Modifications in ornithine decarboxylase and diamine oxidase in small bowel mucosa of starved and refed rats

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SUMMARY Starvation followed by refeeding, which provides a model of intestinal adaptation characterised by proliferative and biochemical changes, was used to clarify the biological roles of ornithine decarboxylase (ODC) and diamine oxidase (DAO) – enzymes involved in polyamines metabolism. Ornithine decarboxylase and DAO were assayed in the proximal and distal small bowel mucosa of 55 rats, starved for four days and then refed. Rats (five per day) were killed after four days' starvation and at days 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 of refeeding. ODC, whose specific activity was similar in both intestinal segments, almost disappeared after starvation and showed a biphasic response during refeeding. High values were found on day 3 of refeeding in the proximal, and on day 4 in the distal small bowel; thereafter, they decreased gradually to be followed by a further significant increase during the last two days of the experiment. Diamine oxidase specific activity *increased* after starvation despite a very low total DAO activity in both intestinal segments. Refeeding induced a gradual recovery of DAO total activity. Diamine oxidase specific activity also reverted gradually to control values after five days of refeeding. These data confirm the prominence of ODC in the replication processes and suggest that intestinal DAO may not play a major role in enterocyte replication.

Several models of intestinal adaptation are associated with biochemical and morphological changes in the small bowel mucosa. Starvation and refeeding – for example, cause marked modifications in the proliferative indices. In particular, starvation induces a marked fall in the crypt cell production rate and in plasma enteroglucagon levels, while refeeding reverses this pattern.¹

In this study we investigated the effects of starvation and refeeding on rat small bowel mucosal ornithine decarboxylase (ODC) and diamine oxidase (DAO) activities – enzymes involved in polyamine metabolism and therefore in cell replication.² The overall importance of intestinal DAO, however remains to be defined.³ In particular, the fact that it is located mainly in the ileum⁴ and is released directly from the enterocytes into the plasma by IV heparin^{5,6} casts doubts on the exclusivity of its role in enterocyte replication. The behaviour of these enzymes in this

experimental model may better clarify their role in the mechanisms involved in intestinal epithelial adaptation. Since the changes in rat jejunum and ileum in response to starvation and refeeding are different,¹ and as DAO activity is higher in the distal rat small bowel than in the proximal part,⁴ we measured the changes in ODC and DAO activities in both segments. Sucrase, a brush border enzyme which is linked to mature enterocytes and whose activity is rapidly affected by food intake,⁷⁻⁹ was also assayed.

Methods

ANIMALS

Sixty male Wistar rats weighing 240–280 g were used. They were divided into groups of five, housed in wire-bottomed cages to prevent coprophagia, and fed a pelleted diet (MINGNINI SpA, Italy). They were weighed daily and their food intake was recorded. For 10 days before the experiment began and throughout the experimental period, the animals were kept in

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constant light conditions to avoid rhythmic changes in food intake and possibly also in sucrase¹⁰ and ODC¹¹ activities.

EXPERIMENTAL DESIGN

All the other groups of rats with the exception of the control group, were starved for four days. One group was killed before refeeding (day 0); the other groups were killed at days 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 of refeeding.

ORNITHINE DECARBOXYLASE, DIAMINE OXIDASE AND SUCRASE ASSAYS

The rats were anaesthetised with diethyl ether and then decapitated. The small bowel was stripped of its mesentery from the ligament of Treitz to the ileocaecal junction and removed. The small intestine was next divided into two segments of equal length, gently washed with cold saline and opened longitudinally.

The mucosa was carefully scraped from each segment, homogenised with a Ystral GmbH homogeniser (West Germany) in 0.1 M/1 sodium phosphate buffer, pH 7.2, and centrifuged at 5000 g for three minutes at 4 °C, with a Beckman TJR centrifuge (USA).

Because of its short half-life¹² the ODC assay was carried out immediately to avoid loss of enzyme activity. The DAO, sucrase and protein assays were done within one week on supernatants stored at -20 °C. Centrifugation, which was of help in the ODC assay, resulted in a slight decrease in DAO and sucrase activities in the supernatant, with respect to the homogenate. Ornithine decarboxylase was assayed according to Djhuhrus,¹³ DAO as described in our previous study,⁴ sucrase by Dahlqvist's method¹⁴ and proteins by Lowry's method.¹⁵

Ornithine decarboxylase units are expressed as pM of putrescine released in one hour at 37 °C, pH 7.2; DAO units as nM of putrescine dihydrochloride oxidised in one hour at 37 °C, pH 7.2; sucrase as μ M of glucose released in one minute at 37 °C, pH 7.4.

STATISTICAL ANALYSIS

A two tailed unpaired Student's *t* test was used. Results, expressed as mean $\pm$ SD were considered to be statistically significant when $p < 0.05$.

Results

FOOD INTAKE

The mean food intake for each rat before starvation was 25.0 ± 4.3 g/day. The mean food intake after refeeding was 25.3 ± 3.1 g/day.

BODY WEIGHT

The mean initial body weight of the rats (262 ± 13 g)

Figure Changes in mucosa wet weight, ODC specific activity, DAO specific activity, DAO total activity, and sucrase specific activity in proximal (●) and distal (○) rat small bowel during four days starvation and after refeeding - mean values $\pm$ standard deviations.

decreased to 204 ± 12 g ($p < 0.001$) after four days starvation. Following refeeding, however, the mean body weight increased steadily returning to basal values after five days' refeeding. It then slowly increased until day 8 but remained unchanged thereafter. The results for small bowel mucosal weight, ODC, DAO and sucrase, are shown in the Figure.

MUCOSAL WEIGHT

The mean mucosal wet weight (MWW) at the beginning of the experiment was 2.7 ± 0.7 g in the proximal, and 2.4 ± 0.5 g in the distal, small bowel and decreased to 1.4 ± 0.05 g and 1.3 ± 0.1 g ($p < 0.001$) respectively, after starvation. After refeeding, the MWW increased consistently, returning to basal values after three days in the proximal, and after seven days in the distal, segment and reached a peak at day 10. At this time, MWW showed a marked increase with respect to control values in both segments, but this was significantly higher ($p < 0.001$) in the proximal (59.8%), than in the distal, segment (20.1%)

ORNITHINE DECARBOXYLASE

After starvation, and during the first two days of refeeding ODC specific activity (sa) was almost zero in both intestinal segments, but a significant increase ($p < 0.001$) was recorded on days 3, 4, 5. The peak value (109 ± 20 U/mg) was found on day 3 in the proximal and on day 4 (156 ± 41 U/mg) in the distal small bowel. Thereafter, the values decreased gradually so that by day 6, an ODC sa very similar to that in control group (proximal: 36.7 ± 4.5 U/mg; distal: 16.5 ± 7 U/mg), was found. Then, over the last two test days of the experiment, a new and significant increase ($p < 0.001$) occurred in both the proximal (peak value: 150 ± 25 U/mg) and distal (peak value: 101 ± 45 U/mg) small bowel mucosa.

DIAMINE OXIDASE

Diamine oxidase sa increased after starvation more so in the proximal (4.0 ± 0.8 U/mg) than in the distal small bowel (4.3 ± 0.5 U/mg). Control values were 2.6 ± 0.5 U/mg in the proximal and 3.8 ± 0.5 U/mg in the distal tract. Nevertheless, DAO *total* activity decreased sharply in both segments after starvation, the percentage decrease being higher in the distal (47.5%) than in the proximal (26.2%) small bowel. During the first three days of refeeding, DAO sa remained high in the distal but decreased in the proximal segment and then returned to control values. On days 5 to 8, DAO sa was higher than control values – mainly in the distal small bowel mucosa. While DAO sa was consistently higher in the distal than in the proximal intestinal tract, DAO *total* activity was comparable in both study segments during the first five days of refeeding. Then, in the following days, higher activities were found in the distal than in the proximal tract.

SUCRASE

After four days' starvation, sucrase sa decreased significantly ($p < 0.001$) in both the proximal (0.075 ± 0.01 U/mg; basal values: 0.12 ± 0.03 U/mg) and distal tract (0.055 ± 0.01 U/mg; basal values: 0.065 ± 0.01 U/mg). After the first day of refeeding, however, the sa of this enzyme increased promptly to exceed basal values on day 2; it then returned to control values on day 3 and remained unchanged thereafter.

Discussion

In this study, the behaviour of ODC in the proximal and distal small bowel is, in some ways, similar to that already described in other experimental models of intestinal adaptation.¹⁶ The modifications in DAO activity are considerable however and suggest that this enzyme is not exclusively involved in cell

replication. Four days' starvation led to a loss of body weight and a reduction in small bowel MWW. After this period, when there is known to be a marked decrease in crypt cell production rate (CCPR) and in plasma enteroglucagon,¹ in both segments studied ODC sa approached zero while DAO sa was significantly increased. Nevertheless, DAO *total* activity was clearly diminished when compared with that found in normally fed rats. Similar results for DAO sa were seen in rats treated with difluoromethyl ornithine, a specific and irreversible ODC inhibitor.¹⁷

These data indicate that the reduction in MWW induced by starvation is associated with almost zero levels of ODC sa. This finding is consistent with the belief that ODC plays a primary role in cell replication. Conversely, the increase in DAO sa and the marked fall in DAO *total* activity suggest that DAO is spared more than other proteins and this argues against an exclusive role for DAO in cell proliferation. ODC sa showed two rises after refeeding – the first within a few days and a second towards the end of the experiment, when a parallel increase in MWW was also recorded. The changes observed over the last few days of the experiment were more intense in the proximal than in the distal tract; further studies are necessary to establish whether these changes are an expression of mucosal hyperplasia. It is interesting to note that a biphasic trend of ODC has been described previously in an experimental model of intestinal carcinogenesis.¹⁸ The present data, therefore, may strengthen the connection between intestinal hyperplasia and carcinogenesis.¹⁹ Throughout the experiment, the changes in DAO sa did not correspond with modifications in any of the other parameters: in particular, the changes in DAO sa were completely different from those for ODC sa. DAO *total* activity was higher in the distal tract than in the proximal, intestinal tract. However, comparable levels of DAO *total* activity were found in both intestinal segments after starvation and during the first few days of refeeding, and this is consistent with a more marked loss of DAO activity in the distal small bowel. Nonetheless, DAO sa was constantly higher in the distal than in the proximal small bowel because of a faster protein recovery in the proximal tract.

The DAO changes in this study were different from those described in other studies in which different models of intestinal adaptation were used.¹⁶ This is probably explained by the fact that during starvation and refeeding, the modifications in cell replication are different from those induced by small bowel resection or chemotherapy.

Sucrose sa decreased during starvation but showed a prompt recovery during refeeding. These findings

confirm that sucrase activity is strictly dependent on food intake – as concluded by others.⁷⁻⁹

In conclusion, our data confirm that ODC plays a specific role in enterocytic replication processes and shows a biphasic response. The first rise is connected with mucosal recovery and the second, probably with mucosal hyperplasia. Conversely, in this experimental model, DAO behaviour was not in keeping with its supposed exclusive role in enterocyte replication.

One suggested function of this enzyme in the small bowel is in the regulation of exogenous and endogenous amine (histamine and putrescine) concentrations. This hypothesis, supported by the unusual intestinal location of DAO and by its releasability by heparin from enterocytes at the villous tips, requires further study.

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