*Acta Physiologica Hungarica, Volume 91 (1), pp. 73–81 (2004)*

# Effect of orally administered plant lectins on intestinal liquor accumulation and amylase activity in rats

K **Baintner**[1], P **Kiss**[2], S **Bardocz**[3], A **Pusztai**[3]

[1] Department of Physiology, Faculty of Animal Science, University of Kaposvár, Kaposvár, Hungary
[2] Department of Agricultural Chemistry, Szent István University, Gödöllő, Hungary
(present address: Ceva-Phylaxia Co. Ltd., Budapest, Hungary)
[3] 6. Ashley Park North, Aberdeen, Scotland, UK

Received: November 11, 2003
Accepted: January 15, 2004

Short-term effects of orally administered plant lectins, with special reference to the *Phaseolus vulgaris* agglutinin (phytohaemagglutinin, PHA), were studied in growing rats.

The orally administered PHA elicited a dose-dependent accumulation of liquor with elevated pH in the proximal small intestine. Although the concentration of α-amylase activity did not change, total α-amylase activity slightly, but significantly increased in the gut. When a panel of plant lectins with different carbohydrate binding specificities was tested at the dose of 100 mg/kg body weight, most of them stimulated the secretion of liquor, but the total α-amylase activity was increased only by PHA, ConA or WGA.

**Keywords:** lectins, rat, amylase, intestinal, phytohaemagglutinin, PHA, RPA, ConA, SBA, GNA, VFA, WGA, TPA

Several plant lectins are consumed in raw or improperly heated form as part of animal feed or human food. Their biological effects depend both on their ability to resist proteolytic degradation, and, depending on their carbohydrate specificity (5, 6), to bind to various receptor moieties in the mucus, the brush border membrane glycoproteins or to bacteria. A small percentage of lectins are internalised from the gut surface (4) and transferred to the circulation (7). The structure and function of plant lectins were extensively reviewed by van Damme et al. (12).

Correspondence should be addressed to
Károly Baintner
Department of Physiology
Faculty of Animal Science
University of Kaposvár
H-7400 Kaposvár, Guba S. u. 40, Hungary
E-mail: baintner@mail.atk.u-kaposvar.hu

0231–424X/$ 20.00 

In the present work the short-term effects of plant lectins on the accumulation of the intestinal liquor and amylase activity are investigated, whereas the origin and mediation of secretion will be dealt with in another paper.

## Material and Methods

### *Lectins and chemicals*

Bovine serum albumin (BSA) were purchased from Sigma. Most lectins were purified by affinity chromatography, then desalted by dialysis, freeze-dried, and finally checked for purity by SDS-PAGE and for activity by haemagglutination of rat blood cells.

Kidney bean phytohaemagglutinin (PHA) was purified by the combination of ion-exchange and affinity chromatography (2). The preparations containing either $L_4$ isolectin or a mixture of $EL_3$, $E_2L_2$ and $E_3L$ isolectins were equally active. If not specified otherwise, the latter preparation (E+L) was used in the experiments. All other lectins were purified also by affinity chromatography: the fava bean (*Vicia faba*) agglutinin (VFA) on Sephadex G-100 (14), the snowdrop bulb (*Galanthus nivalis*) agglutinin (GNA) on agarose-bound mannose (10), the soyabean (*Glycine max*) agglutinin (SBA) on epichlorohydrine crosslinked guar-gum (8), the asparagus pea (*Tetragonolobus purpureas*) agglutinin (TPA) on L-fucose-Sepharose (3), the black locust tree bark (*Robinia pseudoacacia*) lectin (RPA-I) on fetuin-Sepharose (11) and the wheat germ (*Triticum vulgare*) agglutinin (WGA) on N-acetyl-glucosamine-Sepharose column (13). In the latter method the WGA was eluted from the column with 0.5 M acetic acid instead of the 1% N-acetyl-glucosamine. Carbohydrate specificities of the lectins are shown in Table I.

**Table I**

*The lectins used in the present experiments*

| Abbreviation | Latin name | Source | Carbohydrate specificity |
|---|---|---|---|
| VFA | *Vicia faba* | broad bean | glucose/mannose |
| ConA | *Canavalia ensiformis* | jack bean | glucose/mannose* |
| GNA | *Galanthus nivalis* | snowdrop bulb | mannose-(1,3)mannose |
| WGA | *Triticum vulgare* | wheat germ | GlcNAc-β(1,6)Gal-sialic acid |
| SBA | *Glycine max* | soyabean | galactose/GalNAc |
| TPA | *Tetragonolobus purpureas* | asparagus pea | L-fucose |
| PHA | *Phaseolus vulgaris* | kidney bean | complex specificity** |
| RPA-I | *Robinia pseudoacacia* | black locust bark | complex specificity** |

With the exception of fucose, the sugars are in "D" configuration.
* Less specific than VFA.
** Not inhibited by simple sugars.

*Animals and treatments*

120–130 g female HSD Wistar rats were used. After 24 h fasting the lectin dissolved in 0.4 ml saline (usually 100 mg/kg b.w.) was administered through a gastric tube. One hour later the animals were killed by bleeding under ether anaesthesia, the abdominal wall was slit open, the small intestine was removed and flushed with 5 ml physiological saline. The volume of the collected liquor was measured and the volume of the secreted fluid was calculated by subtracting the 5 ml saline from the volume of the effluent. In some of the experiments the solid part of the intestinal content was removed by centrifugation, and only the volume of the clear solution was measured. It was established that this had no influence on the difference between experimental and control groups.

*Analytical procedures*

The outflow of the intestinal wash was collected, homogenised and centrifuged (2000×*g* for 5 min). The clear supernatant, which was free of bacterial cells, was used for the assays.

To determine the amylase activity the Phadebas test (Pharmacia, Uppsala) was used with some modifications: The reaction mixture was adjusted to pH 7.0 with phosphate buffer and the proteolytic activity was suppressed with soyabean trypsin inhibitor (STI). The reaction mixture consisted of 3.1 ml saline, 1.0 ml 0.1 M phosphate buffer, 1.0 mg STI in 0.09 ml and 0.01 ml sample. After pre-heating the mixture at 37 °C in a water bath, the reaction was started by adding a pellet of stained starch (Phadebas) into the mixture. The reaction was stopped by 1.0 ml of 0.5 M NaOH 5 minutes later, then the mixture was centrifuged. One ml liquor was removed and diluted threefold. The absorbance was read at 620 nm and the activity calculated by subtracting the blank and multiplying the value by a coefficient of 2.3. The results were expressed in nkat/ml. To avoid the effect of differences in fluid secretion, the activity of the whole intestinal wash was calculated and expressed in μkat.

The trypsin and chymotrypsin activities were determined with benzoyl-L-arginine ethyl ester or benzoyl-L-tyrosine ethyl ester, as the respective substrates. The reading on the spectrophotometer gave the activity values directly (9).

The $K^+$-ion concentration was determined by flame photometry, while that of $Ca^{2+}$ and $Mg^{2+}$-ions by atomic absorption photometry. The pH was measured by using a Radelkis pH-meter.

SSPS program was used for the analysis of variance (ANOVA).

## Results

*Composition of the intestinal liquor*

PHA ($L_4$ or E+L; 100 mg/kg b.w.; n=6) was administered by oral gavage and the pH of the intestinal wash was measured one hour later. Both the E+L-type (pH 7.1) and the $L_4$-type (pH 7.0) preparations increased the pH of the wash significantly ($p<0.05$), compared

with the saline-treated controls (pH 6.8) or the soyabean trypsin inhibitor treated-rats (pH 6.7). The effect of the two PHA preparations did not differ significantly.

In the same experiment the oral administration of PHA, compared with the saline-treated controls, had no influence on the concentration of $K^+$, $Ca^{2+}$ and $Mg^{2+}$-ions in the intestinal wash. The $Na^+$-ion concentration was not determined, due to the high background values.

In another experiment oral PHA administration did not affect the level of amylase activity, but significantly increased both the volume of the wash ($p<0.01$) and its total amylase activity ($p<0.05$). A different effect was observed with STI, which significantly increased the level of amylase activity ($p<0.01$), but did not stimulate the volume of fluid secretion, resulting in similar total amylase values as in the PHA-treated rats. The trypsin and chymotrypsin activities showed higher variability than that of the amylase, and no PHA-induced changes could be observed.

*Dose-response curves*

Dose-response curves were plotted up to 150 mg/kg b.w. oral PHA. The treatments significantly ($p<0.05$) increased the volume of the intestinal liquor and the response showed a saturation curve that levelled at about 100 mg/kg b.w. (Fig. 1).

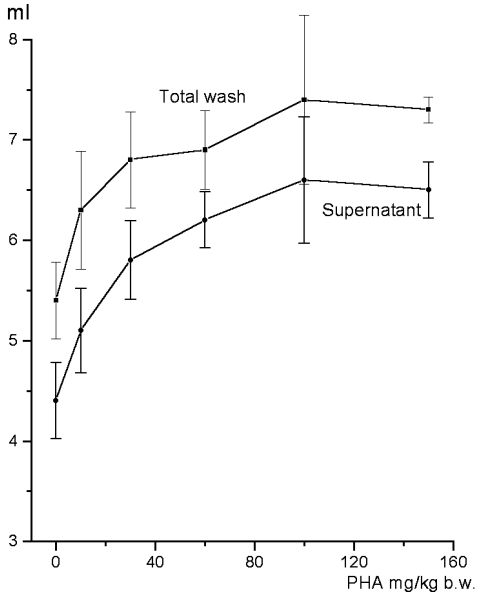


Fig. 1. Effects of the different doses of orally administered PHA on the accumulation of intestinal liquor in growing rats. The effect of the smallest PHA dose (10 mg/kg b.w.) already significantly ($p<0.05$) differs from both the control and the two highest doses. Each point represents mean of 6 values ±standard deviation. The small intestine was flushed with 5 ml physiological saline. The amount of the solid material is represented by the difference between the wash volume and that of the supernatant

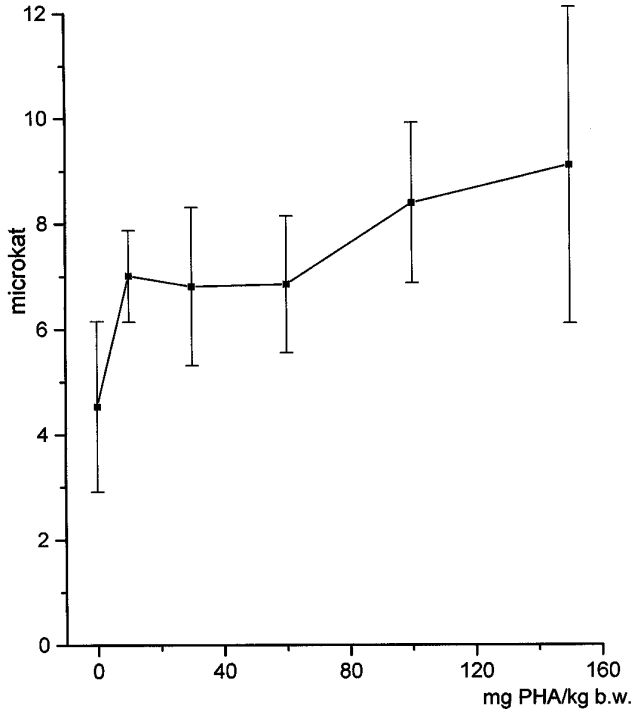


Fig. 2. Effects of different doses of orally administered PHA on the total amylase activity of the intestinal wash. The lowest PHA dose (10 mg/kg b.w.) significantly ($p<0.05$) elevated the activity, but little further increase occurred with the higher doses

Total amylase activity of the intestinal wash was already significantly ($p<0.05$) increased at the lowest dose (10 mg/kg b.w.), as compared to the saline control, but only small further increases could be noticed with higher PHA doses (Fig. 2).

*Comparing of the effects of different lectins*

The effect of a panel of orally administered plant lectins (100 mg/kg b.w. in 0.4 ml saline; n=6) on the accumulation of intestinal liquor was determined (Fig. 3). With the exception of GNA and VFA, all lectins significantly increased ($p<0.05$) the volume of secretion, compared with the BSA control. The active lectins could be grouped according to their efficiency: PHA was the most effective, followed by RPA-I, WGA and ConA, while SBA and TPA had the smallest effect. All groups differed significantly ($p<0.05$).

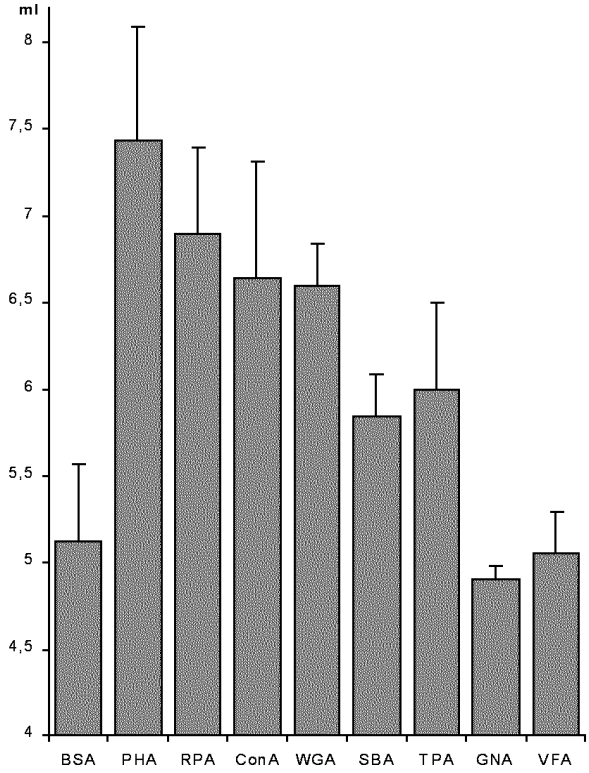


Fig. 3. Effect of a panel of orally administered plant lectins with different carbohydrate binding specificities (100 mg/kg b.w.) on the accumulation of intestinal liquor. Each column represents the mean of 6 values ±standard deviation; n=6. Abbreviations and specificities of the lectins are shown in Table I

In the same experiment, significant elevation of total amylase activity ($p<0.05$) was found with the PHA- and WGA-treated rats, whereas the activities measured with RPA, SBA and TPA, did not differ from the BSA-treated control (Fig. 4).

## Discussion

The orally administered PHA induced liquor accumulation in the proximal small intestine within an hour, and the effect could be seen by naked eye after opening the abdomen. The occurrence of this response was highly consistent in the consecutive experiments. The ionic composition of the liquor was not significantly altered by the treatment, but a small and significant ($p<0.05$) elevation of the pH could be measured.

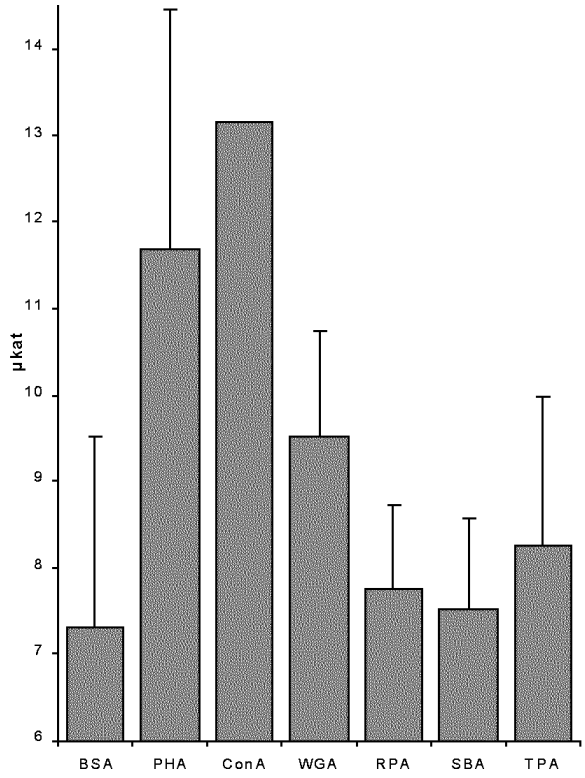


Fig. 4. Effect of a panel of orally administered plant lectins with different carbohydrate binding specificities (100 mg/kg b.w.) on the total amylase activity of the intestinal wash. Each column represents the mean of 6 values ±standard deviation; n=6. Values of the ConA group (n=3) fit into the same range as those of the PHA group. Abbreviations and specificities of the lectins are shown in Table I

The activity of α-amylase did not change, but, due to the higher secreted fluid volume, the total α-amylase activity of the intestinal wash was significantly increased ($p<0.05$). The effects of the two PHA-isolectin preparations did not show significant differences. Soyabean trypsin inhibitor exerted its well-known stimulatory effect on pancreatic amylase secretion, but did not modify the volume of the intestinal wash.

It was expected that the small elevation of total α-amylase activity in the intestinal wash is accompanied by a similar increase of trypsin and chymotrypsin activities. However, the higher standard deviation of these determinations than that of amylase activity, thwarted the demonstration of eventual subtle changes.

The dose-response study of orally given PHA showed a saturation curve for the secreted volume, reaching the maximum at about 100 mg/kg b.w. (Fig. 1). The supernatant of the centrifuged wash showed a very similar curve, but with lower values.

Total amylase activity was already elevated by a low PHA dose (10 mg/kg b.w.), and little increase was observed with higher doses (Fig. 2). Suppression of food consumption required the highest PHA doses applied as it was shown in an earlier study (1).

In the fasted and BSA-treated control rats the volume of the wash approximated that of the introduced liquor. Similar wash volumes were measured in the rats treated with GNA or VFA, due to the lack of intestinal receptors for these lectins (5). In contrast, all lectins which bind to receptors on the gut epithelium induced accumulation of intestinal liquor, and PHA exerted the most marked effect (Fig. 3). It is remarkable that binding of lectins to different carbohydrate moieties resulted in similar secretory responses.

The total α-amylase activity of the intestinal wash (Fig. 4) was elevated by three lectins (PHA, ConA and WGA), whereas three other gut-binding lectins were ineffective (RPA-I, SBA and TPA). This means that binding to the gut surface is not a sufficient requirement for biological effect. A similar phenomenon was noted in the study of lectin-induced suppression of food consumption (1).

Although several lectins occur in our food crops, most often they are inactivated by heat treatment (kidney bean and soyabean) or removed by processing (soya tofu in the Far-East). The concentration of other lectins in the food is low (wheat germ and tomato) or they lack intestinal receptors (fava bean and lentil).

## Acknowledgements

We thank Willy Peumans (Catholic University of Leuven, Belgium) for the GNA and advices and János Kelemen (PTE/EFK, Kaposvár, Hungary) for performing flame photometry. Supported by grants FKFP 503/99 and OTKA T-43541.

## REFERENCES

1. Baintner K, Kiss P, Pfuller U, Bardocz S, Pusztai A: Effect of orally and intraperitoneally administered plant lectins on food consumption of rats. Acta Physiol. Hung. 90, 97–107 (2003)
2. Bardocz S, Grant G, Ewen SWB, Duguid TJ, Brown DS, Englyst K, Pusztai A: Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. Gut 37, 353–360 (1995)
3. Blumberg S, Hildeheim J, Yariv J, Wilson KJ: The use of 1-amino-L-fucose bound to Sepharose in the isolation of L-fucose-binding proteins. Biochim. Biophys. Acta 264, 171–176 (1972)
4. King TP, Pusztai A, Grant G, Slater D: Immunogold localization of ingested kidney bean (*Phaseolus vulgaris*) lectins in epithelial cells of the rat small intestine. Histochem. J. 18, 413–420 (1986)
5. Pusztai A. (1991): Plant Lectins. Cambridge University Press p. 110.
6. Pusztai A, Ewen SWB, Grant G, Peumans WJ, van Damme EJM, Rubio L, Bardocz, S: The relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. Digestion 46 (suppl. 2), 308–316 (1990)
7. Pusztai A, Greer F, Grant G: Specific uptake of dietary lectins into the systemic circulation of rats. Biochem. Soc. Transactions 17, 481–482 (1989)

8. Pusztai A, Watt WB, Stewart JC: A comprehensive scheme for the isolation of trypsin inhibitors and the agglutinin from soybean seeds. J. Agric. Food Chem. 39, 862–866 (1991)
9. Schwert GW, Takenaka Y: A spectrophotometric determination of trypsin and chymotrypsin. Biochim. Biophys. Acta 16, 570–575 (1955)
10. van Damme EJM, Allen AK, Peumans WJ: Isolation and characterization of a lectin with exclusive specificity towards mannose from snowdrop (*Galanthus nivalis*) bulbs. FEBS Letters 215, 140–144 (1987)
11. van Damme EJM, Barre A, Smeets K, Torrekens S, van Leuven F, Rougé P, Peumans WJ: The bark of *Robinia pseudoacacia* contains a complex mixture of lectins. Plant Physiol. 107, 833–843 (1995)
12. van Damme EJM, Peumans WJ, Pusztai A, Bardocz S (1997)*:* Handbook of Plant Lectins*:* Properties and Biomedical Applications. Wiley, Chichester.
13. Vretblad P: Purification of lectins by biospecific affinity chromatography. Biochim. Biophys. Acta 434, 169–176 (1976)
14. Wang JL, Becker JW, Reeke GN jr, Edelman GM: Favin, a crystalline lectin from *Vicia faba*. J. Mol. Biol. 88, 259–262 (1974)