Effects of a panel of dietary lectins on cholecystokinin release in rats

MARK JORDINSON, RAYMOND J. PLAYFORD, AND JOHN CALAM
Gastroenterology Laboratory, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 ONN, United Kingdom

The intestinal hormone cholecystokinin (CCK) is an important regulator of upper gastrointestinal functions, including gallbladder contraction, pancreatic secretion and growth, gastric emptying, and satiety (20).

Raw soybean flour releases CCK in many species, including humans (5), and diets of raw soybean flour stimulate pancreatic growth in rats (6, 21). This was initially attributed to soybean trypsin inhibitors (SBTI) (9, 21), but Grant et al. (9) showed that ingestion of soybean lectin (SBL) reproduces this effect (9). We (14) showed that depletion of lectin from raw soy flour greatly diminishes its CCK-releasing effect. Pure SBL released CCK, thus stimulating pancreatic enzyme secretion in anesthetized rats, and SBTI acted synergistically with the lectin (14).

A normal diet contains many lectins (24). They are generally heat labile, but considerable amounts remain after cooking (31). Once ingested, lectin activity largely persists during passage through the gastrointestinal tract (4, 16, 31). Therefore, we have now asked whether other lectins release CCK.

Lectins were chosen to represent the groups described by Goldstein and Poretz (8). This is based on the finding that lectins that bind to motifs including N-acetyl-d-galactosamine tend to stimulate intestinal cells (18, 19, 31). These include soybean (Glycine max) lectin (SBL), which binds to N-acetyl-d-galactosamine and d-galactose, and peanut (Arachis hypogaea) lectin (PNL), which binds to d-galactose-b-1,3-N-acetyl-d-galactosamine. The latter stimulates proliferation of colonic cell lines as well as normal and diseased colonic epithelia (29, 31).

In contrast, lectins that bind to mannose or glucose tend to have no effects or even inhibit processes such as proliferation (13, 19). Broad bean (Vicia faba) lectin (BBL) is a member of this group. Wheat germ (Triticum vulgaris) lectin (WGL) binds to N-acetyl-d-glucosamine and causes calcium-dependent stimulation of enterocytes (33). This is interesting because elevations of intracellular calcium can release CCK (20, 23).

For in vivo studies we used our anesthetized rat model (14) with bile-pancreatic juice returned to the duodenum. The addition of protein cooked soybean flour (CSF) and SBTI increases the sensitivity of this model to lectins. In view of this, we also examined the stability of lectin activity in bile-pancreatic juice. The role of calcium was examined in an in vitro system based on preparations of perfused intestinal cells (3).

MATERIALS AND METHODS

Chemicals, including lectins, were purchased from Sigma (Pole, Dorset, UK) unless otherwise stated.

Effects of Lectins on Pancreatic Protein Output and Plasma CCK Concentrations in Anesthetized Rats

Preparation of animals. The rat model and experimental procedure were as described previously (14). Briefly, male Sprague-Dawley rats (200–300 g) were lightly anesthetized with halothane, and cannulas were placed in the duodenum, bile-pancreatic duct, and jugular vein. The temperature of the rats was maintained at 37°C throughout the experiment.

Experimental procedure. Intraduodenal test infusions were given at least 90 min after surgery to allow pancreatic output to reach a steady state. The amounts of lectins used were chosen on the basis of our previous finding that either 30 mg of raw soybean flour or the 84 µg of lectin that it contains significantly increase CCK release and pancreatic protein output in the rat model (14). We therefore examined the effects of 84 µg of each lectin. If no response was seen, a dose of 840 µg was then used. The lectin was added to 44 mg of CSF and 0.16 mg SBTI at 37°C and infused into the duodenum in a total volume of 0.6 ml over 15 min. Bile-pancreatic juice was collected continuously in 15-min aliquots into tared 1.5-ml Microfuge tubes, and the volume was determined by weight. We retained 20 µl for protein assay. The remainder was kept at 37°C and slowly returned to the duodenum. Test substances CSF, SBTI, and lectin were mixed with bile-pancreatic juice immediately before reinfusion. Blood samples...
(0.5 ml) were taken from the jugular vein for CCK radioimmunoassay immediately before and every 15 min after the test substance was infused.

Assay of protein and CCK. Protein was measured in bile-pancreatic juice using dye-concentrate solution (Bio-Rad, Hertfordshire, UK) with bovine serum albumin as the standard. Peak pancreatic protein output occurred 30–45 min after the infusion of test substances. One-hour protein responses were calculated as the total protein output during the hour after infusion minus twice that during the 30 min preceding the infusion.

CCK was measured by a specific radioimmunoassay using antibody Dino7, as described previously (14). The concentrations of pure peptides that produced half-maximal inhibition of tracer binding to antiserum Dino7 in this assay are 1.7 pmol/l for CCK-8, 3.2 pmol/l for porcine CCK-33 (Peninsula Laboratories, St. Helen’s, UK), 4.8 nmol/l for sulfated gastrin-17, and 5.7 nmol/l for nonsulfated gastrin-17. The detection limit of the assay, defined as the smallest concentration of CCK-8 per assay tube that could be differentiated from the absence of CCK with 95% confidence, was 0.2 pmol/l. The intra- and interassay variabilities of the assay were 6.2% and 12.1%, respectively.

Effects of Lectins on CCK Release from Perifused Intestinal Mucosal Cells

Perifused intestinal cell preparation. This method was as described by Bouras et al. (3). Briefly, intestinal cells are prepared by incubating everted proximal small intestine of male Sprague-Dawley rats in calcium-free medium with EDTA. Cell viability as assessed by trypan blue exclusion was >95%. The cells were then mixed with Sephadex G50 beads (Pharmacia, Uppsala, Sweden) and supported on a 5-µm nylon filter in columns made from disposable 3-ml syringes (Pharmacia, Uppsala, Sweden) and supported on a 5-µm nylon filter in columns made from disposable 3-ml syringes (105–106 cells/column). Cells were equilibrated for 1 h with N2-hydroxyethylpiperazine-N7-2-ethanesulfonic acid (HEPES) buffer, pH 7.4, at 37°C in a water bath and oxygenated with 100% oxygen. After 1-h equilibration, basal CCK output was measured. Cells were then perfused with 5 ml buffer containing 1, 10, or 100 µg/ml of SBL, PNL, WGL, and BBL. Four 5-min collections were then made. The first was discarded, because the dead space was ~5 ml. The release of CCK (fmol/fraction) was represented as means ± SE of CCK in three 5-min collections from n = 6 experiments determined by radioimmunoassay. CCK peptides were extracted from perifusates using C18 Sep-Pak cartridges (Waters Millipore), as described by Bouras et al. (3). Sep-Pak eluates were lyophilized directly in the radioimmunoassay tubes before CCK assay as described above.

Role of calcium in lectin-mediated CCK secretion. To establish whether cellular responses were dependent on extracellular calcium, we exposed the cells to lectins in a calcium-free medium containing 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N7,N7-tetraacetic acid (EGTA) substituted for CaCl2.

Responses to repeated lectin administration. This was studied to address whether CCK-releasing cells were damaged by exposure to lectin. After initial exposure to the lectins, cells were perfused with HEPES buffer containing the complementary sugar of the lectins (N-acetyl-d-galactosamine for SBL, d-galactose for PNL, N,N7,N7′-triacetylchitotriose for WGL, and mannose for BBL, all at 10 mM) to elute the lectin. The cells were then perfused with HEPES buffer for 15 min and rechallenged with the same lectin. Stability of SBL, PNL, WGL, and BBL in Activated Pancreatic Juice

The method was based on that used by Borgstrom et al. (2). Rat bile-pancreatic juice was collected on ice. Samples (1 ml) were mixed with 600 µl of a tris(hydroxymethyl)aminomethane (Tris)-maleate buffer (50 mmol/l, pH 5.6) containing 10 mmol/l CaCl2 and 84 µg lectin at room temperature. The mixture was then activated by adding 400 µg of enterokinase in 400 µl of the above-mentioned buffer, to give a final pH of 7.4. The mixture was incubated for 0, 30, and 60 min at 37°C, then stopped by adding an excess of SBTI. Remaining lectin activity was measured by agglutination of red blood cells at serial dilutions against lectin standards (15). Group O erythrocytes pretreated with papain were used to detect SBL and WGL. Group O erythrocytes treated with neuraminidase were used to detect PNL and BBL.

Effect of Lectins on Trypsin Activity

Trypsin activity was measured in the presence and absence of lectins based on hydrolysis of Nα-benzoyl-d-arginine p-nitroanilide (BAPNA), as described by Erlanger et al. (7). Bovine trypsin (5 mg) was dissolved in 100 ml of 0.001 M HCl, and a standard curve (10–100 µg) was prepared. Control tubes contained trypsin (100 µg) and water, and sample tubes contained trypsin (100 µg) containing either SBL, PNL, WGL, or BBL (84 µg). All tubes were made up to a total volume of 2 ml with distilled water in triplicate and preincubated for 10 min at 37°C. BAPNA (60 µg) was dissolved in 2 ml of dimethyl sulfoxide and diluted with 200 ml of Tris assay buffer, pH 8.2, containing 0.02 M CaCl2 previously warmed to 37°C. BAPNA (3.5 ml) was added to every tube and kept at 37°C for 10 min. The reaction was stopped by adding 0.5 ml acetic acid (30%, vol/vol) to every tube. Tubes were vortexed and read at 410 nM (ultraviolet visible spectrophotometer, PU 8720, Philips, Cambridge, UK). Trypsin inhibitor activity was then determined from the standard curve.

Data Analysis

Results were compared by Student’s t-test. P < 0.05 was considered significant.

RESULTS

Effects of Lectins on Pancreatic Protein Output and CCK Release in Anesthetized Rats

Effects of lectins on pancreatic protein output. The addition of SBTI to CSF produced a modest rise in the 1-h integrated protein response of 0.7 ± 0.2 mg/h (Fig. 1). This was therefore used as the negative control in statistical analysis. The addition of 84 µg SBL to vehicle (CSF plus SBTI) stimulated a 1-h integrated protein response of 2.5 ± 0.7 mg/h (P < 0.05 vs. vehicle). PNL (84 µg) with vehicle stimulated a 1-h integrated protein response of 1.9 ± 0.6 mg/h (P < 0.05 vs. vehicle). WGL (84 µg) had no significant effect, but increasing the dose to 840 µg stimulated a 1-h integrated protein response of 3.3 ± 1.2 mg/h (P < 0.05 vs. vehicle). In contrast, BBL had no significant effect at 84 or 840 µg/ml.

The effect of the lectins was almost entirely due to a rise in pancreatic protein concentration, and there was no significant effect on volume output. For example, the addition of SBL (84 µg) to vehicle elevated protein output from 11 ± 3 to 18 ± 3 µg/ml (P < 0.01), but...
volume only rose slightly from 1.1 ± 0.1 to 1.2 ± 0.1 ml/h (P = 0.15). Similar results were obtained with PNL and WGL.

Effect of lectins on CCK release in anesthetized rats. Plasma CCK concentrations were significantly elevated 3.1 ± 0.6 pmol/l (P < 0.05 vs. basal) by CSF plus SBTI (vehicle) in a volume of 600 µl (Fig. 2). SBL (84 µg) plus vehicle significantly stimulated CCK release to 8.4 ± 0.6 pmol/l (P < 0.05 vs. vehicle). PNL (84 µg) plus vehicle significant increased plasma CCK concentrations to 7.0 ± 0.6 pmol/l (P < 0.05 vs. vehicle). WGL (840 µg) significantly increased CCK release to 9.7 ± 1.3 pmol/l (P < 0.01 vs. basal). None of the lectins produced a significant response when used at the lower concentration of 1 µg/ml, but all had a significant effect at 10 and 100 µg/ml; SBL (1, 10, and 100 µg/ml) stimulated a CCK output of 2.0 ± 0.7, 7.9 ± 0.4, and 11.2 ± 1.1 fmol, respectively (10 and 100 µg/ml both P < 0.05 vs. basal). PNL (1, 10, and 100 µg/ml) stimulated a CCK output of 2.2 ± 0.4, 5.7 ± 0.6, and 8.4 ± 1.1 fmol, respectively (10 and 100 µg/ml both P < 0.05 vs. basal), and WGL (1, 10, and 100 µg/ml) produced a CCK output of 2.3 ± 0.6, 5.1 ± 0.4, and 8.5 ± 0.9 fmol, respectively (10 and 100 µg/ml both P < 0.05 vs. basal). BBL (1, 10, and 100 µg/ml) had no significant effect on CCK output, which was 1.4 ± 0.5, 1.4 ± 0.6, and 2.0 ± 0.7 fmol, respectively.

Basal CCK release was not significantly altered by removing calcium from the medium, but responses to KCl (50 mM), SBL, PNL, and WGL (100 µg/ml) were significantly reduced when calcium was removed. In a calcium-free medium, CCK release in response to KCl, SBL, PNL, and WGL was 1.1 ± 0.5, 1.1 ± 0.5, 1.2 ± 0.5, and 1.0 ± 0.2 fmol, respectively.

Response to perifused mucosal cells after repeated exposure to lectins. After repeated exposure to the lectins (100 µg), the cells responded with similar values as reported on initial exposure; values for basal, KCl, SBL, PNL, WGL, and BBL were 1.3 ± 0.5, 7.5 ± 0.5, 7.1 ± 0.4, 4.8 ± 0.3, 5.0 ± 0.5, and 1.1 ± 0.6 fmol, respectively. Perifusion of the cells with HEPES containing the corresponding sugars of the lectins restored basal output to normal.

Stability of SBL, PNL, WGL, and BBL in Activated Pancreatic Juice

Incubation of SBL and PNL (84 µg) in activated pancreatic juice diminished their hemagglutinating activity by 5% at 30 min and 15% at 60 min. Incubation of WGL (84 µg) diminished its hemagglutinating activity by 20% at 30 min and 35% at 60 min, whereas the hemagglutinating activity of BBL (84 µg) was diminished by 30% at 30 min and 35% at 60 min.

Effect of Lectins on Trypsin Activity

Incubation of 100 µg trypsin with SBL, PNL, WGL, or BBL (84 µg) had no significant effect on its enzyme activity. Trypsin activity in the presence of the lectins was 97%, 100%, 98%, and 100% of control, respectively.

DISCUSSION

We have already shown that SBL is a potent releaser of CCK and thus stimulates pancreatic output in anesthetized rats (14). The present work confirms this
and shows that some, but not all, lectins have the same effect. CCK release and pancreatic protein output were stimulated by SBL and PNL, which bind motifs including D-galactose and N-acetyl-D-galactosamine, and by WGL, which binds to a motif including N-acetyl-D-glucosamine. BBL, which binds to mannose and glucose, did not release CCK and had no effect on pancreatic protein output. Studies using an isolated perfused cell preparation showed that the CCK response to SBL, PNL, and WGL was clearly dose dependent. In addition, their effects were dependent on the presence of extracellular calcium. The response to the lectins was blocked in the presence of their competing sugars, which indicates that the lectins had to bind to their specific carbohydrate motifs to have an effect.

The CCK-releasing effect of raw soy flour was initially attributed to the trypsin inhibitors that it contains (9, 21). The laboratory of Pusztai (9) produced the first evidence that SBL might be involved. Dietary supplementation with this lectin led to growth and an increase in the polyamine content of the pancreas in rats (9). Pusztai et al. (27) also recently showed that the red kidney bean lectin phytohemagglutinin, which binds to complex motifs including N-acetyl-D-galactosamine, did not release CCK and had no effect on pancreatic juice. SBL and PNL, which bind motifs including N-acetyl-D-galactosamine, also releases CCK.

How lectins release CCK is not clearly understood. Cell damage does not appear to be involved, because CCK release in vitro was not diminished on repeat exposure to lectin. The present study shows that lectin-stimulated CCK-release is dependent on the presence of extracellular calcium.

Liddle et al. (3, 23) have shown that an influx of calcium is involved when a variety of stimuli release CCK from native CCK cells and the CCK-releasing cell line STC-1. Lectins might release CCK by activating mechanisms involved in CCK release such as potassium (35) or calcium channels or receptors for any of the various factors that release CCK (12, 20, 35, 36). CCK-releasing peptides are trypsin sensitive, but the lectins had no effects on the activity of this enzyme. The known effects of lectins on intestinal cells provide further clues. These include elevations in intracellular calcium. A rise in intracellular calcium stimulated by WGL in intestinal 407 cells was largely due to an influx of extracellular calcium (32).

After ingestion, lectins bind to the luminal surface of intestinal cells, especially in the upper small intestine (17), where CCK cells are located (20). Ultrastructural changes occur, particularly affecting the microvilli (11, 17, 22, 34). Responses include increases in the synthesis and uptake of polyamines and elevations of markers of proliferation, such as thymidine uptake, DNA content, and mitotic activity (1). Similar changes in CCK cells might lead to release of CCK peptides.

Responses of intestinal cells are related to the affinity of lectins for the cell membrane (19). Also, the stimulatory effect of lectins on intestinal cells is generally greatest if they bind motifs that include N-acetyl-D-galactosamine and least if they bind to mannose or glucose (8). The present results suggest a similar relationship between lectin specificity and CCK-releasing effect.

The relative CCK-releasing effects of the lectins were also related to their resistance to degradation by bile-pancreatic juice. SBL and PNL, which bind N-acetyl-D-galactosamine, survived best, and BBL, which binds to mannose, was lost most rapidly in activated bile-pancreatic juice. This does not appear to be a general rule however. Pusztai et al. (26) also found that SBL survived better than BBL in the intestine of rats but that snowdrop (Galanthus nivalis) lectin, which binds to mannose, survived even better than SBL. Differences in the degradation of lectins in the intestinal lumen probably did not contribute to the differences in their CCK-releasing effects in the present study, because they were administered with SBTI. Furthermore, their respective potencies were similar in vitro in the absence of digestive enzymes.

Studies in laboratory animals show that ingested lectins have a wide range of effects that might be relevant to human diseases. These include changes in the differentiation (13) as well as the proliferation of...
intestinal flora (28), and bacterial lectins can activate growth of implanted tumor cells can also be affected by intestinal (see above) and colonic cells (29, 30). The G950 LECTINS AND CHOLECYSTOKININ RELEASE


