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Effect of phytohaemagglutinin on intestinal cell proliferation Role of polyamines

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SUMMARY. The polyamines, putrescine, spermidine and spermine, mediate the effects of hormones and growth factors as second messengers. They are necessary for every step of protein, RNA and DNA synthesis and are therefore essential for cell growth and proliferation. As with hormones and peptide growth factors, plant lectins which bind to cell surface receptors of the brush border membrane are powerful extraneous growth factors for the gut and as a result, by interacting with brush border epithelial receptors, induce extensive proliferation and changes in the metabolism of epithelial cells via activation of second messenger pathways. In model experiments with phytohaemagglutinin (PHA, the lectin from the kidney bean, *Phaseolus vulgaris*) it was shown that lectins which bind avidly to the mucosal surface induce dose- and time-dependent and fully reversible hyperplastic and hypertrophic growth of the small bowel. The resulting increase in crypt cell proliferation (CCPR) alters the gene expression in epithelial cells. These metabolic changes require vast amounts of polyamines, mostly spermidine. Thus, one of the first effects of the PHA signal is to stimulate the basolateral polyamine uptake system for the sequestration of polyamines from blood circulation in sufficient amounts to sustain the growth of the tissue. Our data indicate that the main source of polyamines to replenish those taken up by the gut is the diet.

It has been shown repeatedly that, because of intensive cell proliferation, tumour growth requires large amounts of polyamines. PHA or other lectins accelerate normal metabolic reactions in a regulated way while maintaining their full reversibility and without causing irreversible aberrations. The fact that the lectin-induced growth of the gut requires large amounts of polyamines, suggests that lectins are ideal agents to limit the availability of polyamines for unwanted growth such as neoplastic proliferation of tumours. Accordingly, it may be possible to limit the availability of polyamines for tumour growth by inducing a competitive, but fully reversible, controlled growth of the gut tissue. The intensively but reversibly growing gut tissues can slow down tumour growth by sequestering polyamines and nutrients from circulation.

RESUMEN. Efecto de la fitohemaglutinina sobre la proliferación de las células intestinales. Papel de las poliaminas. Las poliaminas, putrescina, espermidina y espermina, actúan como segundos mensajeros mediando los efectos de hormonas y factores de crecimiento. Ellas se requieren en cada paso de la síntesis de DNA, RNA y proteínas y, por lo tanto, son esenciales para el crecimiento y la proliferación celular. A través de su unión a los receptores de la superficie del borde en cepillo, las lectinas de plantas actúan como potentes factores exógenos de crecimiento del intestino y en consecuencia inducen una intensa proliferación celular y cambios en el metabolismo de las células epiteliales por intermedio de la activación de las rutas de los mismos segundos mensajeros. Usando fitohemaglutinina (PHA, la lectina de *Phaseolus vulgaris*), se encontró que aquellas lectinas que se unen ávidamente a la mucosa inducen el desarrollo hipertrófico reversible del intestino delgado, de una manera que es dependiente de la dosis y del tiempo de exposición. El aumento en la proliferación de las células de las criptas (CCPR) altera la expresión de los genes en las células epiteliales, para lo cual se requiere de grandes cantidades de poliaminas, en especial de espermidina. En consecuencia, uno de los primeros efectos de la PHA estriba en incrementar la captación, a través de la membrana basolateral, de poliaminas provenientes de la circulación sistémica, en proporciones suficientes para mantener el crecimiento del tejido. Nuestros resultados indican que la mayor parte de las poliaminas, necesarias para cubrir el déficit producido por el incremento de la demanda intestinal, son de origen dietario.

Se ha demostrado repetidamente que, a causa de su intensa tasa de proliferación celular, los tumores requieren de un suministro incrementado de poliaminas, pudiendo establecerse un proceso de competencia con tejidos normales, que sean estimulados reversiblemente a proliferar, de manera controlada, con PHA u otras lectinas. Por ejemplo, el crecimiento intestinal inducido por lectinas, el cual demanda altas cantidades de poliaminas, sugiere que las lectinas podrían actuar como agentes ideales para limitar la disponibilidad de poliaminas y otros nutrientes de la circulación, disminuyendo, en consecuencia, el crecimiento tumoral.

INTRODUCTION

The epithelium of the small bowel is composed of a monolayer of epithelial enterocytes fulfilling the absorptive and digestive functions of the gut. The epithelium of the intestinal tract has one of the highest cell turnover rates in the body of mammals (1) enabling it to react rapidly to any changes, from dietary influence to bacterial invasion.

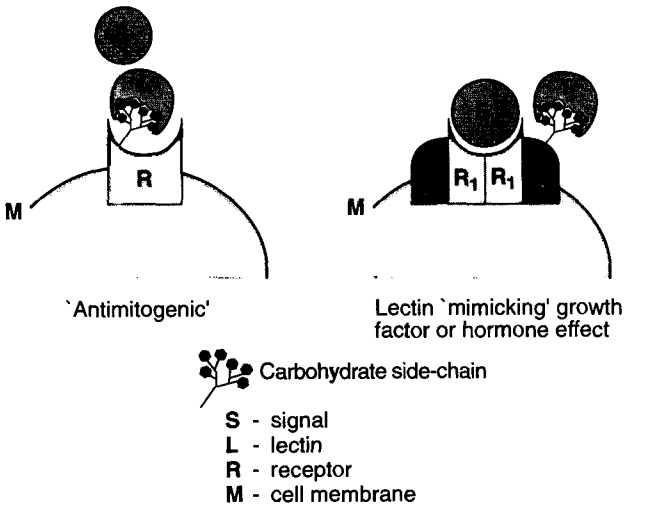
The small intestinal epithelium is organized into two functionally and morphologically distinct compartments: the crypts, where the stem cells proliferate and differentiate, under the influence of growth factors, hormones and cytokines, and the villi, where the differentiated cells mature and migrate toward the tip of the villi for absorption and digestion to occur (1). All cell-surface proteins are glycosylated in order to be transported from the site of synthesis to the plasma membrane (2,3). The pattern of glycosylation varies in different species (4), but within any one species it depends on the stage of differentiation and maturation, on the position of the cells along the crypt-villus axis, on the site of the gastrointestinal tract and also on age and blood group specificity. The great variability in glycosylation may help to explain why lectins differ in their ability to interact with the surface of gut.

Lectins are very potent exogenous growth signals, some can even mimic the action of major metabolic hormones and growth factors. In contrast to dietary proteins which are rapidly degraded during passage through the gut, lectins resist degradation by proteolytic enzymes and also by different bacteria. The almost complete survival of the lectins of *Galanthus nivalis* (GNA), despite the absence of binding to the brush-border in acute exposure, and also of PHA suggests that the molecular structure of some lectins may be intrinsically resistant to proteolytic breakdown (5).

Since most growth factor- and hormone-receptors are glycoproteins or glycoconjugates embedded in the cell surface membrane, interaction between lectins of plant or bacterial origin and the gut depends on specific recognition by the lectin of the membrane glycans projected into the gut lumen. Therefore, the growth factor or hormone-like action of lectins can be explained by their lectin function: through recognition and binding to surface membranes, they send signals and deliver messages into the cells (Figure 1). Receptor proteins are usually composed of more than one subunit, and the subunits exposed on the external side of the membrane are glycosylated.

The process of recognition between lectins and their receptors is instantaneous. The strength of lectin binding is also dependent on the number of unoccupied receptor sites. If there are many carbohydrate side-chains with the 'right' sugar structure the lectin will bind extensively, but if there are just a few binding sites, or the sites are well separated from each other, only weak or no binding occurs. There is evidence that strong binding lectins are readily endocytosed or transcytosed although the signals necessary for these processes are not well understood at the moment.

FIGURE 1

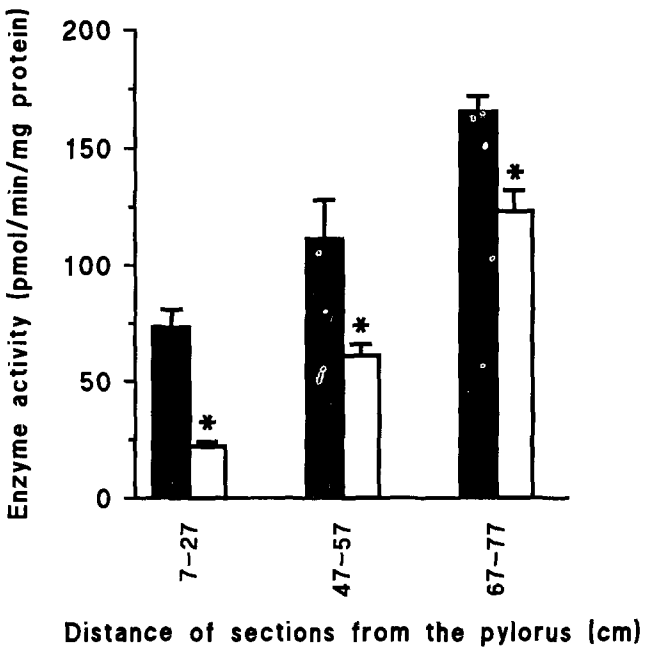


Location of the glycosyl side-chains on the cell-surface proteins in the membrane, and possible interactions of lectins and growth factor- or hormone receptors. Depending on their position, the sugars-structures can be present in or near to the active centre, on the same subunit where the growth factor or hormone-binding site is located or, on another subunit. Accordingly, lectins can bind to the main or other subunit(s) through their glycosyl side-chains and activate them by changing their conformation. These changes can mimic partly or fully the changes induced by the 'proper' biological signal (growth factors, hormones, cytokines, etc...), or, if the carbohydrate side-chain is on another subunit from the signal binding site, they can even attenuate the effects of the signals. In some cases the lectin, by binding to the receptor, can inhibit either the binding of the signal, or prevent some conformational changes in the receptor or in the surrounding membrane domain. These lectins are called 'anti-mitogenic.'

Some carbohydrates are scanty in the small bowel, but present in the large intestine so that lectins which are specific to those receptors will pass through the small bowel and bind to the surface of the large intestine. As a result, most lectins can also affect the metabolism of the large intestine. The growth factor activity of gut lectins is determined mainly by the strength and intensity of their binding (5). Even when this is relatively weak, by cross-linking cell surface receptors it still disturbs the organization of the epithelial membrane (5,6) and induces slight growth of the gut. PHA is a potent growth factor for the mammalian gut and is the best studied model. On lectin-induced small intestinal growth, the length of the villus is rarely significantly affected (5). In contrast, the crypt size, the number of cells they contain and the CCPR are substantially increased (7). These changes correlate well with the effectiveness of the lectins as growth factors. However, the newly produced cells need time to differentiate, and since the migration speed of cells on the villus is also faster, the proportion of immature cells on the villi rises. With continuous

exposure to lectins such as PHA, which binds extensively, the cell turnover time can decrease from 72 to 12 hours. As protein and enzyme patterns of the newly formed cells are typical of the immature cell type, there are significant differences in the capacity of those cells to absorb and digest food. On exposure to PHA, there is a significant increase in the activity of maturation marker enzymes such as diamine oxidase in the gut tissue (Figure 2) and the specific activity of sucrase-isomaltase and alkaline phosphatase (8).

FIGURE 2



Diamine oxidase activity in the small intestine of rats fed either lactalbumin (■) or 42 mg PHA/rat/day (□) diets for 10 days. Values are means ± SE of 9 rats. $P<0.01$ (*) when compared to control (lactalbumin) values.
(These data were produced in collaboration with Drs Perin and Sessa, Universita Delgi Studi di Milano, Italy).

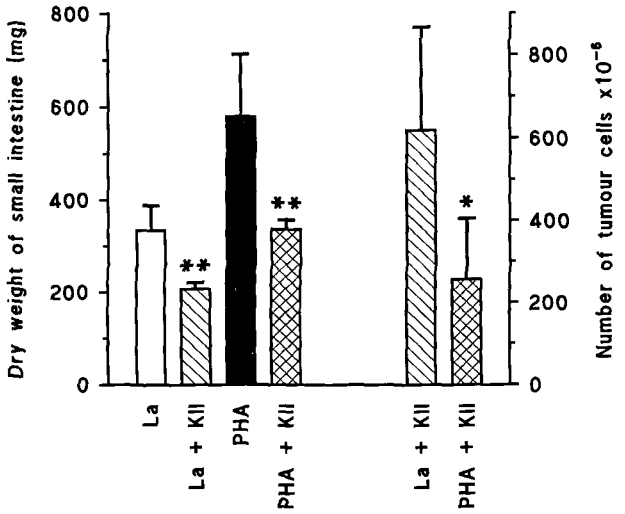
Even a small increase in the size of the gut has a slight nutritional penalty for the animal, in that more of the dietary protein and energy are consumed in the need to renew the gut surface more quickly than under normal conditions. With PHA and the lectins from soya bean (*Glycine max*, SBA) or wheat germ (*Triticum vulgaris*, WGA) which bind avidly to epithelial cells and are more powerful growth factors for the small bowel, the cost in nutritional terms is even more expensive. Indeed, at high dietary intakes of these lectins, most or all of the diet is used by the gut alone leaving other organs starved of nutrients (9,10). Diets containing PHA or raw kidney beans can double the weight of the small intestine within 7 days (11-14). However, the contribution of the growth stimulating activity of the lectins to their nutritional toxicity is relatively slight under most practical conditions.

The most studied example is PHA which recognises and binds to the complex carbohydrate structures present on the intestinal surface of mammals. One of the first changes induced in the epithelium of the small intestine of the rat is the instant stimulation of protein synthesis (15,16). The second and long-lasting increase in protein synthesis is evident after a few hours and results in the synthesis of new RNA (17). These processes require the polyamines putrescine, spermidine and spermine (18,19) which are essential for the adaptational growth of the gut (20,21). The growth of the gut induced in conventional rats by PHA requires the accumulation of large amounts of polyamines, mostly spermidine, in the tissue (17). However, this accumulation occurs without a major increase in the activity in the small intestine of ornithine decarboxylase (ODC), the rate-determining enzyme of polyamine synthesis (11,17) indicating that, in this instance, ODC has little to do with the increase in polyamine concentration. In the growing intestine, part of the polyamine pool originates from the systemic circulation through the basolateral membrane and one of the first steps of this process is the stimulation of the basolateral uptake of polyamines (11,22). Therefore, measurement of the tissue polyamine content and the rate of basolateral uptake of polyamines, mostly spermidine, can be used as markers of the metabolic activity of the intestine and to follow the effects of different antinutritional factors, including the growth factor-like effects of the lectins on gut metabolism.

The recognition that the lectin-induced changes in cellular metabolism are fully reversible has allowed us to use the PHA-induced rat small intestinal growth model as a convenient tool for magnifying and studying the fundamental metabolic reactions of epithelial cell proliferation, differentiation and maturation and ensuing changes in the cellular metabolism of the gut. In recent developments, PHA was used to manipulate body metabolism of tumour-bearing mice with the aim of redirecting nutrients and polyamines away from the tumour by a competing growth signal. PHA is ideal for such an experiment since it induces reversible, polyamine-dependent, hyperplastic growth of the small bowel (11) and has been shown to compete successfully with hypertrophic growth of the skeletal muscle induced by clenbuterol, a ß-adrenoreceptor agonist (16). It was thought, therefore, that PHA might also compete with the putative tumour growth signal(s) and stop, or at least slow down neoplastic proliferation. We have shown that NMR mice fed PHA-containing diets do indeed have fewer tumour cells than the lactalbumin fed control animals, when injected intraperitoneally with Krebs-II ascites cells. As expected, in the PHA-fed animals the weight of the gastrointestinal tract increased while the number of tumour cells in the peritoneum decreased (Figure 3). Earlier observations (23,24) and also evidence presented in Figure 4 show that our approach based

on interorgan competition is valid as PHA was able to slow down the proliferation of Krebs-II ascites cells. Biochemical analysis of the tissues (protein, RNA, DNA and polyamine contents) indicated that interorgan competition between the tumour and vital organs can be used to manipulate the metabolism of tumour-bearing mice, or compete with any unwanted growth.

FIGURE 3



Dry weights of the small intestine and number of tumour cells of tumour-bearing mice fed either on kidney bean (PHA) or control lactalbumin (La) diets. The values and mean ± SD for 5 animals per treatment. ** Significant difference between those mice injected with Krebs-II ascites cells (KII) are their respective controls ($P<0.001$). * Significant difference in number of tumour cells between those mice fed PHA diets and the La controls ($P<0.05$).

ACKNOWLEDGEMENTS

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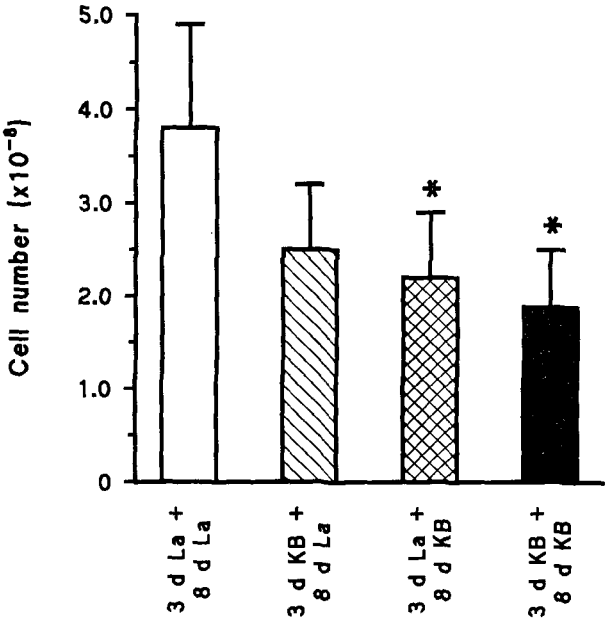
FIGURE 4



Four different groups of NMR mice were injected intraperitoneally with Krebs-II ascites cells. For 11 days each group followed a different combination of the lactalbumin (control) and/or PHA (42 mg/mouse/day) diets. Values are means ± SD of 5 mice. $P<0.05$ (*) when compared to control values.

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