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Effects of human growth hormone (hrGH) treatment on amine metabolism in rats subjected to extensive small bowel resection

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Summary. The effect of human recombinant growth hormone (hrGH) on intestinal adaptation in rats subjected to massive small bowel resection has been followed by monitoring changes in the tissue polyamine system and in red blood cell (RBC) polyamine levels. In parallel, the activities of monoamine oxidase A and B and diamine oxidase, the enzymes that catalyse one of the major routes of biogenic amine metabolism, oxidative deamination, were also examined.

The results suggest that whilst hrGH treatment accelerates adaptive intestinal hyperplasia evoked by the resection, it has no significant effect on RBC polyamine level or gut mucosal DNA concentration as measured 3 weeks post surgery. hrGH treated operated rats exhibited significantly lower amine oxidase activities which implies that GH may alter biogenic amine systems.

Key words: Growth hormone – Intestinal adaptation – Amine metabolism – Erythrocyte polyamines

Introduction

Extensive small intestine resection results in the loss of absorptive surfaces, acceleration of intestinal transit and, as a consequence, in malnutrition, weight loss, diarrhoea and other complications of short bowel syndrome (Vanderhoof et al., 1992). The availability of human recombinant growth hormone (hrGH) and stimulatory effects of GH on gut growth (Leblond and Carriere, 1955; Lehy et al., 1986; Lobie et al., 1990) suggested its use in the treatment of short bowel syndrome (Ellegard et al., 1997). The trophic response of GI tract epithelium to hormones such as GH is mediated by polyamines, which are vital in cell proliferation (Janne et al., 1978). Tissue polyamine concentrations directly reflect growth stimulation or retardation (Seidel, 1986; Hosomi et al., 1987). Likewise, it has been shown that measurement of an erythrocytic polyamine concentration may be diagnostically useful. Polyamines produced and secreted in excess by rapidly growing tissues enter the circulation where they are transported almost exclusively by red blood cells (RBC). Since these cells are unable to synthesise or degrade polyamines, the RBC polyamine level may serve as a marker of cellular proliferation (Moulinoux et al., 1981, 1984, 1991).

Recent studies have shown that in addition to its growth promoting effects, growth hormone exerts multiple metabolic effects. However, with the exception of some data on the polyamine system, there is little available information about amine metabolism.

This study was undertaken in rats to: 1/ evaluate the effects of hrGH by monitoring polyamine and amine metabolism parameters in the adapting short bowel and 2/determine whether erythrocyte polyamine concentrations reliably reflect the proliferative activity of the remaining bowel and may be suitable clinically to follow adaptation processes in children with short bowel syndrome.

Materials and methods

All procedures strictly followed the Polish legislation concerning animal experiments and were approved by the local animal ethics committee.

A 70% resection of the small intestine of Wistar rats was performed under ether anaesthesia leaving equidistant lengths of bowel from pylorus and ileocecal valve. Two controls were employed: rats with only transected small intestine and intact rats.

Recombinant human GH (0.2 IU, s.c., Saizen, Serono, Switzerland) was administered once daily for 5 or 10 days, to randomly selected rats from the second postoperative day onwards.

Animals were sacrificed by guillotine decapitation 8, 13 and 21 days after surgery. Trunk blood was collected for RBC polyamine measurements. Remnant small bowel was freed of attached mesentery, removed, its length and weight recorded for mucosal hyperplasia assessment. Distal ileum of a similar length was dissected from control rats and was handled in a similar manner. After thoroughly flushing with cold saline, the intestinal mucosa was scraped off. The liver, which is the highest source of monoamine oxidase enzymes, was also sampled. All tissue samples were frozen in liquid nitrogen and stored at −70°C until assayed for enzyme activities and amine and polyamine concentrations.

Biochemical analyses

Ornithine decarboxylase was measured with L [1-^{14}C]-ornithine, using the $^{14}CO_2$ trapping method (Kobayashi, 1963). Monoamine oxidase (MAO-A and MAO-B) activities were estimated in tissue homogenates with radioassays (Fowler and Tipton, 1982), employing serotonin (final concentration 200 μM) or beta-phenylethylamine (final concentration 20 μM) and specific inhibitors deprenyl and clorgyline (0.3 μM each), respectively. Diamine oxidase activity was assayed with ^{14}C putrescine (Fogel et al., 1985) and the polyamine oxidase against acetylspermine with homovanilic acid as a fluorogen (Matsumoto et al., 1984). All enzyme activities are expressed in pmol/min/mg protein.

Polyamines, spermidine (SPD) and spermine (SPM), after extraction from erythrocytes or intestinal mucosa with 0.4 M perchloric acid, were dansylated, separated by reversed phase HPLC using a LiChrosorb RP 18 (5 µm) column and quantitated by fluorometry as described by Mates et al., (1992). The concentration of polyamines in erythrocytes is given as nmol/ml of packed RBC, in tissue in nmol/g wet weight. Tissue histamine concentrations were determined by a fluorometric procedure after isolation on a small Cellex P column as described elsewhere (Fogel, 1988). DNA concentrations was assayed using the diphenylamine method (Burton, 1968). Protein was measured according to Lowry et al. (1951).

Statistical analysis

The values are presented as mean ± SD. Differences between groups were assessed with paired or unpaired t-tests as appropriate.

Results

Resection was a powerful stimulus for regenerative processes in the intestine. DNA concentrations and the mucosal hyperplasia index: weight/length ratio were significantly higher in operated vs control rats ($p < 0.01$ and $p < 0.05$, respectively). hrGH treatment, however, had no significant effect, although on 8th postoperative day, hormone-treated rat intestinal mucosa was somewhat thicker (Fig. 1a, b).

As shown in Fig. 2, red blood cell polyamine concentrations were significantly higher in operated versus control rats ($p < 0.01$ at least), with the exception of those belonging to R (resected) 21 days group, indicating that the adaptive growth of intestinal remnant is mirrored by circulating polyamine levels. No significant differences were noted between hrGH-treated and untreated operated rats, although the former tended to have higher polyamine concen-

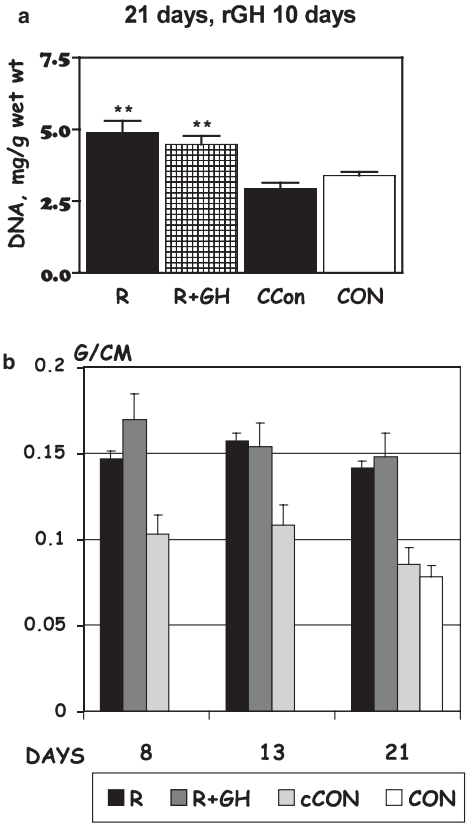

Fig. 1. Adaptive processes after massive small bowel resection; the effect of growth hormone treatment. Panel **a**: Concentration of mucosal DNA. Panel **b**: mucosal hyperplasia index: weight/length ratio. *R*, resection; *R + GH*, resection and growth hormone treatment; *Ccon*, bowel transection control; *CON*, intact rats

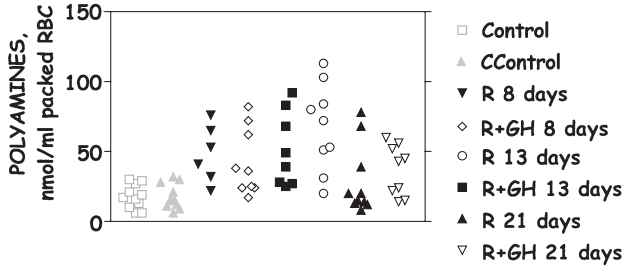

Fig. 2. Red blood cell polyamine concentrations during intestinal adaptation; the effect of growth hormone therapy. *R*, resection; *R + GH*, resection and growth hormone treatment; *CCon*, bowel transection control; *Control*, intact rats

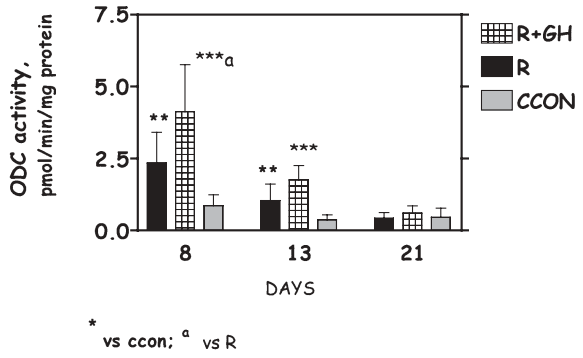

Fig. 3. Changes in the activity of ornithine decarboxylase, the enzyme synthesizing putrescine, polyamine precursor, in intestinal mucosa after massive small bowel resection; the effect of growth hormone treatment. *R*, resection; *R + GH*, resection and growth hormone treatment; *CCON*, bowel transection control; *con*, intact rats

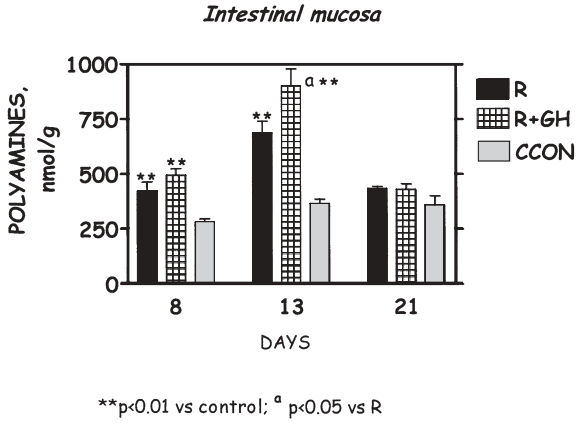

Fig. 4. Mucosal polyamine concentration in rat small intestine adapting after massive bowel resection; the effect of GH therapy. *R*, resection; *R + GH*, resection and growth hormone treatment; *CCon*, bowel transection control

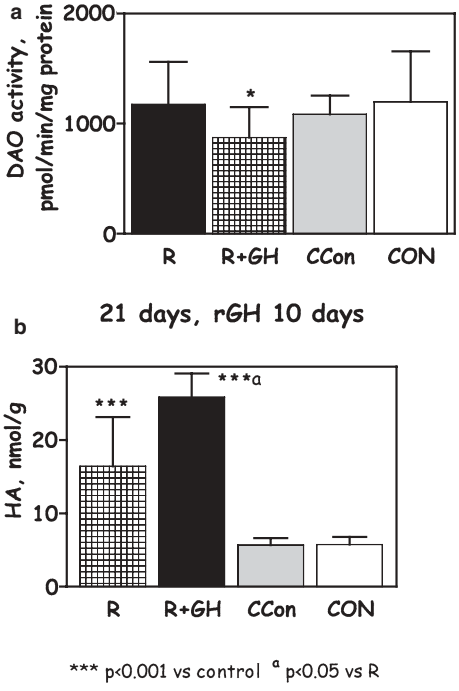

Fig. 5. Adaptational processes in rat small bowel after massive resection; the effect of growth hormone therapy. Panel **a**: diamine oxidase activity. Panel **b**: tissue histamine concentration. *R*, resection; *R + GH*, resection and growth hormone treatment; *CCon*, bowel transection control; *CON*, intact rats

trations. R + hrGH 21 values remained significantly different from the control.

Changes in the activity of ornithine decarboxylase, the enzyme synthesizing putrescine, polyamine precursor, in the intestinal mucosa after massive small bowel resection are depicted in Fig. 3. The additive stimulating effect of hrGH on ODC increases in the intestinal remnant was only seen during the early regeneration phase. Thereafter the two operated groups did not differ. The increases in the tissue ODC activities were followed by an increase in polyamine concentrations as illustrated in Fig. 4. hrGH treated rats have significantly ($p < 0.05$) higher polyamine concentrations than the untreated counterparts on 13th day, following the ODC peak. However, weeks after the surgery the polyamine levels were similar in all groups. Spermidine was the main polyamine in the intestinal mucosa, accounting for >90% (93.1 ± 0.07%) of total tissue polyamines in normal rat small bowel and slightly but not significantly less (88.3 ± 0.13) in regenerating bowel as measured on day 13th post surgery.

Growth hormone treatment had the opposite effect on the putrescine catabolizing enzyme, diamine oxidase, activity. Thus, operated rats that were treated with growth hormone for 10 days had significantly lower DAO activities on the 21st day post surgery, when regeneration processes were already accomplished (Fig. 5a). Reduced DAO activity in the intestinal mucosa coincided with a higher tissue concentration of histamine, the other DAO substrate (Fig. 5b). These animals also had significantly reduced MAO A and B activities in both the intestinal mucosa (Fig. 6a) and liver (Fig. 6b).

Discussion

Several studies have shown that growth hormone stimulates growth of different organs, including the

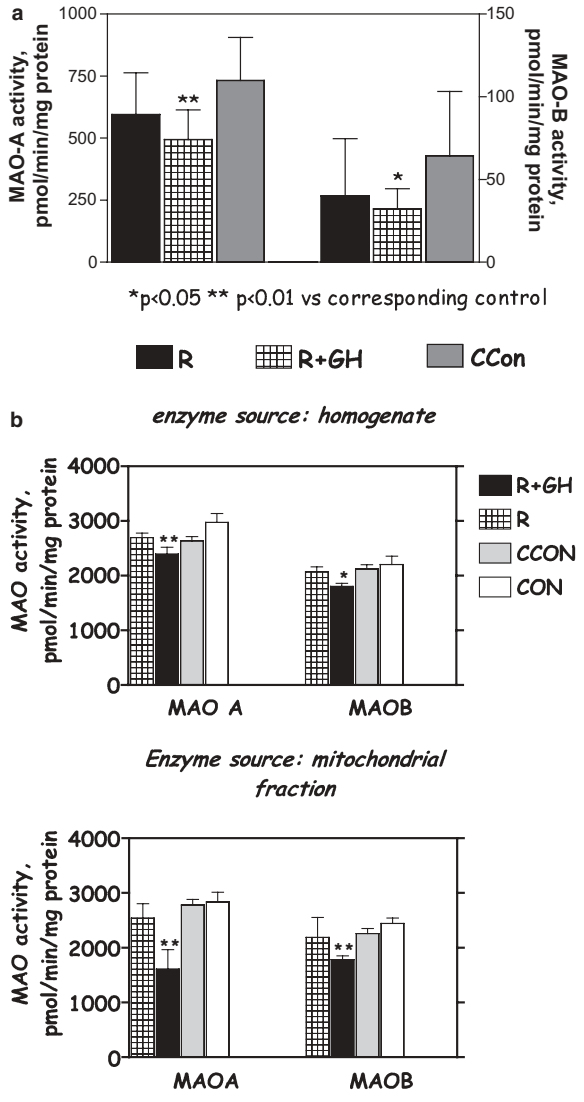

Fig. 6. Activities of monoamine oxidase A (MAO A) and B (MAO B) in after massive small bowel resection; the effect of growth hormone therapy. Panel **a**: intestinal mucosa; Panel **b**: liver. *R*, resection; *R + GH*, resection and growth hormone treatment; *CCon*, bowel transection control; *CON*, intact rats

small intestine (Kaplan, 1990; Leblond and Carriere, 1955; Lehy et al., 1986). Commercial availability of recombinant human growth hormone has triggered interest in its use as a therapeutic measure to support adaptive growth of the intestine after massive small bowel resection. The results of clinical and experimental studies that employed animal model of short bowel syndrome on the effect of GH therapy are inconsistent. Both positive effects and no effects in enhancing the morphological intestinal adaptation have been described (Shulman et al., 1992; Byrne et al., 1995; Liu et al., 1996; Socha et al., 1996; Scolapio et al., 1997; Vanderhoof et al., 1997; Ljungmann et al., 2000). However, it is well established that induction of ornithine decarboxylase, the key enzyme in polyamine synthesis, is a common cellular response to any trophic stimulus and therefore may be used as an index of the trophic response (Janne et al., 1978; Hosomi et al., 1987). In the present study, by monitoring ornithine decarboxylase activity (Fig. 3) at different periods following the resection and GH therapy we observed that at early stage of adaptation, growth hormone added positively to ODC peak with no further stimulatory effect on the enzyme activity later on. This higher ODC activity in hormone treated resected rats as compared to GH untreated counterparts could be regarded as acceleration of adaptative growth of the bowel remnant. It corresponded with the higher mucosal DNA concentration, although the latter did not attain statistical significance when compared with resected GH untreated rats.

We have shown here that intestinal hyperplasia triggered by massive gut resection was reflected by a significant increase in circulating polyamines, justifying use of erythrocyte polyamine concentration measurement as the marker of small bowel proliferative activity. However, growth hormone treatment did not further enhance it to a great extent, treated rats only tended to have higher RBC polyamine levels even if the intestinal polyamine concentration was significantly higher on day 13 (Fig. 2 and Fig. 4).

The finding that hormone treated animals expressed significantly lower activities of the amine oxidative deamination enzymes was unexpected. Taking into account that diamine oxidase activity reflects the maturational state of enterocytes (Baylin et al., 1978; Luk et al., 1980), the lower diamine oxidase activities in GH treated rats (Fig. 5a) may suggest that there are more less differentiated enterocytes in the intestinal epithelium of these rats. Although specific receptors are present in GI tract (Lobie et al., 1990), most of the enterotrophic effects of GH are thought to be mediated by insulin-like growth factor I (IGF-I) (Behringer et al., 1990; Ohneda et al., 1997). A comparison of IGF-1 transgenic mice (Ohneda et al., 1997) with those over expressing GH (Ulshen et al., 1993), led Ohneda et al. (1997) to conclude that while the effects on intestinal length and mass of both factors were similar, IGF-1 stimulates differentiation of intestinal epithelial cells less effectively. Thus, IGF-1 mediation might be a plausible explanation of reduced DAO activity. Of the FAD dependent amine

oxidases, monoamine oxidase A and B, MAO A is evenly expressed in mature and dividing cells but MAO B appears to be lower in crypt cells (Fogel and Maslinski, 1992). So while similar reasoning could be applied to MAO B, it does not explain lower MAO A activity in resected rats receiving hrGH. Moreover, the effect on MAO enzymes was not restricted to small bowel; it was also observed in the liver. Unlike cytosolic diamine oxidase, monoamine oxidases are expressed in mitochondrial outer membranes (Ramsay, 1998). Growth hormone effects on membrane lipid distribution (Clejan and Maddaiah, 1986) might affect the catalytic activities of monoamine oxidase enzymes (Buckman et al., 1983).

While in some cases the alteration in oxidative deamination of amines may produce unwanted reactions e.g. cheese reaction, in some others as for example in depression, could be of benefit.

It is tempting to speculate that GH exerts its effects on appetite, cognitive functions, memory, mood and other brain functions (Nyberg, 2000) at least in part by interfering with biogenic amine metabolism at the level of amine oxidases. However, no measurements were done in this study on brain tissue.

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