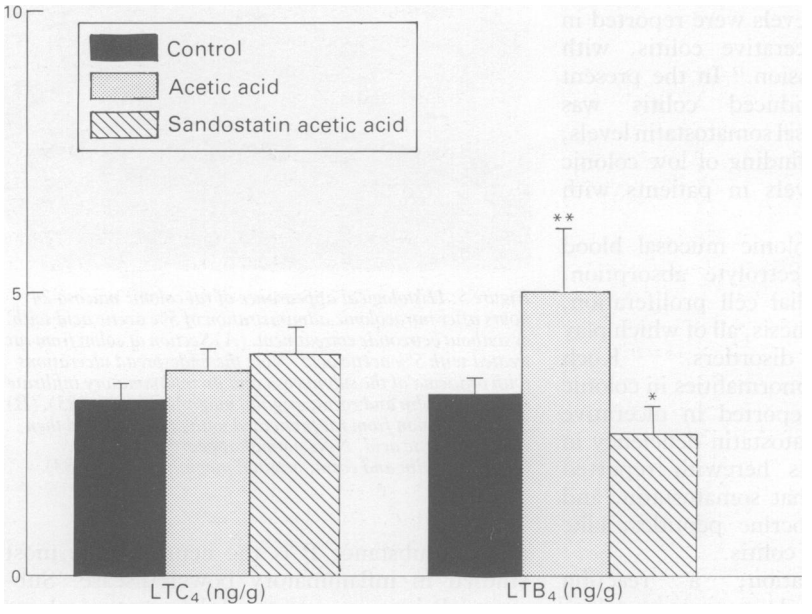

*Figure 3: Mucosal leukotriene B_4 (LTB_4 and leukotriene C_4 (LTC_4) generation 24 hours after intracolonic administration of 5% acetic acid with or without octreotide cotreatment. Results are mean (SEM) of 8 to 24 rats per group. **Significantly different from control rats, p<0·05. *Significantly different from acetic acid treated rats, p<0·05, Mann-Whitney non-parametric test and Students's* t *test.*

Mucosal neuropeptide concentrations 24 hours after intracolonic administration of 5% acetic acid with or without octreotide cotreatment

	Somatostatin	*VIP*	*cGRP*
Control:			
Mean (SEM)	66·0 (2·9)	9·6 (1·1)	1·9 (0·4)
Median	66·0	10·2	1·95
Range	25·1–106 (23)	1·7–21·8 (26)	0·3–3·9 (8)
Acetic acid:			
Mean (SEM)	12·1 (2·9)*	4·7 (0·9)	1·3 (0·3)
Median	8·15	3·7	1·7
Range	3·4–26·6 (8)	0·6–8·2 (6)	0·4–2·3 (7)
Octreotide+acetic acid:			
Mean (SEM)	ND	2·4 (0·3)**	2·2 (0·4)
Median	ND	1·9	1·9
Range	ND	1·2–4·4 (7)	1·1–3·9 (6)

Colonic mucosa (20–30 mg) was frozen in liquid nitrogen, weighed and homogenized in 10 volumes of acetic acid 2 M, containing mercaptoethanol and phenylmethylsulfonylfluoride, as described in Methods. Somatostatin, vasoactive intestinal peptide (VIP) (pg/mg), and calcitonin gene related peptide (cGRP) (fmol/mg) were determined by RIA. No of rats in parenthesis.
* Denotes significantly different from controls, p<0·05.
** Denotes significantly different from acetic acid treated rats, p<0·05.

Morphologically, colonic mucosa of all acetic acid treated rats showed widespread ulcerations with oedaema of the lamina propria and marked polymorphonuclear infiltrate. There were foci of inflammatory cell infiltrates in the muscularis propria and serosa (Fig 5A). In contrast, five out of eight rats treated with octreotide before damage induction had normal looking mucosa without any ulcerations. In the other three rats, the colonic mucosa showed superficial ulcerations with marked oedaema and polymorphonuclear infiltrates into the lamina propria (Fig 5B).

The protection provided by octreotide was accompanied by a significant decrease in diarrhoea, as well as a reduction or return to normal LTB_4 levels: from 5·6 (0·5) in acetic acid treated rats to 2·56 (0·5) ng/g (median 2·4; range 0·76–4·60) in octreotide treated rats, and PAF activity from 40·0 (3·8) to 12·9 (3·8) pg/10 mg (median 9·5; range 0–33), respectively (Figs 3 and 4).

No differences were found in mucosal cGRP levels when compared with acetic acid treated rats. Mucosal VIP levels, which were low in acetic acid treated rats compared with controls, decreased further when rats were pretreated with octreotide (Table).

Discussion

Octreotide (sandostatin) is a synthetic cyclic octapeptide with a common 4-amino acid sequence as somatostatin-14, which is responsible for its biological activity. A synthetic substitution enables the impediment of its degradation and, thus, prolongs its half life to 100 minutes.[2] Octreotide is currently the most

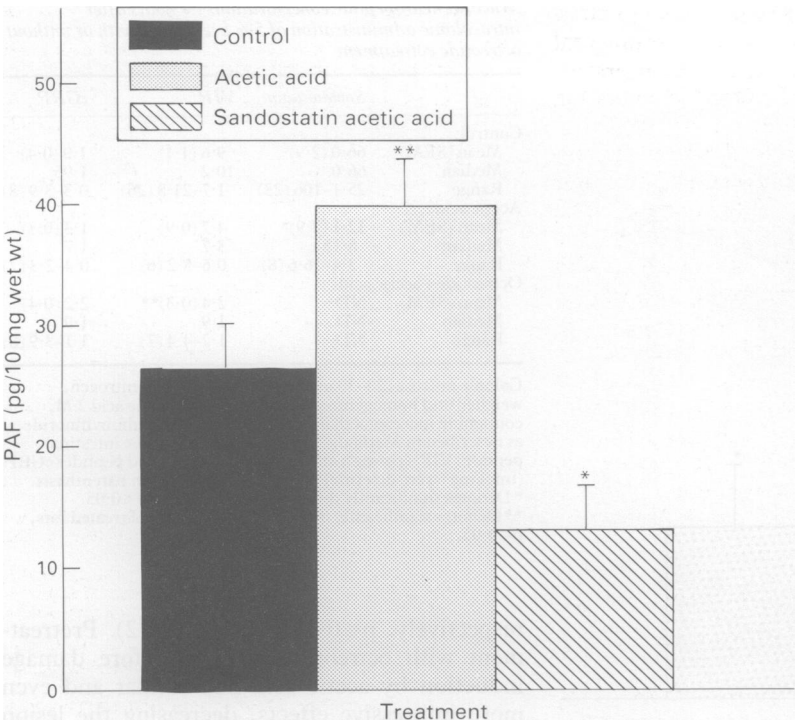

*Figure 4: Mucosal platelet activating factor (PAF) activity 24 hours after intracolonic administration of 5% acetic acid with or without octreotide cotreatment. Results are mean (SEM) of 8 to 11 rats per group. **Significantly different from control rats, $p<0.05$. *Significantly different from acetic acid treated rats, $p<0.05$, Mann-Whitney non-parametric test and Student's t test.*

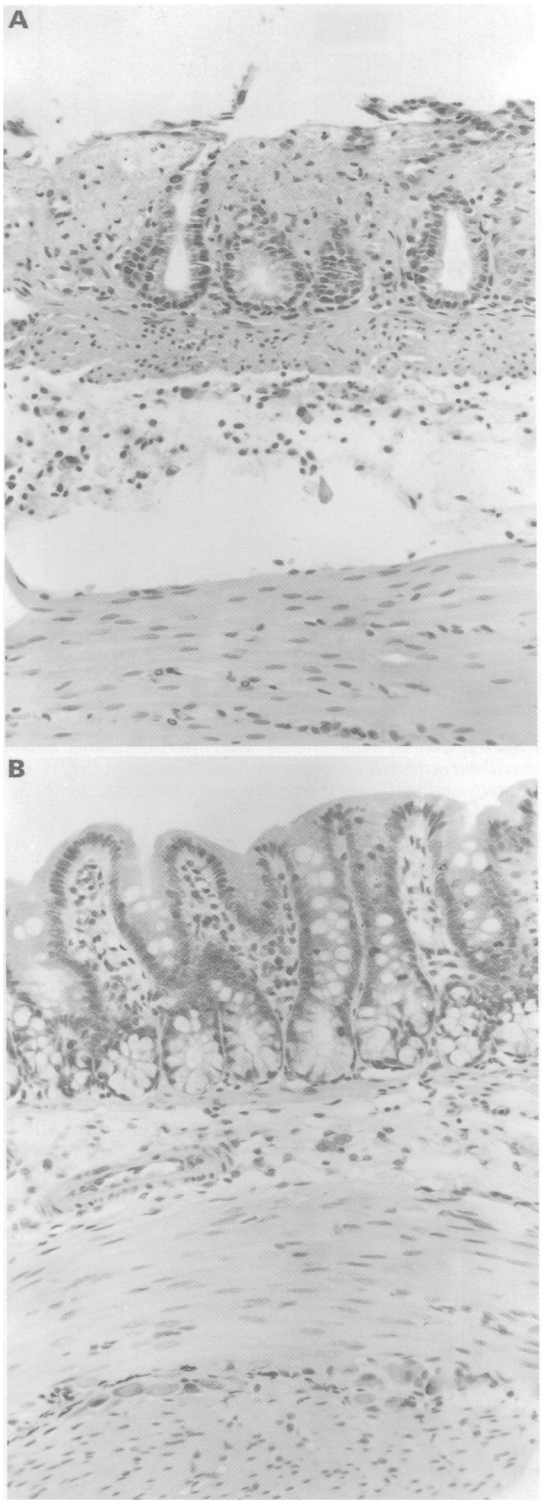

Figure 5: Histological appearance of rat colonic mucosa 24 hours after intracolonic administration of 5% acetic acid with or without octreotide cotreatment. (A) Section of colon from rat treated with 5% acetic acid. Note the widespread ulcerations with oedaema of the submucosa and an inflammatory infiltrate (Haematoxylin and eosin, original magnification, ×183). (B) Section of colon from rat pretreated with octreotide and then with 5% acetic acid. Note normal appearing mucosa. (Haematoxylin and eosin, original magnification, ×183).

effective drug for the treatment of carcinoid syndrome and VIP induced diarrhoea, and is effective in various other forms of diarrhoea, including ileostomy diarrhoea, AIDS diarrhoea, diabetic diarrhoea, and short bowel syndrome.[2 14-19]

In the present study octreotide was found to decrease effectively the severity of rat experimental colitis induced by acetic acid. There have been several conflicting reports on alterations in intestinal endocrine cells and peptide levels in inflammatory bowel disease.[8-11] Raised circulating somatostatin levels were reported in patients with active ulcerative colitis, with normal levels upon remission.[12] In the present study, acetic acid induced colitis was accompanied by low mucosal somatostatin levels, supporting our previous finding of low colonic mucosal somatostatin levels in patients with active ulcerative colitis.[13]

Somatostatin affects colonic mucosal blood flow, fluid secretion, electrolyte absorption, intestinal motility epithelial cell proliferation, and immunoglobulin synthesis, all of which play a major role in diarrhoeal disorders.[5-8 22 23] Koch *et al* speculated that the abnormalities in colonic crypt cell proliferation reported in ulcerative colitis are a result of somatostatin deficiency in the disease.[8] The results herewith reported support the contention that somatostatin, and possibly other neuroendocrine peptides, take part in the pathogenesis of colitis.

Neurogenic inflammation, a reaction including vasodilatation, plasma extravasation, and smooth muscle contraction, is elicited by mediators released from unmyelinated afferent nerve endings. Substance P, VIP, cGRP, and somatostatin are among the candidate mediators.[24] Substance P is the neuropeptide most studied in inflammatory bowel disease. Substance P increases neutrophil lysozyme release and phagocytosis, monocyte chemotaxis, and mast cell secretion, all of which ameliorate inflammatory processes.[24] Mantyh *et al* reported dramatic upregulation of substance P receptor

binding sites in patients with inflammatory bowel disease and we found high colonic mucosal content of substance P in patients with ulcerative colitis, suggesting that it is involved in regulating the inflammatory and immune responses.[25 26] Less is known about the role of VIP in inflammatory bowel disease. VIP is a powerful mediator involved in neurogenic inflammation and intestinal motility. Its concentrations in the mucosal-submucosal layers of patients with active ulcerative colitis were found to be low.[27] Duffy *et al* proposed plasma VIP levels as a laboratory supplement to clinical activity index in human inflammatory bowel disease, and Tien proposed VIP to be involved in the diarrhoea associated with mucosal inflammation in rats.[28 29] We have found that octreotide decreases mucosal VIP levels compared with those found in acetic acid treated rats, thus possibly abolishing its effect on the diarrhoea.

Alpha-calcitonin gene related peptide (cGRP I) is encoded by the calcitonin gene. Patients with medullary thyroid carcinoma have raised plasma cGRP levels and 25% of these patients present with diarrhoea, the candidate mediators for this response being calcitonin and cGRP.[30] Exogenous administration of cGRP I to dogs evoked increased small intestinal ion and water secretion and altered colonic chloride secretion in guinea pigs.[31 32] We did not find changes in mucosal cGRP levels in our model of colitis nor any effect of octreotide on mucosal cGRP levels.

In summary, we have found that octreotide significantly reduces colonic mucosal damage caused by 5% acetic acid. The protective effect was accompanied by reduction in mucosal platelet activating factor activity and mucosal LTB_4 and VIP levels. The effects of octreotide in humans remain to be evaluated.

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