Stimulation of Gallbladder Emptying and Pancreatic Secretion in Chicks by Soybean Whey Protein[1] (36443)

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(Introduced by M. L. Scott)

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Many investigators have studied the effects of trypsin and chymotrypsin inhibitors on the size, enzyme content and enzyme secretion of the pancreas of rats and chicks. In general, these investigations showed that the pancreases of animals fed diets containing sources of proteolytic inhibitors had greater quantities of enzymes after a fast than comparable fasted animals fed control diets (1–5). When pancreatic enzyme levels were measured after animals were fed, those receiving sources of proteolytic inhibitors generally had lower levels of all pancreatic enzymes than animals not receiving the inhibitor (2–4, 6–8). Thus dietary proteolytic inhibitors appear to cause a general stimulation of the pancreas, resulting in both an increased synthesis and a greater secretion of pancreatic enzymes.

Trypsin inhibitors from the soybean (6, 9), the navy bean (10), egg white (5, 9), a synthetic inhibitor, *p*-aminobenzamidine (11), tannins from carobs (12) or peanut skins (13) and a chymotrypsin inhibitor from potatoes (14) all have been shown to stimulate the pancreas. These are chemically very diverse and have in common only the ability to inhibit proteolysis by interfering with one or more of the pancreatic enzymes.

The physiological mechanism whereby these inhibitors stimulate the pancreas is not well understood. Since inhibitors from widely varying sources are able to cause the effects, it appears that the inhibition of proteolysis itself may be the signal to the pancreas rather than some property of the inhibitor molecule *per se*. Snook (5) has proposed that the gastrointestinal hormone, pancreozymin, mediates the response to trypsin inhibitor. Khayambashi and Lyman (15) showed that a factor present in plasma of rats receiving a diet containing crude trypsin inhibitor stimulated pancreatic enzyme secretion. Rothman and Wells (16) showed that repeated administration of pancreozymin caused pancreatic enlargement and increased enzyme synthesis rats.

The gallbladder of chicks seems to respond to proteolytic inhibitors in a manner analogous to the pancreas. Serafin and Nesheim (17) reported that the gallbladders of chicks fed unheated soybean meal contained considerably less bile than those of chicks fed heated soybean meal.

Pancreozymin which stimulates pancreatic enzyme secretion, and cholecystokinin which stimulates gallbaldder emptying, have been shown to be the same hormone (18, 19). Thus if the effects of trypsin inhibitors on pancreatic and gallbaldder secretion are mediated through pancreozymin-cholecystokinin, the parallel effects observed with the gallbladder and pancreas would be expected.

This paper describes experiments which show that soybean whey proteins and purified trypsin inhibitor accentuate the gallbladder emptying and pancreatic amylase depletion caused by feeding.

Experimental procedures. Rhode Island Red × Barred Plymouth Rock male chicks

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used in all experiments were maintained in battery brooders with raised wire floors. Except for those used in Expt. F, Table II, and Expt. G, Table III, chicks were fed a commercial chick starting diet[4] prior to the experiments, and the same diet was used to stimulate gallbladder emptying and pancreatic secretion. The pretreatment diet in the experiment indicated was a semipurified diet containing 15% added fat described by Serafin and Nesheim (17).

Soybean whey was prepared from defatted soybeans by methods described by Garlich and Nesheim (9). Trypsin inhibitor activity in the soybean whey was determined by measuring the inhibition of hydrolysis of *n*-benzoyl-*dl*-arginine *p*-nitroanilide (BAPA) by trypsin according to procedures described previously (20). The purified trypsin inhibitor was 2× crystalline soybean trypsin inhibitor,[5] purchased from a commercial source. It was assayed for activity by the above method prior to use. The two batches of soybean whey prepared for use in these experiments had 320 and 400 units of trypsin inhibitor activity/mg. The heated whey preparations had 2 and 13 units of activity/mg, respectively.

For determination of amylase activity, the pancreas was rapidly removed, weighed and homogenized with 0.02 *M* phosphate buffer (pH 6.9) containing 0.005 *M* $CaCl_2$ and 0.38 *M* NaCl. Amylase was estimated by a modification of the procedure of Bernfeld (21). Incubation was carried out for 5 min at 30°. The amount of amylase catalyzing the appearance of reducing groups equivalent to 1 mg maltose during an incubation period of 1 min at 30° was defined as 1 unit of amylase activity. Amylase units were expressed as units per gram pancreas.

A basic experimental procedure was followed in all experiments. Chicks were fasted overnight to allow gallbladders to fill. The emptying of the gallbladder was then measured by weighing gallbladders 1 hr after treatments were given the chicks. Preliminary experiments had determined that maximal effects were observed on gallbladder weight within this period. In some experiments the pancreases were also removed at this time for enzyme determinations. Differences in gallbladder weights among treatments were primarily due to the amount of bile present since the weight of empty gallbladders makes up a very small part of the total gallbladder weight. Gallbladders from 10 chicks averaging 192 g in body weight averaged 18.5 mg with a range of 14.3 to 23.2 mg after bile was removed. The data obtained were subjected to analysis of variance and differences between treatments were determined by Duncan's multiple range test (22).

Results. The influence of feed and soybean whey proteins on gallbladder emptying are shown in Table I. When water or a water solution containing soybean whey were administered to chicks, no significant effects on gallbladder size were observed (Expts. A and B). However, when 1 g of feed was given, gallbladder weight decreased significantly compared to chicks receiving nothing or water alone. When soybean whey was given to chicks that received food, a further significant reduction in gallbladder weight occurred. Heat-treated whey was not as effective as the undenatured whey (Expt. B). These experiments showed that administration of whey proteins by themselves did not cause gallbladders of the chicks to empty, but when feed was administered to stimulate emptying of the gallbladder, the undenatured whey proteins accentuated the response to feeding.

If the effect of the soybean whey was mediated through pancreozymin-cholecystokinin, the pancreas should respond to the whey proteins in a fashion similar to that of the gallbladder. The results of 3 experiments presented in Table II show the effect of whey proteins on gallbladder contraction and amylase content of the pancreas. The amylase was used as an indicator of pancreatic enzyme release since the release of all enzymes in the pancreas seems to be affected by trypsin inhibitors in a parallel fashion (7). The data show that the pancreatic amylase activity responded to feeding in the same manner as the gallbladder.

[4] Agway, Inc., Syracuse, NY.

[5] Nutritional Biochemicals, Cleveland OH.

TABLE I. The Effect of Feed or Soybean Whey Proteins on Gallbladder Emptying in Fasted Chicks.

Substance orally administered	Wt	
	Body (g)	Gallbladder[a] (mg)
Expt. A		
H_2O, 1 ml	204	280 ± 19 c
Soybean whey, 50 mg	200	270 ± 15 cb
Feed pellets, 1 g	206	229 ± 13 b
Feed pellets, 1 g, + 50 mg soybean whey	197	176 ± 14 a
Expt. B		
H_2O, 0.5 ml	187	209 ± 10 ab
Soybean whey, 25 mg	185	250 ± 17 a
Feed pellets, 1 g, + 25 mg heated soybean whey	188	169 ± 14 b
25 mg soybean whey	185	116 ± 7 c
Expt. C		
None	194	283 ± 27 a
Feed pellets, 1 g	187	156 ± 16 bc
Feed pellets, 1 g, + 50 mg soybean whey	196	119 ± 9 c

[a] Measured 1 hr after oral administration of test substance. In Expt. A each value represents mean of 12 chicks; Expt. B, of 8 chicks; Expt. C, of 9 or 10 chicks. Standard error of mean is also given. Means not followed by same letter are significantly different ($p < .05$) by Duncan's multiple range test.

Although in Expts. D and E the difference in amylase activity in the pancreas of chicks receiving the soybean whey was not significant from the activity of chicks receiving feed alone, the pancreatic amylase levels in chicks receiving whey were numerically lower in both experiments than in chicks receiving feed alone. In Expt. F the effect of the soybean whey administration on the pancreatic amylase activity was significant. Taken as a whole, the data in these experiments are consistent with the hypothesis that the gallbladder and pancreas are responding in a similar manner.

A purified trypsin inhibitor was studied along with the whey in Expts. E and F. In Expt. E, the response of the gallbladder to the trypsin inhibitor was not as marked as the response to the soybean whey. In Expt. F, the soybean whey and the purified inhibitor were equally effective in significantly lowering gallbladder weight and pancreatic amylase compared to feed alone. Chicks in Expt. F received a diet containing 15% fat from hatching and had much fuller gallbladders than chicks in Expts. D and E that received the commercial chick starting diet. We have consistently observed large amounts of bile in gallbladders in fasted chicks previously fed a diet high in fat.

The effect of a commercial preparation of pancreozymin[6] was studied with chicks to determine if injections of this hormone would cause gallbladder emptying in fasted chicks. This preparation did not cause a significant reduction in gallbladder size or pancreatic amylase level when administered by injection directly into the heart or when given intraperitoneally. This is shown by the data in Table III.

In other experiments for which data are not shown, negative results were obtained when the pancreozymin was given orally alone or in conjunction with the soybean whey proteins.

The biological activity of the pancreozymin preparation was tested by injecting it intraperitoneally into 8 fasted rats and measuring its effect on pancreatic amylase levels 1 hr after injection. The amylase activity of

[6] Calbiochem, Los Angeles, CA.

TABLE II. Influence of Soybean Whey and Trypsin Inhibitor on Gallbladder Weight.

Substance orally administered	No. of chicks	Wt		Amylase content (units/g pancreas)[a,b]
		Body (g)	Gallbladder (mg)	
Expt. D (20-day-old chicks)				
Fasted	6	198	225 ± 29 a	2993 ± 547 a
Feed + 50 mg heated soybean whey	7	204	115 ± 9 b	2125 ± 221 ab
50 mg soybean whey	7	205	95 ± 12 b	1342 ± 124 b
Expt. E				
None	16	122	146 ± 8 a	4584 ± 522 a
Feed, 1 g, + 50 mg heated soybean whey	15	119	116 ± 6 b	3148 ± 371 a
50 mg soybean whey	15	116	88 ± 9 c	2798 ± 351 a
50 mg crystalline trypsin inhibitor	15	122	109 ± 8 bc	3268 ± 455 a
Expt. F				
None	11	162	383 ± 26 a	3600 ± 323 a
Feed, 1 g, + 50 mg heated soybean whey	12	163	279 ± 20 b	3800 ± 340 a
50 mg soybean whey	11	158	203 ± 32 c	1613 ± 219 b
50 mg crystalline trypsin inhibitor	12	165	203 ± 20 c	1963 ± 236 b

[a] Values not followed by same letter are significantly different at $p < .01$. Means are shown ± SEM. All values were obtained 1 hr after administration of test substances.

[b] In Expt. E, 10 pancreases were analyzed for amylase activity from each treatment. In Expt. F, all chicks were used for measurement of amylase activity.

the injected rats was 2070 ± 172 units/g of pancreas whereas for controls the activity was 3018 ± 240 units/g. This difference was significant ($p > .01$) when tested by a t test. Thus the pancreozymin preparation seemed to be active with the rat even though no effect was observed when it was administered to chicks either on gallbladder size or on pancreatic amylase concentration.

Discussion. The data obtained in these experiments show that soybean whey proteins or trypsin inhibitors have a parallel action on gallbladder emptying and pancreatic enzyme depletion. When fasted chicks were administered soybean whey proteins by crop intubation, these proteins had little effect on gallbladder emptying or pancreatic amylase activity. However, when these proteins were administered together with a small amount of feed, the response of these organs to the feed was enhanced. This suggests that the whey proteins act by affecting the normal stimulus to gallbladder and pancreatic function. The effect of these proteins on both the pancreas and gallbladder suggests that they are affecting a common stimulator to both of these organs. Since the common thread to stimulators of the pancreas seems to be their ability to inhibit proteolysis, this property may be the mechanism whereby these inhibitors function.

The mucosal sites of production and release of cholecystokinin-pancreozymin are not known (23). If, in the elaboration of this hormone, the hormone or some peptide stimulator of the hormone were released into the duodenum when food was introduced, a mechanism for feedback control of pancreatic secretions could be postualted. The polypeptide, when released, might be reabsorbed and would cause stimulation of the pancreas and gallbladder until sufficient proteolytic activity built up in the duodenum to hydrolyze the peptide prior to its transport to the pancreas. This would then remove the stimulus to pancreatic enzyme release. If a proteolytic inhibitor were introduced into the duodenum, however, the destruction of the peptide

TABLE III. Effect of Pancreozymin on Gallbladder Size of Fasted Chicks.

Mode of administration	No. of chicks	Wt		Amylase content (units/g pancreas)
		Body (g)	Gallbladder (mg)	
Expt. G[a]				
Control, no hormone	10	128	329 + 20	
Pancreozymin[b] in heart	10	133	338 ± 18	
intraperitoneally	10	129	334 ± 42	
Expt. H[a]				
Fasted chicks	10	128	181 ± 11	5076 ± 447
Pancreozymin[b] in heart	10	124	162 ± 9	4100 ± 461

[a] In Expt. G, chicks were fed a diet with 15% fat from hatching; in Expt. H, a commercial chick starter was fed. No significant differences were found in gallbladder weight or amylase content of pancrease in either experiment.

[b] 8.2 Crick units in 0.5 ml saline solution were given.

would be delayed and continued stimulation might result.

These experiments do not offer any direct evidence for such a mechanism but they are consistent with such a possibility. The whey proteins and trypsin inhibitor were not effective stimulators of the pancreas and gallbladder by themselves but when given in conjunction with feed they enhanced the effect of feed in stimulating these organs. A critical test of the above hypothesis awaits the application of sensitive assays for cholecystokinin-pancreozymin.

The experiments with the commercial pancreozymin preparation were intended to test this hypothesis. The soybean whey proteins might be expected to enhance the action of orally administered pancreozymin if it escaped digestion by gastric pepsin. Unfortunately no response in chicks could be elicited from the pancreozymin preparation by any dosage route even though seemingly massive doses were given. The preparation was active with rats. Our experiments may indicate some species specificity with cholecystokinin-pancreozymin.

It is possible that some dietary component is absorbed and stimulates the pancreas and gallbladder when trypsin inhibitors are present in the duodenum. However, we have confirmed the observation of Khayambashi and Lyman (24) that pancreatic hypertrophy occurs even when amino acids supply all the protein in the diet[7] so that undigested components of dietary protein are unlikely stimulators.

[7] Unpublished data.

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