Nutrient-Gene Interactions

Retinoic Acid and Glucocorticoid Treatment Induce Hepatic Glycine N-Methyltransferase and Lower Plasma Homocysteine Concentrations in Rats and Rat Hepatoma Cells1,2

Matthew J. Rowling and Kevin L. Schalinske3

Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011

ABSTRACT Perturbation of folate and methyl group metabolism is associated with a number of pathological conditions, including cardiovascular disease and neoplastic development. Glycine N-methyltransferase (GNMT) is a key protein that functions to regulate the supply and utilization of methyl groups for S-adenosylmethionine (SAM)-dependent transmethylation reactions. Factors or conditions that have the ability to regulate GNMT and the generation of homocysteine, a product of transmethylation, have important implications in the potential perturbation of methyl group metabolism. We showed that retinoid compounds induce active hepatic GNMT, resulting in compromised transmethylation processes. Because retinoids can stimulate gluconeogenesis, a condition known to alter methyl group and homocysteine metabolism, the current study was undertaken to determine the relationship between all-trans-retinoic acid (RA) and gluconeogenic hormones on these metabolic pathways. Intact adrenal function was not required for RA to induce and activate hepatic GNMT; however, treatment of rats with dexamethasone (DEX) was as effective as RA in inducing GNMT in rat liver. As the marked increase in plasma total homocysteine levels observed in adrenalectomized rats was reduced to normal levels by treatment with either RA or DEX, indicating that the transsulfuration and/or remethylation pathways may be enhanced. Moreover, coadministration of RA and DEX had an additive effect on GNMT induction. Similar findings were also observed in a rat hepatoma cell culture model using H4IIE cells. Taken together, these results demonstrate that both RA and DEX independently induce GNMT, thereby having substantial implications for the potential interaction of retinoid administration with diabetes. J. Nutr. 133: 3392–3398, 2003.

KEY WORDS: • glycine N-methyltransferase • methyl groups • homocysteine • retinoic acid • dexamethasone

Glycine N-methyltransferase (GNMT)4 (EC 2.1.1.20) is a key protein in the regulation of methyl group and folate metabolism (1). GNMT catalyzes the S-adenosylmethionine (SAM)-dependent methylation of glycine to generate sarcosine, a compound with no known physiologic function, thereby optimizing the ratio of SAM to S-adenosylhomocysteine (SAH). The ratio of SAM/SAH is an index of transmethylation potential, because SAH inhibits most SAM-dependent methyltransferases (2,3). Moreover, the enzymatic activity of GNMT is allosterically inhibited by the binding of the folate coenzyme 5-methyl-tetrahydrofolate (5-CH3-THF), thereby providing a regulatory signal between SAM-dependent transmethylation and the supply of methyl groups from the folate-dependent one-carbon pool (4,5). Conversely, SAM serves as an allosteric ligand for the inhibition of 5,10-methylene-tetrahydrofolate reductase (MTHFR) activity (6,7), the enzyme that irreversibly catalyzes the synthesis of 5-CH3-THF. After transmethylation, SAH is hydrolyzed to homocysteine and adenosine. Homocysteine can be remethylated to regenerate methionine, a process that requires either 5-CH3-THF or betaine as methyl donors, or it can be catalyzed through the transsulfuration pathway by the initial action of cystathionine β-synthase (CBS). In addition to removing excess homocysteine, transsulfuration is also important in the biosynthesis of essential compounds such as cysteine and glutathione. Thus, regulation of GNMT is a key control point in the optimal function of folate and methyl group metabolism (Fig. 1), interrelated metabolic pathways that when perturbed, are associated with a number of pathologies (8–10).

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3 To whom correspondence should be addressed.
E-mail: kschalin@iastate.edu.
4 The abbreviations used are: ADX, adrenalectomized; Bt2cAMP, dibutylryl-cAMP; CBS, cystathionine β-synthase; DEX, dexamethasone; GNMT, glycine N-methyltransferase; MTHFR, 5,10-methylene-tetrahydrofolate reductase; 5-CH3-THF, 5-methyltetrahydrofolate; PC, phosphatidylcholine; PEPCK, phosphoenolpyruvate carboxykinase; PMSF, phenylmethylsulfonyl fluoride; RA, all-trans-retinoic acid; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.
retinoid compounds induces and activates GNMT, thereby compromising SAM-dependent transmethylation reactions, including the methylation of DNA, RNA, proteins and phospholipids. SAM is an allosteric inhibitor of 5,10-methylenetetrahydrofolate reductase (MTHFR) and positive modulator of cystathionine β-synthase (CBS), whereas 5-methyltetrahydrofolate (5-CH3-THF) is an inhibitory ligand for GNMT. Other abbreviations used are: BHMT, betaine homocysteine methyltransferase; MS, methionine synthase; THF, tetrahydrofolate.

FIGURE 1  Folate, methyl group and homocysteine metabolism. Glycine N-methyltransferase (GNMT) regulates the ratio of S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH), thereby optimizing transmethylation reactions (X → X-Ch3), such as the methylation of DNA, RNA, proteins and phospholipids. SAM is an allosteric inhibitor of 5,10-methylenetetrahydrofolate reductase (MTHFR) and positive modulator of cystathionine β-synthase (CBS), whereas 5-methyltetrahydrofolate (5-CH3-THF) is an inhibitory ligand for GNMT. Other abbreviations used are: BHMT, betaine homocysteine methyltransferase; MS, methionine synthase; THF, tetrahydrofolate.

MATERIALS AND METHODS

Chemicals. Materials were obtained from the following sources: tissue culture reagents, Life Technologies (Rockville, MD); S-adenosyl-L-[methyl-3H]methionine, Perkin Elmer (Boston, MA); goat anti-rabbit IgG horseradish peroxidase, Southern Biotechnology (Birmingham, AL); enhanced chemiluminescence Western blotting detection reagents, Amersham Pharmacia (Piscataway, NJ); S-adenosyl-L-methionine and dexamethasone, Sigma Chemical (St. Louis, MO); and protease inhibitors and RA, Calbiochem (La Jolla, CA). All other reagents were of analytical grade.

Animal experiments. All animal experiments were approved by and conducted in accordance with Iowa State University Laboratory Animal Resources Guidelines. Adrenalectomized (ADX) and sham-operated male Sprague-Dawley (Harlan Sprague Dawley, Indianapolis, IN) rats (125–149 g) were housed in plastic cages and given free access to food and water in a room with a 12-h light-dark cycle. ADX rats received saline (10 g/L NaCl) as drinking water. The composition of the control diet was the same as described previously (23) except the AIN-93G formulation of vitamin and mineral mix was utilized. After rats were acclimated to both the control diet and oral administration of corn oil, they were randomly assigned to various treatment groups (n = 5/group) and given a daily oral dose of either vehicle (corn oil) or vehicle containing RA (30 μmol/kg body weight). Although this represents a pharmacologic dose of RA and we have shown that levels as low as 5 μmol/kg body weight effectively elevated GNMT activity, the maximal saturating effect of RA on GNMT induction was achieved using the higher concentrations (Ozias, M. K. and Schalinske, K. L., unpublished data). For glucocorticoid studies, rats were given a daily intraperitoneal injection of DEX (1 mg/kg body weight) that was dissolved in a vehicle containing propylene glycol:absolute ethanol:glacial acetic acid:ascorbic acid (95:5:0:0.1, v/v/v/v). In preliminary studies, we found that this level of DEX administration was as effective as higher doses (i.e., saturating) and allowed maintenance of normal body weight. After a 5- to 7-d treatment period with RA and/or DEX, rats were anesthetized with a intraperitoneal injection of ketamine:xyazine (90:10 mg/kg body weight) and blood samples were collected via cardiac puncture in heparinized syringes, centrifuged at 4000 X g for 8 min and stored at −20°C until analysis. Liver and pancreatic tissue samples were rapidly removed and homogenized in ice-cold buffer containing 10 mmol/L sodium phosphate (pH 7.2), 0.25 mol/L sucrose, 1 mmol/L EDTA, 1 mmol/L sodium azide and 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF). After centrifugation at 20,000 X g for 30 min, β-mercaptoethanol was added to the resulting supernatants to a final concentration of 10 mmol/L and they were stored at −70°C for subsequent analysis of GNMT activity and abundance as previously described (11,13).

Cell culture. Rat hepatoma H4IE cells (American Type Culture Collection, Manassas, VA) were grown in 150-cm² flasks to 70–75% confluence under 5% CO2 in a humidified incubator at 37°C in DMEM containing 100 mL fetal bovine serum, penicillin (100,000 U/L) and streptomycin (100 mg/L). Cells were given fresh media immediately before treatments. Cells were either treated alone, or with various combinations of DEX (0.1 μmol/L), dibutyryl-cAMP (Br2cAMP, 0.5 mmol/L), glucagon (0.5 μmol/L) and/or RA (10 μmol/L). Preliminary dose-response studies established these levels as optimal for modulating methyl group metabolism. After a 72-h incubation period in the presence of the various treatment reagents, cells were detached with 2.5 g/L trypsin/1 mmol/L EDTA, washed twice in HBSS, and lysed on ice in a buffer containing 10 mmol/L HEPES (pH 7.4), 10 mmol/L sodium pyrophosphate, 50 mmol/L sodium fluoride, 50 mmol/L β-glycerophosphate, 5 mmol/L EDTA, 1 mmol/L sodium...
orthovanadate, 2 mmol/L benzamidene, 100 mg/L leupeptin and pepstatin, 250 mg/L soybean trypsin inhibitor, 0.2 mmol/L PMSF, 24 mg/L p-nitroanilinedibenzoate and 5 mL/L Nonidet P-40. Lysates were centrifuged at 16,000 × g for 8 min after which supernatants were removed and stored at −70°C for subsequent analysis of GNMT protein abundance.

Measurement of GNMT Activity. The enzymatic activity of GNMT was determined in tissue supernatants as described by Cook and Wagner (24) with minor modifications (13). The assay reaction mixture (100 μL) consisted of 200 mmol/L Tris buffer (pH 9.0), 5 mmol/L dithiothreitol, 2 mmol/L glycine and 2 mmol/L [methyl-3H]SAM (47.7 kBq/μmol). The reaction was initiated with 250 μg cytosolic protein, and incubation of the assay mixture was carried out at 25°C for 30 min. The reaction was terminated by the addition of trichloroacetic acid, and activated charcoal was used to remove unreacted radiolabeled SAM by centrifugation (14,000 × g for 5 min). An aliquot of the resulting supernatant containing the radiolabeled product was removed for liquid scintillation counting and GNMT activity was expressed as pmol sarcosine formed/(min·mg protein). All GNMT assays were performed in triplicate.

Analysis of GNMT protein abundance. The abundance of GNMT protein in tissue and cell culture extracts was determined using immunoblotting techniques as previously described (11–13). Briefly, a 10–20% gradient gel was cast for SDS-polyacrylamide gel electrophoresis for determination of the abundance of the 32-kDa monomer subunit of GNMT, which functions enzymatically in its homometamer form. After separation, proteins were electrochemically transferred to a nitrocellulose membrane, followed by incubation with either an affinity-purified polyclonal GNMT antibody (kindly provided by C. Wagner, Vanderbilt University) or a monochlonal GNMT antibody (25) that recognizes a specific peptide sequence within the protein (kindly provided by Y.-M.A. Chen, National Yang-Ming University, Taipei, Taiwan). For either primary antibody, blots were incubated overnight at 4°C followed by a 1-h incubation at room temperature with goat anti-rabbit horseradish peroxidase secondary antibody. GNMT abundance was assessed by chemiluminescence detection and after multiple exposures to Kodak X-Omat AR film, densitometric analysis was performed using Sigma-Gel Software (SPSS, Chicago, IL). For both enzyme activity measurements and Western blot analysis, the total soluble protein concentration in liver and cell extracts was determined by the method of Bradford (26) using a commercial kit (Coomassie Plus; Pierce Chemical, Rockford, IL) and bovine serum albumin as a standard.

Determination of plasma homocysteine concentrations. Total (free + protein-bound) homocysteine concentrations were determined using HPLC with fluorescence detection (27). Plasma samples were derivatized with 7-flouro-2-oxa-1,3-diazole-4-sulfonate, and a standard mixture (100 μL) consisted of 200 mmol/L Tris buffer (pH 9.0), 5 mmol/L dithiothreitol, 2 mmol/L glycine and 2 mmol/L [methyl-3H]SAM (47.7 kBq/μmol). The reaction was initiated with 250 μg cytosolic protein, and incubation of the assay mixture was carried out at 25°C for 30 min. The reaction was terminated by the addition of trichloroacetic acid, and activated charcoal was used to remove unreacted radiolabeled SAM by centrifugation (14,000 × g for 5 min). An aliquot of the resulting supernatant containing the radiolabeled product was removed for liquid scintillation counting and GNMT activity was expressed as pmol sarcosine formed/(min·mg protein). All GNMT assays were performed in triplicate.

Statistical analysis. The mean values from each treatment group were subjected to a one- or two-way ANOVA (28). If the ANOVA was significant (P < 0.05), the means were compared using Fisher’s least significant difference procedure. All statistical analysis was performed using SigmaStat software (SPSS, Chicago, IL).

RESULTS

Intact adrenal function was not required for the induction of hepatic GNMT by RA. The activity and abundance of GNMT was elevated after administration of RA to adrenalectomized rats (Fig. 2). As we showed previously, RA increased both the activity (Fig. 2A) and abundance (Fig. 2B) of hepatic GNMT ~2.0-fold in sham-operated control rats. The immunoblot above the bar graph in Figure 2B is a representative sample from each treatment group with the relative fold-induction indicated under each lane. Removal of the adrenal glands did not alter the basal activity or the abundance of GNMT; administration of RA was equally effective at elevating GNMT activity (1.9-fold) and abundance (2.0-fold) in ADX rats compared with their respective untreated controls. These findings indicate that RA regulates methyl group metabolism independently of adrenal function.

Hepatic GNMT was activated and induced by both RA and glucocorticoid treatment. Although it did not appear that adrenal function was required for GNMT regulation by RA, a primary objective was to determine whether glucocorticoids, alone or in combination with RA, could also stimulate GNMT expression (Fig. 3). For sham-operated rats, treatment with DEX increased the activity of GNMT in a manner similar to that exhibited by RA-treated rats (1.6- and 1.9-fold, respectively). Similar results were observed after treatment of ADX rats with RA and DEX; hepatic GNMT activity was elevated 2.0- and 2.4-fold, respectively. Moreover, when administered together, RA and DEX clearly appeared to have an additive
effect on activating GNMT in both sham-operated (2.6-fold) and ADX (3.6-fold) rats compared with control values. The activation of hepatic GNMT by RA and DEX, alone or in combination, was reflected in the abundance of the protein, as shown by Western blot (Fig. 4A). In contrast, pancreatic GNMT protein abundance was not responsive to either RA, as we have previously reported (12), or DEX treatment in sham-operated (Fig. 4B) or ADX rats (data not shown). The activity of GNMT in the pancreas also remained constant regardless of RA or DEX administration in both sham-operated and ADX rats (data not shown). These results indicate that in addition to RA, the glucocorticoid DEX was equally effective at regulating the expression of GNMT in both sham-operated and ADX rats in a tissue-specific manner; the additive effect of coadministration of RA and DEX suggests that the basis for their action, at least in part, is mechanistically distinct between the two.

**Homocysteine levels were reduced by RA and DEX treatment of adrenalectomized rats.** Recent work showed that a diabetic state (i.e., the absence of insulin) or an equivalent elevation in circulating counter-regulatory hormones (i.e., glucagon and/or glucocorticoids) decreases plasma homocysteine concentrations in rats due to an increase in homocysteine catabolism through the transsulfuration pathway (14–16). We explored the possibility that RA had a similar ability to reduce homocysteine levels, indicating that the excessive production of homocysteine due to GNMT induction resulted in an increase in its metabolism. Plasma homocysteine levels were elevated more than twofold as a result of adrenalectomy (Fig. 5). Moreover, both RA and DEX were effective in reducing homocysteine levels (41 and 74%, respectively) in ADX rats; no further decrease was observed when RA and DEX were provided simultaneously. In contrast, homocysteine concentrations were not reduced by either RA and/or DEX treatment in sham-operated rats.

**Induction of GNMT in rat hepatoma cells.** To assess the ability of RA and hormones to directly induce expression of GNMT protein, we utilized a cell culture system consisting of a rat hepatoma cell line. GNMT is not expressed to a significant degree in most cell lines, including human HepG2 cells; however, in preliminary experiments we found that H4IIE cells did express discernible levels of GNMT, and the regulation of GNMT in this cell line was sensitive to factors shown to be effective in vivo. GNMT abundance in H4IIE cells was regulated in a manner similar to that observed in vivo, with RA and DEX alone or in combination increasing GNMT expression.

**FIGURE 3** Induction of hepatic glycine N-methyltransferase (GNMT) by retinoic acid (RA), dexamethasone (DEX) in sham-operated and adrenalectomized (ADX) rats. Sham-operated and ADX male Sprague-Dawley rats were treated with either a daily dose of RA (30 μmol/kg body weight), DEX (1 mg/kg body weight) or both. After 5 d, liver samples were removed and GNMT activity was determined as described in Materials and Methods. Data are means ± SEM, n = 5; bars with different letters differ, P < 0.05.

**FIGURE 4** Regulation of hepatic and pancreatic glycine N-methyltransferase (GNMT) abundance by retinoic acid (RA) and dexamethasone (DEX) in sham-operated and adrenalectomized (ADX) rats. Tissue samples from the same rats as described for Figure 3 were used for the determination of GNMT abundance by Western blot analysis as described in Materials and Methods. (A) Representative immunoblot of hepatic GNMT abundance in sham-operated and ADX rats treated with RA, DEX or both compounds. The relative fold-induction is indicated under each lane. (B) Representative immunoblot of pancreatic GNMT abundance in sham-operated rats treated with RA, DEX or both compounds. All blots are representative examples from individual rat tissue samples.

**FIGURE 5** Plasma homocysteine concentrations in sham-operated and adrenalectomized (ADX) rats after treatment with retinoic acid (RA), dexamethasone (DEX) or both. Plasma samples from the same rats as described for Fig. 3 were obtained for the assessment of total homocysteine concentrations by HPLC as described in Materials and Methods. Data are means ± SEM, n = 5; bars with different letters differ, P < 0.05.
subject to regulation by RA (Fig. 6) (lanes 1–4). This regulation of GNMT protein abundance by RA in H4IIE cells was similar to what was observed in rat liver (lanes 5–8). For comparative purposes, Figure 6 also demonstrates that the level of expressed GNMT protein, relative to a given amount of total cellular protein, was significantly less in H4IIE cells compared with rat liver. Similar to the rat liver data shown earlier in Figure 3, RA and DEX treatment of H4IIE cells increased GNMT abundance 2.6- and 3.6-fold, respectively (Fig. 7). Moreover, coadministration with both compounds resulted in an additive 4.8-fold stimulation of GNMT expression. These results using a cell culture model demonstrate that both RA and DEX regulate methyl group metabolism directly, in the absence of other physiologic factors. Moreover, pretreatment of H4IIE cells with actinomycin D abolished the ability of both RA and DEX to increase GNMT abundance, indicating that transcriptional mechanisms were involved (data not shown).

In addition to DEX, a number of other components have also been shown to play a potential role in the regulation of GNMT, methyl group and homocysteine metabolism. Thus, H4IIE cells were treated with various combinations of RA, DEX, Bt2cAMP, and glucagon (Fig. 8). As shown earlier in Figure 7, the immunoblot shown in Figure 8A demonstrates that exposure of H4IIE cells to RA or DEX (lanes 2 and 3, respectively, compared with lane 1) induced GNMT protein (2.1- and 1.8-fold, respectively), and cells incubated with both RA and DEX (lane 6) exhibited an elevation of GNMT protein abundance that appeared to be additive (4.8-fold increase). In contrast, incubation with Bt2cAMP alone did not induce GNMT compared with controls (compare lanes 4 and lane 1). Moreover, the addition of Bt2cAMP appeared to attenuate GNMT induction in cell cultures treated with RA and/or DEX (compare lanes 5 and 2, lanes 7 and 3, lanes 8 and 6). Experiments with glucagon-treated H4IIE cells (lane 3) had results similar to those with Bt2cAMP (lane 4) in that no induction was discernible compared with untreated cells (lane 1), in contrast to the 4.5-fold increase exhibited by cells treated with both RA and DEX (lane 2) (Fig. 8B).

**DISCUSSION**

GNMT is a key protein in the regulation of methyl group metabolism by optimizing the ratio of SAM to SAH, thus controlling SAM-dependent transmethylation reactions. This role is supported by evidence that GNMT is tissue specific, abundant, constituting 1–3% of soluble cellular protein, less sensitive to SAH inhibition than other methyltransferases and regulated allosterically by the folate coenzyme 5-CH3-THF (3–5,17). Alterations in GNMT function and methyl group metabolism were documented as a result of numerous conditions including methyl group deficiency or excess, folate deficiency, ethanol administration, gender, age and genetic disorders (12,29–37). Interestingly, GNMT expression is significantly reduced or absent from most cell lines and tumor...
tissue, and its expression has been examined as a potential marker for cancer susceptibility (3,25,38–40). Because the regulation of GNMT and methyl group metabolism has implications for a number of pathologic conditions (8–10), elucidation of nutritional and physiologic factors that modulate its expression is of great importance in health and disease.

We showed in the work presented here, as well as in previous studies (11–13), that retinoid compounds such as RA represent a factor that has the ability to regulate GNMT and methyl group metabolism. A novel finding from this research is that similar to in vivo studies, treatment of H4IIE cells with RA increases the abundance of hepatic GNMT. Previous studies on GNMT regulation by Wagner and co-workers (4,5) focused on allosteric and post-translational mechanisms; 5-CH$_3$-THF serves as a ligand to inhibit its activity, whereas cAMP-dependent phosphorylation of the protein increases GNMT activity without changes in abundance. Although we cannot dismiss these mechanisms, or a decrease in GNMT degradation as contributing factors in its regulation by RA, it appears that the primary mechanism resides in increased expression of the protein.

Although others have reported that the action of retinoids to stimulate the induction of gluconeogenic enzymes or to alleviate methionine toxicity was adrenal mediated (21,22), the ability of RA to regulate GNMT was clearly not dependent on intact adrenal function and presumably the secretion of glucocorticoid compounds. We demonstrated for the first time that the glucocorticoid DEX also has the ability to regulate GNMT; DEX was as effective as RA in inducing active GNMT in rat liver and its abundance in H4IIE cells. In contrast, administration of hydrocortisone to ADX rats did not alter GNMT activity in an earlier study (41). GNMT regulation in a cell culture model indicates that RA and DEX exert their action in the absence of other physiologic factors. Moreover, the coadministration of RA and DEX had an additive effect on GNMT activation, suggesting that distinct cellular mechanisms were utilized, mechanisms that do not appear to be sensitive to the action of Bt$_2$cAMP or glucagon. The specific signaling components involved in RA- and/or DEX-mediated regulation of GNMT remain an area of future investigation. Because GNMT has not been reported to contain a retinoic acid-, cAMP- or a glucocorticoid-response element in its promoter region (41), the effects of either compound on the induction of GNMT expression are likely secondary to other cellular events. A synergistic interaction among DEX, RA and Br$_2$cAMP to induce PEPCK in rat hepatocytes was reported (19); however, the PEPCK promoter contains regulatory regions for all three compounds.

For both RA and DEX, the induction of GNMT would be expected to have a substantial effect on the concomitant production of homocysteine. The marked increase in plasma homocysteine concentrations observed in ADX rats was reduced to normal levels by both RA and DEX, presumably as a result of increased homocysteine metabolism, such as enhanced catabolism via the transsulfuration pathway. These findings are supported by earlier studies demonstrating that the transsulfuration of homocysteine was enhanced by glucocorticoids or a diabetic condition (14–16), as well as the demonstration that retinoid compounds appear to enhance methionine catabolism (23,42,43). An interesting and previously unreported finding was the elevation in circulating homocysteine levels exhibited by ADX rats alone. As indicated by a decrease in relevant enzyme activity (e.g., methionine adenosyltransferase), a lack of adrenal function was reported to decrease methionine catabolism, whereas the administration of glucagon and/or glucocorticoids enhances the catabolism of methionine and homocysteine, including an elevation in the activity of MTHFR (21,44–46). Administration of RA and/or DEX did not reduce plasma homocysteine levels in intact rats; however, we found consistently that younger rats treated with RA for longer time periods (8–10 d) had a significant (~50%) reduction in the concentration of plasma homocysteine (Ozias, M. K. and Schalinske, K. L., unpublished data).

In addition to enhanced transsulfuration, a number of other possibilities must be considered in attempts to explain the reduction in plasma homocysteine concentrations. Although an increase in homocysteine would be predicted as a result of the increase in GNMT activity and therefore necessitate enhanced transsulfuration, it is also logical that a decrease in homocysteine would ensue as a result of inhibition of other SAM-dependent transmethylation reactions and/or an elevation in the remethylation of homocysteine. Together, the synthesis of creatine and phosphatidylcholine (PC) represent the major use of methyl groups from SAM and thus are the largest contributors to homocysteine production. A recent study showed that phosphatidylethanolamine N-methyltransferase, the SAM-dependent hepatic enzyme that catalyzes the synthesis of PC, plays a major role in the regulation of homocysteine levels in the plasma (47). Perturbation of SAM-dependent transmethylation due to GNMT induction is supported by our earlier work demonstrating that RA administration results in hepatic hypomethylation of DNA and diminished creatinine excretion (12,13), as well as the development of hepatic steatosis (48), presumably due to an inability to synthesize adequate PC. The remethylation of homocysteine via the folate-dependent one-carbon pool may also contribute to the enhanced metabolism of homocysteine after RA treatment. We found in preliminary studies that the activity of methionine synthase was increased in RA-treated rats (Ozias, M. K. and Schalinske, K. L., unpublished data). The potential contribution of remethylation as a determinant in explaining the effects of RA and/or DEX on methyl group and homocysteine metabolism is currently being evaluated.

Our findings that both retinoid compounds and glucocorticoids can markedly induce GNMT and alter methyl group/ homocysteine metabolism has important implications on a number of fronts. First, these findings contribute to the growing body of reports demonstrating that gluconeogenic hormones and conditions, such as starvation and diabetes, can result in abnormal changes in the metabolism of methyl groups and homocysteine. In the absence of renal dysfunction, diabetes/hyperglucagonemia is associated with a decrease in circulating homocysteine concentrations and an increase in GNMT activity (14–18,49). Second, the clear interaction that exists between glucocorticoids and RA to induce GNMT may be an important concern for diabetics who may use retinoid compounds. We have found in preliminary studies that diabetic rats were significantly more sensitive to induction of GNMT by treatment with RA (Rowling, M. J. and Schalinske, K. L., unpublished data). Third, the sensitivity of humans to perturbations in methyl group metabolism as a result of retinoid compounds or a gluconeogenic condition may be highly influenced by the presence of the known polymorphisms that exist for a number of key enzymes that function in folate and methyl group metabolism (e.g., MTHFR). Taken together, these findings have important implications for a large number of individuals who have alterations in carbohydrate, folate or methyl group metabolism. Moreover, it should be noted that the use of clinical retinoid compounds
has risen tremendously over the last decade, affecting ~800,000 new individuals each year (50).

LITERATURE CITED


