Rat colon ornithine and arginine metabolism: coordinated effects after proliferative stimuli

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Han, Xiaoli, Michael N. Kazarinoff, Nikolaus Seiler, and Bruce A. Stanley. Rat colon ornithine and arginine metabolism: coordinated effects after proliferative stimuli. Am J Physiol Gastrointest Liver Physiol 280: G389–G399, 2001.—Ornithine decarboxylase (ODC) catalyzes the first step in the polyamine biosynthetic pathway, a highly regulated pathway in which activity increases during rapid growth. Other enzymes also metabolize ornithine, and in hepatomas, rate of growth correlates with decreased activity of these other enzymes, which thus channels more ornithine to polyamine biosynthesis. Ornithine is produced from arginase cleavage of arginine, which also serves as the precursor for nitric oxide production. To study whether short-term coordination of ornithine and arginine metabolism exists in rat colon, ODC, ornithine aminotransferase (OAT), arginase, ornithine, arginine, and polyamine levels were measured after two stimuli (refeeding and/or deoxycholate exposure) known to synergistically induce ODC activity. Increased ODC activity was accompanied by increased putrescine levels, whereas OAT and arginase activity were reduced by either treatment, accompanied by an increase in both arginine and ornithine levels. These results indicate a rapid reciprocal change in ODC, OAT, and arginase activity in response to refeeding or deoxycholate. The accompanying increases in ornithine and arginine concentration are likely to contribute to increased flux through the polyamine and nitric oxide biosynthetic pathways in vivo.

ornithine decarboxylase; arginase; ornithine aminotransferase; tumor promoters; polyamines

INCREASES IN INTRACELLULAR polyamine levels and in the activity of ornithine decarboxylase (ODC), one of the rate-limiting enzymes in the tightly controlled polyamine biosynthetic pathway, are associated with rapid growth states, including tumorigenesis (for review, see Refs. 27, 46, and 64). There is also evidence (2, 41) that the enzyme acts as an oncogene. A variety of tissues, including the colon (40), show elevated ODC activity in tumors compared with normal neighboring tissue.

Previous studies (56) have shown a synergistic induction of ODC activity in rat colon after intrarectal instillation of the colon tumor-promoting bile salt sodium deoxycholate (NaDOC) and starvation and refeeding. In addition to changes in enzyme activity, another possible influence on intracellular polyamine levels, and one given considerably less attention in most studies, is the changing concentration of ornithine, the substrate of ODC. The normal ornithine concentration in tissues is considerably below the Michaelis-Menten constant ($K_m$) of ODC, so that ODC is operating far from maximum velocity ($V_{max}$). The consequence of this is that a change in the intracellular ornithine level will cause a change in flow through the polyamine biosynthetic pathway and that changes in other enzyme activities affecting the ornithine level may also affect flux through the polyamine biosynthetic pathway and ultimately polyamine levels themselves.

Several metabolic pathways affect ornithine levels. The major mammalian source of ornithine is from the cleavage of arginine to ornithine by the enzyme arginase, which exists in at least two tissue-specific, genetically distinct forms (55). The pathways of further ornithine metabolism include the above-mentioned ODC reaction, the conversion of ornithine to glutamate semialdehyde catalyzed by the enzyme ornithine aminotransferase (OAT), and the urea cycle enzyme ornithine transcarbamylase (OTC). It has been previously reported (60, 66), in a series of established slow- to fast-growing hepatomas, that growth rate was correlated to increased ODC activity and to decreased OAT and OTC activity, indicating a stable alteration of ornithine metabolism that would channel relatively more ornithine into the polyamine biosynthetic pathway in the faster growing hepatomas. The basal activity of OAT is reported to be five to ten times higher than that of ODC in the colon and in many other tissues (18, 38, 56, 57), and increased cellular levels of ornithine are seen in human patients with OAT defi-
ciencies (61), as well as in adult mice in which OAT activity is inhibited (48). Therefore a decrease in OAT activity could contribute considerably to an increase in the concentration of ornithine available for the polyamine biosynthetic pathway. Such a result was reported (25), in which the kidneys of testosterone-treated female mice showed decreased OAT activity and elevated ornithine levels, as well as elevated ODC and arginase activity, within 5 days after testosterone treatment. The arginase activity increase would also contribute to elevated ornithine but would tend to decrease arginine availability for nitric oxide (NO) production.

Conflicting results have been reported regarding the involvement of NO and arginase activity in growth of established tumors. Skin tumors with elevated ODC activity have also been shown to have elevated ornithine levels as a result of constitutively elevated arginase activity (compared with normal surrounding skin) (21) and some gastric adenocarcinomas have also shown elevated arginase activity (67). Conversely, increased arginase activity has been shown to inhibit NO production (14), whereas NO has been shown to inhibit apoptosis (22), a crucial part of the proliferative response in establishment of colon tumors (5). Specific inhibition of the inducible form of NO synthase (iNOS or NOS2) has also been shown to slow the growth of solid tumors in vivo (59), and the balance of arginine metabolism between NO and polyamine production has been hypothesized to have a role in angiogenesis during establishment of melanomas (20).

Such alterations in ornithine and arginine metabolism might be a downstream result of the transformation of a normal cell to a tumor phenotype, or the alterations might be crucial components of such a transformation. If such effects are crucial components of the transformation process or the cell proliferation that accompanies tumor promotion, one would expect to see them occur rapidly in response to tumor promotion. With this in mind, the present study tests whether aspects of such short-term coordination of ornithine and arginine metabolism occur in rat colon in response to the proliferative stimulus of refeeding after 2 days of food deprivation or exposure to the tumor-promoting bile salt NaDOC.

MATERIALS AND METHODS

Animals and treatments. Male Sprague-Dawley rats were acclimatized for 14 days before treatment, at which point they weighed 200–280 g, and weight-matched animals were randomly assigned to treatment groups. Animals were fed standard rodent chow (Charles River), which has been shown in other studies (43) to contain (in nmol/g) ~230 putrescine, 560 spermidine, and 110 spermine. The arginine content of the diet has been measured by the manufacturer at 1.07%, but the ornithine content has not been measured.

For starved-refed (S/R) animals, food was removed from the individual cages at the start of the dark cycle and replaced 48 h later (time 0). NaDOC (0.5 ml of 24 mM in 0.15 M saline) was intrarectally instilled with a Foley catheter either at the start of the dark cycle (time 0) or at 8 h into the dark cycle refeeding period. To measure colon protein synthesis rates, animals were injected intraperitoneally with 10 µCi [3,5-3H]leucine in 0.15 M NaCl/100 g body wt (American) 33 min before death. Incorporation of radioactivity into TCA-precipitable protein in colonic mucosa is linear from 10 to 40 min under these conditions. Animals were killed by cervical dislocation. All animal treatments were designed to avoid unnecessary pain, and all protocols conformed to institutional animal welfare guidelines.

Chemicals. Chemicals were of the purest grade commercially available and were obtained from Sigma Chemical (St. Louis, MO), Aldrich (Beere, Belgium), Baker Chemicals (Deventer, The Netherlands), or E. Merck (Darmstadt), unless otherwise noted. Polymamines for standards were from Fluka (Buchs, Switzerland), whereas the acetylated polyamines, 2-(difluoromethyl)-ornithine, 4-acetylenic GABA, and 1,8-diaminooctane-2HCl were synthesized at Merrell-Dow (Strasbourg, France). α-Pthaldialdehyde was from Eastman-Kodak (Rochester, NY). dl-[1-14C]Orotidine (57 µCi/µmol) was from Amersham. Radioactivity was counted in Aquasol-2 or Econofluor from New England Nuclear (Wilmington, DE).

Tissue preparation. After death, the colon was immediately excised from the cecum to the rectum, and fecal matter was flushed out from the cecum to the rectum by a stream of ice-cold 0.15 M saline. Each colon was slit longitudinally opposite the normal medial line of attachment to avoid fatty material. The colon was then spread on an ice-cold glass plate, and the mucosa was rewash to remove all fecal matter.

After the second saline wash, the colons were lightly blotted to remove all saline, and the mucosal layer was scraped off with the edge of a microscope slide. The scrapings were transferred to a preweighed slide, which was then reweighed to determine the weight of the mucosal scrapings. The scrapings were quickly homogenized in ice-cold buffer A (10 mM sodium phosphate, pH 7.4, 4 mM dithiothreitol, and 0.2 mM EDTA) in a machine-driven Thompson Dounce-type homogenizer. Mucosal homogenates were immediately aliquoted as follows.

For polyamine determination, 400-µl aliquots were mixed with 20 µl of 4.2 M HClO4. Samples were centrifuged 15 min in a desktop Microfuge, and supernatants were kept at 4°C until HPLC analysis.

For amino acid and acetylputrescine determinations, 400-µl aliquots were mixed with 40 µl of 2% NaDOC on ice, then 2 ml of 10% TCA were added and vortexed. After 15 min centrifugation at 20,000 g, the supernatants were stored at 4°C until HPLC analysis.

For protein and OAT activity determinations, 50- and 400-µl aliquots, respectively, were frozen in liquid nitrogen and stored at −70°C until analysis. For radiolabeled leucine incorporation into TCA-precipitable protein, 400-µl aliquots were immediately mixed with 40 µl of 2% NaDOC and were incubated on ice for 3 min while other aliquots were taken, and then 2 ml of 10% (wt/vol) TCA were added and mixed well. For ODC activity determinations, remaining homogenates were kept on ice until the end of each death period. After 90 min centrifugation at 100,000 g and 4°C, the supernatants were stored at −70°C until assay. The total time between death and complete aliquoting was <6 min for each animal.

In additional experiments measuring only enzyme activities, a small aliquot of crude homogenates was taken for protein determination. The remainder was frozen in four aliquots at −70°C until assay. One hundred micrograms of protein were assayed as detailed below.
Assay procedures. Total ODC activity was measured in triplicate in a final volume of 400 μl containing 0.11 mM pyridoxal-5'-phosphate, 1.1 mM 4-acetylenic GABA, 4 mM dithiothreitol, and 0.2 mM EDTA in 20 mM sodium phosphate, pH 7.2. 14CO2 released from 1 μCi of DL-[1-14C]ornithine per sealed assay tube was captured by 20 μl of hyamine hydroxide (New England Nuclear) on filter paper. After 2 h at 37°C and 110 rpm, reactions were stopped by the addition of 0.5 ml of saturated citric acid, and tubes were shaken for an additional 45 min. Filter papers were counted in 10 ml of Aquasol-2 plus 100 μl of ethanolamine.

The use of undiluted radiolabeled ornithine at a concentration below Km improves the sensitivity of the ODC assay; however, the amount of enzyme activity is then directly related to fractional conversion (v/S), in which v is the initial velocity of the reaction and [S] is the initial substrate concentration (14CO2 released/initial substrate concentration). The Michaelis-Menten equation denominator. All ODC activities reported are calculated with the actual measured ornithine concentration plus the concentration of ornithine added in the substrate buffer (200 mM glycine-NaOH, pH 9.5, 40 mM unlabeled arginine, and 50 mM MnCl2) and 0.8 μCi guanido-[14C]arginine added just before assay. After the homogenate was added to start the reaction and the tubes were capped, the tubes were then shaken at 37°C for 90 min and boiled for 5 min. 14CO2 was then released from the [14CO2]urea produced in the first part of the reaction by adding 1.6 ml of 0.2 M potassium phosphate (pH 6.6) containing 2 U of jack bean urease, quickly capping the tube with a center well containing 300 μl of hyamine hydroxide to capture released 14CO2, and incubating for 1 h shaking at 37°C. The reaction was stopped by the addition of 0.2 ml of 0.1 N HCl injected through the cap septum, and the additional 14CO2 released by the acidification was collected for another 30 min shaking at 37°C. The center well with the hyamine hydroxide was removed and counted in 10 ml of Econofluor.

Polyamines were determined in 0.2 M HClO4 supernatants by the HPLC separation of their ion pairs with dodecyl sulfate (50). Every eighth run was an external standard used for quantitation.

All protein determinations were performed according to the method of Lowry et al. (23) Duplicate 100-μl aliquots of frozen crude homogenates were precipitated in 0.2 M HClO4, then separated for DNA, RNA, and protein determination as previously reported (53). The DNA determinations from the resulting pellet were performed according to the diphenylamine method of Burton (4) and quantified by comparison with a standard curve of 1–10 μg deoxyribose. Rates of protein synthesis (leucine incorporation into TCA precipitable protein) were calculated by adding an HPLC determination of total leucine concentration to the basic method of radiolabeled leucine incorporation into protein of Watts and Wheldrake (65).

Statistical analyses. For direct comparisons of the effects of starvation (without refeeding) to ad libitum-fed animals (time 0 only), Student’s two-tailed t-test was used. All other comparisons were initially done by ANOVA (2- to 18-h S/R vs. control animals or 4- or 12-h S/R vs. control vs. NaDOC ± S/R). Where the ANOVA F test indicated significant overall differences (P < 0.05) between groups, individual groups were then compared by the Student-Newman-Keuls post-ANOVA multiple comparisons test or, for time series comparisons, by paired t-test (2 tailed). Wherever the initial data analysis indicated that the assumption of equal sample variances could not be met, the nonparametric Mann-Whitney U test (2 tailed) was used instead of parametric statistics.

RESULTS

Macromolecular synthesis. Starvation greatly reduces macromolecular synthesis in the colon, whereas refeeding causes a rapid, synchronized entry of mouse colon cells in S phase (12, 15). Although the DNA-to-protein ratio in colonic mucosa (Fig. 1) is not a direct measure of DNA synthesis, the significant change in
the DNA-to-protein ratio at 2 h in the refed animals (Mann-Whitney U test, \( P = 0.03 \)) is probably indicative of a similar rapid entry of starved cells into S phase on refeeding. Although the starvation period depressed the incorporation of \([3H]\)leucine into protein by \(\sim 40\%\) (time 0 animals), there was no significant difference in the incorporation of \([3H]\)leucine between the control and refed animals at any other time point, whereas the NaDOC-treated animals actually showed slightly less incorporation than control animals (results not shown).

**ODC activity.** Not unexpectedly, starvation significantly depressed ODC activity (Mann-Whitney U test, \( P = 0.002 \) vs. control) as seen in Fig. 2, but the levels rose quickly above those of controls on refeeding, at the same 2-h time point at which colon cells appeared to be synchronously entering S phase. The refeeding was done at the start of the dark period, and again as seen in Fig. 2, there is a peak of ODC activity in the ad libitum-fed controls at 4 h as well. Such a peak of ODC activity 4 h after the start of the dark (heavier feeding) period in ad libitum-fed control animals has been previously reported (16, 29) for rat liver and small intestine. Thus the major effect of refeeding at the early time points seems to have been to make the postprandial peak appear more rapidly, at 2 h after refeeding as opposed to 4 h after normal feeding, and to quickly overcome the depressive effect of starvation on colon ODC activity. However, in the refed animals a second broad peak of activity appears, and from 9 to 18 h refed animals consistently had 1.5- to 2-fold higher ODC activities than control animals. This activity was further increased by concomitant NaDOC exposure, i.e., as previously reported (56) and as seen in Fig. 3, there was a synergistic effect between 12 h of refeeding and 4 h exposure to NaDOC. Because there was no increase in general protein synthesis rate (see above), the observed increases in ODC activity cannot result from general increases in overall protein synthesis rate but must result instead from specific increases in ODC.

*Polyamine levels.* As seen in Fig. 4, putrescine levels in both controls and S/R animals increased in parallel with the initial increases in ODC activity. Although putrescine levels in refed animals are significantly higher overall from 2 to 18 h after refeeding (\( P = 0.016 \)), the putrescine levels at the later time points were only slightly above controls, whereas the refed ODC activity at these points remained significantly elevated. The higher ODC activity levels observed at 12 h in either refed or ad libitum-fed animals treated with NaDOC are also reflected in parallel significant increases in putrescine in these groups. Both spermine and spermidine were significantly depressed in starved animals compared with controls (time 0), and although increased after refeeding, remained significantly lower overall (\( P < 0.01 \), Mann-Whitney U test) throughout the time period of 2–18 h. This may indicate a continued depression of the enzymes (e.g., spermidine and spermine synthase) needed to further metabolize the putrescine to spermidine and spermine and would also oppose the idea that the relatively low putrescine-to-ODC ratio seen at later time points stems from putrescine-to-spermidine conversion. Alternate explanations for this low putrescine-to-ODC ratio include increased putrescine secretion from cells at these time points or increased diamine oxidase (DAO) activity caused by refeeding. DAO is known to be active in colonic mucosa and has been proposed to have a role in the control of putrescine, cadaverine, and histamine levels, all bioactive amines reported (31, 49, 52) to have roles in intestinal cell proliferation. The fact that NaDOC treatment at either time 0 or time 8 led to a large increase in histamine concentration 4 h later but previous refeeding attenuated this response to NaDOC at 12 h (results not shown) is consistent
with an increase in DAO activity at later time points after refeeding, although this activity was not measured in the present experiments.

\(N^1\)-acetyl spermidine was slightly but not significantly higher in the starved animals (time 0, Fig. 5, top). On refeeding, the \(N^1\)-acetyl spermidine levels of the S/R animals dropped, as did the levels for the ad libitum-fed control animals as they entered the heavier feeding dark period; unlike in control animals, however, \(N^1\)-acetyl spermidine values in refeed animals continued to drop throughout almost the entire measurement period. In contrast to \(N^1\)-acetyl spermidine levels, acetyl putrescine was significantly depressed in starved animals compared with controls but quickly doubled in concentration by 2 h, accompanying the increase seen in putrescine concentration (Figs. 4 and 5, bottom). Because the enzyme that acetylates putrescine is a nuclear enzyme (47), this may indicate that more putrescine was entering the nucleus at 2 h postrefeeding and may be linked to the possible entry of cells into S phase at that point. Increased entry of putrescine into the nucleus with a concomitant increase in DNA synthesis has been previously reported in mammalian BSC-1 cells (24). \(N^1\)-acetyl spermine was below detection levels in colons of both control and S/R animals but was detectable 4 h after NaDOC treatment at 5–20 pmol/µg protein (results not shown), which is considerably lower than the \(N^1\)-acetyl spermidine levels in the same NaDOC-treated animals (40–55 pmol/µg, Fig. 5, top).

**Ornithine and arginine levels.** Ornithine and arginine levels were, similar to other amino acids, de-
pressed at the end of a 48-h period of starvation (time 0, Fig. 6). However, unlike other amino acids that remained at or below control levels after refeeding (Fig. 7), both ornithine and arginine levels quickly increased. Ornithine levels were significantly higher overall in refed animals than in control animals 2–18 h after refeeding ($P < 0.0002$ by 2-tailed paired $t$-test), and arginine was significantly higher 2–9 h after refeeding ($P = 0.019$ by 2-tailed paired $t$-test). Because the levels of other amino acids did not increase on refeeding, a simple increase of all amino acids with refeeding cannot explain the increased ornithine and arginine levels. Ornithine levels were also increased 4 h after NaDOC treatment, alone or in combination with refeeding, although much more so at 12 h after refeeding (NaDOC instillation at 8 h, Fig. 8) than at 4 h after refeeding (NaDOC instillation with refeeding at 0 h, results not shown). NaDOC treatment at 8 h but not at time 0 caused a rise in arginine levels 4 h later, but this was not statistically significant (results not shown).

**OAT activity.** In most adult tissues, OAT catalyzes further metabolism of ornithine to glutamate semial-

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**Fig. 6.** Ornithine and arginine levels in rat colonic mucosa after refeeding. Ornithine and arginine levels were measured by HPLC analysis of the supernatants of homogenized colonic mucosal scrapings precipitated in 0.2% NaDOC and 8% TCA. Animals were either starved for 48 h and refed at time 0 or untreated (ad libitum fed). Values represent means ± SE of 4–6 animals. Ornithine levels in the starved-refed group were significantly higher than in the controls ($P = 0.0002$), as were the arginine values from 2 to 9 h after refeeding ($P = 0.019$), as measured by paired $t$-test (2 tailed).

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**Fig. 7.** Amino acid levels in rat colonic mucosa after refeeding. Amino acid levels were measured by HPLC analysis of the supernatants of homogenized colonic mucosal scrapings precipitated in 0.2% NaDOC and 8% TCA. Animals were either starved for 48 h and refed at time 0 or untreated (ad libitum fed). Values represent means ± SE of 4–6 animals.

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**Fig. 8.** Ornithine levels in rat colonic mucosa 12 h after refeeding and/or 4 h after instillation of NaDOC. Ornithine levels were measured by HPLC analysis of the supernatants of homogenized colonic mucosal scrapings precipitated in 0.2% NaDOC and 8% TCA. Treatment groups were either untreated controls (ad libitum fed) or animals starved for 48 h and refed at time 0, ad libitum fed and instilled with 24 mM NaDOC 4 h before death, or starved for 48 h, refed at time 0, and then instilled with 24 mM NaDOC 4 h before death. Values represent means ± SE of 4–6 animals. One-way ANOVA showed significant differences between groups ($P < 0.0001$). Differences between individual groups were determined by Student-Newman-Keuls multiple comparison test.
dehydrate (1, 48). As seen in Fig. 9, OAT activity was depressed in refeed animals compared with controls throughout the period studied. NaDOC treatment of ad libitum-fed animals at time 8 also led to a decrease in OAT activity 4 h later (at time 12, the end of the dark cycle) but did not further decrease OAT activity 4 h later in S/R animals exposed to NaDOC at either time 0 or time 8.

Arginase activity. In contrast to the reports of elevated arginase activities in some established tumors (21, 67) and in testosterone-stimulated kidneys (25), the short-term effect of NaDOC treatment, refeeding, or NaDOC treatment plus refeeding is greatly decreased arginase activity 4 h after NaDOC and 12 h after refeeding (Fig. 10). Arginase activity was not measured at other time points.

OTC activity. In vitro measured OTC activity in rat colon was close to background levels with the colorimetric assay used. At ~25 pmol·min⁻¹·mg protein⁻¹, OTC activity was very low relative to OAT activity at 12 h after refeeding and did not differ significantly between treatment groups (results not shown). Citrulline levels were 25–40% lower in the S/R group and/or NaDOC-treated animals at 12 h after refeeding, which would be consistent with decreased in vivo OTC-catalyzed conversion of ornithine to citrulline; however, the difference at 12 h was not statistically significant (1-way ANOVA, results not shown). Citrulline levels in the S/R group were significantly lower (~25%) compared with controls at all time points after refeeding (P = 0.0013, paired t-test, results not shown).

DISCUSSION

In agreement with previously reported results (56), the present results at 12 h after refeeding demonstrate a clear synergism between NaDOC exposure and refeeding on rat colon ODC activity, and the increases in putrescine concentration that accompanied peaks of ODC activity indicate that the changes in vitro ODC activity represent in vivo changes. It appears that there is at most additive induction of ODC activity at 4 h after refeeding and NaDOC instillation (data not shown), which may indicate that the colon mucosa is not fully competent to respond to NaDOC treatment that soon after the end of the starvation period. Alternately, the large amounts of food consumed by the starved animals during the first few hours of refeeding might greatly dilute the instilled NaDOC, buffering its effects.

In addition to the enhanced ODC activity, ornithine levels were also slightly raised in S/R animals (120% of control values at 12 h) and in NaDOC-treated animals (145% at 12 h). The combined treatments synergistically raised the ornithine levels still further (182% at 12 h) (Fig. 8). At 4 h after refeeding, the S/R animals had an ornithine level that was ~115% of that in controls, and combining that with NaDOC treatment caused only a nonsignificant further elevation to 122% of that in controls (results not shown). Thus there is an interactive effect of refeeding and NaDOC instillation on ornithine levels in the colon at 12 h but not at 4 h after refeeding. Both refeeding and NaDOC lead to changes in the ornithine level, within the same time scale as for the induction of ODC activity, and the fact that NaDOC treatment alone can raise the levels of ornithine opposes this being simply a nutrient effect from refeeding, thus implying another sort of control. Both the observed decreases in OAT and arginase activity and possible changes in ornithine and/or arginine concen-
tration, but the greatly decreased arginase activity makes it unlikely that increased arginine-to-ornithine conversion is contributing to the increase in ornithine levels, because the arginase activity decreases are of significantly greater magnitude than the moderate increases in arginase concentration. The basal arginase activity measured here is of lower magnitude than that measured, for example, in rat kidney, liver (39), or epidermis (21), but what is more interesting is the direct contrast of the decreased arginase activity observed here in response to proliferative stimuli compared with the increases observed in testosterone-stimulated kidney (25) or in papillomas compared with surrounding normal tissue (21).

An increase in ornithine concentration would be expected to lead to an increase in putrescine production, unless ODC is saturated at physiological ornithine concentrations. Assuming that 70% of the mucosal scraping weight was water and that the measured ornithine was evenly distributed in that water space, ornithine concentrations of 5–15 μM were measured in this study, well below the reported $K_m$ of 50–200 μM for ornithine in rats (35, 45). Therefore, it is unlikely that ODC in the colon is saturated with its substrate. An increase in the ornithine pool as a potential source of increased polyamine production has been largely ignored compared with increases in ODC activity (see, however, Refs. 48 and 68). The results presented here suggest that such changes in available ornithine can occur rapidly in response to normal lumen components (NaDOC) and simple changes in eating pattern. In addition to the mass action effects on putrescine production, it has been reported that increased ornithine concentration favors the active dimer form of the ODC enzyme (7), opposing the dissociating, inactivating effect of physiological levels of salts (54).

The observed changes in ornithine levels seem to be the result of short-term coordination of ornithine metabolism, making more ornithine available for polyamine biosynthesis. Similar findings of long-term coordination have been previously reported for hepatomas (17, 60, 66), and, more recently, a coordinated response in female mouse kidneys to testosterone over 5 days has been reported (25). Decreased ornithine catabolism due to lowered OAT activity levels in the S/R and NaDOC-treated animals can account for the higher ornithine levels in these same animals compared with controls and would have the effect of shunting ornithine into polyamine production. Conversely, an increase in OAT activity accompanies the decreased ODC activity and slows growth as colon cells in culture approach confluence (unpublished observations). A similar mechanism of control of ornithine availability to the urea cycle in liver by changes in OAT activity has already been proposed (33).

Previous results (30) with protein deprivation have shown decreased OAT activity in liver and primary hepatocytes; thus the lowered OAT activity levels observed here after refeeding might arise from protein deprivation during the starvation period rather than a specific coordination of ornithine metabolism. However, this same effect of lowered OAT, elevated ornithine, and elevated ODC activity is seen within 4 h of NaDOC instillation, indicating a more specific, rapid change. Rapid changes in liver OAT activity in response to dietary changes have previously been reported (13).

The other important ornithine-metabolizing enzyme, OTC, shows high activity in liver and to a lesser degree in small intestine, whereas evidence both for and against the existence of OTC activity in colon has been found (18, 38). OTC activity measured in this series of experiments was very low compared with OAT activity and did not change significantly with experimental treatment, although citrulline levels tended to be lower in the colons of S/R and NaDOC-treated rats than in those of controls at all time points after refeeding. This tendency toward lowered citrulline levels in S/R and/or NaDOC-treated animals, in spite of the increased ornithine levels, would be consistent with decreased in vivo OTC-catalyzed conversion of ornithine to citrulline and could thus represent another diminished ornithine-metabolizing pathway (along with the decreased OAT activity reported above). However, changes in the activity of NOS (which converts arginine directly into NO plus citrulline) or in the activity of argininosuccinate synthetase (which metabolizes citrulline to argininosuccinate) could also cause the citrulline level changes observed here. Regardless of the source of the lower citrulline levels observed, the quantitative effect of changes in the already low OTC activity on ornithine pools is clearly of lesser importance than the changes in OAT and arginase activity. In the current study, ornithine levels increased in spite of decreased arginase activity and increased ODC activity, which suggests that the decrease in OAT activity was the dominant factor in increasing the ornithine levels in these colonic mucosal cells; however, a potential additional contribution from increased uptake of circulating ornithine and/or arginine cannot be ruled out. Because none of the other amino acids quantitated in the current experiments were increased by starvation and refeeding or NaDOC exposure, any such contributions of increased ornithine and arginine uptake

Table 1. Sequence similarities in urea cycle and ornithine-metabolizing enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sequence similarity</th>
</tr>
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<tbody>
<tr>
<td>OAT gene (−90)</td>
<td>G7ATCCCTGGCCCT</td>
</tr>
<tr>
<td>OTC gene (−92)</td>
<td>GCAOCCCTGGCCCT</td>
</tr>
<tr>
<td>Arginase gene (−312)</td>
<td>GAGOCCTGGCCCT</td>
</tr>
<tr>
<td>Argininosuccinate synthetase gene (−193)</td>
<td>GCOCCTGGCCCT</td>
</tr>
<tr>
<td>ODC gene (−335)</td>
<td>CC GCCCTGGCCC</td>
</tr>
<tr>
<td>AP-2 strong consensus</td>
<td>TCGGCCANGCC</td>
</tr>
<tr>
<td>Alternate bases reported in some AP-2 sites</td>
<td>C G C ODC</td>
</tr>
</tbody>
</table>

The ornithine aminotransferase (OAT) gene sequence is taken from Ref. 32. The sequences of the urea cycle enzymes are taken from Refs. 19, 36, and 58. The ornithine decarboxylase (ODC) sequence is taken from Ref. 62. Numbers given in parentheses indicate the distance from the transcription start sites of each gene. OTC, ornithine transcarbamylase; AP-2, activator protein-2. Bold letters indicate bases in specific positions that are shared across several promoter sequences.
would presumably have to arise from specific upregulation of cationic transporters with specificity for arginine and ornithine [because neither lysine (Fig. 7) nor histidine levels (results not shown) were increased by refeeding], rather than from a simple generalized post-feeding increase in availability of circulating amino acids.

Evidence for the teleological importance to cells of increasing ornithine levels during periods of cell growth (whether normal or abnormal) comes from reports (26) of a flux of ornithine from host livers into transplantable tumors in mice, although other studies (44) have suggested that arginine might be of more importance than ornithine in supporting at least some types of rapid growth. Ornithine has been reported (9) to inactivate ODC in vitro in the presence of O₂ and absence of thiols, but this is not necessarily a good reflection of the physiological control of the enzyme. In cell culture, exogenous ornithine has been shown to inhibit ODC activity and inducibility in Reuber H35 hepatoma cells (68) and CHO-DF3 cells (11). Dietary ornithine has also led to a decrease in ODC activity in chicks, as well as a decrease in arginase activity, the primary endogenous source of ornithine (3). Although seemingly contradictory to the concept that cells increase their ornithine supply under growth conditions or other conditions with which increases in ODC activity are associated, these findings are consistent with the fine regulation of the overall system. In the case of an abundance of ornithine, mass action considerations allow more putrescine to be made with lower ODC activity, and there is no need for arginase activity to help provide ornithine. What one observes in the chicks, hepatomas, or CHO-DF3 cells treated with a high concentration of ornithine is, in fact, higher putrescine levels, and elevated polyamine concentration is the actual antecedent mechanism for the suppression of ODC activity (11, 28). Although excess ornithine can indirectly decrease ODC activity, excess dietary ornithine has been shown to increase OAT activity in the liver (6), another example wherein OAT is regulated in the opposite direction to ODC.

Although NO production was not measured in the current experiments, arguments similar to those given above can be made about the effects of arginine substrate concentration changes on NO production, i.e., changes in substrate arginine concentrations would be expected to directly change NO production rates without any necessity for increased in vitro measured NOS activity. There are several examples of arginine levels affecting NO production in the literature (see, for example, Refs. 8 and 14), and specific inhibition of NO production has been found to slow solid tumor growth in vivo (59).

What are the potential control points in the regulation of these enzymes? The urea cycle enzymes are known to be coordinately expressed, and sequence similarities in the 5′-flanking sequence of the genes of several of these enzymes, including that of arginase and OTC, have been reported (19, 36, 58). A very similar sequence exists just 5′ to the transcription start site (−90) in the human OAT gene (32), and the ODC gene also has a similar site, identified as an activator protein-2 site (Table 1). A single “composite glucocorticoid response element” in the mouse proliferin gene has been reported (10) to modulate response to glucocorticoids in a positive or negative manner depending on the cellular ratio of c-Fos and c-Jun, indicating that similar binding sites and transcription factors might act in either a positive or negative sense based on their interaction with other nearby binding sites or transcription factors. One possibility is that these similar binding sites in ODC and OAT might have opposite effects by analogous interactions with differing nearby transcription factors. In addition to the possibility of such speculative changes in transcription rate, previous reports (30, 34, 37, 42) have indicated that translational control of ODC, OAT, and OTC can occur, as can decreases in ODC degradation rate during proliferative periods. Further experiments are needed to elucidate which of these mechanisms are responsible for the observed coordinated effects on ornithine and arginine metabolism.

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