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NITRIC OXIDE IN THE PATHOGENESIS OF ULCERATIVE COLITIS AND THE POSSIBLE ROLE OF GUT BACTERIA IN ITS SYNTHESIS

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SUMMARY

Nitric oxide is an important biological mediator of muscle relaxation in the cardiovascular system. Following our discovery that nitric oxide was also responsible for tonic relaxation of colonic muscle by the enteric nervous system, we investigated the possibility that its synthesis was increased in ulcerative colitis, which may explain the impaired contractility of colonic muscle associated with this condition.

The effects of human leukocytes on precontracted colonic circular muscle from male Wistar rats was investigated. Muscle strips were mounted in organ baths and mechanical activity measured with isometric force transducers.

Amino acids were measured by high performance liquid chromatography and an amino acid analyser in rectal biopsies from patients with active ulcerative colitis (AUC) and compared with quiescent (QUC) and healthy controls (HC). Citrulline concentration was taken as an indirect measure of nitric oxide synthesis. Nitric oxide synthase (NOS) activity was measured in rectal biopsies from patients with AUC and HCs. The production of ^{14}C-citrulline from ^{14}C-arginine was taken as an index of nitric oxide synthase activity.

Human polymorphonuclear leukocytes and mononuclear cells relaxed precontracted colonic circular muscle strips and this was reduced in the presence of NO antagonists. Citrulline concentrations were significantly greater in rectal biopsies from patients with AUC than QUC or HCs. Incubation of biopsies from AUC with L-NMMA reduced citrulline levels. Constitutive NOS and inducible NOS activities were increased in AUC but were undetectable in HCs. Faeces from AUC but not HCs had constitutive NOS activity.

We conclude that nitric oxide synthesis is increased in ulcerative colitis. Mucosal inducible NOS activity is increased in AUC and probably originates in leukocytes which produce sufficient nitric oxide to relax colonic muscle *in vitro* and may contribute to the motility disturbance in AUC. Faecal constitutive NOS activity is increased in AUC and may arise from an abnormal colonic microflora present as a pathogenic factor in this condition.

INTRODUCTION

Spontaneous mechanical activity of colonic circular smooth muscle is under non-adrenergic, non-cholinergic (NANC) tonic neural inhibition (*Crema*

et al., 1968; *Koch* et al., 1988). Following identification of nitric oxide (NO) as an endothelial derived relaxing factor (*Palmer* et al., 1987), NO has been proposed as a neurotransmitter in both the gastrointestinal tract and the brain (*Bult* et al., 1990; *Knowles* et al., 1990). The enzyme NO synthase (NOS) has been identified in the myenteric nerve plexus (*Bredt* et al., 1990). Synthesis of NO from L-arginine is inhibited by certain L-arginine analogues such as N^G-monomethyl-L-arginine (L-NMMA) (*Rees* et al., 1989). Inhibitory L-arginine analogues block certain NANC mediated motor events, such as muscle relaxation produced by electrical field stimulation of the canine ileo-colonic region (*Boeckxstaens* et al., 1990), and NANC relaxation of the internal anal sphincter (*Tottrup* et al., 1992). NO elevates intracellular cyclic GMP levels by increasing the activity of soluble guanylate cyclase (*Knowles* et al., 1990). Cyclic GMP causes muscle relaxation (*Nakatsu* and *Diamond*, 1987).

In previous work (*Middleton* et al., 1993), we have shown that NO biosynthesis mediates NANC tonic neural inhibition of spontaneous mechanical activity in distal colonic circular smooth muscle. The colonic smooth muscle adjacent to the inflamed mucosa of patients with ulcerative colitis (UC) has diminished spontaneous activity. This leads to a reduction of muscle tone and a loss of colonic segmentation which are associated with diarrhoea (*Kern* et al., 1951; *Garrett* et al., 1967; *Connell*, 1962; *Spriggs* et al 1951). In 1980 Snape et al. showed that the gastro-colonic reflex of patients with UC to be attenuated. This was thought to result from electromechanical disassociation of colonic smooth muscle (*Snape* et al., 1980; *Snape* and *Kao*, 1988). The mediator of these mechanical abnormalities was not however identified.

In this paper we report the results of investigations to elucidate the synthesis of NO by human leukocytes in patients with UC. The activity of NO synthase in human rectal biopsies has been studied and the nature of this enzyme examined in both biopsy material and faeces from both patients suffering from UC and from normal controls.

METHODS

Investigations of the effects of nitric oxide derived from human leukocytes

Materials

Drugs and solutions were prepared on the day of use. The following were obtained from Sigma Chemicals Ltd. and where necessary dissolved in distilled water immediately before use: Acetylcholine, superoxide dismutase, N^G-methyl-L-arginine, N^Gmethyl-D-arginine, methylene blue, tetrodotoxin, FMet-Leu-Phe, and hypaque 1017 and 1119. Indomethacin was initially dissolved in 10mM Na_2CO_3 and further diluted in Krebs-Henseleit solution. Oxyhaemoglobin was prepared from bovine haemoglobin (75% methaemoglobin, Sigma Chemicals Ltd.) and its purity was assessed spectrophotometrically according to the method described by *Martin* et al (1986). Preparations were accepted if their concentrations of oxyhaemoglobin were greater than 90%.

Preparation of Tissue

Male Wistar rats weighing 250-400g were killed and strips of colonic circular smooth muscle were attached to isotonic transducers (Harvard, Kent, England) in 2 ml organ baths and perfused by

oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution, composition: (mM/l) NaCl 118; KCl 4.69; $MgSO_4$ 1.13; $CaCl_2$ 2.56; $NaHCO_3$ 25; $NaHPO_4$ 1.15; glucose 5.5. This had a pH of 7.4 to 7.6 at 37°C. Muscle strips were mounted with the longitudinal axis parallel to the direction of the circular muscle bundles. Temperature was regulated and pH monitored intermittently. Muscle strips were maintained under a tension of 3 g, which produced near optimal contraction and experiments were commenced after a stabilisation period of two hours, which was found necessary in preliminary studies to ensure consistent muscle performance. Muscle strips were precontracted by 10 μM acetycholine and the mean amplitude of steady state contractions was measured for two minutes before and after the addition of leukocytes. Differences between the effects of paired leukocyte samples were tested with student's 't' test for paired data.

Cell Preparation

Venous blood from healthy human volunteers aged between 18 and 70 years was collected with EDTA or glass shot beads (Scientific Furnishings, Macclesfield, England) to remove platelets by defibrination. Blood (5 ml) was layered above an 8 ml bilayer of hypaque 1017 and 1119 in equal volumes and centrifuged at 700 g for 25 minutes. Mononuclear cells (macrophages and lymphocytes with or without platelets) and granulocytes (neutrophils, basophils, and eosinophils) were aspirated from two distinct layers. Leukocytes were washed twice in l0 ml Krebs-Henseleit solution (37°C) containing l00 nM/l indomethacin (KHI) to inhibit prostaglandin synthesis, centrifuged at 200 g for 10 minutes and resuspended in 5ml KHI. Leukocyte suspensions were accepted if red cell corpuscles were <5% of total and viability was >95% as judged by a tryphan blue exclusion test (Sigma Chemicals Ltd., England). This test was used to ensure a 100% death rate of cells frozen in liquid nitrogen. Cell suspensions were centrifuged at 200g for 10 minutes and the pellet of cells added to the organ bath of which the bathing fluid was used to resuspend them for transfer.

Experimental controls consisted of a similar procedure without the presence of cells.

Nitric oxide synthesis in human rectal mucosa

Patients were selected if, at sigmoidoscopy, they had macroscopically active or quiescent UC or normal rectal mucosa. Rectal biopsy specimens were frozen in liquid nitrogen or placed in short term tissue culture within 30 seconds of collection.

Concentrations of 40 amino acids were measured in biopsy samples from 6 patients with histologically active UC and 7 with normal histology by high performance liquid chromatography after derivitisation with 9-fluorenylmethyl chloroformate (*Einarsson* et al., 1983). Forty-one amino acids were measured with an amino acid analyser (LKB, Biochrom) in specimens from 5 patients with histologically active disease and 5 with histologically quiescent disease. In the short term tissue culture experiment, paired rectal biopsy samples from 8 patients with active UC were incubated for 2 hours in oxygenated modified Krebs-Henseleit at 37°C with either L-NMMA or the inactive isomer D-NMMA (200 μmol/l). After incubation specimens were immediately frozen in liquid nitrogen and amino acids measured by HPLC after derivitisation with 9-fluorenylmethyl chloroformate. Amino acid concentrations in inflamed and non-inflamed mucosa were compared by the Wilcoxon rank-sum test and, after incubation with L-NMMA or D-NMMA, by

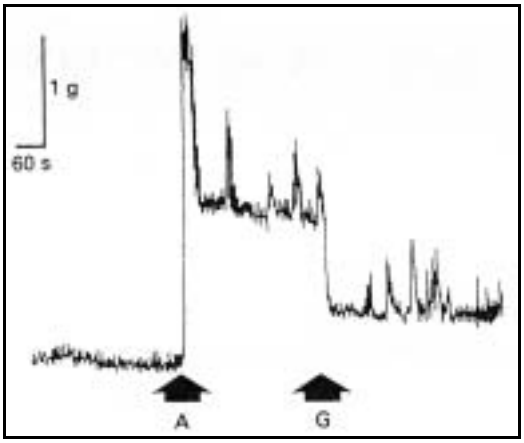

Figure 1: Typical chart recording. Distal colonic circular smooth muscle contracted by acetylcholine (l0 μM) (A) and relaxed to granulocytes (5x 10^8 cells/l) (G).
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the Wilcoxon matched pairs signed-ranks test.

Measurement of mucosal nitric oxide synthase activity

Rectal mucosal biopsies were taken at routine sigmoidoscopy from 11 randomly selected patients (5 distal colitis, 4 left sided colitis, 2 total colitis, mean [SD] age 42 ± 9.8, 2 female, 9 male) attending the gastroenterology clinic at Addenbrooke's Hospital. All had macroscopically active UC, subsequently confirmed histologically. Control biopsies were taken from 10 patients (mean age 38 ± 10.5, 4 female, 6 male) with minor symptoms who had histologically normal mucosa and did not subsequently develop inflammatory bowel disease.

NO is produced by NOS from L-arginine with the liberation of equimolar amounts of citrulline (*Hibbs* et al., 1987a). The inhibitory effects of

Table 1: Substances with known effects on the NO-cGMP pathway affected relaxation of precontracted distal colonic circular smooth muscle by granulocytes

	Cells 1^{-1}	Mean muscle relaxation (SEM) (%)	No of samples (pairs)	p-value
Viable	5 x $l0^8$	78 (11.7)		
Non-viable	5 x $l0^8$	0.37 (3.3)	8	<0.005
Control	1 x $l0^8$	50 (9.5)		
Oxyhaemoglobin	1 x $l0^8$	25 (5.9)	8	<0.005
N^G-monomethyl-D-arginine	1 x $l0^8$	53 (11.8)		
N^G-monomethyl-L-arginine	1 x $l0^8$	1.8 (3.9)	9	<0.02
Control	5 x $l0^8$	73 (13.5)		
Methylene blue	5 x $l0^8$	24 (6.1)	7	<0.01
Control	7.5 x $l0^7$	37 (11.6)		
Superoxide dismutase	7.5 x $l0^7$	96 (3.6)	7	<0.005

Mean relaxations are compared with controls by Student's t test for paired data.
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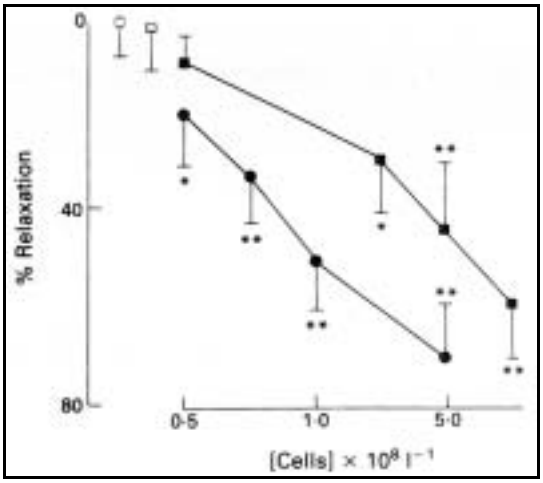

Figure 2: Granulocytes (●) and mononuclear cells (■) relaxed precontracted distal colonic circular smooth muscle in a concentration dependent manner. Mean percentage relaxations of muscle (±SEM) are shown. Collective means and standard errors of controls for both groups of cells are indicated by open symbols. Data were analysed with Student's t test for paired data (*p<0.05; **p=0.01). This figure is reproduced with permission from: Gut 34: 814-817 (1993).

N^G-methyl-L-arginine (L-NMMA), the specific inhibitor of both NOS enzymes (*Palmer* and *Moncada*, 1989) on the conversion of ^{14}C-arginine to ^{14}C-citrulline in the presence of calcium (*Marletta* et al., 1988), was used as an index of total (TNOS) activity and in the absence of calcium, as an index of inducible (INOS) activity (*Busse* and *Mulech*, 1990). The ^{14}C-citrulline was separated by using thin layer chromatography (TLC) using a method modified from *Hibbs* et al. (1987a).

Measurement of faecal nitric oxide synthase activity

Faecal samples were collected from 11 patients with macroscopically active UC (3 distal, 5 left sided, 3 total, 5 female, 6 male, mean age [SD] 41 ± 9.4) who had histologically active disease on a rectal biopsy taken within the previous month. Control samples of faeces were collected from 9 healthy volunteers (mean age [SD] 45 ± 11, 4 female, 5 male) with no history of gastrointestinal disturbance. Measurements of NOS activity were performed by the same method used for mucosal specimens. The investigation of faecal NOS activity was qualitative.

Differences in ^{14}C-citrulline production between paired incubations of mucosal biopsies and faeces in the presence of L- or D-NMMA were tested by students t-test for paired data and the Wilcoxon matched-pairs signed-ranks test respectively and significance taken as $p<0.05$.

RESULTS

Investigation of the effects of nitric oxide derived from human leukocytes

Granulocytes and mononuclear cells produced concentration dependent relaxations of circular smooth muscle precontracted with 10 μM/l acetylcholine (Figures 1 and 2). Non-viable leuko-

Table 2: Substances with known effects on the No-cGMP pathway affected relaxation of precontracted distal colonic circular smooth muscle by mononuclear cells

	Cells 1^{-1}	Mean muscle relaxation (SEM) (%)	No of samples (pairs)	p-value
Viable	5 x $l0^8$	46 (13.8)		
Non-viable	5 x $l0^8$	(23% contraction) (12)	7	<0.005
Control	8 x $l0^8$	63 (8.3)		
Oxyhaemoglobin	8 x $l0^8$	25 (7)	9	<0.005
NG-monomethyl-D- arginine	2 x $l0^8$	33 (12.1)		
NG-monomethyl-L-arginine	2 x $l0^8$	7.7 (8.7)	12	<0.05
Control	5 x $l0^8$	43 (20)		
Methylene blue	5 x $l0^8$	(3.5% contraction) (15)	7	<0.01
Control	5 x $l0^7$	12 (7.6)		
Superoxide dismutase	5 x $l0^7$	54 (11.7)	15	<0.02

Mean relaxations are compared with controls by Student's t test for paired data.
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cytes did not relax muscle. Removal of platelets did not alter relaxation of precontracted muscle by $1x10^8$/l mononuclear cells which was 22 (7%) with platelets and 26 (10%) without ($p>0.1$, n=7 pairs). Therefore platelets were not removed from mononuclear cell suspensions in the subsequent experiments as this process reduced cell yield. Substances known to affect the NO-cGMP pathway affected muscle relaxation by leukocytes (Tables 1 and 2). Addition of 200 nM oxyhaemoglobin and 10 μM methylene blue to the organ bath, one and 10 minutes before cells respectively, reduced muscle relaxation. Incubation of cells for 45 minutes with 100 μM N^G-monomethyl-L-arginine reduced muscle relaxation, but 100 μM N^G-monomethyl-D-arginine had no effect. Superoxide dismutase (60 units/ml) added one minute before leukocytes produced an increase in muscle relaxation. Tetrodotoxin (l00 nM) did not affect muscle relaxation by leukocytes (n = 5, not shown). Leukocytes were activated by incubation for one hour with FMet-Leu-Phe 100 nM. Activated mononuclear cells (5 x 10^7/l) caused a mean muscle relaxation of 43.6 (15%) compared with 8.3 (4%) by paired non-activated cells (n = 10 pairs, $p<0.05$). Activation of granulocytes did not increase muscle relaxation (mean relaxation by activated granulocytes 21.3 (10%) compared with 18.4 (6%) by non-activated cells (n = 12 pairs, $p=0.7$).

Nitric oxide synthesis in human rectal mucosa

Citrulline, arginine and cystine were the only amino acids whose concentrations differed significantly between groups. Citrulline was higher in active than in quiescent colitis ($p<0.05$) or normal mucosa ($p<0.05$). Significantly higher concentration of arginine and cystine were found in patients with active colitis. Citrulline concentrations were significantly lower in biopsy specimens incubated with L-NMMA than in those incubated with D-NMMA (Figure 3, Table 3).

Measurement of mucosal nitric oxide synthase activity

NOS activity was not detected in histologically normal mucosa. Inflamed mucosa produced ^{14}C-citrulline from ^{14}C-arginine at a mean rate (±SE) of 2.1±0.75 nM/mg/min in the presence of

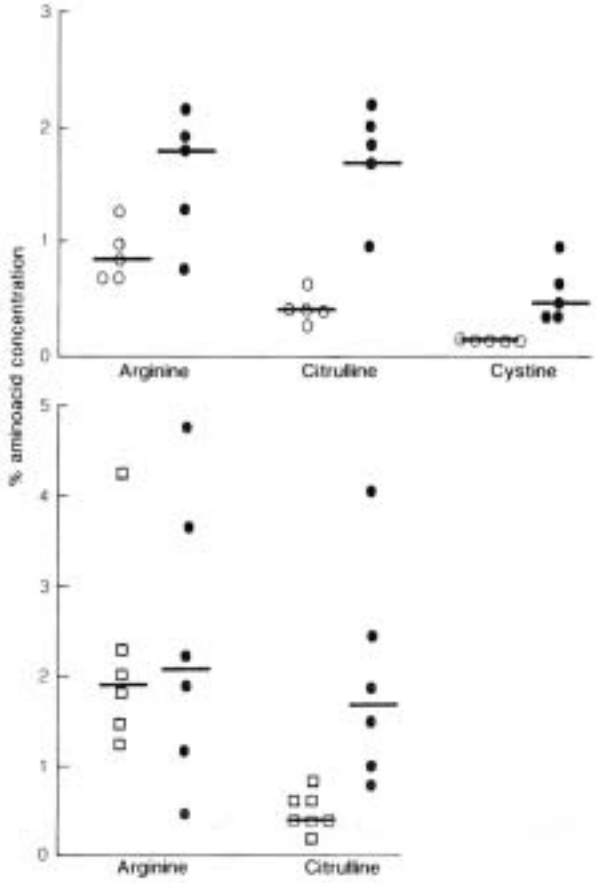

Figure 3: Aminoacid concentrations in rectal biopsy specimens. Concentrations shown as percentage of total aminoacids measured. Concentrations in patients with active ulcerative colitis (●) were compared with those in patients with quiescent disease (O) (upper) or histologically normal mucosa (□) (lower). Bars = medians.
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control (D-NMMA) which was reduced to 1.55±0.4 nM/mg/min by L-NMMA. In the absence of calcium mean ^{14}C-citrulline production was 1.5±0.3 nM/mg/min with D-NMMA falling to 1.28±0.3 nM/mg/min with L-NMMA. The difference between TNOS and INOS activity was taken as constitutive (CNOS) activity.

Removal of calcium reduced mean ^{14}C-citrulline production from 2.2±0.75 nM/mg/min to 1.5±3 nM/mg/min which corresponds to calcium dependent ^{14}C-citrulline product of 0.7±0.08 nM/mg/min.

Production of ^{14}C-urea was not affected by either L-NMMA or removal of calcium.

Measurement of faecal nitric oxide synthase activity

L-NMMA reduced ^{14}C-citrulline production by faeces from patients with UC but did not affect faeces of healthy subjects in this manner. In the absence of calcium L-NMMA did not reduce ^{14}C-citrulline production. Production of ^{14}C-urea by faeces was not affected by L-NMMA or removal of calcium.

Table 3: Aminoacids* in paired rectal biopsy specimens after incubation with L-NMMA or D-NMMA (200 μmol/l)

Patients	With D-NMMA		With L-NMMA	
	Arginine	Citrulline	Arginine	Citrulline
1	3.8	1.7	5.6	1.1
2	1.8	5.0	0.6	0.3
3	6.7	3.1	4.7	2.7
4	8.4	1.9	0.5	0.5
5	5.4	6.2	2.1	1.0
6	1.4	6.4	0.9	3.7
7	..	12.8	..	7.0
8	7.2	1.7	..	1.1

*Values are percentages of total aminoacids measured.
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DISCUSSION

Granulocyte and mononuclear cells relax colonic circular smooth muscle strips, precontracted by acetylcholine. The mediator of muscle relaxation is unlikely to be a prostanoid as leukocytes were incubated with 100 nM indomethacin. Leukotrienes produce contraction of this tissue and thromboxane has no effect (*Middleton* and *Hunter*, 1992). Muscle relaxation was increased by superoxide dismutase and reduced by oxyhaemoglobin and pre-incubation with methylene blue. Incubation of effector phagocytes with N^G-monomethyl-L-arginine reduced muscle relaxation, whereas incubation with N^G-monomethyl-D-arginine had no effect. Only viable mononuclear cells and granulocytes caused muscle relaxation, suggesting that the relaxing factor is not stored by these cells. Muscle relaxation was not affected by tetrodotoxin and therefore unlikely to be mediated by neural elements. These findings strongly support the suggestion that effector phagocytes relax circular smooth muscle by the release of NO.

Our results are in agreement with those of others who found that activated and non-activated macrophages (*Salvemini* et al., 1989; *Stuehr* et al., 1989; *Marletta* et al., 1988) and granulocytes (*McCall* et al., 1989; *Schmidt* et al., 1989) relax vascular smooth muscle by release of NO that is synthesised from L-arginine by a stereo-specific enzyme, NO synthase. Release of NO is increased by activation of macrophages (*Iyengar* et al., 1987) but not granulocytes (*Wright* et al., 1989), possibly because of the simultaneous increase in production of superoxide anions that react with NO.

Leukocytes, forming part of the inflammatory infiltrate of ulcerative colitis and at other sites of inflammation in the gastrointestinal tract, may produce smooth muscle relaxation via release of NO. Diffusion of NO through the submucosa might be facilitated by the formation of a stabilising adduct with a carrier molecule such as cysteine, or a thiol containing protein such as albumin (*McCall* and *Vallance*, 1992; *Thornbury* et al., 1991; *Ignarro*, 1990). Formation of these S-nitrosothiol compounds has been shown to increase the biological half life of NO in physiological solutions from three to five seconds (*Palmer* et al., 1987; *Harbison* et al., 1986) to

about 40 minutes (*Stamler* et al., 1992). Pacemaker cells are located on the submucosal surface of the circular muscle (*Smith* et al., 1987). These cells not only produce electrical slow wave pacemaker activity responsible for the spontaneous mechanical activity of circular smooth muscle but also form a regenerative surface that propagates this activity. Damage to these cells reduces electrical pacemaker activity and impedes its propagation (*Sanders* et al., 1990). NO or its adduct may inhibit this pacemaker activity or the response of myocytes to it, thus reducing spontaneous mechanical activity and causing a reduction in smooth muscle tone. In severe inflammation where the muscularis propria is infiltrated by leukocytes, profound dilatation may occur such as that seen in toxic megacolon (*Heppell* et al., 1986). It has recently been shown that NO relaxes the human internal anal sphincter (*Burleigh*, 1992). This may contribute to the urgency to stool, often associated with ulcerative colitis, if inflammatory cells release NO in sufficient amounts to affect sphinteric function.

Smooth muscle relaxation by NO is mediated by raising intracellular cGMP concentration, which inhibits the release of calcium from intracellular stores (*Nakatsu* and *Diamond*, 1989; *Arnold* et al., 1977; *Middleton* et al., 1992) and may produce mechanical changes without alterations in membrane potential (*Ito* et al., 1980). This may explain the electromechanical disassociation found by *Snape* et al. (1980) as the cause of reduced gastrocolonic reflex in patients with ulcerative colitis.

It is possible that the increased NO synthesis in our patients with UC was mediated by infiltration of the mucosa by leukocytes. Because citrulline concentrations in the rectal mucosa differed in all the experiments during the measurement of mucosal nitric oxide synthase activity, it is most unlikely that the increase in citrulline occurred by chance. Since L-NMMA has no effect on arginase or arginine decarboxylase activity (*Moncada* et al., 1991), increase citrulline biosynthesis must be a consequence of NO synthase activity which simultaneously results in the production of equimolar amounts of NO (*Hibbs* et al., 1987b). The increased concentrations of cysteine in active UC (Figure 3) can be explained because NO is known to combine with cysteine to form S-nitrosocysteine which liberates NO yielding cystine (*Yeates* et al., 1985).

The inhibitory effect of L-NMMA on the formation of ^{14}C-citrulline suggests that inflamed rectal mucosa in UC has increased NOS activity. This was reduced by approximately 60% on removal of calcium implying that 60% of total mucosal NOS activity is due to the constitutive enzyme. Removal of calcium had a greater inhibitory effect on ^{14}C-citrulline production than could be explained by CNOS inhibition alone implying that calcium restriction may also inhibit enzymes other than CNOS.

Increased mucosal INOS activity in ulcerative colitis can be explained by the presence of leukocytes, which do not contain the constitutive enzyme whose increased activity is not therefore easily explained. Enteric bacteria were seen contaminating mucosal biopsy specimens under light microscopy, and samples of faeces from patients with active UC had CNOS activity whereas those from healthy controls did not. Leukocytes shed into the lumen in ulcerative colitis are present in faeces but do not contain CNOS. This must therefore have another origin, and may be present in as yet unidentified microorganisms. Sulphate reducing bacteria are more common in faeces from patients with UC (*Florin* et al., 1990) and it is possible that CNOS is present in these or other microorganisms. Several enteric

bacteria have been shown to produce NO in micromolar concentrations in anaerobic culture at pH similar to that of the colonic lumen in UC (*Raimundo* et al., 1992).

Low concentrations of NO cause relaxation of colonic circular smooth muscle (*Middleton* and *Hunter*, 1992) and increased NO synthesis in UC may contribute to the associated smooth muscle dysfunction (*Spriggs* et al., 1951; *Kern* et al., 1951) which produces an attenuated gastrocolonic reflex (*Snape* et al., 1980), impaired contraction of colonic smooth muscle (*Cohen* et al., 1986) and the profound dilatation seen in toxic megacolon (*Heppell* et al., 1986). NO is directly toxic to host and target cells (*Billiar* et al., 1989) and, together with the superoxide anion, may under certain circumstances give rise to the formation of the peroxynitrite radical which may lead to the generation of highly toxic hydroxyl radicals (*Beckman* et al., 1989)

In certain experimental models of inflammation, NO may be cytoprotective, possibly by limiting microvascular damage (*Boughton-Smith* et al., 1992). It may also be involved in defence against microorganisms (*Granger* et al., 1986), although many bacteria are resistant to it (*Saito* et al., 1991). However, in ulcerative colitis production of NO may also lead to host tissue damage.

The formation of carcinogenic nitrosamines by leukocytes is NO dependent (*Grisham* et al., 1992) and is favoured by the low pH of the colonic lumen in UC (*Marletta* 1988). This may contribute to the associated risk of colonic neoplasia (*Collins* et al., 1987, *Korelitz* 1983).

Thus, NO biosynthesis has been shown to mediate NANC tonic neural inhibition of spontaneous mechanical activity in distal colonic smooth muscle. NO may be important in producing many of the clinical effects known to be associated with UC, such as diarrhoea, smooth muscle dysfunction, toxic megacolon or neoplasia. NO has been shown to be produced by human leukocytes and infiltration of inflammatory cells may be a factor in increasing NO production in inflamed colonic mucosa. However, the increased activity of NO synthase that we have demonstrated in the mucosa involves not only the inducible but also the constitutive form of the enzyme. As constitutive NO synthase is not found in leukocytes, it seems likely that some faecal CNOS is derived from other sources such as colonic bacteria. NO synthase activity was not demonstrated in faeces from healthy human volunteers and it seems possible that an abnormal colonic microflora, which produces excessive amounts of NO, may contribute to the disease process in ulcerative colitis. Further studies on the role of the intestinal microflora in the production of NO are currently underway.

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