Polyamine regulation of ornithine decarboxylase and its antizyme in intestinal epithelial cells

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Yuan, Qing, Ramesh M. Ray, Mary Jane Viar, and Leonard R. Johnson. Polyamine regulation of ornithine decarboxylase and its antizyme in intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 280: G130–G138, 2001.—Ornithine decarboxylase (ODC) is feedback regulated by polyamines. ODC antizyme mediates this process by forming a complex with ODC and enhancing its degradation. It has been reported that polyamines induce ODC antizyme and inhibit ODC activity. Since exogenous polyamines can be converted to each other after they are taken up into cells, we used an inhibitor of S-adenosylmethionine decarboxylase, diethylglyoxal bis(guanylhydrazone) (DEGBG), to block the synthesis of spermidine and spermine from putrescine and investigated the specific roles of individual polyamines in the regulation of ODC in intestinal epithelial crypt (IEC-6) cells. We found that putrescine, spermidine, and spermine inhibited ODC activity stimulated by serum to 85, 46, and 0% of control, respectively, in the presence of DEGBG. ODC activity increased in DEGBG-treated cells, despite high intracellular putrescine levels. Although exogenous spermidine and spermine reduced ODC activity in DEGBG-treated cells close to control levels, spermine was more effective than spermidine. Exogenous putrescine was much less effective in inducing antizyme than spermidine or spermine. High putrescine levels in DEGBG-treated cells did not induce ODC antizyme when intracellular spermidine and spermine levels were low. The decay of ODC activity and reduction of ODC protein levels were not accompanied by induction of antizyme in the presence of DEGBG. Our results indicate that spermine is the most, and putrescine the least, effective polyamine in regulating ODC activity, and upregulation of antizyme is not required for the degradation of ODC protein.

antizyme; putrescine; spermine; S-adenosylmethionine decarboxylase; diethylglyoxal bis(guanylhydrazone)

POLYAMINES ARE PRESENT in all living cells and are essential for cell proliferation, differentiation (41), and migration (27). Depletion of polyamines results in the inhibition of cell proliferation and migration and the failure of embryonic development (17), whereas accumulation of polyamines causes apoptosis (44, 56) or cell transformation (1, 22). Cells maintain intracellular polyamines at optimal levels by regulating synthesis or degradation and by uptake or release of polyamines from or to the exterior. Ornithine decarboxylase (ODC) is the key regulatory enzyme in the synthetic pathway of polyamines. ODC activity increases rapidly in response to growth factors, amino acids such as asparagine or glutamine (5, 32, 60, 61), and hypotonic stress (24, 43, 58). ODC activity is tightly regulated at four levels, namely gene transcription, mRNA degradation, mRNA translation, and protein degradation (9, 10, 42, 50). ODC antizyme was first discovered by Heller et al. (12) in rat liver and several cultured cell lines. Antizyme is induced by polyamines and inhibits ODC activity by forming a complex with the enzyme (12). The active form of ODC is a homodimer composed of two 53-kDa subunits. The active site is formed at the interface of the two monomers, the monomers having no enzymatic activity (6, 57). Many in vitro studies indicate that ODC is degraded by a 26S proteasome and that this process is accelerated by antizyme. The COOH-terminal region of ODC is recognized and attacked by the 26S proteasome. A conformational change in ODC is induced by binding with antizyme, which results in the exposure of the COOH terminus to the 26S proteasome (11). The second function of antizyme is to mediate the rapid feedback inhibition of polyamine uptake into cells (30).

Polyamines in the intestinal lumen come from dietary intake, gastrointestinal secretion, sloughed cells, or bacterial synthesis. Intestinal cells are exposed to polyamines at relatively high concentrations in the postprandial state. Polyamines are transported into enterocytes through specific carriers, which are energy dependent (48). Polyamines absorbed by and synthesized in the villous cells might reach crypt cells via local circulation or by a paracrine effect (17). Putrescine effectively inhibits the increase in ODC in intestinal epithelial crypt (IEC-6) cells stimulated by asparagine or serum (7, 16). Among the three naturally occurring polyamines, spermidine is regarded as the most potent feedback inhibitor of ODC (10). However, many in vitro studies show that spermine is the most, and putrescine the least, effective in supporting various other biological processes (4, 49, 51, 52).
Polyamines are interconvertible after they are taken into cells (Fig. 1). S-adenosylmethionine decarboxylase (SAMe-DC) catalyzes the decarboxylation of S-adenosylmethionine, which provides aminopropyl groups for synthesis of spermidine and spermine. Intracellular putrescine is converted to spermidine by spermidine synthase and spermidine to spermine by spermine synthase. In a backward conversion, spermine is transformed to $N^\epsilon$-acetylspermine by $N^\epsilon$-spermidine/spermine acetyltransferase ($N^\epsilon$-SSAT) and then oxidized by polyamine oxidase to produce spermidine. Catalyzed by the same enzymes, spermidine is converted to putrescine (8, 40). Therefore, the inhibition of ODC activity by one exogenously added polyamine does not necessarily mean it is inhibited by that specific polyamine. In this study, by blocking the synthesis of spermidine from putrescine and spermine from spermidine with diethylglyoxal bis(guanylhydrazone) (DEGBG), an inhibitor of SAMe-DC, we characterized the inhibitory effect of specific polyamines on ODC activity in IEC-6 cells. Antizyme protein levels during the process of ODC degradation were examined as well.

MATERIALS AND METHODS

Chemicals and supplies. Biochemicals were purchased from Sigma Chemical (St. Louis, MO). L-[1-14C]ornithine was obtained from New England Nuclear (Boston, MA). Media, balanced salt solutions, and insulin were purchased from GIBCO (Grand Island, NY). Disposable cultureware was purchased from Corning Glass Works (Corning, NY). RIPA buffer was obtained from Boehringer Mannheim. DEGBG was synthesized and kindly provided by Dr. Patrick J. Rodrigues and Dr. Mervin Israel (Dept. of Pharmacology, University of Tennessee). Briefly, aminoguanidine bicarbonate (0.14 mol) was dissolved in 32 ml of water, to which 8.9 ml of 9 M H$_2$SO$_4$ were added dropwise. The solution was heated with stirring, and after 10 min at 80°C, 3,4-hexanedione (0.07 mol) in ethanol (8:6:32) was added dropwise. The formation of DEGBG crystals began when ~75% of the 3,4-hexanedione had been added. The solution was refluxed for 2.5 h and then cooled on ice. The DEGBG crystals were filtered off, washed with acetone, ethanol, and ether, and then dried. ODC antibody was kindly provided by Dr. Mari Haddox (University of Texas Medical School, Houston, TX). ODC antizyme antibody was kindly provided by Dr. John Mitchell (Southern Illinois University).

Cell culture. The IEC-6 cell line (CRL 1592) was derived from normal rat small intestinal crypt cells developed and characterized by Quaroni et al. (45) and was obtained from the American Type Culture Collection (Rockville, MD) at passage 13. The stock was maintained in T-150 flasks in a humidified 37°C incubator in an atmosphere of 90% air-10% CO$_2$. The stock medium was DMEM with 5% heat-inactivated fetal bovine serum (FBS), 10 μg/ml insulin, and 50 μg/ml gentamicin. The stock was passaged weekly at 1:5 dilution and fed three times a week. Passages 16–21 were used in the experiments. The cells were routinely checked for mycoplasma and always found to be negative.

Measurement of ODC activity. ODC activity was determined as previously reported (59). Briefly, dishes were placed on ice, and cells were washed with ice-cold Dulbecco’s PBS (DPBS). To each dish was added 0.5 ml of SAMe-DC buffer (1.0 mM Tris·HCl, 1.0 mM EDTA, 0.05 mM pyridoxyl 5-phosphate, and 5 mM dithiothreitol, pH 7.4), and the cells were frozen at −80°C until assayed on the following day. The cells were thawed on ice, scraped from the dishes, transferred to microfuge tubes, sonicated, and then centrifuged at 12,000 g for 10 min at 4°C. The cell supernatants (300 μl) were incubated in stoppered vials containing 20 μl of a 1:5 dilution of the labeled ornithine stock for 15 min at 37°C. The released $^{14}$CO$_2$ was trapped on a piece of filter paper impregnated with 20 μl of 2 N NaOH suspended in a center well above the reaction mixture. The reaction was stopped by the addition of 10% TCA and shaken for an additional 10 min to allow complete absorption of labeled CO$_2$. The trapped $^{14}$CO$_2$ was measured by liquid scintillation spectrophotometry. Enzyme activity was expressed as picomoles of CO$_2$ per milligram of protein per hour. Protein was assayed by the method of Bradford (3).

Fig. 1. Pathway for polyamine synthesis showing point of inhibition by diethylglyoxal bis(guanylhydrazone) (DEGBG). ODC, ornithine decarboxylase; SAMe-DC, S-adenosylmethionine decarboxylase; SSAT, spermidine/spermine $N^\epsilon$-acetyltransferase; PAO, polyamine oxidase.
Assay of intracellular polyamines. Cells were cultured and treated as described for the ODC activity assay. Intracellular putrescine, spermidine, and spermine were analyzed by HPLC, as previously described (27). Briefly, after the cell monolayers were washed with DPBS, 0.5 ml of 0.5 M perchloric acid was added, and then the monolayers were frozen at −80°C until all samples were ready for dansylation, extraction, and HPLC. The standard curve encompassed the range from 0.31 to 10 μM. Polyamine content was expressed as nanomoles per milligram of protein.

Western blot. Cells were washed twice with cold DPBS, and 300 μl of RIPA buffer were added to the dish. The cells were then harvested using a rubber scraper, transferred to microfuge tubes, incubated on a rotator at 4°C for 30 min, and centrifuged at 14,000 g at 4°C for 10 min. One hundred micrograms of total cell protein from supernatant were separated on 15% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. Membranes were blotted with 3% bovine albumin in PBS containing 3% Triton X-100 and then probed with antibody against ODC or ODC antizyme. The immunocomplexes on the membrane were reacted for 1 min with a chemiluminescence reagent. Finally, the filters were placed in a plastic sheet protector and exposed to autoradiography film for 30 or 60 s. The density of bands was quantitated by NIH Image software.

Statistics. Values are means ± SE. Statistical analysis was performed using ANOVA and appropriate post hoc testing. Absence of error bars in figures indicates that the SE was too small to be seen as separate from the mean. P < 0.05 was regarded as significant.

RESULTS

ODC activity in cells treated with 10% FBS in the presence of exogenous polyamines. IEC-6 cells were plated in 60-mm dishes and grown for 2 days to confluence. ODC activity was measured 3 h after cells were treated with 10% FBS in the presence of various extracellular concentrations of putrescine, spermidine, or spermine (Fig. 2). At 0.1 μM, putrescine did not inhibit ODC activity, whereas spermidine and spermine decreased ODC activity to 85 and 68% of control, respectively. At 1 μM, putrescine, spermidine, and spermine reduced ODC activity to 70, 37, and 0% of control, respectively. None of the polyamines inhibited ODC activity at 0.01 μM, but ODC activity was completely inhibited by all three at 10 μM. In the presence of 1 mM DEGBG, ODC activity induced by 10% FBS was inhibited to 85% of control by 10 μM putrescine and to 47% by 10 μM spermidine and was completely inhibited by 10 μM spermine (Fig. 3).

ODC activity and protein levels in cells treated with DEGBG and DEGBG plus exogenous polyamines. After cells were grown in medium containing 1 mM DEGBG for 2 days, intracellular putrescine increased by 15-fold and spermidine and spermine levels were decreased to 57 and 50% of control, respectively (Fig. 4). When cells were treated with DEGBG along with 1, 2, and 3 μM spermidine, putrescine levels increased over control by 8.7-, 5-, and 3.1-fold, respectively. Each of these was a significant decrease compared with DEGBG alone. Spermidine levels increased to 95, 133, and 175% of control, respectively. Spermine levels remained at ~42% of control after treatment with DEGBG plus the three concentrations of spermidine. When cells were treated with 1 mM DEGBG plus exogenous 1, 2, and 3 μM spermine, putrescine levels were increased only 6.1-, 2.2-, and 1.6-fold over controls, respectively. Therefore, spermine was even more effective than spermidine in preventing the rise in putrescine caused by DEGBG alone. Spermidine levels were 65, 60, and 65% of controls, respectively, and spermine levels were 89, 102, and 121% of control, respectively. Exogenous spermidine at 1 μM and exogenous spermine at 2 μM restored intracellular spermidine and spermine to control levels, respectively. There was no significant increase in intracellular spermine levels as the exogenous spermidine concentrations increased. Intracellular spermidine levels did not change as the exogenous spermine concentrations increased.

ODC activity and protein levels in cells treated with DEGBG and DEGBG plus exogenous polyamines. After cells were treated with 1 mM DEGBG for 2 days, ODC activity increased by 24-fold (Fig. 5). When cells were treated with 1 mM DEGBG along with 1, 2, and 3 μM exogenous spermidine for 2 days, ODC activity increased 17.6-, 9.3-, and 3.2-fold, respectively. ODC activities in cells treated with 1 mM DEGBG along with
1, 2, and 3 μM exogenous spermine were 15.6- and 3.8-fold and 58% of control, respectively. Thus exogenous spermidine and spermine suppressed the stimulation of ODC activity that occurred in response to DEGBG. Spermine was more effective than spermine, and 3 μM spermine totally inhibited the large increase stimulated by DEGBG.

The level of ODC protein was increased to 321% after 2 days of incubation in the presence of 1 mM DEGBG (Fig. 6). The addition of 3 μM putrescine to the incubation medium had no effect on this increase. Spermidine (3 μM), however, reduced the increase in ODC protein to 145% of control, and the same concentration of spermine totally prevented the increase in ODC protein level (78% of control).

ODC activity and ODC and antizyme protein levels after cells were exposed to exogenous polyamines. When cells were treated with 10 μM exogenous putrescine, spermidine, or spermine for only 1 h, ODC protein levels were 95, 86, and 83% of control, respectively (Fig. 7A). Antizyme protein levels of the same cells remained unchanged (Fig. 7B). After cells were grown in 1 mM DEGBG for 2 days to confluence, 3 μM exogenous spermidine or spermine was added to the medium. After 3 h of incubation, ODC activities were measured at 0, 3, and 6 h and expressed as a percentage of the activity in DEGBG-treated cells at time 0.
ODC activity of DEGBG-treated cells decreased gradually to 86% at 3 h and 78% at 6 h. After 3 μM spermidine was added to the medium, the ODC activity at 0, 3, and 6 h was 93, 68, and 17% of that of DEGBG-treated cells at time 0. After 3 μM spermine was added to the medium, the ODC activity of cells at 0, 3, and 6 h was 88, 12, and 0% of that of DEGBG-treated cells at time 0, respectively. ODC and antizyme protein levels of those cells were measured at 0, 1.5, and 3 h and were expressed as percentage of control. After spermine was added and cells were incubated for 3 h, ODC protein levels at 0, 1.5, and 3 h were 295, 242, and 157% of control, respectively (Fig. 8B). The ODC protein level of DEGBG-treated cells at 0 h was 326% of control. Antizyme protein levels remained unchanged during the time course (Fig. 8C). ODC antizyme protein was induced 3 h after 10 μM polyamines was added to the medium (Fig. 9A). The antizyme protein levels induced by putrescine, spermidine, and spermine were 2.6-, 5.2-, and 5.1-fold higher than control, respectively. ODC protein levels were markedly decreased after 3 h of treatment with exogenous polyamines (Fig. 9B).

DISCUSSION

The amino groups of the polyamines are protonated at physiological pH and form ion pairs with negatively charged molecules. The functions of the polyamines depend on their electrostatic interactions with DNA, RNA, proteins, and negatively charged membrane constituents. Increasing with the number of charges, binding energy is higher for spermine than for spermidine, which is higher than for putrescine (47). Many studies have shown that spermine is the most, and putrescine the least, effective in influencing various biological processes in which polyamines are involved. GTPase activity of purified GTP-binding protein (Gɔ/Gi) from calf brain reconstituted into phospholipid vesicles is stimulated by polyamines. The order of potency is spermine, spermidine, putrescine (4). In a study of the role of polyamines in nuclear factor-κB (NF-κB) binding to NF-κB response elements by electrophoretic mobility shift assays, addition of 1 mM spermine or spermine caused four-and sixfold increases in NF-κB-NF-κB response element binding, respectively. At 2 mM, putrescine induced a twofold increase (49). Glycogen synthase (casein) kinase-1 in bovine kidney is stimulated 7-, 2-, and 0.5-fold by spermine, spermidine, and putrescine, respectively (51). Addition of spermine to postnuclear extract from U937 cells induces cytochrome c exit from mitochondria and the onset of caspase activity. Spermine is more effective than spermidine, whereas putrescine has no effect (53).

Consistent with the above results, our data in Fig. 2 indicated that spermine, not spermidine, was the most
effective in inhibiting ODC activity stimulated by 10% FBS. DEGBG is a potent inhibitor of SAMe-DC and has been shown in leukemia 1210 (L1210) cells to specifically inhibit SAMe-DC activity and not to alter the activity of ODC or SSAT (55). It effectively inhibits SAMe-DC activity in IEC-6 cells as well (63). When the synthesis of polyamines was blocked by DEGBG, only 10 μM spermine completely inhibited ODC activity, suggesting that the inhibition of ODC by putrescine or spermidine observed in previous studies can be attributed, in part, to their conversion to spermine. Since 1 mM DEGBG inhibits SAMe-DC activity in IEC-6 cells by 85%, as shown in our previous study (63), some putrescine might still be converted to spermidine or spermine in the presence of DEGBG. Therefore, the actual inhibition of ODC by putrescine is even less than 15%, as shown in Fig. 3, suggesting that putrescine, at the concentrations administered, is not effective in inhibiting ODC in cultured IEC-6 cells. There is an equilibrium between free and bound forms of polyamines. Polyamines exert their biological effects by binding to intracellular macromolecules (47). The binding ability of putrescine is less than that of spermine, because it has fewer positive charges. Therefore, putrescine is ineffective partially because of its inability to bind to proteins involved in regulating ODC. Furthermore, since the number of charges and molecular length differ between putrescine and spermine, putrescine bound to proteins may not have the same regulatory functions as spermine.

ODC activity in cells without serum stimulation is low, especially after the cells reach confluence. We studied ODC activity in unstimulated cells by depleting intracellular spermidine or spermine with DEGBG. Putrescine was accumulated in DEGBG-treated cells, whereas spermidine and spermine levels were decreased (Fig. 4). The accumulation of putrescine in those cells could be attributed to several factors. First, inhibition of SAMe-DC prevented the conversion of putrescine to spermidine and spermine. Second, synthesis of putrescine increased because ODC activity was high in those cells (Fig. 5). In addition, putrescine would be able to bind to polyamine-binding sites that were available after spermidine and spermine were depleted. Binding of putrescine to those macromolecules would reduce the level of free putrescine and prevent it from being exported out of the cells. ODC was not inhibited, despite the accumulation of putrescine, suggesting that putrescine was ineffective in feedback inhibiting ODC. Increases in exogenous spermidine or spermine only increased intracellular spermidine or spermine, respectively, suggesting that interconversion of polyamines was prevented. Synthesis of spermine from spermidine was indirectly blocked by DEGBG. \(N^\delta\)-SSAT, a regulatory enzyme in the backward conversion of polyamines, is induced only when polyamines are in excess (47). In a study of L1210 cells, exogenous spermine increased intracellular spermine levels only at 1 mM (21). Since exogenous spermine at 3 μM only increased intracellular spermine levels to 121% of control and intracellular spermidine was not altered by addition of exogenous spermine, we speculated that \(N^\delta\)-SSAT was not markedly induced. ODC activity decreased as intracellular spermidine or spermine increased (Fig. 5). When intracellular spermidine was restored to control levels by 1 μM exogenous spermidine, ODC activity was ~17.6-fold higher than control. ODC activity remained at 3.8-fold higher than control when intracellular spermine was restored to control levels by 2 μM exogenous spermine. ODC activity was decreased to the same level when the intracellular concentration of spermidine reached as high as 175% of control. The decay of ODC activity in DEGBG-treated cells induced by addition of spermine was more dramatic than that induced by addition of spermidine (Fig. 8A). The above results further support the conclusion that spermine is more effective than spermidine in inhibiting ODC activity.

ODC protein levels increased when ODC activity increased, suggesting that translational regulation was involved in DEGBG-treated cells (Fig. 6). ODC mRNA levels were not changed by DEGBG treatment (data not shown), indicating that polyamines did not regulate the transcription of the ODC gene. After adding spermidine and spermine to the ODC assay mixture containing cell extracts from DEGBG-treated cells, we did not see any decrease in ODC activity (data not shown). This result indicated that polyamines did not allosterically regulate ODC activity.

Induction of antizyme by polyamines and its enhancement of degradation of ODC have been well studied (9, 10). Antizyme accelerates the degradation of ODC in Chinese hamster ovary cell extracts (38), reticulocyte lysate (37), and ODC-overproducing variant cells (18). Transfected antizyme gene expression results in the rapid decay of ODC activity and ODC protein levels in hepatoma tissue culture cells (35). Interleukin-1 decreases the ODC activity of human melanoma cells, and this downregulation of ODC is blocked by expression of the antisense RNA of human antizyme in those cells, suggesting that antizyme mediates the degradation of ODC (62). However, in our study, when cells were treated with 10 μM polyamines for 1 h, decreases in ODC protein levels were not associated with increases in antizyme levels (Fig. 7). We also examined antizyme protein levels during the degradation of ODC in DEGBG-treated cells. ODC activity and the level of ODC protein decreased markedly within 3 h after spermine was added to DEGBG-treated cells (Fig. 8B), but the level of antizyme protein remained unchanged (Fig. 8C). These results suggest that decreases of ODC activity and ODC protein in IEC-6 cells did not require upregulation of ODC antizyme. A recent study shows that dexamethasone reduces ODC activity in lung tissue of newborn rats without altering antizyme protein levels, also suggesting that antizyme induction is not necessary for decreased ODC protein levels (2). Multiple forms of antizyme have been described. Rat hepatoma (HTC) cells expressed one minor antizyme band of 29.5 kDa and one major band of 24 kDa (31). DH23b cells, a variant of the HTC line, exhibited two additional forms of
antizyme from AZI and, thus, enhance the formation of ODC from antizyme suppression in vitro. ODC was reactivated in mouse fibroblasts on transient transfection with an AZI-expressing plasmid construct (39). Excessive polyamines may lead to the dissociation of antizyme from AZI and, thus, enhance the formation of the ODC-antizyme complex and the degradation of ODC.

Subcutaneous administration of dexamethasone increases ODC activity in the liver of rat pups but inhibits the ODC activity in lung, suggesting that the regulation of ODC is tissue specific (2). Intestinal cells are unique, in that they carry out absorptive functions. Excessive antizyme inhibits polyamine transport and, consequently, may impair the absorption of polyamines from the intestinal lumen. Diamine oxidase, catalyzing the oxidation of putrescine, is found primarily in intestinal cells (47), suggesting that the metabolism of polyamines in intestinal cells is different from that in other cell types. The means of regulating the degradation of ODC in intestinal cells may be different from that in other cell types as well. In IEC-6 cells, polyamines may decrease ODC activity by inhibiting the translation of ODC protein and/or enhancing the formation of ODC-antizyme complex without inducing antizyme.

Antizyme mRNA is expressed constitutively in various tissues of the rat, and the level of antizyme mRNA is not increased by polyamines (26). Antizyme mRNA contains two open reading frames (ORFs). The initiating frame (ORF1) is short, encoding 68 amino acids and a stop codon. The second, main frame (ORF2) is in the +1 frame with respect to ORF1 and lacks possible initiator codons. Addition of polyamines evokes translational frame shifting from ORF1 to ORF2, thereby resulting in the translation of the entire antizyme gene (25, 46). Antizyme was induced (Fig. 9A) and ODC proteins were barely detectable (Fig. 9B) after cells were treated with 10 μM polyamines for 3 h. Exogenous putrescine was much less effective than spermidine and spermine in the induction of antizyme. Since interconversion of polyamines was not blocked, the induction of antizyme protein by exogenous putrescine may be, in part, attributed to spermidine and spermine converted from putrescine. Accumulation of putrescine in DEGBG-treated cells did not induce antizyme, suggesting that high putrescine level cannot substitute for spermidine or spermine in the induction of antizyme (Fig. 8C). Exposure of ODC-overproducing cells to micromolar levels of spermine or spermidine causes an abnormal accumulation and polyamine toxicity. Transfection of cells with rat antizyme cDNA not only leads to a decrease in the amount of ODC but also a decrease in polyamine transport activity and release of the cells from growth inhibition (54). Thus antizyme appears to be responsible for the protection of cells against the excessive accumulation of polyamines. Since the basal level of ODC protein was quite low and started to decrease before the induction of antizyme, the newly synthesized antizymes after 3 h of exposure to 10 μM polyamines may function primarily to inhibit polyamine uptake.

Our results indicated that spermine was the most potent polyamine in inhibiting ODC activity. Putrescine was almost without effect in decreasing ODC activity and protein levels and in increasing antizyme protein levels. The upregulation of antizyme was not
required for the decay of ODC activity and the reduction of ODC protein levels induced by polyamines in IEC-6 cells. We also showed that polyamines did not allosterically regulate ODC. To our knowledge these findings have not been described previously.

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