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Regulation of ornithine aminotransferase gene expression and activity by all-transretinoic acid in Caco-2 intestinal epithelial cells☆

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Abstract

Ornithine aminotransferase (OAT) is a crucial enzyme in the synthesis of citrulline and arginine from glutamine/glutamate and proline by enterocytes of the small intestine. However, a role for OAT in intestinal polyamine synthesis and cell growth is not known. All-transretinoic acid (RA), an active metabolite of vitamin A, regulates the activity of several metabolic enzymes related to OAT, including ornithine decarboxylase and arginase, which may influence the function of OAT through effects on substrate (ornithine) availability. The objective of the present study was to test the hypothesis that RA regulates OAT mRNA expression and enzymatic activity in intestinal epithelial cells. Caco-2 cells were cultured for 12–72 h in the presence of 0, 0.01 and 1 μM RA and then used for measurements of OAT mRNA levels and enzyme activity as well as ornithine and polyamines. Treatment with RA induced increases in OAT gene expression and enzymatic activity, which resulted in decreased intracellular concentrations of ornithine and polyamines (putrescine, spermidine and spermine) in a dose-dependent manner. These changes occurred concomitantly with a decrease in the total number of cells, and the increase in OAT activity was due to increased OAT mRNA expression. In cells treated with 1 μM RA, addition of 10 μM putrescine to culture medium restored both cellular levels of polyamines and cell numbers to the values for the control group (without addition of RA). We conclude that exposure of Caco-2 cells to RA induces OAT expression for increasing ornithine catabolism. This leads to a reduced availability of intracellular ornithine for polyamine synthesis, thereby decreasing cell proliferation. These novel findings indicate a functional role for OAT in regulating intestinal polyamine synthesis and growth.

Keywords: Retinoic Acid; Ornithine aminotransferase; Polyamines; Intestinal cells

1. Introduction

Ornithine aminotransferase (OAT; EC 2.6.1.13) is a nuclear-encoded, mitochondrial matrix enzyme that catalyzes the interconversion of L-ornithine and L-Δ^1-pyrroline-5-carboxylate (P5C) (Fig. 1). This protein is widely distributed in animal tissues [1]. Studies with neonatal mice [2] and pigs [3] have implicated OAT as a crucial enzyme in the synthesis of citrulline and arginine from glutamine/glutamate and proline by small-intestinal enterocytes. Because the milk of most mammals is relatively deficient in arginine, intestinal synthesis of citrulline and arginine plays an important role in arginine homeostasis of the neonate [4]. Thus, a deficiency of OAT in infants [2], neonatal pigs [3] and OAT-knockout mice [2] results in hypoornithinemia, hypocitrullinemia and hypoargininemia. Additionally, OAT modulates the proliferation of colonocytes [5] and vascular smooth muscle cells [6], as well as extracellular matrix formation [6], by regulating intracellular concentrations of ornithine. Expression of OAT has been reported for fetal porcine and rat small intestinal epithelium [7,8], as well as the endoderm of the developing rat jejunum

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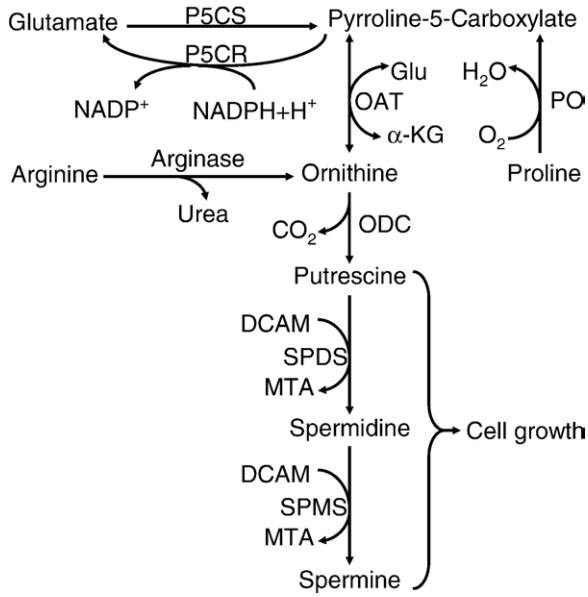

Fig. 1. Pathways for ornithine and polyamine synthesis via ornithine aminotransferase and ornithine decarboxylase in cells. DCAM, decarboxylated S-adenosylmethionine; MTA, methylthioadenosine; PO, proline oxidase; P5CS, pyrroline-5-carboxylate synthase; P5CR, pyrroline-5-carboxylate reductase; SPDS, spermidine synthase; SPMS, spermine synthase.

[7]. These findings suggest that OAT plays an important role in fetal development by regulating the provision of proline, ornithine, citrulline and arginine for use by the small intestine and/or by the entire fetus.

OAT activity and gene expression are regulated temporally and spatially in small intestines of fetal [7,8], suckling [7,9,10] and postweaning [11,12] mammals. Previous work by Shull et al. [13] indicates that all-transretinoic acid (RA), an active metabolite of vitamin A, up-regulates hepatic OAT mRNA expression by increasing transcription. However, the effects of RA on OAT activity and mRNA expression in the small intestine have not been characterized. Our interest in studying the effects of RA on small intestinal OAT activity and mRNA expression are based on several observations. First, OAT is expressed to a greater extent in differentiated villus cells [8] than in less differentiated crypt cells, and RA stimulates differentiation of function and morphology in the small intestine, including fetal small intestine [14]. Second, RA regulates the activity of several metabolic enzymes related to OAT, including ornithine decarboxylase [15] and arginase [16], which may influence the function of OAT through effects on substrate availability.

Retinoic acid can influence the intestinal epithelium by acting directly on the epithelium or through indirect, paracrine effects mediated through actions on the underlying mesenchyme [14]. Caco-2 cells were used for this study because they provide a well-characterized model of small intestinal epithelium [15,17]. McCormack et al. [15] demonstrated that, in Caco-2 cells, RA induced a differentiated phenotype, including increased sucrase and maltase activities, decreased cell proliferation and decreased ornithine decarboxylase (ODC) activity. Additionally, Caco-2 cells express nuclear retinoic acid receptors RARα, RARβ, RARγ and RXRα, as well as cellular retinoic acid binding protein [17]. The objective of the present study was to test the hypothesis that RA regulates OAT mRNA expression and enzymatic activity in intestinal epithelial cells.

2. Materials and methods

2.1. Cell culture and experimental treatment

Caco-2 cells (human colon adenocarcinoma cell line; American Type Culture Collection) were grown in MEM (GIBCO, Grand Island, NY, USA) supplemented with L-glutamine (2 mM), all other protein amino acids (0.1 mM each) and 15% fetal bovine serum. Stock cells were maintained in T-75 flasks, grown until approximately 90% confluent and subcultured after trypsinization. All cell cultures were performed at 37°C in a humidified atmosphere of 5% CO_2–95% air. At the initiation of all experiments, cells were counted with a hemocytometer and plated at a density of 2×10^4 cells/cm^2. After 72 h in culture, cells were treated with 0, 0.01 or 1.0 μM RA at time points ranging from 6 to 72 h. In some experiments, cells were cultured for 72 h in medium containing 0, 1 μM RA or 1 μM RA plus 10 μM putrescine. RA was dissolved in 100% ethanol and added to culture medium at a 1:1000 dilution. The control group (without addition of RA) received the same amount of diluted ethanol. Preliminary experiments using 0.1 nM, 0.01 μM and 1.0 μM RA indicated that 0.1 nM RA had no measurable effect on OAT enzyme activity or mRNA expression; therefore, only 0.01 and 1.0 μM RA were used for subsequent investigations. For determination of cell number after experimental treatment, cells were trypsinized and an aliquot was counted using a Coulter Counter (Beckman Coulter, Fullerton, CA, USA).

2.2. Measurement of ornithine aminotransferase activity

OAT enzymatic activity was determined using a modification of a previously reported method [3]. Cells were washed with cold phosphate-buffered saline (PBS), scraped into tubes and pelleted by centrifugation at 500*g* for 5 min at 4°C. The cells were lysed in PBS containing 1% Nonidet P40 and subjected to three cycles of freezing in liquid nitrogen and thawing at 37°C. Cell lysates were centrifuged at 10,000*g* for 1 min at 4°C, and the supernatant was used for assay of OAT activity. The assay mixture consisted of 75 mM potassium phosphate buffer (pH 7.5), 20 mM ornithine, 0.45 mM pyridoxal phosphate, 5 mM *o*-aminobenzaldehyde, 0 or 3.75 mM α-ketoglutarate and cell supernatant (0.03–0.074 mg protein). The assay mixture was incubated at 37°C for 15 min, and the reaction of P5C with *o*-aminobenzaldehyde was measured colorimetrically at 440 nm. Protein concentration of samples was determined using a

modification of the Bradford method (Pierce Coomassie Plus, Pierce, Rockford, IL, USA) and bovine serum albumin as the standard.

2.3. Determination of intracellular levels of polyamines and ornithine

Polyamines (putrescine, spermidine and spermine) and ornithine in Caco-2 cells were analyzed by high-performance liquid chromatography (HPLC) methods involving precolumn derivatization with *o*-phthaldialdehyde, as previously described [18,19]. Briefly, Caco-2 cells were washed twice with ice-cold PBS, scraped into centrifuge tubes and centrifuged at 500*g* for 5 min at 4°C. Cell pellets were acidified with 1.5 mM $HClO_3$ and neutralized with 2 mM K_2CO_3. The neutralized samples were centrifuged at 3000*g* for 15 min and then used for HPLC analysis of ornithine and polyamine concentrations [18,19].

2.4. RNA isolation and Northern blot analysis

Total RNA was extracted from cultures using TriPure Isolation Reagent (Roche Molecular Biochemicals, Indianapolis, IN, USA) according to the manufacturer's recommendations. The resulting RNA pellet was suspended in 10 mM tris(hydroxymethyl)aminomethane (Tris)–HCl (pH 8.0) containing 1 mM EDTA, and the concentration of RNA was determined from its absorbance at 260 nm. For Northern blotting, total RNA (30 μg) was denatured and separated on a 1.2% agarose gel containing 3% formaldehyde and transferred by capillary blotting to positively charged nylon membranes (Schleicher and Schuell, Keene, NH, USA) overnight. Blots were prehybridized for 1 h at 65°C in hybridization buffer (50% formamide, 50 mM Na_2PO_4, 5×SSC (1×SSC is 150 mM NaCI and 15 mM sodium citrate), 0.1% SDS, 1 mM EDTA, 0.5×Denhardt's solution, and 200 μg/ml herring sperm DNA). A cRNA probe for rat OAT [20] (cDNA kindly provided by Dr. David Valle, The Johns Hopkins University School of Medicine) was generated by in vitro transcription using ^{32}P-UTP as the label as described previously [8]. The blots were hybridized for 16 h at 65°C. Blots were washed twice in 2×SSC/0.1% sodium dodecyl sulfate (SDS) for 10 min at room temperature, twice in 0.2×SSC/0.1% SDS for 10 min at room temperature, and twice in 0.1×SSC/0.1% SDS for 15 min at 68°C. After the final wash, blots were placed on an InstantImager (Packard, Meriden, CT, USA) for quantitative analysis of radioactive signals. Each mRNA signal was standardized to nonspecific binding of probe to the 28S transcript.

2.5. Statistical analysis

Data from Northern blots and studies on cell number and OAT activity were subjected to least squares analysis of variance (ANOVA) using the general linear models (GLM) procedures of SAS for Windows (SAS Institute, Cary, NC, USA). The model used tested for effects of time, dose and time×dose. Data for effects of RA on intracellular concentrations of polyamines and ornithine was analyzed as percentage of control using generalized linear modeling and Fisher's least significant difference. Data from Northern blot analysis were normalized using the 28S rRNA to correct for differences in loading. $P \leq .05$ were considered to be statistically significant.

3. Results

3.1. Effect of RA on OAT activity

To determine whether RA treatment affected enzymatic activity of OAT, which plays a central role in ornithine metabolism, we measured the activity of OAT in Caco-2 cells after treatment with RA. OAT activity in control cells increased over time (time, $P<.001$). RA increased (time×dose, $P<.001$) OAT activity over the 72 h treatment in a dose-dependent manner (Fig. 2).

3.2. Effect of RA on cell number

To assess the effect of RA on cell proliferation, we determined cell numbers after RA treatment. Total cell number in all treatment groups (Fig. 3) increased over time (time, $P<.001$). Exposure of Caco-2 cells to RA for 12 to 72 h reduced cell number in a dose-dependent manner (time×dose, $P<.01$).

3.3. Decrease in intracellular concentrations of ornithine and polyamines after exposure to RA

Intracellular concentrations of ornithine and polyamines (putrescine, spermidine and spermine) were measured to

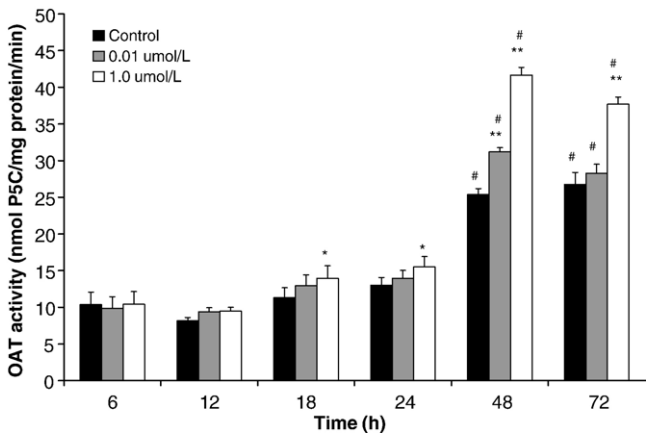

Fig. 2. OAT activity in Caco-2 cells treated with or without RA. Caco-2 cells were exposed to 0, 0.01 or 1.0 μM RA for 6, 12, 18, 24, 48 or 72 h. OAT activity was determined spectrophotometrically. Data shown are mean±S.E.M. (n=9). OAT activity of control cells increased over time (time, $P<.001$), RA treatment increased OAT activity in a dose-dependent manner (time×dose, $P<.001$). "*" and "**" indicate differences ($P<.01$ and .001, respectively) relative to respective 0 μmol/L RA control; "#" indicates difference ($P<.001$) relative to corresponding 24 h treatment.

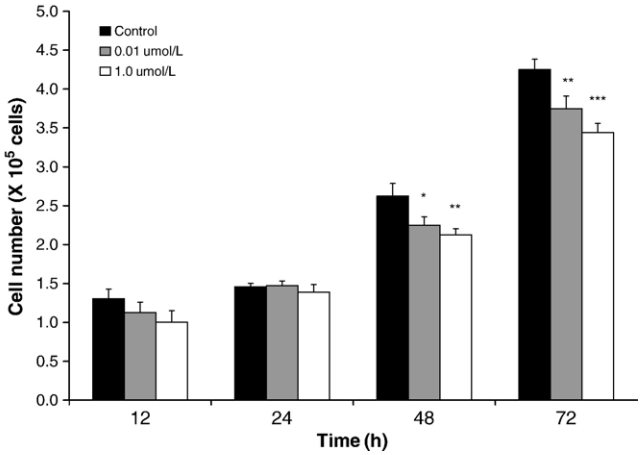

Fig. 3. Growth of Caco-2 cells treated with or without RA. Caco-2 cells were treated with 0, 0.01 or 1.0 μM RA as described in Fig. 2. After 12, 24, 48 or 72 h or treatment, cells were counted. Data shown are mean±S.E.M. (n=9). Control cell numbers increased over time (time, P<.001). RA treatment reduced cell numbers in a dose-dependent manner (time×dose, P<.01). "*", "**" and "***" indicate differences (P<.05, .01 and .001, respectively) relative to respective 0 μmol/L RA control.

assess effects of RA on ornithine availability and polyamine synthesis. Intracellular ornithine concentrations were increased (P<.05) over the control value after 12 h of exposure to 1 μM RA and then decreased (P<.01) after 24, 48 and 72 h of exposure to 1 μM RA (Table 1). Similarly, treatment with 1 μM RA to culture medium for 48 and 72 h reduced (P<.05) intracellular concentrations of polyamines at 48 and 72 h.

Table 1
Concentrations of ornithine and polyamines in Caco-2 cells treated with or without RA

RA (μM)	Ornithine	Putrescine	Spermidine	Spermine
12 h				
0	0.64±0.02^{b}	0.53±0.02	2.38±0.13	3.22±0.11
0.01	0.69±0.04^{b}	0.52±0.03	2.23±0.12	3.10±0.14
1	0.78±0.03^{a}	0.54±0.02	2.17±0.10	2.93±0.15
24 h				
0	0.65±0.03^{a}	0.52±0.03	2.26±0.08	3.10±0.14
0.01	0.55±0.02^{b}	0.48±0.02	2.15±0.10	2.92±0.12
1	0.46±0.01^{c}	0.48±0.03	1.96±0.07	2.79±0.09
48 h				
0	0.62±0.02^{a}	0.52±0.02^{a}	2.30±0.09^{a}	3.15±0.12
0.01	0.43±0.02^{b}	0.44±0.01^{b}	1.96±0.07^{b}	2.98±0.10
1	0.24±0.01^{c}	0.37±0.01^{c}	1.67±0.06^{c}	2.86±0.13
72 h				
0	0.61±0.02^{a}	0.51±0.01^{a}	2.26±0.08^{a}	3.08±0.14^{a}
0.01	0.34±0.01^{b}	0.39±0.01^{b}	1.83±0.06^{b}	2.72±0.12^{ab}
1	0.19±0.01^{c}	0.28±0.01^{c}	1.46±0.05^{c}	2.41±0.09^{b}

Data are means±S.E.M., n=8 independent experiments. Values are expressed as nmol/mg protein. Caco-2 cells were treated with 0, 0.01 or 1 μM RA for 12, 24, 48 or 72 h. Intracellular concentrations of putrescine, spermidine, spermine and ornithine were determined by HPLC. Means sharing different superscript letters within a column of each time period differ (P<.05), as analyzed by 1-way ANOVA.

3.4. Effect of exogenous putrescine on polyamine levels and cell number in RA-treated cells

When Caco-2 cells were cultured in the presence of 1 μM RA, addition of 10 μM putrescine to the medium increased (P<.05) cellular concentrations of putrescine, spermidine and spermine to the values observed for the control group (without addition of RA) (Table 2). The putrescine treatment also prevented (P<.05) a decrease in cell number brought about by exposure to 1 μM RA (Table 2).

3.5. Effect of RA on OAT mRNA expression

Northern analysis was performed on total RNA from control and RA treated Caco-2 cells to determine effects of RA on OAT gene expression and showed one major 2.1-kb transcript. Northern analysis indicated that OAT mRNA expression was increased (dose×time, P<.05) by 1 μM RA (Fig. 4) throughout the 72 h treatment. Treatment with 0.01 μM RA did not stimulate OAT mRNA expression above control values. In order to determine if the effect of RA on OAT mRNA expression was mediated at the transcriptional level, we investigated the formation of new OAT mRNA transcripts in nuclei of treated and untreated in Caco-2 cells by nuclear run-on analysis; however, no stimulation of new OAT mRNA transcription was detected (data not shown).

4. Discussion

This is the first report, to our knowledge, that demonstrates the effect of RA on OAT mRNA expression and activity in intestinal epithelial cells. Our results indicate that RA, which stimulates differentiation of intestinal epithelial cells, induces an increase in OAT enzyme activity in conjunction with decreased intracellular concentrations of ornithine and polyamines as well as decreased cell numbers

Table 2
Effects of putrescine on polyamine concentrations and Caco-2 cell numbers in cultures treated with RA

Treatment	Cellular concentrations (nmol/mg protein)			Cell numbers (×10^5 cells)
	Putrescine	Spermidine	Spermine	
0 μM RA	0.54±0.03^{a}	2.41±0.10^{a}	3.37±0.16^{a}	4.57±0.23^{a}
1 μM RA	0.31±0.02^{b}	1.53±0.07^{b}	2.65±0.12^{b}	3.49±0.17^{b}
1 μM RA+10 μM Putrescine	0.62±0.05^{a}	2.60±0.14^{a}	3.51±0.23^{a}	4.52±0.21^{a}

Data are means±S.E.M., n=8 independent experiments. Caco-2 cells were treated with 0, 1.0 μM RA or 1.0 μM RA plus 10 μM putrescine for 72 h, as described in text. At the end of the 72-h culture period, cell numbers and intracellular concentrations of putrescine, spermidine and spermine were determined, as described in text. Means sharing different superscript letters within a column differ (P<.05), as analyzed by 1-way ANOVA.

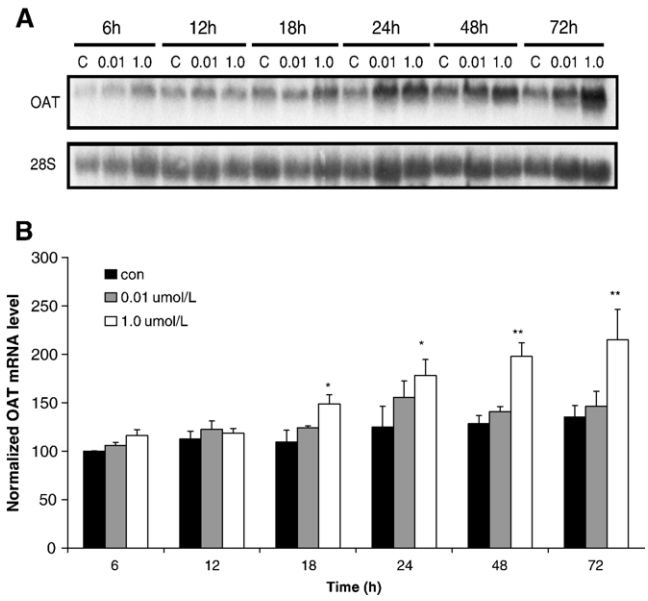

Fig. 4. Expression of the OAT gene in Caco-2 cells treated with or without RA. Treatment with RA increases expression of OAT mRNA in Caco-2 cells. (A) Representative autoradiogram of a Northern blot hybridized with a radiolabeled rat OAT cRNA probe. Total RNA was extracted from Caco-2 cells that were treated as described in Fig. 2. Hybridization signals for OAT mRNA and 28S rRNA are shown in upper and lower panels, respectively. (B) Three experiments for OAT and 28S mRNA hybridization signals were directly counted. Normalized OAT signals are shown as percentage of 6 h control values as mean±S.E.M. (n=3 independent experiments). Treatment with 1.0 μmol/L RA increased steady-state levels of OAT mRNA (dose, P<.05). "*" and "**" indicate differences (P<.05 and .01, respectively) relative to respective 0 μmol/L RA control.

in Caco-2 cultures. Expression of OAT has been detected in vivo in the endoderm of the fetal rat jejunum as early as day 14 of gestation [21]. Additionally, Dekaney et al. [8] and De Jonge et al. [7] have reported changes in spatial expression of OAT in the mucosal epithelium of fetal pig and fetal rat small intestine, respectively, during the course of gestation and during neonatal development. Although this suggests that OAT is important during fetal small intestinal development, the mechanism(s) by which these changes are regulated is not understood. The mucosa of the fetal pig small intestine begins morphological and functional differentiation early in gestation [22–24]. During this same time, OAT mRNA expression becomes compartmentalized within the epithelium, changing from expression throughout the epithelium to being expressed primarily in the differentiated villus enterocytes [8]. There is also evidence for compartmentalization of related metabolic enzymes, including carbamoylphosphate synthase, ornithine carbamoyltransferase, argininosuccinate synthase and argininosuccinate lyase in fetal and neonatal small intestine [7,25]. The functional significance of this compartmentalization may be for supplying the whole fetus with citrulline and arginine synthesized by the small intestine [26] because the small intestine of most neonatal mammals is the major source of endogenous arginine [27]. However, these changes in expression of the aforementioned enzymes may also reflect local metabolic needs for arginine and its metabolites by enterocytes during gestation.

The fetal intestine is exposed to RA, which plays a role in intestinal morphogenesis and differentiation during fetal development [14]. Results of this present study indicate that RA mediates a direct effect on OAT activity in cultured intestinal epithelial cells. The fetal murine small intestine expresses RARβ [28] and RARγ [14], as well as cellular retinol-binding proteins CRBP I and II and CRABP I in epithelial and mesenchymal compartments [14]. The effects of RA on animal cells are both direct and indirect, mediated by modulation of RA-sensitive genes [29] and by up-regulating autocrine and paracrine factors such as transforming growth factor (TGF)-β [30] and by altering extracellular matrix components [14], respectively. Expression of OAT in vascular smooth muscle cells is increased by TGF-β [6,31]. Interestingly, in the liver, extracellular matrix proteins have been postulated to alter OAT expression [32]. The fetal human small intestine expresses the TGF-β 1, 2 and 3 isoforms, as well as TGF-β receptors I and II [33], as reported for rodents [34,35]. Furthermore, extracellular matrix proteins are key regulators of epithelial–mesenchymal interactions in the small intestine during gestation and play an important role in modulating proliferation and differentiation in fetal enterocytes during development [36]. Therefore, RA may be regulating OAT mRNA expression in enterocytes along the crypt-villus axis in fetal small intestine by multiple mechanisms.

An RA-induced increase in transcription was considered as a mechanism which resulted in increased steady-state levels of OAT mRNA [29]. However, nuclear run-on analysis revealed no increase, compared to controls, in newly formed OAT transcripts from nuclei of Caco-2 cells exposed to RA. This is not completely unexpected, because in other tissues, expression of OAT is regulated at the transcriptional [37–39], translational [40–42] and pre-mRNA splicing [38] levels. In addition, investigation of the 5′-flanking regions of exon 1 of human, mouse and rat *OAT* identified several AGGTCA-like motifs, corresponding to consensus binding sites for steroid receptor superfamily members such as RAR [43] that were present in both rat and mouse *OAT* genes but absent in the human *OAT* gene [44], which is the species of origin of Caco-2 cells. Although the mechanism for the RA-mediated increase in OAT expression is not understood, one potential mechanism may be a RA-induced increase in OAT mRNA stability. This idea is further supported by previous reports of RA-mediated increases in mRNA stability of bone-type alkaline phosphatase [45,46], surfactant proteins [47] and IGF-binding proteins [48]. Additional studies are necessary to fully elucidate the mechanism by which RA increases OAT expression.

The observed RA-stimulated increase in OAT activity in Caco-2 cells is an inverse response to that reported for proliferative stimuli on rat colonocytes [5]. Han et al. [5] demonstrated that, in the rat colon, the presence of

proliferative stimuli (e.g., sodium deoxycholate), increased ODC activity and concomitantly decreased OAT activity. Accompanying the changes in enzymatic activity were increased levels of putrescine and ornithine, suggesting that sodium deoxycholate modulated ODC and OAT activities to accumulate ornithine for use in polyamine synthesis. This mechanism of providing ornithine for polyamine synthesis has also been reported for renal [49] and hepatic tumors [50], as well as porcine intestinal epithelial cells [18,51].

Results of this study demonstrate that RA induces an increase in OAT expression and activity while decreasing intracellular ornithine and polyamine concentrations and cell numbers in Caco-2 cell cultures. Park et al. [52] also reported that RA inhibited the growth of human colon cancer cells. These results, together with previously reported RA-mediated decreases in ODC activity [15], suggest that RA coordinately regulates OAT and ODC to modulate cell proliferation by altering intracellular ornithine and polyamine concentrations. Although specific investigation of polyamine transport was beyond the scope of this current study, it should also be noted that the RA effect on polyamine concentrations could be due to a decrease in polyamine uptake, as has been observed in cultured mouse hepatocytes treated with RA [53]. Arginase II may also be part of this group of enzymes, which modulates cell proliferation and responds to RA [54]. Hypertrophic female mouse kidneys exhibit coordinate increases in ODC and arginase II activities and a decrease in OAT activity [49]. Notably, arginase II and ornithine are essential for proliferation of Caco-2 cells [55]. The addition of ornithine to Caco-2 cells treated with an arginase II inhibitor stimulated proliferation to levels characteristic of untreated cells [55]. Although arginase II activity is reduced by retinoic acid in other tissues [16], further studies are necessary to determine the effect of RA on intestinal arginase activity and to determine if RA coordinately regulates ODC, OAT and arginase II in intestinal epithelial cells. Likewise, the effect of RA on in vivo expression of OAT, ODC and arginase II during fetal and neonatal development remains unknown.

The reason for the transient increase in intracellular ornithine concentrations of cells treated with RA for 12 h (Table 1) may be explained by an initial increase in arginase activity [49] and a concomitant decrease in ODC activity [15]. The half-life of ODC is approximately 30 min and its expression is down-regulated by RA [15]. Our data indicate that OAT activity is not increased until 18 h of treatment. Although speculative, it is possible that ODC activity rapidly decreases to a level that uses little ornithine, while activities of arginase II (a source of intracellular ornithine in Caco-2 cells) and OAT (the enzyme that diverts ornithine away from ODC) respond more slowly to RA, resulting in increased intracellular concentrations of ornithine. However, when intracellular concentrations of ornithine were reduced substantially, it becomes a limiting factor for polyamine synthesis [1,54].

A decrease in intracellular concentrations of polyamines may impair DNA and protein synthesis, as well as the proliferation of intestinal epithelial cells [56]. In support of this notion, we found that addition of 10 μM putrescine to the culture medium effectively prevented the decreases in both intracellular polyamine concentrations and cell numbers in RA-treated Caco-2 cell cultures (Table 2), as previously reported for endothelial cells treated with DL-α-difluoromethylornithine [57]. Others have shown in a rat intestinal epithelial cell line (IEC-6) that polyamine depletion leads to arrest of the cell cycle at G_1 via a p53-dependent mechanism involving p21 [58]. However, in Caco-2 cells, which have a mutated, inactive p53, this mechanism is not as clear.

In summary, the results of this study demonstrate that RA induces OAT activity and mRNA expression in Caco-2 intestinal epithelial cells. Furthermore, this increase in OAT activity and expression occurs concomitantly with decreases in intracellular concentrations of ornithine and polyamines as well as cell number in the Caco-2 cultures. Our findings indicate a functional role for OAT in regulating intestinal polyamine synthesis.

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