S-Adenosylmethionine: Guanidinoacetate *N*-Methyltransferase Activities in Livers from Rats with Hormonal Deficiencies or Excesses[1] (37512)

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The dietary and hormonal regulation of the first enzyme in the creatine biosynthetic pathway, arginine:glycine amidinotransferase (transamidinase) has been investigated extensively (1–4). Little information has been published about the dietary and hormonal regulation of the second enzyme in the creatine biosynthetic pathway, *S*-adenosylmethionine:guanidinoacetate *N*-methyltransferase (GA-methyltransferase). The only data reported about GA-methyltransferase activities were obtained with rats fed diets supplemented with either creatine or guanidinoacetic acid. Livers from rats fed a complete diet supplemented with guanidinoacetic acid had twice the GA-methyltransferase activities found in livers from rats fed the complete diet without added guanidinoacetic acid (5, 6). Livers from rats fed a complete diet supplemented with creatine had GA-methyltransferase activities similar to those of livers from rats fed the complete diet without added creatine (6). The purpose of this investigation was to ascertain whether liver GA-methyltransferase activities are under hormonal regulation.

Materials and Methods. Weanling albino rats were purchased from Hormone Assay Laboratories, Inc., Chicago, IL for Expts I through IV, inclusive, and from Sprague-Dawley, Madison, WI, for Expt V. All rats were housed individually.

The thyroid-parathyroidectomized rats received injections ip of 5 μCi ^{131}I/g body weight at approximately 24 hr after surgery,[3] a modification of the procedure used by Evans *et al.* (7) to assure complete removal of thyroid tissue. Durbin *et al.* (8) have reported that liver tissue from rats that received doses of ^{131}I greater than 10 μCi/g body weight had no damage that was apparent when examined histologically.

All thyroid-parathyroidectomized and sham-thyroidectomized rats had access to 0.7% calcium lactate in tap water and iodine-deficient diet,[4] *ad libitum,* for a period of 6 days after surgery. During the balance of the experiment, all thyroxine-treated thyroidectomized rats had access to 0.3% calcium lactate in tap water *ad libitum.* Adrenalectomized rats had access to 0.15 *M* NaCl in tap water *ad libitum.* At all other times and in all other experiments the rats had access to complete purified diet (9) and tap water *ad libitum.*

Bovine growth hormone[5] was dissolved in

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[3] Carrier-free Na^{131}I in NaOH purchased from Cambridge Nuclear Corp., Cambridge, MA, was diluted with 0.15 *M* NaCl to contain 250 μCi in 0.5 ml. Sham-thyroidectomized rats received 0.5 ml of 0.15 *M* NaCl.

[4] Salts, mixed in the same proportions as recommended in USP XIV, with chloride substituted for iodide was used in preparation of the purified diet.

[5] Bovine growth hormone (NIH-GH-B14) was a gift from the Endocrinology Study Section, NIAMD, National Institutes of Health, Bethesda, MD.

slightly alkaline 0.15 *M* NaCl (500 μg/0.1 ml). Estradiol (10 μg/0.1 ml), testosterone (4 mg/0.2 ml), and cortisol[6] (2 mg/0.1 ml) were dissolved or suspended in sesame oil. Insulin,[7] 80 units/cm³, was used as supplied. Solutions of sodium *l*-thyroxine[8] in 0.15 *M* NaCl (16 μg/0.1 ml) were used within 2–3 hr after preparation. The large doses of thyroxine were given every fourth day starting 24 days prior to sacrifice and the large doses of all other hormones were given daily starting 4 days prior to sacrifice. For Expt IV only, the concentration of sodium *l*-thyroxine was 0.5 μg/0.1 ml, and the injections were given daily starting at once after surgery. All injections were administered subcutaneously in the dorsal neck region.

The rats were killed by decapitation. The livers were immediately excised and chilled in beakers on ice. Ten percent liver homogenates were prepared with 0.06 *M* Na_2HPO_4–KH_2PO_4 buffer (pH 7.4), using a Waring blender, and the homogenates were assayed for GA-methyltransferase activities by a method previously reported (6). Enzyme activities were expressed as micromoles of creatine formed per gram wet weight liver per 2 hr incubation at 37°. The kidney transamidinase activities were determined by a method previously reported (10).

Results and Discussion. The liver GA-methyltransferase activities are listed in Table I.

The mean GA-methyltransferase activity of livers from rats 22 days after the adrenals were removed was slightly lower (−12%, $p > 0.01$) than the mean activity of livers from sham-operated rats.

The mean GA-methyltransferase activity of livers from intact female rats was lower (−19%, $p > 0.01$) than the mean activity of livers from intact male rats. The mean activity of livers from male rats 45 days after castration was slightly lower (−6%, $p > 0.1$) than the mean activity of livers from intact male rats, and the mean activity of livers from female rats 45 days after castration was slightly higher (+8%, $p > 0.1$) than the mean activity of livers from intact female rats. In an additional experiment (data not shown), castrated rats in groups of five were given small doses of hormones (2 μg estradiol or 500 μg testosterone/100 g body wt) daily for 24 days. The activities of livers from rats receiving estradiol were slightly lower and the activities of livers from rats receiving testosterone were slightly higher than the activities of livers from rats that did not receive the hormone injections, but the differences were not statistically significant.

The mean activity of livers from rats 11 days after hypophysectomy was slightly lower (−17%, $p > 0.1$) than the mean activity of livers from sham-operated rats. Further, the activities of livers from a group of five hypophysectomized rats that received 20 μg growth hormone/day for 1 wk in another experiment were found to be the same as activities of livers from hypophysectomized rats that received no hormone replacement (data not shown).

The mean activity of livers from rats 43 days after thyroid-parathyroidectomy was lower (−14%, $p < 0.01$) than the mean activity of livers from sham-operated rats. Activities of livers from thyroid-parathyroidectomized rats that received 0.5 μg T_4/100 g body wt daily from surgery to sacrifice were slightly higher (+5%, $p > 0.1$) than activities of livers from untreated thyroid-parathyroidectomized rats and were slightly lower (−19%, $p > 0.1$) than activities of livers from sham-operated animals.

GA-methyltransferase activities of livers from rats that received large doses of various hormones (Expt V) are shown in Table

[6] Testosterone (mp 153–154°, specific rotation +101.5° in 1% dioxane at 23°, sodium D line, ϵ = 16,150 at 240 nm) and 17β-estradiol, a product of Preparations Laboratories, Inc., Huntington Station, NY and cortisol, a product of Ikapharm, Ramat-Gan, P.O. Box 31, Israel, were gifts from Dr. Frank Ungar.

[7] Semilente Iletin, insulin zinc suspension prompt, USP, 80 units/cm³, was purchased from Eli Lilly and Co., Indianapolis, IN.

[8] Synthroid, sodium *l*-thyroxine combined with mannitol in the ratio of 1:20 (w/w), was purchased from Flint Laboratories, Morton Grove, IL, for Expt IV. Sodium *l*-thyroxine, B grade, was purchased from Calbiochem., Los Angeles, CA, for Expt. V.

TABLE I. Guanidinoacetate *N*-Methyltransferase Activties in Livers from Rats After Removal of Certain Endocrine Glands, or After Administration of Certain Hormones.

Treatment	Days post-operative	No. of rats	Body wt at sacrifice (g)	Methyltransferase activities (μmoles creatine/g liver/2 hr)
Expt I				
Sham-adrenalectomy[a]	22	10	179 ± 14[b]	1.6 ± 0.2[b]
Adrenalectomy	22	9	141 ± 18	1.4 ± 0.1
Expt II				
Male rats				
None	—	8	325 ± 18	1.6 ± 0.2
Castration	45	9	278 ± 20	1.5 ± 0.1
Female rats				
None	—	7	190 ± 9	1.3 ± 0.2
Castration	45	8	277 ± 21	1.4 ± 0.2
Expt III				
Sham-hypophysectomy	11	9	99 ± 7	1.8 ± 0.1
Hypophysectomy	11	7	68 ± 5	1.5 ± 0.2
Expt IV				
Sham-thyroid-parathyroidectomy	43	9	295 ± 15	2.2 ± 0.2
Thyroid-parathyroidectomy	43	18	124 ± 10	1.9 ± 0.2
Thyroid-parathyroidectomy + T_4[c]	43	13	260 ± 24	2.0 ± 0.2
Expt V				
None		10	195 ± 14	2.2 ± 0.1
Insulin, 4 units/day		10	194 ± 16	2.4 ± 0.6
Estradiol, 10 μg/100 g/day		10	174 ± 8	1.9 ± 0.5
Testosterone, 4 mg/day		9	193 ± 14	1.9 ± 0.2
Cortisol, 2 mg/100 g/day		10	161 ± 11	1.9 ± 0.5
Thyroxine, 16 μg/100 g/every 4th day		10	198 ± 11	1.8 ± 0.4
Growth hormone, 500 μg/100 g/day		10	196 ± 13	1.8 ± 0.3

[a] Male rats were used in all experiments except where female rats are specifically indicated.
[b] Mean ± 1 SD.
[c] 0.5 μg T_4/100 g body wt was injected daily starting at once after thyroidectomy.

I. The mean activity of livers from rats that received large doses of insulin were slightly higher (+9%, $p > 0.1$) than the mean activity of livers from control animals, and the mean activity of livers from rats that received large doses of either estradiol or cortisol were slightly lower (−14%, $p > 0.05$) than the mean activity of livers from control rats. The mean activities of livers from rats that received injections of testosterone, thyroxine, or growth hormone were 86, 82, and 82%, respectively ($p < 0.01$ for all) of the mean activity of livers from untreated rats. The transamidinase activities of kidneys from the rats in Expt V were also determined. Injections of either growth hormone, testosterone, or insulin were without any effect on the kidney transamidinase activities. Kidneys from rats given injections of either estradiol, cortisol, or thyroxine had 69, 58, and 85%, respectively, of the activities found in kidneys from intact rats that received no hormone injections.

Growth hormone or thyroxine induces kidney transamidinase activities in hypophysectomized or thyroidectomized rats, respectively (2). Now, as a result of the present work, it is found that livers from rats known to have low kidney transamidinase activities (as a result of hormonal imbalance) had normal

GA-methyltransferase activities.

Summary. *S*-Adenosylmethionine:guanidinoacetate *N*-methyltransferase activities were determined in livers from rats after removal of either adrenals, pituitaries, gonads, or thyroids and parathyroids and in livers from intact rats given large doses of either insulin, estradiol, testosterone, cortisol, thyroxine, or growth hormone. The only differences that were statistically significant ($p < 0.01$) were between the activities of livers from intact rats and the slightly lower ($\sim$20%) activities of livers from rats with their thyroid and parathyroid glands removed or from intact rats given large doses of either testosterone, thyroxine, or growth hormone. Further, liver GA-methyltransferase activities were virtually unaffected by replacement of either thyroxine to thyroidectomized rats, growth hormone to hypophysectomized rats, or testosterone or estradiol to castrated rats. Therefore, direct hormonal regulation of rat liver GA-methyltransferase is contraindicated. Thus, the second enzyme involved in creatine synthesis (GA-methyltransferase) differs greatly from the first enzyme (arginine:glycine transamidinase) in that transamidinase activities have previously been reported to be altered markedly by removal of certain endocrine glands or by injections of large doses of certain hormones into intact rats.

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