Pulsatile growth hormone secretion decreases S-adenosylmethionine synthetase in rat liver

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Received 5 May 2000; accepted in final form 16 October 2000

Oscarsson, Jan, Cissi Gardmo, Staffan Edén, and Agneta Mode. Pulsatile growth hormone secretion decreases S-adenosylmethionine synthetase in rat liver. Am J Physiol Endocrinol Metab 280: E280–E286, 2001.—S-adenosylmethionine synthetase (AdoMet synthetase) is responsible for the synthesis of the major methyl donor S-adenosylmethionine. The AdoMet synthetase gene was identified by subtractive suppressive hybridization as being expressed at higher levels in the liver of rats continuously exposed to growth hormone (GH) than in rats intermittently exposed to the hormone. Further studies on the regulation of AdoMet synthetase showed that the activity and mRNA levels were higher in female than in male rats. Hypophysectomy increased AdoMet synthetase mRNA in both male and female rats. Combined thyroxine and cortisol treatment of hypophysectomized rats had no effect on AdoMet synthetase mRNA levels. Two daily injections of GH for 7 days, mimicking the male secretory pattern of GH, decreased AdoMet synthetase activity and mRNA levels. A continuous infusion of GH, mimicking the female secretory pattern of GH, had small or no effects on AdoMet synthetase activity and decreased the mRNA levels to a lesser degree than two daily injections. It is concluded that the lower AdoMet synthetase activity in male rats is due to an inhibitory effect of the male characteristic pulsatile secretory pattern of GH on AdoMet synthetase mRNA expression.

S-ADENOSYL METHIONINE SYNTHETASE (AdoMet synthetase) (EC2.5.1.6) is responsible for the first and preferred reaction in the catabolism of methionine. The product, S-adenosylmethionine, is the major methyl donor in all cells. Moreover, it is necessary for synthesis of polyamines and takes part in the synthesis of glutathione and phosphatidylcholine (3, 24). The AdoMet synthetase gene is expressed in all living cells and is one of the gene products necessary for independent life (11). In mammals, there are two different genes: one gene is liver specific (MAT1A), and the other gene is ubiquitously expressed (MAT2A) (24). The liver is quantitatively the most important organ for methionine metabolism. In the adult liver, MAT1A gives rise to two different isoforms of AdoMet synthetase: a dimer with a high Michaelis-Menten constant (Km, MAT III) and a tetramer with a low Km (MAT I). Thus the MAT III isoform in the liver allows continued synthesis and utilization of AdoMet when there is a large flux of methionine to the liver.

The AdoMet synthetase activity was early found to be sexually dimorphic, with higher activity in adult female rats than in adult male rats (27). Gonadectomy was found to increase the activity in male rats, an effect that was reversed by testosterone treatment (27). Moreover, AdoMet synthetase activity decreases in male rats after puberty (2, 9). Gonadectomy of female rats decreases the activity, and subsequent estrogen treatment restores it (30). Estrogen treatment also increases the AdoMet synthetase activity in intact male rats (9, 30).

The effects of gonadal steroid hormones on hepatic protein expression, liver functions, and susceptibility to chemically induced liver adenoma have been attributed to their effects on the secretory pattern of growth hormone (GH) in the rat (15, 19, 21, 25, 28, 29, 33, 36, 40). In male rats, GH secretion is pulsatile, with high peaks intervened by low trough levels, whereas in female rats the secretion is more frequent and irregular, resulting in a continuous presence of the hormone (8, 38). The effects of the male pattern of GH secretion can be mimicked in hypophysectomized rats by giving one or two daily subcutaneous injections of GH, and the effects of the female pattern of GH secretion can be mimicked by a continuous delivery of GH (15, 18, 25, 40).

Studies on the effect of GH on AdoMet synthetase activity are limited and inconsistent (4, 9, 31). GH given as one daily injection at a high dose to hypophysectomized female rats has been found to slightly decrease the AdoMet synthetase activity (31). On the other hand, GH given as daily injections to intact male rats has been shown to increase the activity (9), whereas no effect was observed when GH was administered to very young rats (4).

In an attempt to find out which hepatic genes show a higher expression during a continuous compared with intermittent GH exposure, intact male rats were given a continuous infusion of GH. The genes exhibiting a higher expression in response to continuous GH were

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identified by suppression subtractive hybridization. One of the gene products identified was AdoMet synthetase (17). Because the activity of AdoMet synthetase has been shown to be higher in female than in male rats, we decided to investigate the importance of the sexually dimorphic secretory pattern of GH in the regulation of AdoMet synthetase activity and mRNA levels.

MATERIALS AND METHODS

Animals and hormone therapy. Six-week-old male and female Sprague-Dawley rats obtained after hypophysectomy and age-matched control rats (Mellegaard Breeding Center, Ejby, Denmark) were maintained under standardized conditions of temperature (24–26°C), humidity (50–60%), and light (lights on between 0500 and 1900). The rats had free access to standard laboratory chow (rat and mouse standard diet, B&K Universal, Sollentuna, Sweden) and water. The initial mean weights of the male and female hypophysectomized rats were 145 and 121 g, respectively. The corresponding weights for age-matched male and female control rats were 226 and 171 g, respectively. A weight gain of >0.5 g/day among hypophysectomized rats during a 4-day observation period before hormone therapy started was regarded as a sign of remaining pituitary tissue and used as an exclusion criterion (29). Hormone therapy began 7 days after hypophysectomy. All of the hypophysectomized rats were given cortisone acetate (Cortavit) 1 mg/kg and levothyroxine (T4; 10 µg·kg⁻¹·day⁻¹; Nycomed, Oslo, Norway) as a daily subcutaneous injection (0.8 mg/kg) (18, 29, 37, 39). Recombinant bovine GH was a generous gift from American Cyanamid (Princeton, NJ). The daily dose of GH was 0.7 mg/kg to hypophysectomized rats and 0.5 mg/kg to intact male rats. GH was given either continuously by means of an Alzet osmotic mini-pump 2001 (Alza, Palo Alto, CA) or as two daily subcutaneous injections for 6–7 days (28, 29). The osmotic mini-pumps were implanted subcutaneously on the backs of the rats with the animals under light anesthesia. The rats were killed by decapitation between 1000 and 1200. The livers were cut in pieces, processed directly, or immediately frozen in liquid nitrogen and stored at −70°C until assays. The Ethics Committee of Göteborg University and the Stockholm South Ethical Committee of the Swedish National Board for Laboratory Animals approved this study.

Suppression subtractive hybridization and cloning. Suppression subtractive hybridization (SSH) was performed using the Clontech PCR-Select cDNA Subtraction Kit (Clontech, Palo Alto, CA), essentially according to the manufacturer’s instructions. cDNA made from “feminized” male rat liver mRNA samples, i.e., male rats treated with GH (0.5 mg·kg⁻¹·day⁻¹ for 6 days) as a continuous infusion using osmotic mini-pumps (Alzet 2001), was used as the tester. cDNA made from normal male hepatic mRNA was used as the driver. The subtracted cDNA products were cloned into an A/T vector (AdvanTage PCR Cloning Kit, Clontech). Sequence analysis of expressed cDNA products was performed using cycle sequencing with dye-labeled nucleotides (Big-Dye Terminator, Perkin-Elmer, Norwalk, CT), and the gels were run at Cybergene (Huddinge, Sweden). At the KISAK facility at Karolinska Institutet, FastA was used to search the EMBL database for homologies with known genes and sequences.

Northern blot. Total RNA was isolated from liver samples according to Chomczynski and Sacchi (6). Twenty micrograms of RNA were run in formaldehyde containing 1% agarose gel. The RNA was blotted onto Hybond N nylon membranes (Amersham) and covalently linked to the membrane by ultraviolet (UV) irradiation (UV Stratalinker 2400, Stratagene). The membranes were prehybridized at 50°C for ≥3 h in a solution containing 5× saline–sodium phosphate–EDTA buffer (SSPE), 50% formamide, 5× Denhardt’s solution, 1% SDS, 10% dextran sulfate, and 150 g of denatured salmon sperm (ss) DNA per milligram. The same solution without ssDNA was used for hybridization. The template for the AdoMet synthetase probe was the rat sequence nt. −161 to nt. 292 (access no. X15734) (17). As internal standard, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was measured. The GAPDH sequence was the almost full-length cDNA (10). The probes were labeled using a random prime kit, Strip-EZ DNA (Ambion, Austin, TX), and [³²P]dATP (Amersham). The final wash of the membranes was carried out in a solution containing 0.05× SSPE and 0.1% SDS at 65°C for 60 min. Autoradiography and phosphoimaging (Fuji Bas 2000, Fuji) were used for detection. The bands were quantified using the Image Gauge software (Fuji).

AdoMet synthetase assay. The assay used is a slight modification of the procedure of Wong et al. (41), initially described by Mudd et al. (26) and Chou and Lombardi (7). The method is based on the conversion of [¹⁴C]methionine to S-[¹⁴C]adenosylmethionine, which are differentially eluted from a cation exchanger. Liver was homogenized in 0.03 M potassium phosphate buffer containing 2.5 mM dithiothreitol (Sigma, St Louis, MO), pH 6.9, for 30 s at 0°C (Polytron homogenizer, Kinematica, Lucerne, Switzerland). After homogenization, samples were centrifuged at 15,000 g for 20 min. The supernatant (50 µl) was assayed in duplicate or triplicate for AdoMet synthetase activity by addition of 250 µl of a solution containing 8 mM MgCl₂, 200 mM KCl, 1 mM glutathione, 32 mM ATP, 5 mM L-(methyl-³²P)methionine (0.02 µCi/ml; 58 mCi/mmol, Amersham, Ailsbury, Buckinghamshire, UK), and 160 mM Tris-HCl, pH 7.8. L-methionine, reduced glutathione, and ATP were obtained from Sigma and were of reagent grade. The incubations were conducted at 37°C for 15 min in a shaking water bath, during which substrate conversion is proportional to time and enzyme concentration. Putting the samples in an ice bath terminated the reaction. Immediately after termination of the reaction, 200 µl of a solution containing S-adenosyl-L-[(methyl-³²P)]methionine (0.4 µCi/ml, 15 Ci/mmol, Amersham) in triethanolamine buffer, pH 6.6, were added. Four hundred microliters of a total volume of 500 µl were applied to a 0.5 × 6-cm column of DEAE-cellulose (Cellex-P, Bio-Rad, Richmond, CA), prepared according to the instructions from the manufacturer and equilibrated with triethanolamine buffer, pH 6.6. The column was washed with 4-ml portions of the triethanolamine buffer. The portions were collected in scintillation vials and counted in 15 ml of Opti Phase (Hi-Safe, LKB, Sweden). Two peaks were recovered. The first peak corresponded to L-methionine and the second peak to S-adenosylmethionine. Recovery was estimated by determining [³H] and [¹⁴C] activities separately and was 70–80%. Recovery was corrected for breakdown of S-adenosyl-L-[(methyl-³²P)]methionine during storage in each assay. Protein concentration was determined according to the method of Lowry et al. (22). Values were given as nanomoles of S-adenosylmethionine produced per minute per milligram protein. Intra-assay coefficient of variation was 5.6% as determined in 20 duplicates or triplicates.

Statistics. Values are expressed as means ± SE. Comparisons between groups were made by Student’s t-test or one-way analysis of variance (ANOVA), followed by the Student-
Newman-Keuls multiple range test between individual groups. The values were transformed to logarithms when appropriate.

RESULTS

In a search for liver genes expressed at a higher level in rats continuously exposed to GH compared with rats intermittently exposed to the hormone, we used suppressive subtractive hybridization. Genes previously known to be upregulated by a continuous pattern of GH secretion, such as the CYP2C12 gene (21), were identified among the cloned sequences and hence served as positive controls for the experiment. Among the sequences identified as corresponding to known genes but previously not recognized as regulated by the pattern of GH secretion, we found the AdoMet synthetase gene (17). Treatment of adult male rats with 0.5 mg·kg⁻¹·day⁻¹ of GH as a continuous infusion to feminize the GH secretory pattern resulted in a higher expression of AdoMet synthetase mRNA (Fig. 1). This observation confirmed that GH is involved in the regulation of AdoMet synthetase mRNA and indicated that the sex-specific secretion of GH determines the sex difference in its activity. This finding prompted us to further investigate the importance of the sexually dimorphic secretory pattern of GH in the regulation of the sex-differentiated expression of AdoMet synthetase.

The activity of AdoMet synthetase was ~50% higher in female rats than in male rats. A similar difference in expression of AdoMet synthetase mRNA was found (Fig. 2). The effects of hypophysectomy and treatment with GH, either as two daily injections or as a continuous infusion, on AdoMet synthetase mRNA levels were investigated in male and female rats. As shown in Fig. 3, the mRNA levels increased in both male and female rats after hypophysectomy. GH given as two daily injections decreased the mRNA level, whereas a continuous infusion seemed to have small or no effects (Fig. 3). Because all hypophysectomized rats were given T₄ and cortisol therapy, the effect of this substitution on AdoMet synthetase mRNA levels was investigated in a separate experiment. The mRNA level expressed as a ratio of AdoMet synthetase to GAPDH mRNA was 0.048 ± 0.006 (n = 3) in untreated hypophysectomized rats and 0.050 ± 0.001 (n = 3) in T₄- and cortisol-treated hypophysectomized rats. Thus no difference between these groups was observed, indicating that the increase in AdoMet synthetase mRNA after T₄ and cortisol administration to the hypophysectomized rats was not due to this hormone therapy.

The effect of hypophysectomy in male and female rats on AdoMet synthetase activity was also investigated. In hypophysectomized male rats given T₄ and cortisol, the activity was 128 ± 6% (n = 4) compared with 100 ± 12% (n = 5) in intact male rats, P = 0.09. In hypophysectomized female rats given T₄ and cortisol, the activity was 120 ± 4% (n = 6) compared with 100 ± 6% (n = 5) in intact female rats, P = 0.02. These results indicate that both mRNA and enzyme activity increase after hypophysectomy. Thus a pretranslational downregulation of AdoMet synthetase activity by GH is inferred.

In subsequent experiments, the effects of GH treatment as two daily injections or as a continuous infusion on both AdoMet synthetase activity and mRNA levels were assessed in hypophysectomized female and male rats (Figs. 4 and 5). In hypophysectomized female rats, two daily injections of GH decreased AdoMet synthetase activity, whereas a continuous infusion of GH had no effect (Fig. 4A). AdoMet synthetase mRNA decreased after GH treatment irrespective of the mode of administration. However, GH given as two daily injections resulted in a more marked decrease than a
continuous infusion of the hormone (Fig. 4B). In hypophysectomized male rats, GH given as two daily injections decreased AdoMet synthetase activity, whereas a continuous infusion increased the activity (Fig. 5A). AdoMet synthetase mRNA levels decreased after GH treatment as two daily injections, but a continuous infusion did not significantly alter the mRNA level. Moreover, the difference between the two modes of GH administration did not reach statistical significance in the hypophysectomized male rats (Fig. 5B).

**DISCUSSION**

The present results show that GH decreased AdoMet synthetase activity and mRNA levels most efficiently when it was given as two daily injections, mimicking the male GH secretory profile. A continuous infusion of GH, mimicking the female secretory pattern of GH, either had no effect or increased AdoMet synthetase activity. In contrast to the activity data, GH decreased AdoMet synthetase mRNA levels irrespective of the mode of GH administration. However, in line with activity data, AdoMet synthetase mRNA levels decreased more efficiently when the hypophysectomized rats were given GH as two daily injections than as a continuous infusion. Together, the results suggest that the sex difference in AdoMet synthetase activity is most closely related to an inhibitory effect of a pulsatile GH secretion on AdoMet synthetase mRNA levels. In line with this conclusion, a continuous infusion of GH to intact male rats increased AdoMet synthetase mRNA levels.

The sex difference in AdoMet synthetase activity was observed by using a high methionine concentration in the assay. Previous investigators describing the sex difference or effects of gonadal steroids on AdoMet synthetase activity also measured the high K_m form (MAT III) (4, 9, 27). The sex difference in AdoMet synthetase activity was similar in magnitude to the sex difference in AdoMet synthetase mRNA levels, indicating that the sex difference in activity is mainly due to differences at the mRNA level.

One previous study describes a small increase or no effect of hypophysectomy on AdoMet synthetase activity, depending on at what time after hypophysectomy the measurements were performed (31). The increase in AdoMet activity after hypophysectomy in this study is in line with the results of Pan and Tarver (31). Perturbation of glucocorticoid and thyroid hormone levels caused by hypophysectomy appeared not to be responsible for the increase in AdoMet synthetase activity.
mRNA levels. The doses of cortisol and T₄ administered to the animals have previously been shown to be within the physiological range with respect to effects on longitudinal bone growth (18, 39) and plasma concentrations (37). However, higher doses of T₄ and glucocorticoids have been shown to have opposite effects on AdoMet synthetase activity (31), indicating that small and opposite effects of these hormones would have eliminated each other. Few studies have addressed the hormonal regulation of AdoMet synthetase mRNA. However, in line with activity data (31), glucocorticoids have been shown to increase the mRNA levels (13). Therefore, it is conceivable that the increase in mRNA levels after hypophysectomy may at least partly be due to the loss of the suppressive effect of GH. However, the increased AdoMet synthetase activity after hypophysectomy was not decreased by the continuous infusion of GH. This finding indicates that either another inhibitory pituitary-dependent factor exists or that continuous GH exerts a stimulatory effect on AdoMet synthetase at a translational or post-translational level.

The present findings are of importance for the understanding of the diverse effects of GH on hepatic metabolism. GH treatment has previously been shown to stimulate the liver content of polyamines and to stimulate ornithine decarboxylase activity in hypophysectomized rats (20, 34), effects also observed in bovine GH transgenic mice (14). Because the polyamine synthesis requires S-adenosylmethionine, the pulsatile secretion of GH in male rats may feed the polyamine synthesis with fewer propylamine groups than the continuous secretion in female rats.

The stepwise methylation of phosphatidylethanolamine by phospholipid N-methyltransferase results in the formation of phosphatidylethanolamine (PC) (3). The activity of the methylation pathway of PC synthesis in the liver is higher in female than in male rats. This difference has not been attributed to a sex difference in phospholipid N-methyltransferase (2); rather, the higher activity of AdoMet synthetase in female rats results in enhanced methylation of phosphatidylethanolamine (3). The methylation pathway results in PC containing more stearic and arachidonic acids and less palmitic and linoleic acids in female rat livers compared with male rat livers (23, 28). We have previously observed that a continuous infusion of GH, but not daily injections of GH, to hypophysectomized rats increases the proportion of stearic and arachidonic acids in the hepatic PC fraction (28). The suppressive effect of pulsatile GH on AdoMet synthetase cannot be the sole explanation for the effects of the secretory pattern of GH on sex differences in fatty acid composition of PC. However, it is likely that the lower activity of AdoMet synthetase after treatment with GH in a pulsatile fashion would contribute to the lower activity of the methylation pathway in male rats.

It has been shown that female rats are less sensitive than male rats to the effects of choline deficiency on lipid synthesis (3, 27). Furthermore, the secretory pattern of GH has been shown to be of importance for the development of chemically induced adenomas in choline-deficient rats. A choline-deficient diet given to diethylnitrosamine-treated rats results in more altered liver foci in male livers compared with female livers (33). Furthermore, transplantation of an ectopic pituitary under the kidney capsule, which releases GH continuously, protects male rats from the development of liver changes. A plausible mechanism for this effect of GH was suggested to involve steps in the methylation pathway (33). Our results suggest that a higher activity of the AdoMet synthetase enzyme as a result of continuous GH exposure may contribute to the potentially protective effect of a higher methylating capacity.

A high AdoMet synthetase activity may also be detrimental under certain conditions. It has been shown that a 2.3-fold increase in AdoMet synthetase activity by stable transfection in Chinese hamster ovary cells made these cells more sensitive to oxidative stress (35). The reason for this effect is most likely that the AdoMet synthetase reaction consumes a large amount of ATP and NAD, resulting in depletion of these molecules in the cell. Therefore, the lower AdoMet syn-
The regulation of AdoMet synthetase may have implications for the understanding of the role of GH in such diverse processes as polyamine synthesis, phosphatidylcholine synthesis, and susceptibility to chemically induced liver adenoma. Moreover, because methylation is involved in capping of mRNA, methylation of DNA, and nuclear hormone receptor co-activators (5), the GH regulation of AdoMet synthetase may have implications for the understanding of how GH takes part in the regulation of gene transcription.

We thank Elisabeth Wiersma-Larsson for skilful technical help. This work was supported by Grants 8269 and 72XS-13146 from the Swedish Medical Research Council, the Novo Nordisk Foundation, the regulation of gene transcription. The molecular mechanism(s) by which pulsatile GH downregulates the AdoMet synthetase gene can only be speculated on. The effect of a pulsatile mode of GH on gene regulation has been shown to be dependent on activation of the transcription factor STAT 5b (12, 32). The promoter of the liver-specific AdoMet synthetase gene has been partially characterized (1). These authors showed binding of NF-1 and HNF-3 to the AdoMet synthetase promoter by using nuclear extracts from rat liver, but they did not report on STAT binding. However, using a transcription factor binding site profile database (“TFSEARCH: Searching Transcription Factor Binding Sites”, http://www.rwcp.or.jp/papia/) (16), three potential STAT binding sites (score 92.2 to 85.6) were revealed in the cloned promoter. It will be of interest to investigate whether STAT 5b is the mediator of the GH downregulation of the AdoMet synthetase gene and whether it occurs via any of these sites.

In summary, we found that GH given as two daily injections decreased both AdoMet synthetase activity and mRNA, whereas a continuous infusion of GH had small effects on the activity and decreased AdoMet synthetase mRNA in female hypophysectomized rats to a lesser extent than two daily injections of the hormone. These results may have implications for the understanding of the role of GH in such diverse processes as polyamine synthesis, phosphatidylcholine synthesis, and susceptibility to chemically induced liver adenoma. Moreover, because methylation is involved in capping of mRNA, methylation of DNA, and nuclear hormone receptor co-activators (5), the GH regulation of AdoMet synthetase may have implications for the understanding of how GH takes part in the regulation of gene transcription.

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