Mechanisms of secretion and absorption  F222–F229

INTERLEUKIN 1 STIMULATES ANION SECRETION IN MAMMALLAN COLON

H Arla, L-D Vermeulen, A. Epithelial Membrane Research Centre and Department of Medicine, Hope Hospital, Salford, Manchester, M6 8HD, U.K.

Interleukin 1 may have an important role in the pathophysiology of diarrhoeal diseases, since the eicosanoid activity of this cytokine is involved in intestinal secretion. We therefore investigated the secretory effect and mechanism of action of interleukin 1 in isolated rat distal colon mounted as sheets in modified flux chambers. Both surfaces were bathed in Ringers small bowel buffer. Changes in transmucosal short circuit current (Isc), potential difference (pd) and conductance were measured following the basolateral addition of interleukin 1 (10^{-15} - 10^{-8} M), in either chloride free or chloride containing buffer, in the presence of one of the following: (a) no additives, (b) indomethacin 10^{-5} M (cyclooxygenase inhibitor), (c) ICI 207968 10^{-5} M (a lipxygenase inhibitor), (d) combined (b) and (c), and (e) mepacrine 10^{-5} M (a phospholipase A2 inhibitor).

Interleukin 1 produced a dose dependent increase in Isc (EC50 = 2 x 10^{-8} M; peak = 47.1 ± 4 μA cm⁻²) and a modest rise in pd and conductance when added to the basolateral, but not the apical half chamber. The change in Isc was attenuated in the absence of chloride (47.1 ± 4 ν 9.4 ± 1.1 μA cm⁻², p<0.001). The Isc was reduced by pretreatment with either indomethacin (cyclooxygenase inhibition) or ICI 207968 (lipxygenase inhibition) (47.1 ± 4 ν 23.8 ± 3.4; 37.1 ± 4.4 μA cm⁻², respectively, p<0.01). Moreover, combined pretreatment produced a greater fall in Isc (47.1 ± 4 ν 15.3 ± 2.9 μA cm⁻², p<0.001), but this was further reduced by mepacrine inhibition of phospholipase A2 (47.1 ± 3 ± 1.5 μA cm⁻², p<0.001).

In conclusion interleukin 1 stimulates chloride secretion in mammalian colon. This effect is predominantly mediated by arachidonic acid metabolites, however other phospholipase A2 derivatives, in particular platelet activating factor, may also be implicated.

WATER SECRETION DURING GUT ANAPHYLAXIS IS REVERSED BY 5-HYDROXYTRYPTAMINE (5-HT) TYPE 2 AND 3 RECEPTOR ANTAGONISTS. PH Mourid, LJ O'Donnell, E Oguma, IA Dias, MIG Farthing. Dept. Gastroenterology, St Bartholomew's Hospital, London, UK

Exposure of sensitized intestine to specific allergen leads to marked reduction in water and electrolyte absorption. Previously we have demonstrated that 5-HT3, blockade can partially inhibit this effect. In order to determine whether 5-HT3 receptors also play a role in this process we studied the effects of 5-HT3 as well as 5-HT1 blockade using ketanserin (KET) and granisetron (GRA), singly or in combination, on water flux during gut anaphylaxis.

Hooded Lister rats were inoculated ip with 10 μg ovalbumin (OVA) with alum adjuvant; sensitization was confirmed by specific IgG titres of >1:8. Intestinal water and electrolyte movement was assessed at 10 min intervals by in situ jejunal perfusion, 12 days after sensitization, either with plasma electrolyte solution (PES = Na 140, K 4, Cl 104, HCO3 40mM/l) or PES+OVA 20mg/l.

Twenty min after exposure to PES + OVA, net water secretion was observed, compared to absorption with PES alone (median -20 μl/min/g) (interquartile range -43 to -51); n=11 vs 107 [56 to 113], n=10; p<0.01). Pre-treatment with KET 200μg/kg sc (n=7) or GRA 300μg/kg sc (n=8) partially inhibited the secretory response to PES + OVA (18 [11 to 48] and 13 [6 to 32] respectively; both p<0.01 compared to PES + OVA control); simultaneous administration of KET and GRA had no additive effect (25 [13-44], n=10). After 40 min perfusion with PES + OVA, the comparison of net water movement was less pronounced (24 [-3 to 43]) and neither KET nor GRA had any effect (KET 48 [28 to 87], GRA 41 [32 to 83]; NS). Na and Cl movement paralleled that of water.

Thus, the profound water secretion which occurs in the early stages of intestinal anaphylaxis is mediated by 5-HT3 and 5-HT1 pathways. Other mediators may also play an important role especially in the late phase of anaphylaxis.
ELECTROGENIC ION TRANSPORT INDUCED IN VITRO BY BETHANECHOL IN DISTAL COLON FROM FED, STARVED AND UNDERNOURISHED MICE HAS NEURAL AND NON NEURAL COMPONENTS.

V. Sagnamie, R. I. Levin
Department of Biomedical Science, University of Sheffield, Sheffield.

Electrogenic ion transport measured in vitro as the short-circuit current (Isc, µamp/cm² serosal area) across intact distal colon removed from fed, 48 h starved and undernourished (4 days at 50% normal food intake) Swiss mice showed a biphasic response on serosal addition of the muscarinic agonist bethanechol (1 mM). A transient decrease in the basal Isc, which returned to the baseline within 30 seconds, was followed by a large increase which plateaued at the maximum for at least 10 minutes. The starved colon had significantly greater negative and positive components compared to the fed and undernourished colons (P < 0.01, unpaired t-test). Serosal bethanechol acting on colons pretreated with 1 µM serosal tetrodotoxin (TTX) did not induce a significant decrease of the Isc in fed or starved colons while that in the undernourished was practically abolished. The maximal Isc increases induced by bethanechol were all reduced, especially in the fed (P < 0.01) and starved colons (P < 0.001) which also showed decay of their Isc after the maximum had been reached unlike the plateau observed in the absence of TTX. Mucosal amiloride (0.1 mM) had no effect on the biphasic responses of the Isc to bethanechol indicating that they did not involve changes in electrogenic Na⁺ absorption. Pretreatment with mucosal diphenylamine-2-carboxylic acid (2.5 mM), a Cl⁻ channel blocker, surprisingly reduced, however, both the increase and the decrease in the Isc. Bethanechol thus activates electrogentic ion transport in fed and dietary-deprived distal colon by neural and non-neural mechanisms. The initial decrease in the basal Isc appears to be neurally mediated while the increase in Isc has both non-neural (direct action on colonocytes?) and neural components. The latter influences not only the maximum response but also its duration.

CALCIUM ABSORPTION AND BONE METABOLISM IN CROWN’S DISEASE (CD)

Divisione di Gastroenterologia Istituto di Semeiotica Medica, Laboratorio Centrale, Università di Padova.

The aim of this study was to investigate the occurrence of metabolic bone disease and its pathophysiology in selected patients with CD. Methods: We investigated 16 male adults (age 25-50) with long-standing (over 5 years) ileal or ileo-cecal CD (n=8), or with ileal or ileo-cecal resection (n=9) for CD. All patients had no symptoms of bone disease and had an inactive CD (CDAI<150), with no evidence of malabsorption. The study was performed in late autumn and bone metabolism was investigated by measuring S-biochemical indicators (Alkaline phosphatase, osteocalcin), minerals (Calcium, Phosphate, Magnesium) and relevant hormones (PTH, 1,25- OH and 1,25-(OH)²vit.D, nephrogenic 1,25(OH)²vit.D). Calcium absorption was evaluated by means of 45Ca kinetics after an oral dose, and LT-4 vertebral mineral density (BMD) was measured by Dual Energy X-ray Absorptiometry (Bone densitometry WAC LUN 1000).

Results: 7 patients (5 of whom resected) had a reduced Ca-intestinal absorption. Although a frank decrease of BMD was present only in 4 cases (2 score less than -2.5, 8 patients (75%) showed a BMD below 1 SD from normal mean (2 score less than -1). No correlation between Calcium absorption and either vit.D metabolites or BMD was found.

Conclusions: 1. A high proportion (64%) of patients with inactive CD have reduced Ca absorption or low bone density without symptoms of bone disease. 2. The reduction of Calcium absorption seems to be independent of vit D metabolism.

IMPACT OF MOLECULAR GEOMETRY ON PERMEATION RATES OF IN VIVO PERMEABILITY PROBES. K. Thunon, IS
Menzies, I. Barnason. Department of Clinical Biochemistry, King's College School of Medicine and Dentistry, Chemical Pathology, St Thomas's Hospital, London.

It has been particularly difficult to explain why PEG 400 permeates the intestine 100 times more efficiently than lactulose and 51CrEDTA in vivo as these substances are all water soluble, of similar mol. wt. and chemically inert. One suggestion is that PEG polymers are elongated, as opposed to globular and therefore slip through small 'pores' which do not accommodate the other markers. We tested this by assessing permeation rates of 3-O-methyl-D-glucose (mol. wt. 184), D-xylose (150), L-rhamnose (184), lactulose (342), 51CrEDTA (340) and PEG (200-500) through a V-lumen dialysis membrane in an Ussing chamber. Incubations were hourly for 11 hours on separate occasions. Permeation rates were calculated as conc (low side)/conca (high side) multiplied by the square root of the molecular weight so that if diffusion was unrestricted all markers would yield the same values (Grahms law). Results show linear permeation rates of 11 hours. Permeation rates of monoosaccharides were similar but more than twice that of the other markers. Five polymers of PEG were clearly separated but lactulose permeation rates were by comparison exactly as expected on the basis of mol. wt. Permeation rates of 51CrEDTA were significantly lower in all experiments presumably because of its negative charge at pH 7. This shows that the membrane restricts diffusion according to mol. wt. and results do not support the suggestion that the greater permeation rates of PEG 400 in vivo is due to an elongated structure.

Fibre supplemented polymeric enteral diets are currently being prescribed widely in the UK. These data show that mineral absorption may be adversely affected by the addition of fibre to the normal diet. As a result, this prospective study was carried out to quantitate and compare small intestinal absorption of the minerals Ca, Zn, Mg, Fe, Cu, and P during continuous intragastric infusion of: 1) polymeric enteral diet (PD) (6.3gN/l; 1xкаl/ml), or 2) polymeric enteral diet (6.3gN/l; 1xкаl/ml), supplemented with a soy polysaccharide (20g/l) fibre source (PDSF).

Thirteen normal subjects (PDSF n = 7, PD n = 6) were intubated with a multilumen tube, the distal end being positioned just proximal to the cecum. A 20 cm segment of terminal ileum was infused at 1ml/min with normal saline containing a non absorbable marker (0.5μCi/l H3-PS), in order to quantitate steady state colonic inflows of minerals during continuous (7 h) intragastric infusion (82 ml/hr) of enteral diet. Total small intestinal absorption values (% of infused load ± SEM measured by flame photometry) for PD and PDSF respectively were: Ca 82.7 ± 2.1 vs 94.1 ± 1.0; Zn 60 ± 9.14 vs 83.5 ± 4.6; Mg 70.2 ± 2.77 vs 93.6 ± 1.85; Fe 65.3 ± 5.2 vs 67.5 ± 6.3; Cu 48.9 ± 8.99 vs 62.7 ± 3.2; P 90.9 ± 3.6 vs 102 ± 7.58 (% ± SEM) (17% vs 82%).

These data show that the addition of 20g/l soy polysaccharide to polymeric enteral diet has no adverse effect on the absorption of Fe, Cu or P but significantly increases the absorption of Ca, Zn, and Mg.

ALTERATIONS IN ENZYME ACTIVITIES FOLLOWING CHRONIC LOW-FREQUENCY ELECTRICAL STIMULATION OF HUMAN GRACILIS MUSCLE. D. O'Sullivan, M. F. Gras, B. D. George, N. S. Williams. Surgical Unit, The London Hospital Medical College, The Royal London Hospital, London E1 1BB UK.

Chronic low-frequency electrical stimulation (LFES) of fast-twitch, fatigable skeletal muscle has been shown in experimental animals to result in transformation into a slow-twitch, fatigue-resistant muscle. This principle has been applied to the gracilis sling procedure for the treatment of patients with faecal incontinence in an attempt to achieve the fatigue-resistance appropriate for sphincteric function. This study aimed to determine whether the metabolic activity of the human gracilis muscle may be modified by LFES.

Muscle biopsies were taken from 5 patients undergoing the gracilis neosphincter procedure before and after a period (7-16 weeks) of LFES (2-10 Hz). Succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities were assayed using a photometric technique. The results are expressed as the mean (SD) of 4 separate determinations on each biopsy.

<table>
<thead>
<tr>
<th>SDH</th>
<th>µmolNADH/min/mg prot</th>
<th>LDH</th>
<th>µmolNADH/min/mg prot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Pre LFES</td>
<td>Post LFES</td>
<td>Pre LFES</td>
</tr>
<tr>
<td>1</td>
<td>1.49 (0.11)</td>
<td>1.79 (0.09)</td>
<td>0.60 (0.04)</td>
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<td>2</td>
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<td>0.51 (0.04)</td>
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<td>0.32 (0.01)</td>
<td>0.70 (0.08)</td>
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<tr>
<td>5</td>
<td>0.67 (0.03)</td>
<td>0.86 (0.11)</td>
<td>0.66 (0.01)</td>
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</table>

These results indicate a significant increase (P=0.02, paired T test) in SDH activity but no significant overall change in LDH activity. This is consistent with a shift from predominantly anaerobic to aerobic metabolism, which would be expected to accompany an improvement in fatigue-resistance. The results also demonstrate the wide inter-patient variability in enzyme activities which has previously been reported.

This study demonstrates that the metabolic activity of the human gracilis muscle is modified by LFES.

Constipation and incontinence F230-F235


The short term results of postanl repair for idiopathic faecal incontinence are satisfactory but data on long term outcome is lacking. This study was carried out to document the short and long term results and to determine whether pre-operative tests predict outcome.

Method: 36 patients (33 F, mean age 57 yrs) with idiopathic faecal incontinence were operated on by one surgeon between Sept 1985 and March 1991. Patients had resting (RP) and voluntary contraction (VCP) anal pressures and pudendal nerve terminal motor latencies (PNTML) measured pre-operatively. Symptoms were evaluated at 6 months after operation and again at a mean of 32 months (range, 6 - 72) in all 36 patients. Symptoms were classified as: Group A, no improvement; or worse; Group B, minor improvement; Group C, marked improvement.

14 patients were available for post-operative physiology.

Results: At 6 months there were 6 (17%) patients in Group A, 12 (33%) in Group B and 18 (50%) in Group C. At final follow up there were 17 (47%) in Group A, 9 (25) in Group B and 10 (28%) in Group C. There were no significant differences in the pre-operative tests between the 3 groups at 6 months. Comparison of the pre-operative data in the final outcome groups showed (mean ± SEM): Group A vs Groups B & C; RP: 41 ± 12 cmH2O vs 24 ± 8 (P=0.2), VCP: 12 ± 3 vs 27 ± 7 (P=0.07), PNTML 3.3 ± 0.44 ms vs 3.16 ± 0.4 (P = 0.8). Mean differences between post-operative results: RP 39 ± 9 cmH2O (P=0.09), VCP 26 ± 10 (P=0.22), PNTML -0.2 ± 0.5 ms (P=0.2). In 10 of the 14 patients tested the RP and VCP was increased postoperatively.

Conclusion: At 6 months 83% of patients had obtained some benefit from postanl repair but only 53% maintained this improvement with only 28% markedly better. There was a trend towards a more favourable outcome in patients with greater squeeze pressures pre-operatively but other tests were not of long term predictive value.


Neuropeptides are important in control of intestinal motility and might play a central role in disease states. Little is known of their role in the normal internal anal sphincter (IAS) and whether abnormalities exist in incontinence.

Method: IAS from 16 women with IIF (mean age 58 yrs, 26-83) undergoing post-anal repair and 12 cancer controls (age 61 yrs, 51-74; 7F) were fixed in 4% paraformaldehyde. Cryostat sections (10μm) were incubated with primary antisera for 15 hrs. Rabbit antisera to vasoactive intestinal peptide (VIP), neuropeptide-Y (NPY), galanin, calcitonin -gene related peptide (CGRP), substance P and peptide histidine isoleucine (PHI) were used. Sections were incubated in goat-anti-rabbit fluoroeosiniothiocyanate (FITC)-conjugated 2* antisera. Sections were examined with a Zeiss microscope equipped for viewing fluorescence.

Results: Tissues obtained smooth muscle and some adjacent submucosa, but no longitudinal layer or myenteric plexus. Immunoreactivity was typically seen in nerve fibres between muscle bundles and in the submucosa. Generally the innervation was sparse with a greater density of innervation by VIP than of NPY or galanin, less PHI and very few CGRP or substance P-immunoreactive fibres. In sections from incontinent patients the distribution of peptides was the same and there was no significant difference in the amount of immunoreactivity.

Conclusion: There is no alteration in the density or distribution of neuropeptide immunoreactivity in idiopathic incontinence. In both controls and patients there is a greater density of VIP, NPY and galanin containing nerve fibres than PHI, CGRP or substance P-immunoreactive fibres.