Abstracts

On the participation of protein kinase C in regulatory processes in the exocrine pancreas and other exocrine glands

HANS-DIETER SÖLLING, ULRIKE PADEL, WERNER FEST, ESTELLA MACHADO-DE DOMENECH, CARLOS DOMENECH, AND WERNER BOLL (Abtig Klin Biochemie, Univ Göttingen, D-3400 Göttingen, FRG) Stimulation of pancreatic exocytosis by pancreozymin, caerulein or carbacholcholine as well as stimulation of secretion in the parotid gland by carbacholcholine is associated with an activation of protein kinase C. This is indicated by our following findings: (1) Stimulation of secretion leads to a rapid translocation of protein kinase C from the cytosolic to the membrane compartment, the shift being completed within less than five minutes in isolated pancreatic and in less than 45 seconds in isolated parotid gland lobules. (2) The stimulation of secretion is associated with an increased multi-site phosphorylation of the ribosomal protein S6 and the pattern of phosphorylated sites is characteristic for a phosphorylation by protein kinase C. Moreover, the same phosphorylation pattern can be induced in intact cells by phorbol esters. (3) Phorbol esters, known activators of protein kinase C, induce exocytosis in guinea pig pancreatic cells without measurable changes in cellular free calcium.

Up to now it is not clear yet which phosphorylation process catalysed by protein kinase C is directly associated with stimulation of exocytosis. We shall, however, present new data obtained by 2-D-electrophoresis which show that the phosphorylation of hitherto undescribed phosphoproteins is changed following stimulation of exocytosis. We shall demonstrate further, that stimulation of secretion in pancreatic lobules as well as in isolated parotid acini is associated with an enhanced synthesis of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholines, compounds which have been shown to have a secretory effect in these organs. We shall present data which indicate that this is because of a rapid activation of a specific acetyltransferase and that the activation of protein kinase C is associated with this event.

Mucosal polyamine profile in normal and adapting (hypo- and hyperplastic) intestine: effects of DFMO treatment

M. HOSOMI, F. LIRUSSI, N. H. STACE, S. VAJA, G. M. MURPHY, AND R. H. DOWLING (Gastroentology Unit, Guy's Hospital Medical School, London SE1 9RT) The polyamines (PA) putrescine, spermidine and spermine are intracellular second messengers implicated in normal, adaptive and neoplastic growth. Polyamine synthesis is rate limited by ornithine decarboxylase (ODC) which is competitively inhibited by difluoromethylornithine (DFMO) while PA degradation is controlled by diamine oxidase (DAO). To extend previous studies of PA metabolism in intestinal adaptation we first measured mucosal polyamine profiles at different sites in the normal rat intestine (duodenum, 5 x 10 cm segments of jejunum, 5 of ileum, and colon) and compared the results with those obtained in adaptive hypoplasia (seven days parenteral nutrition, TPN, n = 6), in the adaptive hyperplasia of two weeks after 90% small bowel resection (SBR, n = 5) or pancreaticobiliary diversion (PBD, n = 6). We then examined the effects of DFMO (2% in drinking water, daily from two days before surgery) on mucosal PA and the adaptive response to PBD. RESULTS: (i) In the fasting (20 h) normal animals, the concentrations of each of the PA's decreased from duodenum to colon, following the gradient indicated by the usual indices of mucosal mass. Mucosal DAO activity increased progressively from jejunum to ileum. (ii) In the hypoplasia of TPN there were modest decreases whereas in the hyperplasia induced by PBD or SBR, there were significant increases. (iii) DFMO markedly inhibited PA synthesis in the jejunum and ileum as evidenced by subnormal putrescine, spermidine and spermine levels, and significantly inhibited the mucosal adaptive response even to the point of extinction.

These results support the concept that changes in polyamine metabolism are important in intestinal mucosal adaptation. They also suggest that by manipulating intracellular second messengers, adaptive mucosal growth can, for the first time, be manipulated/controlled.

Involvement of polyamines in the development and growth of duodenum and pancreas in neonatal rats

MORISSET (Centre de recherche sur les mécanismes de sécrétion, Université de Sherbrooke, Sherbrooke, Québec, Canada) To evaluate the role of ornithine decarboxylase (ODC) and the polyamines in duodenum and pancreas growth and development, neonatal rats were given daily sc injections of 500 mg/kg α-difluoromethylornithine (α-

DFMO), a specific, irreversible inhibitor of ODC. Body weight and growth of the duodenum and pancreas were estimated in saline control and DFMO treated rats at 7, 14, and 21 days. In each organ, total contents of DNA, RNA and protein were determined as well as contents of amylase and chymotrypsin in the pancreas. Concentrations of these parameters were also established on a DNA basis. Body weights were significantly reduced after seven days of treatment but maximally after 14 days (−30%). In the pancreas, the major changes were observed after 21 days of DFMO with significant decreases in total weight (−14%) and content of DNA (−19%), RNA (−15%), protein (−19%), amylase (−55%) and ChTg (−88%). Of interest, after seven days, pancreatic weight remained unaffected, DNA content was significantly reduced (−17%) while those of protein, amylase and ChTg were significantly increased by 20, 24 and 54%. The duodenum also showed increased weight (15%) and content of DNA (10%) and protein (40%) after seven days of treatment but was the most affected after 14 days of DFMO with significant reductions in weight (−59%) and total content of DNA (−45%), RNA (−53%) and protein (−63%). These data suggest that the ODC/polyamine system does serve as a modulator of duodenum and pancreas growth during neonatal rat development and that differences exist among these two organs in the degree and time course of dependence of growth on polyamines.

Polyamine synthesis inhibitor, difluoromethylornithine (DFMO) does not prevent the pancreatic hyperplasia induced by pancreaticobiliary diversion (PBD) in the rat

N. H. STACE, M. HOSOMI, S. VAJA, G. M. MURPHY, AND R. H. DOWLING (Gastroenterology Unit, Guy's Hospital, London) Polyamines are thought to regulate cell division and growth; inhibition of polyamine synthesis, therefore, may reduce these processes. Ornithine decarboxylase (ODC) the rate limiting enzyme in polyamine synthesis which converts ornithine to putrescine, is specifically and irreversibly inhibited by DFMO. Thus DFMO in tissue culture reduces cell division and when given orally inhibits adaptive growth in intestine and, in one study at least, the pancreas (Morisset et al. 1983; Dig Dis Sci, 28: 943). To see if DFMO also modifies the pancreatic hyperplasia induced by PBD (interposition of 50 cm of jejunum between stomach and duodenum) eight pairs of rats
were given either drinking water alone or 2% DFMO in water beginning two days before PBD.

Both food intake and BW decreased significantly in the DFMO treated rats so that 14 days after PBD, mean BW in the DFMO group (197±SEM6g) was less (p < 0.001) than that in the controls (250±6). As expected, PBD increased pancreatic weight by approx 70% in the controls but surprisingly, mean pancreatic weight, corrected/100 g BW, was significantly greater in the DFMO group (847±59 mg) than in the controls (634±26; p < 0.01). Similarly, mean pancreatic protein (mg 100 g BW⁻¹) was also 33% greater after DFMO (138±12) than in the rats given water (103±4; p < 0.02). Pancreatic DNA (mg 100 g BW⁻¹), although greater after DFMO (50±0.4) than in controls (42±0.3), was not significantly different. DFMO also increased mean testicular weight/100 g BW by 23% (p < 0.001); it did not affect the weight of heart, kidney, liver or spleen, when related to BW. When pancreatic and testicular weights were uncorrected for BW, however, there was no difference between the two groups.

These results show that the pancreatic hyperplasia induced by PBD is not inhibited by DFMO. Rather it inhibits food intake and body weight, emphasising the need for pair feeding in studies with DFMO. This finding challenges the concept that inhibition of ODC reduces adaptive growth in the pancreas.

Changes in keratins expression during intestinal cell differentiation

ANDREA QUARONI (Cornell University, Ithaca, NY, USA) Like most other epithelial cells of vertebrates, the cells covering the intestinal villi, and in the crypts, are known to contain relatively large amounts of proteins immunologically related to epidermal prekeratin, which form a complex cytoplasmic network of tonofilaments, extending into the terminal web, where they appear to be linked to the actin microfilament bundles of the microvilli. To correlate the presence of specific cytoskeletal components with different stages of intestinal cell differentiation, we have produced and characterised eight monoclonal antibodies against detergent extracted intestinal brush borders. These antibodies were found to recognise cytoskeletal proteins of Mr 40000 to 53000 previously identified as keratin polypeptides. By 2-dimensional slab gel electrophoresis, the eight antibodies were found to have distinct, but partially overlapping, polypeptide specificities. These antibodies were used for immunohistochemical staining of frozen sections of small and large intestine, and Western blot analysis of cytoskeletal proteins obtained from intestinal cells separated as a villus-to-crypt gradient. Marked differences between crypt and villus cells were observed, including: (a) the presence of a 46 kD component (recognised specifically by the BBC3/48/5 antibody) exclusively in the differentiated cells; (b) the presence of a set of 48 kD polypeptides predominantly in the crypt cells, and of a 53 kD component in relatively larger amounts in the villus cells; (c) a general shift to less acidic isoelectric points for the major cytokeratin components with cell differentiation. These results have shown that specific changes in keratin gene expression and/or post-translational modification occurred during migration of the epithelial cells along the villus axis, and identified a 46 kD component which may be regarded as a molecular marker for intestinal cell differentiation. If indeed the apical intestinal tonofilaments are involved in the organisation and function of the terminal web, the observed differences in keratins composition may be, at least in part, responsible for the absence of this intracellular structure in the proliferative crypt cells.

Food viscosity as nutritional factor in the adaptation of rat small intestinal absorptive and digestive functions

B ELSHENHANS (Walther-Straub-Institut für Pharmakologie und Toxikologie der Ludwig-Maximilians-Universitäts München, FRG) Dietary constituents such as dietary fibre may affect small intestinal absorption and digestion of nutrients by altering food consistency. Studies using dietary fibre may, however, lead to contradictory results on its physiological and pharmacological effects because of its varying and complex composition. Therefore, the present studies were carried out with defined carbohydrate gelling agents -- that is, viscosity-enhancing polysaccharides (PS). In acute in vitro (tissue accumulation method) and in vivo (single pass perfusion) experiments, PS inhibited intestinal absorption of sugars, amino acids and bile acids as well as luminal hydrolysis of disaccharides and dipeptides. Inhibition was only dependent on the PS viscosity. The importance of the food-consistency changing properties of the PS for adaptive changes in the small intestine was shown by long term feeding studies. Reduced (alk. phos-

Enteroglucagon in two experimental models of intestinal hyperplasia

M GREGOR, H MENGE, C T GERMER, R STÖSSEL, AND E O RIECKEN (Department of Internal Medicine, Klinikum Steglitz, FU Berlin, Fed Rep of Germany) It has been suggested on the basis of indirect evidence that enteroglucagon (EG) may act as a growth promoting factor on the intestinal mucosa. In order to further evaluate this hypothesis we investigated two different experimental conditions which are known to induce intestinal hyperplasia not being associated with an increase in the nutritional load of the intestine. The three dimensional architecture and the proliferative activity of the small intestinal mucosa have been evaluated. Plasma and tissue EG concentrations were measured by RIA.

(1) Germ free rats were conventionalised with a thermoburc flora and were held thereafter as open conventional animals (RC). When compared with germ free control rats (CO) four weeks after conventionalisation there was significant increase of mitotic activity (mitoses/crypt: CO 16±0.8, RC 26±0.6, p < 0.002) and crypt length (CO 96±7.2 µm, RC 119±3.3 µm, p < 0.02) in the ileum but not in the jejunum. The basal EG plasma levels were unchanged two and four weeks after conventionalisation. Furthermore the ileal and colonic tissue levels of EG (pmol/g) showed a significant decrease in the contaminated group but were unchanged in the jejunum (jejunum: CO 708±97; RC 81±89, ns ileum: CO 162±8; RC 95±6.2, p < 0.01, colon: CO 113±7.9; RC 71±2.8, p < 0.05).
Adaptational hyperplasia in ileum limits atrophy in self-emptying jejunal loops

G P Young, and C Morton (University of Melbourne, Dept. of Medicine, The Royal Melbourne Hospital, Victoria, Australia)

The relative importance of luminal nutrients and circulating factors in maintenance/stimulation of intestinal growth was compared in rats subjected to varying degrees of jejunoileal bypass. If circulating factors cause hyperplasia in this model, then atrophy in the self-emptying loop (SEL) should be less marked in extensive bypass. We studied 3 groups: 1) 85% bypass of intestine (85% BYP), n = 2; 2) limited 25% bypass (25% BYP), n = 3; 3) controls which underwent sham surgery, n = 7. Morphology (including villus area index-VAI) and markers of villus cell differentiation (including surace) were measured in directly comparable segments of intestine 12 weeks after surgery.

In ileum, villus hyperplasia was most marked after extensive bypass (85% BYP, see below), yet atrophy was most marked in the short SELs (25% BYP) when the ileum was much less hyperplastic. Changes in villus height and crypt depth paralleled changes in VAI.

Doubling of ileal VAI to levels normally encountered in jejunum was not accompanied by a significant increase in surace specific activity (see above), indicating that adaptational hyperplasia does not in itself bring about a rise in that differentiation marker. Surace activity increased in SELs even though hyperplasia occurred.

Despite the greater distance from luminal contents of the longer SELs in 85% BYP rats, there was significantly less atrophy than in the shorter 25% BYP SELs (13% fall in VAI + 24%, p < 0.05). This suggests that a nonluminal growth factor, possibly blood-borne, is released in extensive bypass and that this factor limits atrophy in the SEL despite the absence of luminal nutrients.

Carbohydrate induced mucosal growth depends on substrate load and 'work of absorption'

E Weser, M Hoban, and A Van Harper (Medical service, VA Hospital and The University of Texas Health Science Center, San Antonio, Texas, USA) Infusion of carbohydrates into mid-small bowel will stimulate mucosal growth in rats maintained on total parenteral nutrition (TPN). In this study the dose response of adaptive mucosal growth to infused monosaccharides was compared with disaccharides. Male Sprague-Dawley rats maintained on TPN, were infused continuously (2 ml/h) via a cather placed in the mid-small bowel with the following solutions: glucose, 5–20% ; galactose, fructose, maltose, lactose, lactulose, 5%; and sucrose, 10% with added acarbose, an α-glucosidase inhibitor (1–30 mg/g sucrose). Control rats were infused with 0-9% saline. After seven days rats were killed, the small bowel removed and divided into eight segments (segment 1–duodenum, segment 8–terminal ileum). The cather segment was discarded. Segment weight, mucosal weight, DNA, and protein concentration per cm segment were measured. In addition liver, kidney, and empty caecum weights were obtained in most animals. All rats gained in body weight during the experimental period. There were no significant differences in liver or kidney weights although mean liver weight tended to be highest in animals receiving 20% glucose or sucrose. Mucosal mass increased proportionately to the amount of sugar infused, not only in segments adjacent and downstream from the infusion site but also in the remote proximal bowel segments. Disaccharides undergoing hydrolysis consistently produced about 30% greater adaptive growth (in segments 5 and 6) than equal amounts of monosaccharides. In fact, 5% monosaccharide infusions (delivering 100 mg sugar/hour) did not differ from control saline infusions whereas equal amounts of disaccharide consistently resulted in adaptive growth. Infusion of lactulose, a non-hydrolysable disaccharide or inhibition of disaccharide hydrolysis by acarbose abolished mucosal growth. In these instances caecum weight increased reflecting adaptive growth by the unabsorbed sugars reaching the colon. These results suggest that the mechanism by which intraluminal sugars stimulate intestinal adaptation is dependent on both substrate dose and work-energy required in the absorptive process.

Secretory dose response studies in the pancreas made hyperplastic by pancreateo-biliary diversion (PBD) or 90% small bowel resection (SBR) in the rat

N S Stace, S Vaja, A Butt, G M Murphy and R H Dowling (Gastroenterology Unit, Guy’s Hospital Medical School, London) Pancreatic hyperplasia may be induced experimentally by several methods but little is known about the secretory potential of these large glands. PBD, by transposing 30 cm of jejunum to lie between pylorus and ampulla and 90% small bowel resection causes pancreateo-hyperplasias, probably due to associated hypercholecystokininaemia. Therefore in PBD and transected controls (TRE), 14 days after surgery, we measured vol, protein and trypsin, amylase and lipase outputs in pairs of fasted (24 h), anaesthetised, bile duct ligated rats during a basal hour and over 6 x 30 min iv infusion periods with doubling doses of CCK-OP (0-25-80 μg/kg/h) given alone or together with background secretin (20 CU/kg/h; n = 7 pairs, exp. 2). Similar dose-response studies were performed eight weeks after surgery on triplets of PBD, SBR and TREC. n = 8, exp. 3).

Although BW’s of TREC and experimental rats were comparable, pancreatic wet weight (mg 100 g/bw) increased by 52%, 71% and 66% (PBD), and by 26% (SBR) over controls in exp. 1, 2, 3 respectively. In all
Biphasic pattern of the trophic effect of caerulein on the rat exocrine pancreas

B M MIAZZA, C PANOW, AND E LOIZEAU
(Division de Gastroentérologie, HCU Geneva, Switzerland)

Cholecystokinin and its analogues stimulate dose dependently pancreatic enzyme secretion, with a characteristic biphasic pattern: the secretion increasing to reach a maximum then declining with greater doses of the secretagogue. The purpose of the present study was to investigate whether such a phenomenon does exist for the trophic effect of caerulein. Seventy male Wistar rats (250–300 g) were randomly assigned to seven treatment groups. The animals received three daily subcutaneous injections of 10% hydrolysed gelatin, as carrier plus saline (control group) or caerulein (0.25, 0.5, 1.0, 2.0, 5.0, or 10.0 μg × kg⁻¹ × day⁻¹) for seven days. The weights of the secreting part of the stomach and of duodenal and ileal segments were recorded. Pancreatic weight, DNA, RNA, and protein contents were measured. All doses of caerulein induced a small but significant increase in stomach weight (6–12% above control value, p < 0.05–0.001). A similar effect was observed in both duodenal and ileal segments, but only with the higher doses (2.0–10.0 μg × kg⁻¹ × day⁻¹, p < 0.05–0.001). The pancreatic mass increased dose-dependently up to the 2.0 μg dose, then declined with 5.0 and even more with 10.0. All values were nevertheless significantly greater than in controls (p < 0.05–0.001). DNA, RNA, protein contents showed a similar biphasic pattern of results. However, the pattern of DNA concentration was inverted in mirror: declining with increasing the dose (up to 1.0 μg), then returning towards control value.

In conclusion, the results of this study show that the trophic effect of caerulein is very similar to the secretagogue effect; it also disclosed the characteristic biphasic pattern.

Time course of pancreatic growth and role of hormonal factors after bile and pancreatic juice diversion in rats

B M MIAZZA, T NICOLET, W F HAHNE, J A CHAYVIALLE, AND E LOIZEAU
(Division de Gastroentérologie, HCU Geneva, Switzerland, and Inserm U 45 Hôp Edouard Herriot, Lyon, France)

Pancreatic-biliary diversion (PBD) provokes the growth of the exocrine pancreas in rats. This study investigates the kinetics of structural changes and its relationship to pancreatic hormonal factors. The effect of surgical PBD was studied in 72 male Wistar rats (200–250 g) and compared with 43 controls at eight postoperative intervals (2, 4, 6, 8, 15 days and 6, 8, 18 weeks). Pancreatic weight, DNA, RNA, protein contents, DNA synthesis (H²TdR incorporation) and plasma levels of pancreatic polypeptide (PP), secretin (S), cholecystokinin (CCK) and gastrin (G) were measured.

After PBD, all parameters of pancreatic mass (weight, DNA, RNA, protein contents) increased to reach a higher plateau on day 15 (p < 0.01–0.001), DNA concentration was significantly lower (p < 0.05–0.001) from day 6, while RNA concentration raised from day 2 (p < 0.05–0.01). H²TdR incorporation increased transiently with a peak at day 6, before returning to control values between day 15 and week 6. Pancreatic polypeptide and secretin plasma concentrations did not change, but CCK was significantly higher than in controls throughout the study (p < 0.05–0.01). On the contrary, G concentrations were significantly lower in PBD rats from day 6 to week 6 (p < 0.05–0.001). In conclusion, the results of the present study confirm that PHD provokes a rapid and persistent increase of the pancreatic mass in the rat. This phenomenon is characterised by both hypertrrophy (increased RNA and decreased DNA concentrations) and hyperplasia (increased DNA content). The rise in CCK plasma concentrations is most likely responsible for the greater cell proliferation.

Reference

The British Society of Gastroenterology

Reversible desensitisation and cycle of VIP receptors in human carcinoma colonic cells

C BOISSARD, J C MARIE, C HEJBLUM, C GESCHAP AND G ROSELLIN
(Inserm U.55, Centre de Recherches Saint-Antoine, Paris 12°, France)

Human carcinoma colonic cells in culture (HT-29) possess specific receptors to VIP which are coupled to the production of cyclic AMP through stimulation of adenylate cyclase. After pretreatment with VIP (10⁻⁶ M), there is a considerable decrease of the cyclic AMP-mediated signals in response to the rechallenge with this neuropeptide. This time and dose dependent process in receptor mediated as shown by the pharmacological specificity of the desensitisation, its association with a considerable decrease of the potentiative action between VIP and forskolin, and its correlation with the disappearance of the VIP receptor. There is a close temporal relationship between the VIP induced cyclic AMP desensitisation and the disappearance of the VIP receptor from the cell surface. Total inhibition of binding and desensitisation are obtained in the same experimental conditions. Both phenomena are critically time- and temperature-dependent and are reversible. The rate of reappearance of the cell surface binding sites increases with temperature and is related to the resensitisation of the cells. In optimal conditions, recycling of the binding sites to the cell surface and resensitisation did not exceed 75% of the control value. Our results indicate that upon addition of VIP, the ligand and the receptor are immediately internalised together and also suggest that internalised receptors follow two different processes: one related to a rapid recycling, the other to a partial destruction.

Postheparin plasma diamine oxidase in man: a non-invasive marker of adaptive mucosal growth in segmental intestinal diseases?

T ROKKAS, S VAJA, G M MURPHY, AND R H DOWLING
(Gastroenterology Unit, Division of Medicine, UMDS of Guy’s and St Thomas’ Hospitals, London) Polyamine synthesis, of importance in intestinal adaptive growth, is stimulated by ornithine decarboxylase and inhibited by diamine oxidase (DAO). Diamine oxidase is a unique enzyme which in animals is largely confined to the small bowel villus tips (Luk et al 1971; 1980: 66: 66) particularly in the ileum (Hosomi et al. Clin Sci, 1984; 66: 66) but little is known about its distribution in man and how this is
affected by segmental bowel diseases in which adaptive changes are known to occur in non-affected or residual intestine. We therefore measured DAO activity (mU/g protein) in gastric (n = 12), jejunal (n = 16) and colonic (n = 18) mucosal biopsies and found significantly (p < 0.001) more DAO in jejunum (190±3.37) than in stomach (1.7±0.60) or colon (9.4±0.84).

In man, plasma DAO is at, or below, assay detection limits but DAO can be 'released' into the circulation by low-dose (5000U) IV heparin (Rokkas et al. Clin Sci, 1985; 69: 36) and in 10 individuals found to have normal jejunal histology, there was a significant (p = 0.001) linear relationship (r = 0.872) between jejunal DAO (mU/g mucosa) and the 2h postheparin plasma concentration/time curves (AUC's: mU/1/2 h). In 14 controls, AUC results (35.7±6.13) overlapped with those in six untreated and 11 treated coeliacs, 6 patients with ulcerative colitis and 3 with ileectomy but were significantly greater (p < 0.01) than those in 14 patients with ileocolonic Crohn’s (11.8±2.78). There was an inverse relationship (r = -0.775; p < 0.01) between the Crohn’s disease activity index and the AUC’s.

In man, DAO activity is higher in small bowel than in stomach or colon. Postheparin plasma DAO correlates with intestinal mucosal DAO and may provide a non-invasive marker of active ileal Crohn’s. Preliminary results in coeliacs responding to a gluten-free diet and in TPN patients resuming oral feeding, suggest that it may also indicate mucosal regeneration and/or adaptive intestinal mucosal growth.