Synergistic Effect of Cortisol and Growth Hormone on Hepatic Ornithine Decarboxylase Activity[1] (36011)

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The rate-limiting step in the synthesis of the polyamines is thought to be the conversion of ornithine to putrescine, a reaction which is catalyzed by ornithine decarboxylase (ODC). Luteinizing hormone, estrogen, and testosterone have been shown to stimulate ODC activity in the ovary, oviduct, and prostate, respectively (1–3). Epithelial growth factor stimulates ODC activity in cultures of chick embryo epidermis (4). In the rat liver, ODC activity is increased by partial hepatectomy (5, 6) or by a single injection of growth hormone (7).

This report describes studies undertaken to further characterize the hormonal control of hepatic ODC activity in the rat. Enzyme activity was measured in livers of intact, hypophysectomized, and adrenalectomized rats to determine the interrelationship between growth hormone and adrenal steroids in regulating ODC activity. We have confirmed the stimulatory effect of growth hormone, and have observed similar effects with hydrocortisone. A synergistic enhancement of ODC activity was observed with the simultaneous injection of both hormones. Testosterone, epinephrine, insulin, *l*-thyroxine, estradiol, or glucagon, administered to hypophysectomized rats, had only minor effects on enzymatic activity.

Materials and Methods. Animals. Normal and hypophysectomized male Sprague-Dawley rats, weighing 50–100 g, were obtained from Hormone Research Laboratories, Chicago, IL. Hypophysectomies were carried out on day 25 of life. Adrenalectomies were performed in our laboratory through a dorsal incision. All animals were fed standard laboratory chow *ad libitum*. Hypophysectomized and adrenalectomized rats were given 5% glucose or 5% glucose in 0.9% sodium chloride solution, respectively, in place of drinking water.

Materials. DL-Ornithine-1-^{14}C-hydrochloride (2.7–37 mCi/mmole) was obtained from New England Nuclear Corporation and from Amersham-Searle. 25 ml erlenmeyer flasks, fitted rubber stoppers and polyethylene center wells were obtained from Kontes Glass Company. Human growth hormone,[3] insulin (Squibb), sodium levothyroxine (Synthroid injection, Flint), epinephrine (Adrenalin chloride, Parke-Davis), testosterone (4-androsten-17β-ol-3-one, Mann Research Laboratories), estradiol [1,3,5 (10)-estratrien-3,17β-diol, Steroids, Inc.], glucagon hydrochloride (Lilly), hydrocortisone sodium succinate (Solu-Cortef, Upjohn) were given by the intraperitoneal route.

Tissue preparation. The hypophysectomized and adrenalectomized rats were used for these studies 4–21 days and 72 hr after surgery, respectively. The respective hormones (or buffer in the case of control animals) were injected intraperitoneally between 9 and 10 a.m. and, unless otherwise noted, the animals were sacrificed 4 hr later by cervical fracture. Each liver was washed in cold saline, blotted dry, weighed, and minced. Homogenization in 5 vol of 0.05 *M* Na–K phos-

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phate buffer (pH 7.2), with 0.1 *M* Na–EDTA was performed in an ice bath with 5 strokes of a motor driven glass–Teflon homogenizer. Homogenates were centrifuged at 20,000*g* for 20 min at 2°. The supernatants were assayed within 1 hr.

Analytical methods. Enzymatic activity was assayed, with minor modifications, by the method of Russell and Snyder (5). To each flask were added 0.5 μCi of DL-ornithine-1-^{14}C-hydrochloride with specific activity of 37 mCi/mmole unless otherwise stated, 0.1 μm pyridoxal phosphate, and buffer (as described above) to make a final volume of 2.0 ml. The flasks were stoppered; and the reaction was initiated by the injection of 0.1–0.8 ml of the 20,000*g* supernatant. Enzymatic activity was measured at 3 concentrations in most experiments. After incubating at 37° for 30 min in a shaking water bath, 1 ml of 2 *M* citric acid was injected into the reaction mixture and 0.2 ml of a 2:1 mixture of ethanolamine and 2-methoxyethanol was injected into the center well. After agitating at 25° for an additional 30 min, the wells were removed and placed in a scintillation vial containing 10 ml of toluene with 0.4% PPO and 0.01% dimethyl POPOP and 2 ml of absolute ethanol.

Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer. Counting efficiency was determined by either the internal or external standard method. Counting efficiency was 73 ± 2% by both methods. Results were calculated on the IBM 1130 computer and expressed as nanocuries of $^{14}CO_2$ liberated/gram of wet weight of liver/30 min incubation. The amount of $^{14}CO_2$ liberated from flasks containing heat-inactivated enzyme and/or no enzyme was subtracted from all other samples prior to the correction for weight. Comparable results were obtained by both methods.

Results. Preliminary studies demonstrated that, over the range assayed, enzyme activity was proportional to the concentration of supernatant added. The rate of $^{14}CO_2$ production was linear for at least 30 min. The activity measured after combining supernatants from control and growth hormone-treated rats was additive. Although whole liv-

TABLE I. Hormonal Regulation of Hepatic Ornithine Decarboxylase Activity.[a]

	Intact		Hypophysectomized		Adrenalectomized	
	No HGH	HGH	No HGH	HGH	No HGH	HGH
No HC	13.30 ± 4.93[b] (11)[c]	58.18 ± 8.54 (5)	1.46 ± 0.53 (12)	13.91 ± 3.16 (12)	14.96 ± 4.81 (5)	33.94 ± 7.06 (6)
HC	34.47 ± 9.62 (5)	219.14 ± 51.80 (6)	81.60 ± 23.76 (6)	225.88 ± 32.17 (4)	99.31 ± 12.20 (4)	—

[a] HGH = human growth hormone; HC = hydrocortisone.
[b] Nanocuries of CO_2/gram of liver/30 min incubation ± SEM.
[c] Number of rats per group.

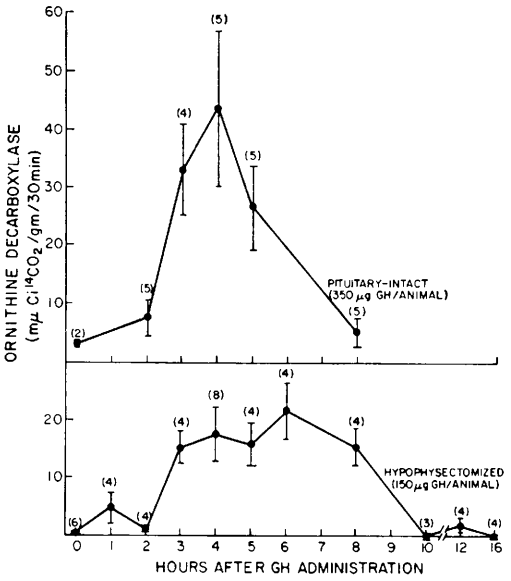

FIG. 1. The time course of ornithine decarboxylase activity in intact and hypophysectomized rats: Animals were sacrificed at varying time intervals after a single intraperitoneal injection of growth hormone in the dose stated. Each point represents the mean ± SEM. The number of rats in each group is shown in parentheses. The specific activity of the substrate was 2.7 mCi/mmole.

er homogenates were far more active in the assay than their corresponding supernatant fractions, only the supernatant fractions were responsive to hormonal stimulation. Most of the decarboxylation by the homogenate was accounted for by the 600*g* pellet. Observations demonstrating that this does not represent true ODC activity will be the subject of a later report.

Basal levels of ODC. There was no significant difference between the levels in buffer-treated intact and adrenalectomized rats (Table I). The levels were significantly reduced ($p<.025$) following hypophysectomy.

Time course of response to growth hormone treatment in intact and hypophysectomized rats. Intact rats were sacrificed at 2, 3, 4, 5, and 8 hr after a single intraperitoneal injection of 350 μg (5 mg/kg) of growth hormone. A significant increase ($p<.025$) in liver ODC was seen after 3 hr (Fig. 1). After administration of 150 μg (about 2 mg/kg) to hypophysectomized rats, a significant increase in ODC was again seen after 3 hr ($p<.01$). Thereafter, enzyme activity plateaued until 8 hr and fell to basal levels by 10 hr.

Dose–response to growth hormone in intact and hypophysectomized rats. In intact rats, no increase in ODC activity occurred with growth hormone dosages up to 4 μg/animal. A linear log-dose–response curve was obtained between 4 and 150 μg of growth hormone (Fig. 2). In hypophysectomized rats, a significant ($p<.05$) increase over basal ODC activity was observed at a dosage of 1.0 μg of growth hormone/animal (Fig. 2).

Effect of adrenalectomy. The administration of growth hormone to adrenalectomized

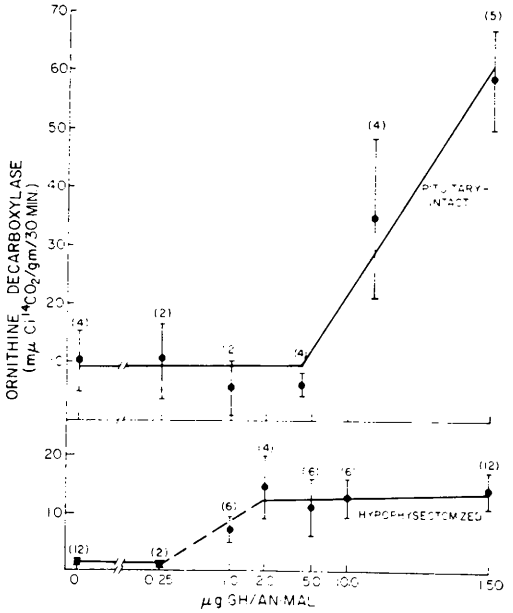

FIG. 2. The effect of different growth hormone dosages on ornithine decarboxylase activity in intact and hypophysectomized rats. Animals were sacrificed 4 hr after the administration of a single intraperitoneal hormone injection. Control animals were injected with buffer. Each point represents the mean ± SEM. The number of rats in each group is shown in parentheses. (—) calculated by the method of least squares; (- -) the probable shape of the curve. The specific activity of the substrate used was 37 mCi/mmole except for a few hypophysectomized animals which were assayed at a specific activity of 26.7 mCi/mmole. Results were comparable, and a calculation was made to correct all data to a specific activity of 37 mCi/mmole.

rats caused a smaller increase in ODC activity than in intact animals (Table I). The absolute increases over control levels were comparable to those in hypopsectomized rats after growth hormone.

Effect of hydrocortisone in adrenalectomized, intact, and hypophysectomized rats. Administration of hydrocortisone in a dosage of 5 mg/rat induced striking stimulation of ODC activity in both hypophysectomized and adrenalectomized rats (Table I). Increments over basal levels in intact rats were smaller than in the other two groups.

Combined growth hormone and cortisone treatment in intact and hypophysectomized rats. When growth hormone and hydrocortisone were administered simultaneously in the same dosages as when they were given alone, a synergistic response was observed. The maximum levels attained after both hormones together were the same in hypophysectomized and intact rats (Table I).

Effect of other hormones on liver ODC activity. To determine the specificity of enzymatic induction of ODC in the liver, hypophysectomized rats were treated with pharmacologic doses of testosterone, epinephrine, insulin, *l*-thyroxine, estradiol, and glucagon. Since these hormones induced only very small increases in ODC activity, these studies were not pursued (Table II).

Discussion. These studies demonstrate that hepatic ODC activity is dependent upon both growth hormone and adrenal corticosteroids. Hypophysectomy is followed by reduction in hepatic ODC activity to almost undetectable levels. Although ODC induction is detected in hypophysectomized rats at a lower growth hormone dosage than in intact rats, the maximum observed response was less than in intact animals treated with growth hormone. This difference may be attributable to the diminished adrenocortical steroid production of the hypophysectomized rat since pharmacological dosages of hydrocortisone, given concomitantly with growth hormone, produce very high levels of hepatic ODC activity. The magnitude of this response is far greater than the sum of the effect measured after each hormone given alone.

TABLE II. Hormonal Regulation of Ornithine Decarboxylase in Liver of Hypophysectomized Rats.

Treatment	(nCi of $^{14}CO_2$/g of liver/30 min ± SEM)
Buffer	1.46 ± 0.530 (12)[a]
Testosterone, 5 mg	1.43 ± 0.02 (2)
Epinephrine, 100 μg	2.82 ± 1.17 (2)
Insulin, 143 mU	3.79 ± 2.18 (2)
l-Thyroxine, 25 μg	6.96 ± 5.41 (4)
Estradiol, 10 μg	6.97 ± 0.22 (2)
Glucagon, 150 μg	8.70 ± 3.91 (4)
HGH, 150 μg[b]	13.91 ± 3.16 (12)
HC, 5 mg[c]	81.60 ±23.76 (6)
HGH + HC	225.88 ±32.17 (4)

[a] Number of rats per treatment group.
[b] Human growth hormone.
[c] Hydrocortisone.

The precise physiological role of adrenal steroids in maintaining hepatic ODC activity is difficult to assess from these studies since control levels were not reduced 72 hr after adrenalectomy. A greater interval after surgery might be required to demonstrate a decrease in activity. This seems unlikely, however, since the enzyme has an exceedingly rapid turnover time (8). Moreover, even 2 weeks after adrenalectomy, there is no change in the ODC response to partial hepatectomy, whereas prior hypophysectomy both delays and reduces the magnitude of the ODC response (9).

Since, in the whole animal, glucocorticoids are catabolic and antagonize the anabolic effects of growth hormone, it was unexpected that these hormones would be synergistic on a process related to cell growth (10). Synergism between growth hormone and glucorcorticoids has also been demonstrated on the induction of hepatic glucokinase (11) in hypophysectomized rats and on the *in vitro* induction of lipolysis and synthesis of cyclic AMP in white fat cells (12). Daily treatment with glucocorticoids increases liver weight, microsomal protein and RNA content (13) in a manner similar to that seen in the livers of growth hormone-treated hypophysectomized rats (14). Therefore, both hormones may be considered to be anabolic for liver cells. Additional studies are required to fully delineate

the complex hormonal control of ODC levels in various tissues and to clarify the biologic significance of this enzyme in tissue growth and other metabolic processes.

Summary. Liver ornithine decarboxylase activity is stimulated by hydrocortisone as well as by growth hormone. Hypophysectomy lowers basal levels but adrenalectomy does not. Simultaneous administration of both growth hormone and hydrocortisone increases the activity more than the sum of the increase due to each hormone alone. Pharmacological dosages of testosterone, epinephrine, insulin, *l*-thyroxine, estradiol, and glucagon produced only minor alterations in hepatic ODC levels.

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