Gastrointestinal responses to a panel of lectins in rats maintained on total parenteral nutrition

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TOTAL PARENTERAL NUTRITION (TPN) is valuable in medical care but causes important clinical complications. Gastrointestinal atrophy during TPN causes malabsorption and diarrhea when enteral feeds are reintroduced (8, 9). Atrophy may also cause sepsis through translocation of bacteria across a weakened mucosal barrier (13).

Therefore, agents that prevent gastrointestinal atrophy during TPN may be useful. Plant lectins are present in normal diets, and some lectins strongly stimulate proliferation of gastrointestinal cells (31, 35, 37, 40, 42). This might be through direct binding of the lectin to epithelial cells. However, certain lectins also stimulate release of regulatory peptides, and we have shown that growth of the excocrine pancreas in response to phytohemagglutinin (PHA) (25) and soybean lectins and stimulation of pancreatic exocrine secretion are mediated by CCK (29). TPN causes a generalized reduction in release of gastrointestinal hormones (12, 19). Administration of regulatory peptides diminishes intestinal hypoplasia in rats on TPN (23) and diminished bacterial translocation in mice on elemental diets (23). Therefore, in this study, we asked whether a panel of lectins can reverse the gastrointestinal atrophy produced by lectins and whether lectins alter hormone release in ways that could explain changes in proliferation.

We used our model of TPN-fed rats. TPN diminishes proliferation of gastrointestinal epithelia in these animals to a basal level within 3 days (21). We chose lectins that bind to different carbohydrate groups and are known to stimulate proliferation in other systems. Peanut agglutinin (PNA; Arachis hypogaea) binds to Gal-β-1-4GalNAc and stimulates proliferation of human colonic epithelium and colonic carcinoma cell lines (31, 40, 42). Concanavalin A (ConA, Canavalia ensiformis), the lectin present in J-jack beans, binds to mannos and glucose residues and caused small intestinal growth in rats (35). Wheat germ agglutinin (WGA; Triticum vulgare), which binds to N-acetylgalactosamine and sialic acid, and lectin present in red kidney beans (Phaseolus vulgaris agglutinin, PHA), which binds strongly to complex carbohydrate groups, are trophic to the small intestine and pancreas of rats (36, 37). In normally fed animals these lectins resist digestion and are largely passed intact in stools (10, 35, 40).

We report here that all the lectins tested are capable of reversing atrophy and stimulating gastrointestinal hormone release. However, each affects distinct regions of the gastrointestinal tract and specific hormones.

MATERIALS AND METHODS

Chemicals were purchased from Sigma (Poole, Dorset, UK) unless otherwise stated. PNA was supplied by Prof. J. Rhodes (University Hospital, Liverpool, UK), ConA and WGA were from EY Laboratories (Leicestershire, UK), and PHA was...
produced by Prof. A. Pusztai (Rowett Research Institute, Bucksburn, Aberdeen, UK).

Preparation of Rats

Male, 180–200 g, Sprague-Dawley rats were anesthetized with 0.1 ml of hypnorm and 0.1 ml diazepam. A Silastic cannula was tied into the right external jugular vein. The cannula was tunnelled subcutaneously to the back of the neck and connected through a stainless steel skin button and tethered to a two-channel fluid swivel joint (Linton Instrumentation, Norfolk, UK). Rats were housed individually in wire-bottomed cages. The TPN diet was delivered to the rats via the right external jugular vein. The TPN solution was kept in bottomed cages. The TPN diet was delivered to the rats via the right external jugular vein. The TPN solution was kept refrigerated, but the line and pump were outside so that the solution entered the animals at room temperature at a rate of 60 ml·rat⁻¹·day⁻¹, giving 1.8 g nitrogen, 6.0 g lipid, 8.5 g glucose, and 1,047 kJ·kg⁻¹·day⁻¹.

Experimental Protocol

Groups of rats (n = 6/group) received TPN for 2 days alone. Beginning on the third day lectins were infused into the rat stomachs via the intragastric cannula with vehicle (0.5 ml saline). Each rat received a single bolus dose of lectin (25 mg/ml saline·rat⁻¹·day⁻¹) at 0900 for a total of 4 days. One group of control rats was also surgically prepared and received TPN and vehicle alone. A further group of rats was fed orally ad libitum on rat chow for comparison. On the final day rats were injected with vincristine sulfate (1 mg/kg ip; David Bull Laboratories, Warwick, UK). One hour later a final dose of lectin was administered, and after another hour the rats were killed with pentobarbitone and blood was then taken by cardiac puncture for measurements of plasma hormones by RIA. The wet weights of the stomach, small intestine, pancreas, cecum, and colon were recorded, and samples of the small intestine and the colon at 10, 50, and 90% of length were fixed in Carnoy’s fluid and stored in 70% (vol/vol) ethanol. Formalin-fixed tissue from all regions was embedded in paraffin, and sections were stained with hematoxylin and eosin for histological examination. Apoptotic cells were visualized by light microscopy.

Assessment of Proliferation by Metaphase Arrest

Pieces of fixed tissue were hydrated, hydrolyzed in 1 M HCl at 60°C for 10 min, and then stained in Schiff’s reagent (the Feulgen reaction). The crypts were displayed by microdissection (18). The number of arrested metaphases in 10 small intestinal crypts and 20 colonic crypts was counted in each specimen. The number of cells arrested in metaphase was used in the subsequent analyses.

Hormone RIAs

Blood was taken by cardiac puncture for hormone assay. Plasma levels of gut hormones were measured by RIAs as previously described for gastrin (7), CCK (29), enteropeptidase (16), glucagon-like peptide-1 (GLP-1) (33), peptide YY (PYY) (1), and insulin (44). Enteroglucagon was calculated by subtracting specifically measured pancreatic glucagon (COOH-terminal immunoreactivity measured with the antisera RCS5) from total NH₂-terminal glucagon immunoreactivity (measured with antisera R59). The assays were capable of detecting gastrin (2 pmo/l), CCK (0.2 pmo/l), enteroglucagon (5 pmo/l), pancreatic glucagon (2 pmo/l), GLP-1 (2 pmo/l), insulin (10 pmo/l), and PYY 2.5 (pmo/l) with 95% confidence.

Statistical Analysis

All results are presented as the group means ± SE. Data were tested as appropriate by two-tailed t-test or by ANOVA.

RESULTS

Effect of Lectins on Total Body Weight

The orally fed group of rats gained about 5% (P < 0.05) of their initial body weight. TPN animals lost on average 5% of their initial body weight (P < 0.05), and there was no significant difference between the lectin-treated groups and TPN control.

Effects of Lectins on Wet Weight of Gastrointestinal Tract

Stomach. Rats that had an intragastric cannula had heavier stomachs compared with orally fed animals (Fig. 1). This finding is likely to be due to adhesions and edema caused by the surgical procedure. No specific effects of lectins were observed on light microscopy.

Small intestine. The wet weights of the small intestines as percentage of total body weight of rats fed PHA (P < 0.001) and WGA (P < 0.05) were greater than those of TPN control animals. ConA and PNA had no significant effect.

Cecum. The wet weight of the cecum in animals fed ConA (P < 0.05), PHA (P < 0.05), and WGA (P < 0.01) was heavier than that of control animals. PNA had no significant effect.

Large intestine. The wet weight of the large intestine in animals fed WGA (P < 0.001) and animals fed PNA (P < 0.001) was heavier than that of control animals. ConA and PHA had no significant effect.

Pancreas. The wet weight of the pancreas was greater in the orally fed than in TPN controls. The addition of PHA (P < 0.001), WGA (P < 0.0001), or PNA (P < 0.0001) to TPN significantly elevated the wet weight.

Comparisons with orally fed animals. The small intestine, cecum, colon, and pancreas of orally fed animals were significantly heavier than those of TPN controls. In the small intestine PHA elevated the weight to close to that of orally fed animals. In the cecum none of the lectins had this effect but in the colon WGA and PNA produced mean weights greater than those of orally fed animals. In the pancreas PHA, WGA, and PNA produced weight close to or above those of orally fed animals.

Effects of Lectins on Proliferation of Stomach

PHA increased proliferation of the gastric fundus (P < 0.05) but not the antrum (Fig. 2). None of the other lectins tested had any effect on either region.

Effects of Lectins on Proliferation of Small Intestine

Proximal small intestine. ConA (P < 0.005), PHA (P < 0.0005), WGA (P < 0.0005), and PNA (P < 0.05) all significantly increased proliferation of the proximal...
small intestine when compared with TPN control rats (Fig. 3).

Middle small intestine. ConA ($P < 0.0005$), PHA ($P < 0.0005$), WGA ($P < 0.0005$) all significantly stimulated proliferation of the middle small intestine above that of TPN control animals. PNA had no significant effect.

Distal small intestine. PHA ($P < 0.0005$) and WGA ($P < 0.05$) significantly stimulated proliferation of the distal small intestine. ConA and PNA had no significant effect.

Comparison with orally fed animals. Proliferation rates in all parts were higher in orally fed than in TPN controls. PHA produced proliferation rates close to those of orally fed animals in the proximal and mid small intestine but not in the distal small gut.

Effects of Lectins on Proliferation of Colon

Proximal colon. PHA ($P < 0.05$), WGA ($P < 0.0005$), and PNA ($P < 0.0005$) all significantly stimulated proliferation of the proximal large intestine compared with TPN control animals. ConA had no significant effect.

Middle colon. PHA and PNA significantly increased proliferation of the middle large intestine compared with TPN control animals ($P < 0.05$ and $P < 0.0005$, respectively). ConA and WGA had no effect on this region.

Distal colon. There was no significant difference in proliferation between TPN control animals and orally fed animals. PHA and PNA appeared to increase prolif-
eration but this did not reach significance. ConA significantly inhibited proliferation in the distal large intestine (P < 0.05).

Comparison with orally fed animals. In the proximal and mid colons proliferation was significantly greater in the orally fed than in the TPN control animals. PNA produced proliferation rates close to those in orally fed animals in both regions, and PHA had this effect in the mid colon.

Effects of Lectins on Gastrointestinal Hormone Release

Gastrin. Plasma gastrin concentrations were significantly lower in the TPN- than in the orally fed rats (Fig. 4). ConA and PHA had no effect, and WGA produced an insignificant rise in plasma gastrin concentrations. PNA approximately doubled plasma gastrin levels to values similar to those seen in orally fed animals (P < 0.05).

CCK. There was no significant difference in CCK levels between TPN- and orally fed rats. All the lectins studied tended to increase plasma CCK concentrations, and this was significant for PNA (P < 0.05), ConA (P < 0.05), and PHA (P < 0.01).

Enteroglucagon. Plasma levels of enteroglucagon were very much reduced by TPN (P < 0.05). ConA and PHA had little effect, but PHA and WGA increased plasma enteroglucagon levels by about 80 and 60%, respectively (both P < 0.05).

GLP-1. GLP-1 levels were also much reduced in TPN-compared with the orally fed rats (P < 0.05). WGA significantly elevated GLP-1 (P < 0.05), whereas the other lectins did not.

Insulin. Rats on TPN had significantly higher levels of insulin compared with orally fed animals (P < 0.001). Feeding lectins to TPN animals had no further effect on insulin levels.

Peptide tyrosine-tyrosine (PYY). Although PYY levels were lower in the TPN controls than the orally fed rats, this was not significant. None of the lectins had any significant effect on the release of PYY.

Comparison with orally fed animals. Plasma concentrations of gastrin, enteroglucagon, and GLP-1 were significantly greater (all P < 0.05 in orally fed than in TPN animals). At least one lectin elevated the plasma concentration of each of these hormones. However, only gastrin was restored to fed levels by PNA.

Histology

No abnormalities in epithelial morphology were seen in hematoxylin and eosin-stained sections from any region of the gut after administration of any of the lectins. Apoptotic cells were no more numerous in control rats receiving TPN alone compared with animals receiving lectins when visualized by light microscopy.

DISCUSSION

The present study aimed to determine whether lectins can prevent the gastrointestinal atrophy produced by TPN and if so whether the lectins released gut hormones that might cause these changes. Our results confirm that TPN inhibits gastrointestinal proliferation. Tissue weight was greater in orally fed than in TPN rats in all regions of the gut. Similarly, epithelial proliferation measured by metaphase arrest was greater in orally fed than in TPN-fed animals in all regions, except for the distal colon.

All of the lectins tested fully or partially reversed the atrophy produced by TPN, but their effects were restricted to specific regions of the gut. PHA was most effective in preventing atrophy in the gastric fundus and in the proximal and mid small intestine, but PNA most effectively restored cell proliferation in the colon. We previously reported that PHA stimulates proliferation in the small intestine of rats (37), and Banwell et al. (4) found, as in the present study, this effect is greater in the proximal than in the distal small gut. We are unaware of any previous report that PHA, or any other lectin, stimulates proliferation of the gastric fundus.

The proliferative effect of ingested PNA was first reported in the small intestine of rats by Henney et al. (24). In the present study of TPN-fed rats, the proliferative response of the small intestine was weak, restricted to the proximal part, and less than the response of the colon. Ryder et al. (39, 42) have studied the proliferative response of human colonic mucosa to PNA in some detail. It is currently unclear why differ-
ent regions of the gastrointestinal tract respond differently to individual lectins. This might be because expression of the relevant carbohydrate group differs between cell types. Alternatively, different cell types might respond differently to lectin binding. This is plausible because various colon cancer cell lines respond differently to the same lectin (31, 41). Finally, regional responses might reflect the different effects of regulatory peptides on specific epithelial cells in the gastrointestinal tract. For example, transgenic mice show proliferation of gastric or colonic mucosa, depending on whether the peptide produced is gastrin or progastrin (see below) (46).

TPN significantly diminished plasma concentrations of gastrin, enteroglucagon, and GLP-1. PHA, which mainly stimulated proliferation of the small intestine, significantly elevated plasma concentrations of CCK and enteroglucagon. There is no evidence that CCK has a direct trophic effect on the intestine, but an indirect effect was possible because CCK increases pancreaticobiliary secretions that may have a trophic effect on rat intestine (2). TPN causes pancreatic atrophy with reduced pancreaticobiliary secretions (17, 26). PHA caused pancreatic growth in the present study of TPN-fed rats. In a previous study of normally fed rats, PHA and soybean lectin both caused pancreatic growth and small intestinal proliferation (25). The effect on the pancreas was removed by CCK-A receptor blockade but the effect on the intestine was not, so we conclude that the proliferative effect of PHA on the small intestine is not mediated by CCK. PHA produced a small but significant elevation in plasma enteroglucagon. A patient with an enteroglucagon-secreting tumor had massive intestinal hypertrophy. Subsequent work suggested that enteroglucagon itself does not stimulate intestinal growth (11, 22, 28, 45) but that another proglucagon-derived peptide (GLP-2) does so strongly (14). We have shown that GLP-2 can reverse intestinal atrophy in parenterally fed rats (20) but were unable to measure GLP-2 in the present study for technical reasons.

**Fig. 3.** Effects of intragastric administration of lectins on proliferation of regions of small intestine and colon measured by metaphase arrest in rats receiving TPN. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. TPN alone. Data are means ± SE; n = 6 animals per group.
reasons. However, enteroglucagon levels did not rise to near those seen in orally fed animals, and GLP-1 levels did not rise significantly. Also Banwell et al. (4) found no significant change in enteroglucagon levels in rats fed a normal diet plus PHA. Therefore, it seems unlikely that proglucagon-derived peptides are involved in the proliferative response to PHA. WGA stimulated proliferation of the small intestine and elevated enteroglucagon and GLP-1 levels, but again the circulating concentrations of these peptides did not approach those seen in orally fed animals.

PNA, which mainly stimulated proliferation in the colon, significantly elevated plasma CCK and gastrin concentrations. There is no evidence that CCK peptides have a trophic effect on the colon. Gastrin peptides do have trophic effects on the colon, but this appears to be chiefly an effect of glycine-extended gastrin. Transgenic mice, which express gastrin in their pancreatic islets, produce fully processed gastrin and have increased proliferation in the stomach but not in the colon. Conversely mice expressing gastrin in their liver produce progastrin and develop proliferation of colonic but not gastric mucosa (46). Based on this, and since the gastrin released by PNA was presumably of gastric origin and therefore processed, it seems unlikely that gastrin peptides caused the proliferative effect of PNA on the colon. Hormonal responses might have been underestimated because blood was collected 1 h after the last dose of lectin, whereas peak plasma hormone concentration often occurs earlier. However, only a prolonged response would be likely to affect gastrointestinal proliferation.

Intraluminal lectins might act via direct effects on the epithelial cell of the gastrointestinal tract rather than via hormones. PHA binds to the brush border of enterocytes after intragastric administration to rats (37), and PNA binds to colonocytes after oral administration to humans (39). Furthermore, lectins directly stimulate proliferation of epithelial cells of the gastrointestinal tract in vitro. PHA stimulated proliferation of the enterocyte-like colonic cancer cell line Caco-2 (32). PNA stimulated proliferation of explants of normal human colonic mucosa and HT-29 colonic cancer cells (42). Low concentrations of WGA and ConA stimulate, but high concentrations of these lectins inhibit, proliferation of colonic cancer cell lines (31, 41). WGA and ConA stimulated proliferation in the small intestine of TPN-fed rats. WGA also stimulated proliferation of the

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**Fig. 4. Effects of intragastric administration of lectins on plasma concentrations of regulatory peptides in rats receiving TPN.**

- **Gastrin**: *P < 0.05 and **P < 0.01 vs. TPN alone.* Data are means ± SE; n = 6 animals/group.
proximal colon, but ConA inhibited proliferation of the distal colon in the present study. In addition, the proliferative effects of lectins on gastrointestinal cells are associated with increased uptake and synthesis of polyamines (6, 32).

The present results raise the possibility that lectins could be given to patients receiving TPN to prevent mucosal atrophy and its associated complications. Administration by nasogastric tube would reduce loss through binding to oral and esophageal epithelium but may be unnecessary for all lectins because some such as ConA and WGA remain active as they pass through the gastrointestinal tract and are excreted in feces (35, 40). That lectins can be given intraluminally may be an advantage over growth factors that need to be given intravenously (21). Our results indicate that it may be necessary to give a combination of lectins such as PHA plus PNA to protect the small and large intestines, respectively. The hormonal effects of lectins may be clinically important. For example, lectin-stimulated CCK release may prevent accumulation of sludge in the gallbladder during TPN (30), but release of this hormone might theoretically exacerbate pancreatitis (15). One potential advantage of preventing atrophy during TPN is to prevent sepsis due to transmigration of bacteria across eneefled epithelia (13). This may explain why early refeeding diminishes sepsis in acute pancreatitis (34, 43). In this context it is relevant that PHA promotes bacterial overgrowth in the small intestine (5, 38). PHA and PNA have already been administered to humans (3, 27, 39). Therefore, our future studies aim to further define the proliferative and adverse effects of different lectins in the gastrointestinal atrophy produced by TPN as a prelude to clinical trials.

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