HORMONAL STIMULATION OF HEPATIC ORNITHINE DECARBOXYLASE*

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SUMMARY.—Hepatic ornithine decarboxylase activity in adrenalectomized rats was increased by a variety of hormones, including hydrocortisone, growth hormone, insulin, glucagon, and thyroxin. Repeated treatment with either hydrocortisone or growth hormone, the most effective of the hormones tested, failed to maintain the elevated enzyme levels attained within a few hours after the initial injection.

Reports by Russell and Snyder (1) and Jdime and Raina (2) that the enzyme ornithine decarboxylase (L-ornithine carboxy-lase, E.C.4.1.1.17) undergoes a rapid and extensive increase in activity in the residual liver of partially hepatectomized rats have provided the impetus for much recent experimental investigation of the role of this enzyme in growth processes and of the factors that regulate its activity in tissues. The hypophyseal growth hormone has been implicated as a significant regulatory factor, since ornithine decarboxylase is markedly increased in both normal and adrenalectomized rats treated with this hormone (3–5). However, the increase in the enzyme after hepatectomy is not abolished but rather delayed in hypophysectomized rats (6). This result suggests that growth hormone cannot be considered as an essential factor in elevation of ornithine decarboxylase levels. We have examined the effect of a variety of hormones on hepatic ornithine decarboxylase activity. Nearly all those tested increase activity to some extent, but the most effective is hydrocortisone, the adrenal steroid. Like the growth hormone, however, this steroid is unable to maintain elevated levels of the enzyme, suggesting the primary involvement of an endogenous agent which has not yet been identified.

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Male Sprague-Dawley rats weighing 160 to 250 g were used in all experiments. Since preliminary experiments showed that hydrocortisone was effective in elevating ornithine decarboxylase levels in intact rats, all the animals used in these experiments were adrenalectomized and maintained on 1% NaCl as drinking water for 2 to 5 days before experiments began. After the treatments indicated, livers were homogenized in 5 volumes of 0.05 M TES buffer [N-Tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid], pH 7.0, supplemented with 0.1 mM EDTA. Assays were made on supernatant solutions obtained after centrifugation for 35 minutes at 105,000 g.

Ornithine decarboxylase activity was determined by the $^{14}CO_2$-release method of Russell and Snyder (6), with some modifications. Reaction mixtures contained 0.05 µmoles pyridoxal phosphate, 0.1 µmoles dithiothreitol, and 0.25 µCi DL-ornithine-1-[^{14}C] (1.6 µCi/mole) dissolved in 1.8 ml of TES-EDTA buffer. After enzyme addition (0.2 ml of liver supernatant fraction) mixtures were incubated at 37° C for 10 minutes before the reaction was initiated by addition of the isotope. After 30 minutes of incubation, the reaction was terminated with 1 ml of 2 M citric acid, then shaking was continued for 30 minutes to collect radioactive CO_2. This was collected in 0.2 ml of NCS reagent (Nuclear Chicago) and counted as described by Russell and Snyder (6). Radioactivity detected when heated enzyme controls were substituted was subtracted from that obtained with experimental flasks. Under these conditions the reaction was found to be linear for at least 30 minutes, to be linear with respect to protein added up to 4 mg, and to have a pH optimum of 7.0. Dithioreitol (in reaction mixtures) and EDTA (in both reaction mixtures and the homogenization buffer) were found to enhance activity significantly. To ensure that $^{14}CO_2$ release was not via oxidative metabolism of ornithine, we added glutamate (7.5 µmoles) to reaction mixtures at random; this addition had no effect on $^{14}CO_2$ evolution with the soluble liver fractions employed here. Tyrosine transaminase was assayed as described by Kenney (7) and protein by the biuret procedure (8).

The effects of various hormones on the levels of hepatic ornithine decarboxylase, measured 4 hours after treatment, are shown in Table 1. Hydrocortisone was clearly the most effective of these agents, increasing ornithine decarboxylase twice as much as either growth hormone or insulin, which had comparable effects. Animals treated with insulin were also given glucose to combat hypoglycemic shock, but this sugar alone did not change the enzyme level. Glucagon and thyroxin were effective to a limited extent. Testosterone caused a barely significant increase, and 17ß-estradiol was ineffective. The lack of effect of estradiol on the liver enzyme

TABLE 1

Effects of Various Hormones on Hepatic Ornithine Decarboxylase Activity in Adrenalectomized Rats

Assays were made 4 hours after intraperitoneal injections of hormones dissolved or suspended in 1 ml of 0.15 M NaCl (saline). Amounts given per 100g body weight were: hydrocortisone and testosterone, 2.5 mg; 17ß-estradiol, 0.25 mg; growth hormone, insulin, glucagon, and thyroxine, 100 µg each. Glucose, 1 ml of a 20% solution, was also given to rats treated with insulin. Data are the mean ± S.E.; the number of animals is given in parentheses.

Treatment	Ornithine decarboxylase activity [(mµmoles $^{14}CO_2$/mg protein/min) X 10]
Saline	0.05 ± 0.01 (13)
Hydrocortisone	2.95 ± 0.29 (4)
Growth hormone	1.55 ± 0.38 (5)
Insulin	1.31 ± 0.30 (8)
Glucagon	0.70 ± 0.18 (5)
Thyroxine	0.47 ± 0.18 (3)
Testosterone	0.10 ± 0.01 (4)
17ß-estradiol	0.05 ± 0.02 (4)
Glucose	0.04 ± 0.01 (4)

is of particular interest, as Cohen et al. have recently described a marked increase in ornithine decarboxylase of chick oviduct exposed to this hormone in vivo or in vitro (9).

The time course of the response of ornithine decarboxylase to repeated administration of hydrocortisone in shown in Fig. 1. Activity reached a peak 3 hours after the first hormone injection, but then declined precipitously despite repeated treatment at 4-hour intervals. In a comparable experiment the hydrocortisone-inducible enzyme tyrosine transaminase, which is known to be directly responsive to the steroid (10), was maintained at an elevated level in rats repeatedly injected with hydrocortisone (Fig. 1, insert). Repeated treatment with the hypophyseal growth hormone was not sufficient to maintain high activity levels of ornithine decarboxylase (Fig. 1).

Thus a variety of apparently unrelated hormones can bring about marked elevation of ornithine decarboxylase activity in the livers of adrenalectomized rats. The most effective of these hormones are not capable of maintaining high decarboxylase levels,

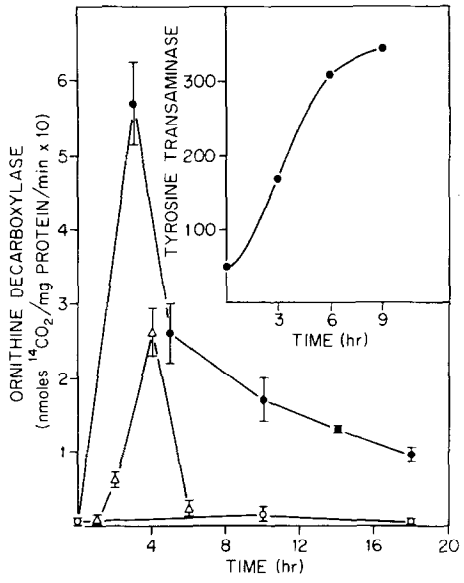

FIGURE 1. Response of hepatic ornithine decarboxylase to repeated injections of hydrocortisone or growth hormone in adrenalectomized rats. Hormones were given at zero time and again at 4-hour intervals to the remaining animals. Solid circles, hydrocortisone (2.5 mg per 100 g); triangles, growth hormone (100 µg per 100 g); open circles, untreated controls. Data are the mean ± S.E. for four rats at each point. Insert: Response of tyrosine transaminase to repeated injections of hydrocortisone at 3-hour intervals. Each point represents a single animal; transaminase activity expressed as units (µg p-hydroxyphenylpyruvate/10 min) per mg protein.

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in contrast to hormonal induction of tyrosine transaminase, for which the direct action of the hormone in hepatic cells is well established. These observations indicate that none of the hormones acts directly to increase ornithine decarboxylase. Rather, the data suggest the involvement of a mediator substance, which appears in limited amounts in response to a variety of hormonal stimuli.

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