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INDUCTION OF ORNITHINE DECARBOXYLASE IN COLON AND LIVER BY STARVATION AND REFEEDING: A COMPARISON OF EFFECTS ON TOTAL AND HOLOENZYME

Bruce A. Stanley and Michael N. Kazarinoff

Division of Nutritional Sciences and the
Section of Biochemistry, Molecular and Cell Biology of the
Division of Biological Sciences, Cornell University
Ithaca, New York 14853

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In rats, short-term starvation and subsequent refeeding induced both holo- and total ornithine decarboxylase (EC 4.1.1.17) levels in colon 5-fold over ad lib fed controls. Starvation alone led to a significant decrease in total ornithine decarboxylase level in colon, while no change was seen in the holoenzyme level. There are only two reported short-term chemical inducers of colon ornithine decarboxylase, and this is the first demonstration of dietary induction in this tissue. Liver total ornithine decarboxylase was also induced by starvation-refeeding, but no holoenzyme effects were seen in any treatment.

INTRODUCTION

Ornithine decarboxylase (ODC), a pyridoxal 5'-phosphate (PLP)-dependent enzyme, catalyzes the rate-limiting step in the synthesis of the polyamines, putrescine, spermidine, and spermine (1). An increase in both ODC activity and polyamine concentration is one of the first events seen during induction of rapid growth or regeneration in a variety of tissues (2), and inhibition of growth of carcinoma cells in culture after inhibition of ODC by the specific suicide inhibitor, α-difluoromethyl ornithine, suggests that this increase in ODC activity is not coincidental but is, in fact, a necessary step for rapid growth (3). The polyamines are known to affect both transcription (4) and translation (5). Recent preliminary evidence indicates a direct involvement of ODC in transcription of rRNA in Physarum polycephalum (6).

There are many known short-term inducers of liver ODC, including chemical, surgical, and hormonal treatments (1), all of which yield a peak of ODC activity 3-5 hr after application; however, intrarectal instillation of bile salts or N-methyl-N-nitro-N-nitrosoguanidine (MNNG) (7) are to date the only reported

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short-term inducers of colon ODC. Since previous studies have found increased ODC activity in liver, small intestine, and skeletal muscle after starving and refeeding (8-10), we investigated the effects of starving-refeeding on levels of both total and holo-ODC in the colon and compared these induction effects with those in the liver. Starvation and refeeding significantly induced colon ODC in a different pattern than that found in the liver.

MATERIALS AND METHODS

Chemicals and biochemicals were the purest available from commercial sources. L-[1-^{14}C]Ornithine·HCl (59 μCi/μmol, 0.847 mM in 2% ethanol) was obtained from Amersham. Male, Sprague-Dawley rats weighing between 230 and 275 g were obtained from Blue Spruce Farms (Altamont, NY). Rats were randomized by weight into starved-refed, starved, or ad lib control groups. All rats were individually housed in stainless steel cages, with a 7 a.m. to 7 p.m. light cycle. A lab chow diet was fed for five days, at which point differential treatments began. At the time of sacrifice, starved-refed rats had been starved for 48 hr, refed for 12 hr, and again been without food for the 6 hr previous to sacrifice. Starved rats had been without access to food for 66 hr, and ad lib rats had been without access to food for the previous 6 hr. Rats were sacrificed by cervical dislocation between 1:30 and 2:30 p.m. The colons were excised, immediately washed with ice-cold normal saline, slit longitudinally, placed on an ice-cold glass plate, washed again with saline, and blotted lightly. The colonic mucosa was then scraped off with a glass microscope slide, and the scrapings (~0.2 g) were placed into 2 ml of freshly prepared ice-cold Buffer A (0.1 M sodium phosphate, pH 7.4, 4 mM dithiothreitol, 2 mM EDTA). Each sample was homogenized 15 sec at setting 7 on a Brinkmann Polytron® equipped with a microprobe. Samples were kept in the dark on ice until being centrifuged at 100,000×g for 1 hr at 4°C. Livers were also immediately excised; 1 g of the left lobe was placed in 3 ml of Buffer A, homogenized for 30 sec on setting 7 of a Polytron®, and stored on ice in the dark until being centrifuged at 30,000×g for 1 hr at 4°C. After filtering through glass wool, both colon and liver supernatants were stored at -70°C until assay.

Ornithine decarboxylase activity was determined by measuring $^{14}CO_2$ released from radioactive ornithine. To reduce blank values, commercial L-[1-^{14}C]ornithine was acidified with 6 N hydrochloric acid in the presence of a CO_2 trapping agent, barium hydroxide, suspended in a center well (Kontes) and then neutralized with sodium hydroxide. The stock ornithine mixture was 0.58 mM L-[1-^{14}C]ornithine in Buffer A. The assays were run as follows: 0.2 ml of thawed supernatant was placed in a 16×100-mm glass test tube on ice with 0.18 ml of the appropriate ice-cold buffer. Buffer A was used to assay holo-ODC activity. For measuring total ODC activity, 0.22 mM PLP was added to Buffer A. A doubled 3.2×2-cm filter paper wick was wedged into the top of the tube. The mixture was preheated at 37°C at 110 rpm in a Metabolyte shaker bath for 15 min. L-[^{14}C]Ornithine stock (20 μl) was added to start the reaction, and 20 μl of saturated barium hydroxide solution was pipetted onto the filter paper wick. The tubes were capped with rubber stoppers and placed in the shaking bath. After 60 min, the reaction was stopped by the injection of 0.1 ml of 100% trichloroacetic acid. After microdiffusion was allowed to occur for 45 min, the filter paper was removed with tweezers and placed in 10 ml of scintillation fluid (3.78 ℓ toluene, 15 g 2,5-diphenyloxazole, 0.4 g 1,4-bis[2-(5-phenyloxazoyl)]benzene) and counted in a Beckman LS7000 counter. A zero-time blank was obtained by injecting 100 μl of 100% trichloroacetic acid into the tubes just before adding the reaction mixture and incubating this mixture for 1.75 hr. Since the holoenzyme and total enzyme buffers yielded identical zero-time values, one average zero-time value was subtracted

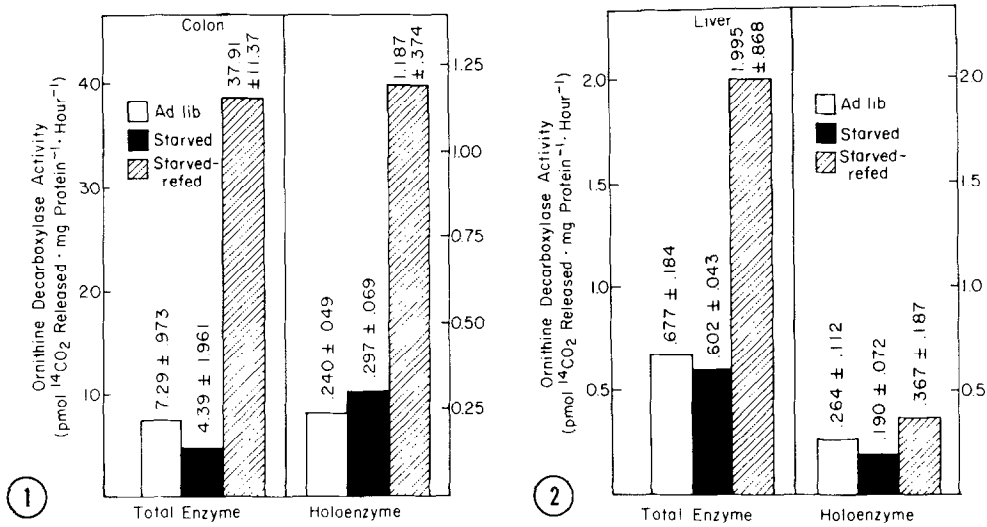

Figure 1. Colon: Total and Holo-ODC Levels After Starvation or Starvation-Refeeding. Rats were fed a lab chow diet as outlined under Methods. Starved-refed rats (n=6) were starved 48 hr, refed 12 hr, and had food removed again 6 hr prior to sacrifice by cervical dislocation. Starved rats (n=5) had been starved 66 hr at sacrifice, while ad lib control rats (n=6) had access to food until 6 hr prior to sacrifice. Numerical values indicated are means ± S.E.M.

Figure 2. Liver: Total and Holo-ODC Levels After Starvation or Starvation-Refeeding. Conditions were the same as indicated for Fig. 1, except that n=5 for the starved group.

from both holo- and total enzyme assays. Protein was determined by the biuret method for the livers (11) and by the Lowry method for the colons (12). All statistical analyses were done using Student's t-test (13).

RESULTS AND DISCUSSION

Starving and refeeding significantly ($p<0.03$) induced both the total and holo-ODC levels in the colon (Fig. 1). This is the first demonstration of induction of ODC in colon by dietary treatment. The induction is not as great as the previously reported 20-fold increase after intrarectal instillation of bile salts or MNNG (7), but it is greater than the reported daily increase in small intestine after a meal (10). The significant ($p<0.04$) decrease in total ODC in colon after brief starvation is similar to previously reported effects in the liver (8). No effect of starvation was seen in colon holo-ODC.

In the liver (Fig. 2), total ODC level changes were similar to the changes seen in colon, with starvation-refeeding inducing ODC 3-fold ($p<0.005$). The levels dropped upon starvation alone but not significantly. Neither starvation-refeeding nor starvation alone had any effect on the holo-ODC levels in liver,

unlike in the colon where both total and holo-ODC levels were induced by starvation-refeeding. It is important to note these differences in holo- vs. total levels of the enzyme, since a 5-fold increase in the total ODC in a tissue may or may not be accompanied by any changes in functional ODC levels.

The observed 3-fold increase in total liver ODC is lower than the 7-fold increases previously reported (8,9). This difference may be explained by differences in the time at which the ODC induction was measured. In the previous studies, ODC was measured between 3 and 5 hr after initiation of refeeding. Other work has shown a daily increase in liver and small intestine ODC levels 3-5 hr after a meal, regardless of whether the rats had been previously starved or had constant access to food (10,14). This effect in liver, and the possibility of a similar effect in colon, confounds measurements taken at early time points. Since we measured ODC levels after 12 hr of refeeding and an additional, short (6 hr) period without access to food, the 3-fold increased observed probably reflects specific starvation-refeeding effects divorced from immediate feeding effects.

It is known that starvation decreases protein and RNA synthesis (15), and refeeding may simply lead to a compensatory increase in general macromolecular synthesis which is reflected first in proteins, such as ODC, which have shorter half-lives and, hence, reach new steady state levels most rapidly (16). On the other hand, there is evidence that tumor promotion leads to an increase in levels of specific proteins and not to an increase in general synthesis (17). It will be important to see if the increase in ODC caused by starving-refeeding is a specific induction or simply reflects an increase in general protein synthesis. Since bile salts are suspected tumor promoters in colon (18), a comparison between bile salt and starving-refeeding effects on ODC levels should provide further information which will answer this question.

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