Cortisol and Estradiol: Nongenetic Factors for Hyperhomocyst(e)inemia

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A low plasma homocyst(e)ine concentration in premenopausal and pregnant women compared with postmenopausal women and men suggests that steroid hormones are nongenetic factors affecting homocysteine metabolism. This hypothesis was tested by determining plasma homocyst(e)ine levels in adult male rats treated with cortisol, estradiol, or a combination of both. Mean plasma homocyst(e)ine concentrations were 3.71 ± 0.71, 5.26 ± 1.76, and 4.28 ± 0.84 nmol/mL in cortisol-treated, estradiol-treated, and cortisol plus estradiol–treated groups, respectively. These values were substantially low compared with the level of 7.32 ± 0.89 nmol/mL plasma homocyst(e)ine in the control group, indicating a significant effect of steroid hormones on homocysteine metabolism.

HOMOCYSTEINE is a normal intermediary metabolite in methionine metabolism. This sulfhydryl amino acid is readily oxidized to homocysteine–homocysteine disulfide (homocystine), cysteine–homocysteine disulfide, and protein-bound homocyst(e)ine. All forms of homocysteine and its derivatives that are identified as homocysteine after reduction are defined as total homocysteine or homocyst(e)ine.[1,2] Plasma homocyst(e)ine concentration is controlled by various factors that may be considerably different between individuals.[1-4] Hence, it is sometimes difficult to demonstrate the effect of a single factor on the plasma homocysteine level.

Plasma homocyst(e)ine in pregnant women decreases to almost half that in nonpregnant women.[5,6] In contrast, the difference in plasma homocyst(e)ine concentration between premenopausal women and postmenopausal women or men is not pronounced; therefore, variable degrees of difference including negative results were reported by different investigators.[7-15] These results suggest a close relationship between homocysteine metabolism and endocrinological status.

Plasma estrogen and progesterone levels are higher in premenopausal women compared with postmenopausal women and men. Pregnancy causes a further increase of these hormones. Plasma cortisol and deoxycorticosterone levels are also increased during pregnancy.[16] Previously, Finkelstein et al[17,18] observed that cortisol and estrogen caused increased activity of liver betaine–homocysteine methyltransferase and kidney methionine synthase, respectively. Therefore, we postulated that steroid hormones are nongenetic factors affecting homocysteine metabolism. To minimize individual genetic and nongenetic variance, including endocrinological status, we chose inbred adult male rats as study subjects. To maximize the hormonal effect, animals were treated with pharmacologic doses of cortisol, estradiol, or a combination of both. With this system, we demonstrated a significant decrease in plasma homocyst(e)ine concentration with administration of the steroid hormones, supporting the hypothesis that cortisol and estradiol are nongenetic factors influencing homocysteine metabolism.

MATERIALS AND METHODS

Twenty-two male Sprague-Dawley rats aged 78 to 120 days and weighing 330 to 410 g (Cestui, Omaha, NE) were used in this study. The rats were housed in 10 stainless steel cages in a room with controlled temperature (21° ± 1.0°C) and lighting (12-hour light cycle). Each rat was identified by punching and notching of the ears. To maintain the basal level of plasma homocyst(e)ine within close range of the mean plasma homocyst(e)ine value, all animals were fed Teklad 10% mouse breeder (Teklad, Madison, WI) beginning 3 weeks before the experiment. The levels of vitamin B_{12}, choline, folic acid, and pyridoxine in this diet are, respectively, 18.3%, 30.6%, 40.4%, and 25% of the amounts contained in other commercial diets.

Animals were divided into four groups. Groups A, B, and C received estradiol, cortisol, and estradiol plus cortisol, respectively. Depo-estradiol (estradiol cypionate injection USP; Upjohn, Kalamazoo, MI) was diluted into 10 μg/mL with cottonseed oil, and estradiol 15 μg/kg body weight was administered intramuscularly once at the beginning of the experiment. A single intramuscular injection of Depo-estradiol is sufficient to maintain a steady serum concentration of estradiol for at least 19 days (Upjohn Depo-estradiol Study, personal communication). Hydrocortisone acetate suspension (Merck Sharp & Dohme, West Point, PA) was injected subcutaneously at a daily dose of 15 mg/kg body weight for 2 weeks. Group A also received a daily saline injection 0.6 mL/kg body weight, and group B also received cottonseed oil 1.5 mL/kg body weight at the beginning of the experiment. Control animals received both saline and cottonseed oil.

The body weight and food consumption of each animal were measured daily throughout the experiment. Five milliliters of blood was obtained by cardiac puncture after 14 days of treatment. Blood was collected into a heparinized tube, and plasma was separated within 1 hour. Animals were exsanguinated after being anesthetized with methoxyflurane, and tissues were excised, sliced, and stored at −80°C.

Plasma total homocyst(e)ine and liver methylenetetrahydrofolate reductase levels were determined as previously described.[19,20] Plasma amino acids were determined by an ion-exchange analyzer[19] (model 6300; Beckman Instruments, Palo Alto, CA).

The chi-square test and Student's *t* test were used to examine differences between groups.

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RESULTS

The control group showed a mean plasma total homocyst(e)ine level of 7.32 ± 0.89 nmol/mL (Table 1). In contrast, the mean plasma total homocyst(e)ine level was 3.71 ± 0.71 nmol/mL in the cortisol-treated group, 5.26 ± 1.76 nmol/mL in the estradiol-treated group, and 4.28 ± 0.84 nmol/mL in the cortisol plus estradiol–treated group (Table 1). This indicates that plasma total homocyst(e)ine concentrations in cortisol-treated, estradiol-treated, and cortisol plus estradiol–treated groups were 50.7%, 71.9%, and 58.5% of the mean levels of the control group, respectively. All experimental groups showed a significant decrease in plasma homocyst(e)ine levels ($P < .05$). Compared with the other two treatments, the effect of estradiol is least pronounced. Simultaneous administration of cortisol and estradiol appears to have an antagonistic effect on reduction of the plasma homocysteine level. Liver methylenetetrahydrofolate reductase activity showed no statistically significant difference between the control group and any hormone-treated group (data not shown).

During the 2-week course of experiment, the body weight of the control group increased 10%, whereas that of estradiol-treated, cortisol-treated, and estradiol plus cortisol–treated groups decreased 5%, 10%, and 20%, respectively. The mean daily food consumption per animal was 22, 18, 18, and 17 g in control, cortisol-treated, estradiol-treated, and cortisol plus estradiol–treated groups, respectively. There was a positive correlation between body weight and food consumption. Plasma protein and amino acid levels were determined for evaluation of reduced food consumption (Table 2). Fasting or reduced food intake causes a decrease of plasma concentrations of threonine and proline and an increase of plasma glycine.[21-25] Plasma branched-chain amino acid concentrations were increased by fasting and decreased by reduced food intake for a prolonged period.[21-25] In this study, a significant decrease in plasma threonine was found only in the cortisol-treated group, and the proline level was decreased in cortisol-treated and estradiol plus cortisol–treated groups. A decrease of the leucine level was found in groups treated with cortisol and cortisol plus estradiol, but not in the group treated with estradiol. A decrease of isoleucine levels was found only in the group treated with both hormones. Plasma glycine was decreased in all hormone-treated groups. A statistically significant decrease in plasma methionine was only observed in animals treated with both cortisol and estradiol.

Table 1. Plasma Homocyst(e)ine Concentration in Rats Treated With Cortisol, Estradiol, or a Combination of Cortisol and Estradiol

Group	No. of Rats	Plasma Homocyst(e)ine (nmol/mL) Mean ± SD	Range	Percentage of Control Value	Significance (*P*)*
Control	6	7.32 ± 0.89	6.21-8.39	100.0%	
Cortisol	5	3.71 ± 0.71	2.71-4.52	50.7%	<.01
Estradiol	6	5.26 ± 1.76	3.95-8.95	71.9%	<.05
Combination	5	4.28 ± 0.84	2.92-5.21	58.5%	<.01

*Student's *t* test.

Table 2. Plasma Total Protein and Amino Acid Concentrations in Rats Treated With Estradiol, Cortisol, or a Combination of Estradiol and Cortisol

Protein and Amino Acids*	Control	Estradiol	Cortisol	Combination
Total protein	6.5 ± 0.8	6.2 ± 0.5	7.4 ± 0.4	7.5 ± 0.9
Threonine	54.7 ± 8.9	60.8 ± 9.7	39.2 ± 6.9†	46.7 ± 3.3
Serine	51.1 ± 6.6	51.9 ± 8.3	39.7 ± 5.6†	37.1 ± 1.0†
Glycine	52.0 ± 9.3	36.0 ± 6.7†	29.2 ± 6.7†	18.9 ± 3.1†
Alanine	98.2 ± 15.1	102.8 ± 18.6	99.0 ± 18.8	93.2 ± 8.0
Proline	50.7 ± 3.7	56.6 ± 9.5	32.0 ± 10.1†	31.1 ± 3.2†
Valine	23.9 ± 5.8	15.3 ± 7.1	14.9 ± 7.7	18.8 ± 1.9
Methionine	8.9 ± 3.3	5.5 ± 1.6	5.7 ± 0.9	4.1 ± 1.1†
Isoleucine	13.5 ± 2.9	12.6 ± 2.5	11.3 ± 3.3	8.6 ± 1.6†
Leucine	29.4 ± 6.2	24.9 ± 3.1	19.5 ± 3.7‡	16.0 ± 3.1†
Phenylalanine	10.4 ± 2.2	9.0 ± 1.3	10.6 ± 1.4	8.7 ± 1.2

*Total plasma protein and amino acid concentrations were expressed as g/dL and μmol/dL, respectively.

†$P < .01$.

‡$P < .05$.

DISCUSSION

The plasma homocyst(e)ine level is reflected by the status of genetic and nongenetic factors. Known genetic defects affecting homocysteine metabolism are estimated to occur in 6% or more of the general population.[26] Each nongenetic factor affects the plasma homocyst(e)ine level and also modifies the magnitude of moderate hyperhomocyst(e)inemia caused by other factors.[1-4] Nongenetic factors include subnormal or low-normal serum vitamin B_{12}, choline, folate, and pyridoxine; high serum creatinine, uric acid, and liver enzymes; other diseases, such as chronic renal failure, liver disease, non–insulin-dependent diabetes mellitus, cancer, psoriasis, etc.; medications; and high protein (methionine) intake.[26] Therefore, a decrease of plasma homocyst(e)ine in premenopausal women compared with postmenopausal women and men may be variably modified or masked by a number of genetic and nongenetic factors.

This study used inbred male rats with a similar age, weight, and nutritional status to minimize individual variance. Most commercial pellet diets are fortified with vitamins, causing a subnormal concentration of plasma homocyst(e)ine.[27] In contrast, this study used a special diet, Teklad 10% mouse breeder, to maintain plasma homocyst(e)ine at least within 1 SD of the mean. Hence, plasma homocyst(e)ine levels of rats in this study are effectively modified by the injection of hormones.

It was reported that the plasma homocyst(e)ine concentration is reduced to 60% of the mean control value in midterm pregnancy.[5,6] In this study, a plasma homocyst(e)ine concentration less than 70% of the normal mean level was observed in animals treated either with cortisol or with the combination of cortisol and estradiol, but not with estradiol only. This suggests that cortisol is more critical than estradiol in the control of the plasma homocyst(e)ine level during pregnancy. A difference in plasma homocyst(e)ine concentrations between pregnant and premenopausal women is probably due to a difference in plasma cortisol rather than estradiol concentration. There is a progressive increase in plasma levels of cortisol and deoxycorticosterone during pregnancy.[16] Most of the elevation of cortisol levels

is due to the estrogen-induced increase in cortisol-binding globulin levels, but the bioactive free fraction is also elevated threefold during pregnancy.[16,28] If the estrogen-induced increase in cortisol-binding globulin exceeds the increase in free cortisol, a less significant homocyst(e)ine decrease may be observed in rats treated with a combination of cortisol and estradiol versus rats treated only with cortisol.

Plasma amino acid patterns found in this study were considerably different from those caused by fasting or found in patients with anorexia nervosa[21-25] (Table 2). This suggests that administration of hormone(s) rather than reduced food intake plays a key role in homocysteine metabolism.

Previously, Finkelstein et al[17,18] reported a systematic evaluation of hormonal effects on the enzyme activities of homocysteine metabolism. A 300% increase of hepatic betaine–homocysteine methyltransferase activity was found in cortisol-treated animals, strongly suggesting a homocysteine-lowering effect of cortisol.[18] In estradiol-treated animals, there was a greater than 150% increase in kidney methionine synthase activity, which is fourfold greater than the activity of liver enzyme.[17] This appears to be related to the reduction of plasma homocyst(e)ine concentration in estradiol-treated animals. Alternatively, the effect of estrogen may be associated with the transamination of methionine, which has been proposed based on an increased concentration of transamination metabolites after methionine loading in premenopausal women versus men.[29]

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