Modulation of Methyl Group Metabolism by Streptozotocin-induced Diabetes and All-trans-retinoic Acid

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Abstract

The hepatic enzyme glycine N-methyltransferase (GNMT) plays a major role in the control of methyl group and homocysteine metabolism. Because disruption of these vital pathways is associated with numerous pathologies, understanding GNMT control is important for evaluating methyl group regulation. Recently, gluconeogenic conditions have been shown to modulate homocysteine metabolism and treatment with glucocorticoids and/or all-$trans$-retinoic acid (RA)-induced active GNMT protein, thereby leading to methyl group loss. This study was conducted to determine the effect of diabetes, alone and in combination with RA, on GNMT regulation. Diabetes and RA increased GNMT activity 87 and 148%, respectively. Moreover, the induction of GNMT activity by diabetes and RA was reflected in its abundance. Cell culture studies demonstrated that pretreatment with insulin prevented GNMT induction by both RA and dexamethasone. There was a significant decline in homocysteine concentrations in diabetic rats, owing in part to a 38% increase in the abundance of the transsulfuration enzyme cystathionine β-synthase; treatment of diabetic rats with RA prevented cystathionine β-synthase induction. A diabetic state also increased the activity of the folate-independent homocysteine remethylation enzyme betaine-homocysteine S-methyltransferase, whereas the activity of the folate-dependent enzyme methionine synthase was diminished 52%. In contrast, RA treatment attenuated the streptozotocin-mediated increase in betaine-homocysteine S-methyltransferase, whereas methionine synthase activity remained diminished. These results indicate that both a diabetic condition and RA treatment have marked effects on the metabolism of methyl groups and homocysteine, a finding that may have significant implications for diabetics and their potential sensitivity to retinoids.

Disciplines

Food Chemistry | Food Microbiology | Food Science | Human and Clinical Nutrition | Molecular, Genetic, and Biochemical Nutrition

Comments

Modulation of Methyl Group Metabolism by Streptozotocin-induced Diabetes and All-*trans*-retinoic Acid*

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The hepatic enzyme glycine *N*-methyltransferase (GNMT) plays a major role in the control of methyl group and homocysteine metabolism. Because disruption of these vital pathways is associated with numerous pathologies, understanding GNMT control is important for evaluating methyl group regulation. Recently, gluconeogenic conditions have been shown to modulate homocysteine metabolism and treatment with glucocorticoids and/or all-*trans*-retinoic acid (RA)-induced active GNMT protein, thereby leading to methyl group loss. This study was conducted to determine the effect of diabetes, alone and in combination with RA, on GNMT regulation. Diabetes and RA increased GNMT activity 87 and 148%, respectively. Moreover, the induction of GNMT activity by diabetes and RA was reflected in its abundance. Cell culture studies demonstrated that pretreatment with insulin prevented GNMT induction by both RA and dexamethasone. There was a significant decline in homocysteine concentrations in diabetic rats, owing in part to a 38% increase in the abundance of the transsulfuration enzyme cystathionine β-synthase; treatment of diabetic rats with RA prevented cystathionine β-synthase induction. A diabetic state also increased the activity of the folate-independent homocysteine remethylation enzyme betaine-homocysteine *S*-methyltransferase, whereas the activity of the folate-dependent enzyme methionine synthase was diminished 52%. In contrast, RA treatment attenuated the streptozotocin-mediated increase in betaine-homocysteine *S*-methyltransferase, whereas methionine synthase activity remained diminished. These results indicate that both a diabetic condition and RA treatment have marked effects on the metabolism of methyl groups and homocysteine, a finding that may have significant implications for diabetics and their potential sensitivity to retinoids.

Methyl group and folate-dependent one-carbon metabolism are interrelated pathways that provide for the subsequent transmethylation of various molecules using *S*-adenosylmethionine (AdoMet)[1] (1). An adequate supply of methyl groups via the diet or the folate-dependent one-carbon pool and regulation of these pathways are essential in preventing associated pathologies such as cancer, vascular disease, and neural tube defects (2–4). Glycine *N*-methyltransferase (GNMT) (EC 2.1.1.20) is an abundant, tissue-specific protein that plays a key role in the regulation of hepatic methyl group metabolism by the enzymatic conversion of glycine and AdoMet to *S*-adenosylhomocysteine (AdoHcy) and sarcosine (1, 5, 6). GNMT functions to optimize the AdoMet/AdoHcy ratio, an indicator of transmethylation potential, because AdoHcy is a potent inhibitor of most AdoMet-dependent methyltransferases (5, 7). Following hydrolysis of AdoHcy, the resulting homocysteine can undergo remethylation to methionine or be irreversibly catabolized by the transsulfuration pathway. Folate-dependent remethylation occurs with the donation of a methyl group by 5-methyltetrahydrofolate (5-CH$_3$-THF) through the action of B$_{12}$-dependent methionine synthase (MS) (8). In hepatic tissue, betaine derived from the oxidation of choline can also serve as a folate-independent source of methyl groups for homocysteine remethylation via the enzyme betaine-homocysteine *S*-methyltransferase (BHMT). Transsulfuration to cysteine occurs through the activity of two vitamin B$_6$-dependent enzymes, cystathionine β-synthase (CBS) and γ-cystathionase.

The regulation of hepatic GNMT represents an important mechanism for controlling both the folate-dependent supply of methyl groups as well as their utilization in AdoMet-dependent transmethylation reactions. GNMT activity is regulated in response to changes in methyl group status as the result of allosteric inhibition of 5,10-methylenetetrahydrofolate reductase by AdoMet (9, 10) and inhibition of GNMT by 5-CH$_3$-THF (11, 12), the enzymatic product of 5,10-methylenetetrahydrofolate reductase. This allows methyl groups to be conserved under conditions of deficiency by decreasing GNMT activity, whereas elevations in its activity function to dispose of excess methyl groups. In addition to allosteric control, phosphorylation of GNMT represents another posttranslational mechanism to increase the activity of GNMT and regulate methyl group metabolism (12).

Recent studies have identified both nutritional and hormonal factors that alter methyl group metabolism by targeting the key enzymes involved. All-*trans*-retinoic acid (RA) has been shown to induce hepatic GNMT activity and protein abundance, resulting in the loss of methyl groups for subsequent transmethylation reactions (13–16). Moreover, dexamethasone

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[1] The abbreviations used are: AdoMet, *S*-adenosylmethionine; GNMT, glycine *N*-methyltransferase; AdoHcy, *S*-adenosylhomocysteine; 5-CH$_3$-THF, 5-methyltetrahydrofolate; MS, methionine synthase; BHMT, betaine-homocysteine *S*-methyltransferase; CBS, cystathionine β-synthase; RA, all-*trans*-retinoic acid; DEX, dexamethasone; STZ, streptozotocin; HPLC, high pressure liquid chromatography.

(DEX) was just as effective as RA in the induction of GNMT activity in rat liver and hepatoma cells, and the co-administration of both DEX and RA induced GNMT in an additive fashion (17). A diabetic state characterized by insufficient circulating concentrations of insulin and elevated levels of the counter-regulatory hormones glucagon and glucocorticoids has also been reported to alter enzymes involved in methyl group metabolism. An elevation in the activity of GNMT has been reported in alloxan-induced diabetic sheep and rats (6, 18); however, little is known about the mechanistic basis for this increase. Brosnan and co-workers have shown that under diabetic conditions, the catabolism of homocysteine was enhanced by transcriptional regulation of CBS, and these changes were prevented by treatment with insulin (19–21). Although we have shown that DEX can significantly alter methyl group and homocysteine metabolism (17), the focus in this study was to evaluate these pathways in a diabetic rat model, alone and in combination with RA, as well as determine the effect of insulin on preventing these alterations.

EXPERIMENTAL PROCEDURES

Chemicals—Reagents were obtained as follows: *S*-adenosyl-L-[*methyl*-³H]methionine, PerkinElmer Life Sciences; chemiluminescence Western blotting detection reagents, Amersham Biosciences; *S*-adenosyl-L-methionine and streptozotocin, Sigma; protease inhibitors and RA, Calbiochem; goat anti-mouse IgG horseradish peroxidase, goat anti-rabbit IgG horseradish peroxidase, and rabbit anti-sheep IgG horseradish peroxidase, Southern Biotechnology (Birmingham, AL). GNMT and CBS antibodies were kindly provided by Yi-Ming Chen, National Yang-Ming University, Taipei, Taiwan (22), and Jan Kraus, University of Colorado Health Sciences Center, Denver, CO, respectively. All other chemicals were of analytical grade.

Animals—All animal experiments were approved by and conducted in accordance with Iowa State University Laboratory Animal Resources Guidelines. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) (125–149 g) were housed in individual plastic cages in a room with a 12-h light/dark cycle and allowed free access to food and water. Rats were randomly assigned to treatment groups (6 rats per group) and acclimated to the control diet (17) for 5 days. Following the acclimation period, rats received a single intraperitoneal injection of either streptozotocin (STZ, 60 mg/kg body weight) in the vehicle (10 mM citrate buffer, pH 4.5) or the vehicle alone. The following day animals received a daily oral dose of RA (30 μmol/kg body weight) or vehicle (corn oil) alone. Following a 5-day treatment period with RA, rats were anesthetized and whole blood samples were collected via cardiac puncture. An aliquot of whole blood was used to assess blood glucose concentrations using a commercial kit (Sigma), whereas the remaining blood was centrifuged at 4,000 × g for 6 min, and the resulting plasma fraction was stored at -20 °C for subsequent analysis of homocysteine concentrations. Liver samples were removed and homogenized in 4 volumes of ice-cold phosphate-buffered (10 mM, pH 7.0) sucrose (0.25 M) containing 1 mM EDTA, 1 mM sodium azide, and 0.1 mM phenylmethylsulfonyl fluoride. Following centrifugation at 20,000 × g for 30 min, supernatants were stored at -70 °C with 1 mM β-mercaptoethanol for subsequent enzymatic and protein abundance measurements. Total soluble protein concentrations were determined based on the method of Bradford (23) using a commercial kit (Coomassie Plus, Pierce) and bovine serum albumin as a standard. Additional liver samples were homogenized in 2 volumes of 0.4 M perchloric acid for the determination of AdoMet and AdoHcy.

Cell Culture—Rat pancreatic AR42J and hepatoma H4IIE cells were obtained from the American Type Culture Collection (Manassas, VA). All cells were grown in 150-cm² flasks in a humidified incubator with 5% CO_2 and a temperature of 37 °C until they were 70–75% confluent. H4IIE cells were cultured in minimum essential medium containing 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (0.1 mg/ml). AR42J cells were grown in F-12K nutrient mixture containing 20% fetal bovine serum as well as penicillin and streptomycin. Cell lines received fresh media prior to the initiation of any treatments. H4IIE and AR42J cells were treated with either a vehicle of 0.01% dimethyl sulfoxide (Me₂SO, control), 10 μM RA, 0.1 μM DEX, or both RA and DEX as described previously (17). A parallel group of AR42J and H4IIE cells was preincubated with 100 nM insulin for 24 h prior to the addition of RA and/or DEX. Following a 72-h incubation period, cells were detached using 0.25% trypsin/1 mM EDTA, washed with Hanks' balanced salt

solution, and lysed on ice in a buffer containing 10 mM HEPES (pH 7.4), 10 mM sodium pyrophosphate, 50 mM sodium fluoride, 50 mM β-glycerophosphate, 5 mM EDTA, 1 mM sodium orthovanadate, 2 mM benzamidine, 100 μg/ml leupeptin and pepstatin, 250 μg/ml soybean trypsin inhibitor, 0.2 mM phenylmethylsulfonyl fluoride, 24 μg/ml *p*-nitroguanidinobenzoate, and 0.5% Nonidet P-40. Following centrifugation at 16,000 × g for 8 min, supernatants were stored at -70 °C for analysis of GNMT protein abundance.

GNMT Activity Analysis—The enzymatic activity of GNMT was determined using the method described by Cook and Wagner (24) with minor modifications, and performed in triplicate. The assay mixture (100 μl) consisted of 0.1 M Tris buffer (pH 9.0), 1 mM glycine, 5 mM dithiothreitol, 1 mM *S*-adenosyl-L-[*methyl*-³H]methionine, and was initiated with 250 μg of protein followed by incubation at 25 °C for 30 min. Trichloroacetic acid (10%) was added to terminate the reaction followed by the addition of activated charcoal and centrifugation (14,000 × g) to remove radiolabeled AdoMet. Aliquots of the supernatants were removed for liquid scintillation counting.

Determination of GNMT and CBS Protein Abundance—GNMT protein abundance was determined using immunoblotting techniques as previously described (17). A 10–20% gradient SDS-polyacrylamide gel was used to quantify the 32-kDa monomer subunit of GNMT. Following separation, proteins were transferred to a nitrocellulose membrane and incubated overnight at 4 °C with a 1:4,000 dilution of the monoclonal GNMT antibody (22). The membrane was then incubated with goat anti-mouse IgG horseradish peroxidase secondary antibody for 1 h at room temperature. CBS protein abundance was analyzed using a similar method as described above to separate the 63-kDa CBS subunit. A 1:20,000 dilution of the polyclonal CBS antibody was used, which was followed by incubation with a goat anti-rabbit IgG horseradish peroxidase secondary antibody. GNMT and CBS protein abundance were detected with chemiluminescence and exposed to Kodak X-Omat AR film. Densitometric analysis was performed using SigmaGel software (SPSS, Chicago, IL). For both GNMT and CBS, three samples from each treatment group were randomly chosen for analysis on a single immunoblot.

Determination of Hepatic AdoMet and AdoHcy Concentrations—Perchloric acid homogenates were centrifuged at 9,000 × g for 10 min, and the resulting supernatants were neutralized and applied to a Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA) to obtain AdoMet and AdoHcy (25, 26). AdoMet and AdoHcy were separated and quantified by reversed-phase HPLC and UV detection (254 nm) using a mobile phase containing 30% methanol in 5 mM octane sulfonic acid (pH 4.0) operated isocratically at 1.2 ml/min.

Analysis of BHMT and MS Activity—BHMT activity was measured as described previously by Garrow (27). The standard BHMT assay contained 5 mM DL-homocysteine, 2 mM betaine (0.1 μCi), and 40 μg of total protein. The activity of MS was determined as described previously (28). The assay reaction mixture containing sodium phosphate buffer (500 mM, pH 7.5), cyanocobalamin (1.3 μM), dithiothreitol (1 M), AdoMet (10 mM), β-mercaptoethanol (82.4 mM), homocysteine (100 mM), and [*methyl*-¹⁴C]-THF (15 mM, 0.17 μCi/μmol) was added to liver supernatants and incubated for 1 h at 37 °C. Ice-cold water was added to terminate the reaction, and the assay mixture was immediately applied to a AG 1-X8 (chloride form) resin columns. Effluent fractions (3 ml of deionized water) were collected for subsequent liquid scintillation counting. For both the BHMT and MS assay, homocysteine was prepared fresh daily from a thiolactone derivative.

Plasma Homocysteine Analysis—Total plasma homocysteine concentrations were determined using HPLC and fluorescence detection (29). For derivatization, 10% tributylphosphine in dimethylformamide was added to 300 μl of plasma samples and subsequently incubated at 4 °C for 30 min. The reaction was terminated with ice-cold trichloroacetic acid containing 1 mM EDTA. Following centrifugation at 1,000 × g for 5 min, supernatants were added to a solution containing borate buffer (0.125 M, pH 9.5), sodium hydroxide (1.55 M), and 4-fluoro-7-sulfobenzofurazan (ammonium salt, 0.1%). *N*-Acetylcysteine (1 mM) was added to the plasma samples prior to derivatization as an internal standard. Samples were injected onto a μBondapak C_{18} Radial-Pak column (Waters Associates) equilibrated in a mobile phase consisting of 4% acetonitrile in 0.1 M potassium phosphate buffer (pH 2.1).

Statistical Analysis—SigmaStat software (SPSS) was used for all statistical analyses. The mean values of each treatment group were subjected to a two-way analysis of variance. When the analysis of variance was significant ($p < 0.05$), means were compared using Fisher's least significant difference procedure.

TABLE I
*Cumulative weight gain and blood glucose concentrations
in rats treated with STZ and/or RA*

Values are expressed as mean ± S.E. ($n = 6$). Values within a column
with different letter superscripts are significantly different, $p < 0.05$.

	Weight gain	Blood glucose
	g	*mg/dl*
Control	77 ± 2[a]	147 ± 17[a]
STZ	18 ± 3[b]	539 ± 19[b]
RA	73 ± 1[a]	165 ± 17[a]
STZ + RA	26 ± 5[b]	435 ± 51[c]

RESULTS

*STZ Resulted in Elevated Blood Glucose and Significantly
Less Weight Gain Regardless of RA Treatment*—Cumulative
weight gain for diabetic rats was 23 and 36% of respective
non-diabetic values, whereas RA treatment was without effect
(Table I). STZ clearly induced a diabetic state in rats as blood
glucose concentrations were elevated 3.7-fold compared with
control values. Rats treated with RA had no significant effect
on circulating levels of glucose compared with control values,
and RA attenuated the hyperglycemia exhibited by diabetic
rats.

Both Diabetes and RA Treatment Induced Active GNMT—
STZ-treated rats exhibited a 1.9-fold increase in hepatic GNMT
activity compared with the control values (Fig. 1*A*). RA in-
creased GNMT activity 2.5-fold compared with control rats and
was significantly greater than the degree of induction in STZ-
treated rats. Hepatic GNMT activity was highest in the rats
receiving both STZ and RA (2.6-fold), although this increase
was not significantly different from RA treatment alone. The
changes in GNMT activity in diabetic and RA-treated rats were
also reflected in the abundance of the protein, where a 5.3- and
4.8-fold increase was observed, respectively (Fig. 1*B*). More-
over, co-administration of STZ and RA significantly induced
GNMT protein abundance (6.8-fold) to a greater extent than
either treatment alone. Thus, both RA treatment and a diabetic
condition modulate the expression of active GNMT in rat liver.

*Induction of GNMT by RA and/or DEX was Prevented by
Insulin Treatment*—As reported previously (17), both RA and
DEX alone or in co-administration resulted in induction of the
GNMT protein in H4IIE rat hepatoma cells (Fig. 2). Incubating
cells with insulin for 24 h prior to treatment with RA and/or
DEX prevented GNMT induction. Similarly, DEX-mediated in-
duction of GNMT in AR42J cells was also prevented by insulin;
however, RA was not an effective signal to induce GNMT in
these cells.

*STZ Treatment Reduced Plasma Homocysteine Concentra-
tions and Induced CBS Abundance*—Plasma homocysteine
concentrations were significantly reduced ~48% in both groups
of STZ-treated rats regardless of RA administration (Fig. 3).
Although we have found previously that RA alone was effective
at lowering circulating homocysteine levels (14), the 26% de-
crease observed under these experimental conditions did not
reach statistical significance. The hypohomocysteinemia exhib-
ited by diabetic rats appears to be due, in part, to a 38%
increase in the abundance of CBS, the initial enzyme in the
irreversible catabolism of homocysteine (Fig. 4). However, CBS
activity is regulated allosterically, and thus flux studies con-
ducted *in vivo* would be required to evaluate the transsul-
furation pathway. Interestingly, RA treatment of diabetic rats
prevented CBS induction (Fig. 4), even though plasma homo-
cysteine concentrations remained diminished (Fig. 3).

*STZ and RA Treatments Differentially Stimulate Homocys-
teine Remethylation Enzymes*—In addition to transsulfuration,
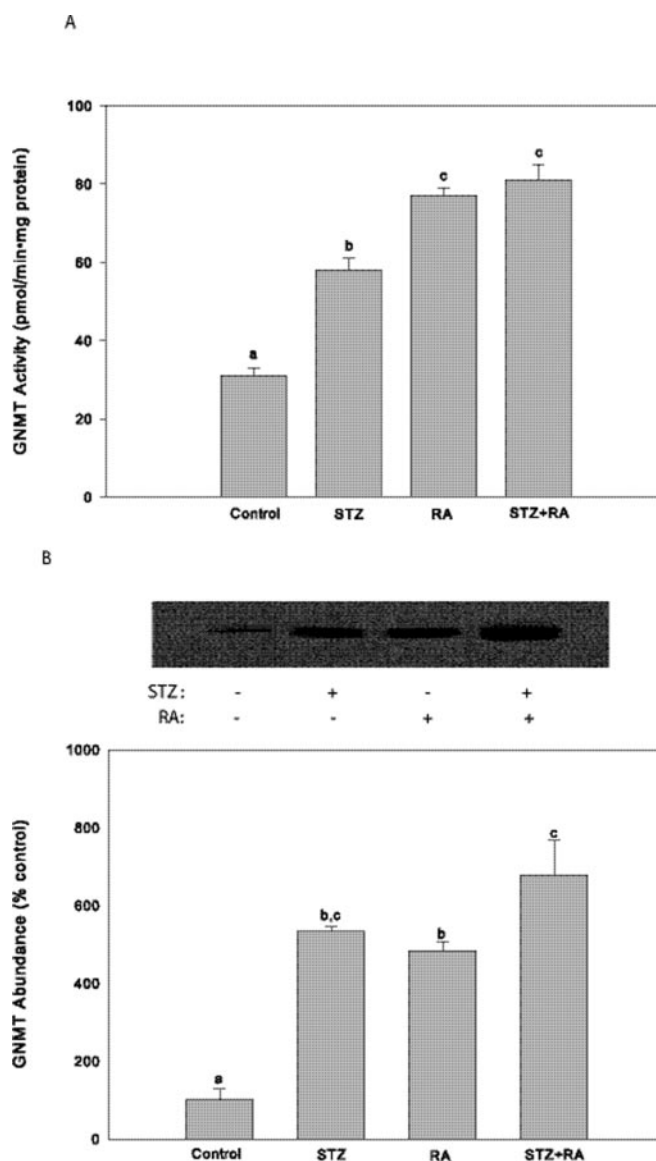the reduced plasma homocysteine levels also appear to reflect

A

FIG. 1. **Induction of hepatic GNMT activity and abundance in
STZ-mediated diabetic and RA-treated rats.** Diabetic (single dose
of STZ, 60 mg/kg body weight) rats were treated daily with RA (30
μmol/kg body weight) for 5 days. Liver samples were removed and the
activity and abundance of GNMT was determined as described under
"Experimental Procedures." *A*, GNMT enzyme activity in diabetic and
non-diabetic rats following administration of RA or corn oil. Data are
expressed as means ± S.E. ($n = 6$), and *bars* denoted with different
letters are significantly different ($p < 0.05$). *B*, GNMT protein abun-
dance in diabetic and non-diabetic rats following administration of RA
or corn oil. A monoclonal GNMT antibody (22) was used for Western
blot analysis, and a representative immunoblot is shown. Data are
expressed as means ± S.E. ($n = 3$), and *bars* denoted with different
letters are significantly different ($p < 0.05$).

changes in the activity of enzymes involved in homocysteine
remethylation. BHMT activity was significantly increased 3.3-
fold in diabetic rats, whereas the activity of MS was reduced
52% (Table II). RA did not significantly alter either enzyme,
although there was a trend ($p = 0.061$) for increased MS activ-
ity, similar to our previous observations (14). RA was effective
at reducing the activity of BHMT in diabetic rats, but did not
prevent the STZ-mediated decrease in MS activity.

*STZ-mediated Elevations in the Hepatic Concentrations of
AdoMet and AdoHcy Were Prevented by RA Treatment*—A dia-
betic condition resulted in a significant increase in the hepatic
concentrations of AdoMet (169%) and AdoHcy (90%) (Table III).
Although RA treatment did not significantly alter AdoMet and
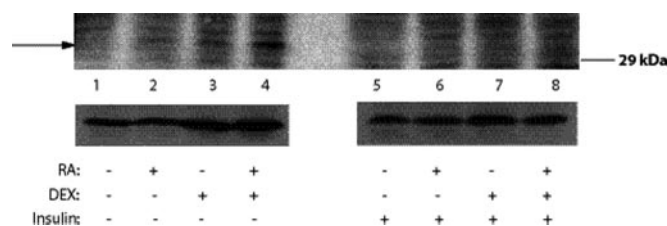
FIG. 2. **Regulation of GNMT expression in rat hepatoma and pancreatic cell lines.** Rat hepatoma H4IIE (*upper panel*) and pancreatic AR42J (*lower panel*) cells were treated with the following: 0.01% Me$_2$SO (control, *lane 1*), 10 μM all-*trans*-retinoic acid (RA, *lane 2*), 0.1 μM dexamethasone (DEX, *lane 3*), or both RA and DEX (*lane 4*). A parallel group of cells was preincubated with 100 nM insulin for 24 h prior to the respective treatments (*lanes 5–8*). Following a 72-h incubation period, cells were lysed and used to determine GNMT abundance via Western blotting as described under "Experimental Procedures."
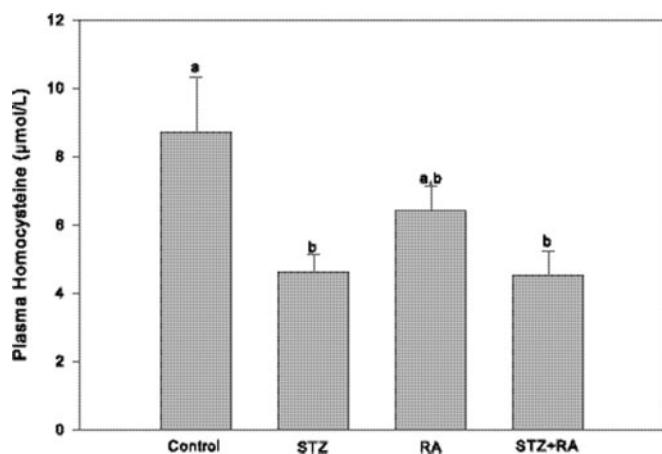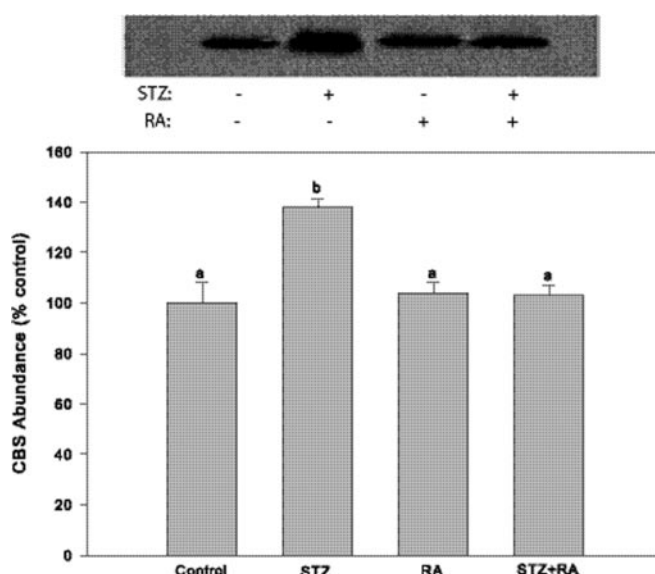

FIG. 3. **Plasma homocysteine concentrations in STZ-mediated diabetic and RA-treated rats.** Plasma samples from the same rats as described for Fig. 1 were obtained for the determination of total homocysteine concentrations by HPLC and fluorometric detection as described under "Experimental Procedures." Data are expressed as means ± S.E. (*n* = 6); *bars* with different *letters* are significantly different ($p < 0.05$).

FIG. 4. **The abundance of CBS was elevated in STZ-mediated diabetic rats and abrogated following treatment with RA.** Hepatic tissue samples from the same rats as described for Fig. 1 were used to determine CBS abundance by Western blot analysis as described under "Experimental Procedures." A representative *immunoblot* is shown above the *bar graph*. Data are means ± S.E. (*n* = 3); *bars* denoted with different *letters* are significantly different ($p < 0.05$).

TABLE II

Activity of remethylation enzymes methionine synthase MS and BHMT in the livers of rats treated with STZ and/or RA

Values are expressed as mean ± S.E. (*n* = 6). Values within a column with different letter superscripts are significantly different, $p < 0.05$.

	BHMT	MS
	units/mg of protein	*pmol/min·mg of protein*
Control	40 ± 3[a]	86 ± 11[a]
STZ	132 ± 11[b]	41 ± 5[b]
RA	51 ± 4[a]	119 ± 19[a]
STZ + RA	100 ± 8[c]	36 ± 6[b]

TABLE III

Hepatic concentrations of AdoMet and AdoHcy from rats treated with STZ and/or RA

Values are expressed as mean ± S.E. (*n* = 5–6). Values within a column with different letter superscripts are significantly different, $p < 0.05$.

	AdoMet	AdoHcy	AdoMet/AdoHcy
		nmol/g of liver	
Control	42.7 ± 2.3[a]	34.9 ± 2.1[a]	1.24 ± 0.08[a]
STZ	114.9 ± 22.5[b]	66.3 ± 10.2[b]	1.77 ± 0.34[a]
RA	38.9 ± 3.1[a]	34.5 ± 4.1[a]	1.20 ± 0.15[a]
STZ + RA	43.6 ± 3.7[a]	40.3 ± 4.3[a]	1.14 ± 0.12[a]

AdoHcy concentrations in non-diabetic rats, it did prevent their accumulation in diabetic animals. Because AdoMet and AdoHcy are allosteric regulators of a number of enzymes involved in folate, methyl group, and homocysteine metabolism, it is difficult to determine the collective metabolic effect of these changes in diabetic rats in the absence of flux measurements using isotopic tracers.

DISCUSSION

Methyl groups, homocysteine, and the folate-dependent one-carbon pool represent interrelated metabolic processes that are important in health and disease. Because GNMT is a vital regulatory protein that functions to control the supply and utilization of methyl groups, it is paramount to understand how nutritional and/or hormonal factors modulate its expression. Our earlier work demonstrated that RA and DEX were independent signals to induce active GNMT in rat liver and in cultured hepatoma cells (17). Here, we have extended these findings by demonstrating that a diabetic condition, mediated by treatment with STZ, also has a profound impact on the activity of hepatic GNMT. Previous rat studies using alloxan-induced diabetes reported similar increases (85%) in GNMT activity (6), whereas treatment with glucagon were less pronounced (23%) (20); however, the mechanistic basis for this increase in GNMT activity has not been thoroughly explored. A novel aspect of our studies demonstrates that for both diabetes and RA, the abundance of GNMT protein was markedly ele-

vated, indicating that GNMT was regulated by transcriptional and/or translational mechanisms or possibly at the level of degradation of the protein. It is not known if regulation of GNMT expression in a diabetic state is the result of an increase in glucocorticoid levels or a lack of insulin. The cell culture studies shown here clearly demonstrate that pretreatment with insulin prevented GNMT induction by both DEX and RA. It will be important in future studies to more fully characterize this diabetic condition with alterations in methyl group metabolism. Interestingly, the responsiveness of GNMT expression to RA and DEX appears to be cell type specific. We have found in other studies that DEX was a more universal signal than RA for GNMT induction across a number of tissues and cell lines, including those that do not express detectable GNMT, such as

the human hepatoma cell line HepG2.[2]

It is not clear how RA, DEX, and/or a diabetic state alter the expression of GNMT. Previous studies on the regulation of GNMT activity have found it to be at the posttranslational level by phosphorylation and allosteric inhibition by $5-CH_3-THF$ (11, 12, 30, 31). Based on the known actions of glucocorticoids and RA with respect to regulation of gene expression, it is likely that the induction of GNMT is the result of increased transcription. In support of this hypothesis, we have found that pretreatment of hepatoma cells with actinomycin D prevented GNMT induction by DEX and RA, and abundance of the protein appears to reflect changes in its synthesis rate.[3] However, GNMT has not been reported to contain a retinoic acid- nor a glucocorticoid-response element in its promoter region (32). Therefore, it is likely that additional intracellular signals are required to directly mediate regulation of GNMT expression. Nonetheless, we cannot exclude the possibility that increases in phosphorylation of the protein have a role in increasing GNMT activity, particularly as glucagon and glucocorticoids can exert their action via alterations in protein kinase activity.

Homocysteine concentrations reflect the collective balance between production from AdoMet-dependent transmethylation reactions, remethylation to methionine, and catabolism via the transsulfuration pathway. As has been reported (19, 20), we also found that a diabetic state was characterized by a reduction in circulating homocysteine levels. This change in homocysteine homeostasis appears to reflect an increase in the activity of BHMT and the abundance of CBS, even though the activity of MS was reduced. Similarly, previous diabetes/hyperglucagonemia studies have attributed the hypohomocysteinemia to up-regulation of CBS and γ-cystathionase, whereas the activity of hepatic BHMT and MS were not significantly altered (19–21). However, in the absence of flux studies it is difficult to determine homocysteine catabolism because CBS is regulated allosterically by other factors such as AdoMet, a known allosteric stimulator of CBS activity (33). In support of the increase in CBS abundance that we reported, we also found that the hepatic concentration of AdoMet was elevated in agreement with previous studies using hyperglucogonemic rats (20). Elevations in hepatic AdoMet levels may be the result of increased expression of methionine adenosyltransferase, as it is up-regulated by glucocorticoids (34). An increase in hepatic AdoMet concentrations would also be expected to reduce $5-CH_3-THF$ levels as a result of allosteric inhibition of 5,10-methylenetetrahydrofolate reductase (9, 10). The lack of $5-CH_3-THF$ acting as an inhibitory ligand for GNMT (11), like phosphorylation, may be a contributing factor for the increase in its activity in diabetic rats. An interesting aspect of our work was that the administration of RA to diabetic rats prevented CBS induction and AdoMet accumulation and partially reduced the activity of BHMT, whereas MS activity was unaffected. In contrast to diabetes, RA alone had no affect on CBS abundance or the activity of BHMT and MS, although we have found in previous studies that RA does increase MS when provided for a longer period of time (14). However, it should be noted that the activity and abundance of GNMT remained elevated in rats treated with RA. This further illustrates the need for metabolic flux measurements under *in vivo* conditions to determine the outcome of these interactions.

The implications of these results for humans are profound, as diabetes and its complications are a significant health problem and the use of pharmacological retinoid compounds has risen dramatically in recent years (35). In addition to folate, optimal metabolism of methyl groups and homocysteine is dependent on a number of B-vitamins including B_{12}, B_6, and riboflavin, as well as an adequate source of methyl groups such as methionine and choline. Moreover, it is well known that the human population has a significant prevalence of polymorphic enzymes important in methyl group, homocysteine, and folate-dependent one-carbon metabolism (36). Taken together, these nutritional, genetic, and hormonal factors underscore the need for understanding the relationship between these metabolic pathways and diabetes. As most type I diabetics control their disease by the use of insulin, there are nonetheless likely many individuals who do not adequately monitor their condition. Moreover, many of the changes noted in our study were recently demonstrated in a type II diabetes (Zucker diabetic fatty rat) model, including an increase in hepatic CBS, BHMT, and AdoMet (37).

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[3] M. J. Rowling, K. M. Nieman, and K. L. Schalinske, unpublished data.

Modulation of Methyl Group Metabolism by Streptozotocin-induced Diabetes and All- *trans*-retinoic Acid

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