Nature of the Increase in Renal Ornithine Decarboxylase Activity after Cycloheximide Administration in the Rat

(Pharmacological stress/pituitary hormones/hypophysectomy/adrenalectomy/
L-[4C]Leucine incorporation)

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ABSTRACT The present study was designed to determine whether the increase in rat renal ornithine decarboxylase (l-ornithine carboxy-lyase, EC 4.1.1.17) activity after cycloheximide administration was a primary effect on the kidney or was a secondary effect of adrenal or pituitary hormones released in response to the drug. Renal ornithine decarboxylase activity was reduced approximately 70% 1 hr after intraperitoneal administration of doses of cycloheximide that also inhibited renal protein synthesis by 60-90% within 1 hr. Protein synthesis began to recover by the second hour, accompanied by a rise in decarboxylase activity that reached a peak about six times greater than pretreatment values at 8 hr, then gradually declined to preinjection levels by 16 hr. Peak ornithine decarboxylase activity was directly proportional to cycloheximide doses up to 250 μg; larger doses, which almost abolished protein synthesis for 8 hr, were inhibitory. Plasma corticosterone rose rapidly after cycloheximide, reached a peak at 2 hr, then fell to baseline by 8 hr. Corticosterone response was also dose-dependent up to 250 μg, but larger doses were inhibitory. Adrenalectomy did not reduce decarboxylase activity response to cycloheximide, nor did cortisol administration enhance it. Hypophysectomy greatly reduced baseline renal decarboxylase activity within 9 hr and all but abolished the increase in enzyme activity normally seen after cycloheximide administration to the intact rat. The hypophysectomized animal exhibited apparent increased sensitivity to cycloheximide, since a smaller dose of the drug caused a reduction in renal protein synthesis similar to that seen with a larger dose in the intact rat. As protein synthesis was recovering in the hypophysectomized animals, renal decarboxylase activity responded adequately to the injection of a crude pituitary extract. These data suggest that renal ornithine decarboxylase turnover is rapid, that baseline activity is maintained by new protein synthesis, and that the increase in renal enzyme activity after cycloheximide is in large part dependent upon pituitary hormone action.

Previous studies have demonstrated that administration of inhibitors of protein synthesis will prevent the increase in activity of the enzyme, ornithine decarboxylase (l-ornithine carboxy-lyase, EC 4.1.1.17), that is normally observed in a variety of experimental conditions (1–11). Recently, however, Beck et al. (12, 13) reported a marked increase in hepatic ornithine decarboxylase activity after administration of puromycin to the intact rat. In addition to discussing various hepatocellular events by which puromycin might have caused the observed increase in enzyme activity, the authors considered the possibility that the effect of the drug might have been mediated by extrahepatic events, such as the release of hormones known to increase hepatic decarboxylase activity.

In the present study, we have examined the effect of cycloheximide, another inhibitor of protein synthesis, on rat renal ornithine decarboxylase activity. We have sought to determine whether the increase in decarboxylase activity observed after administering the drug was mediated primarily by intracellular events within the kidney, or was secondary to the action of pituitary or adrenocortical hormones whose release was stimulated by the antimitabolite.

MATERIALS AND METHODS

Cycloheximide (Nutritional Biochemicals Corp.) was dissolved in distilled water and administered intraperitoneally. Ovine pituitary tissue which had been lyophilized, pulverized, and stored at −20° was generously provided by Dr. Robert Bates, Hormone Distribution Officer of the National Institute of Arthritis, Metabolism, and Digestive Diseases. The pituitary powder was dissolved in 0.1 M NH4HCO3 by three successive 30-sec homogenizations with a Waring blender at 16,500 rpm at 4°. The homogenate was centrifuged for 20 min (48,000 × g, 4°), the precipitate was homogenized and centrifuged again as above, and the second supernate was added to the first supernate. The pooled supernate was stored at −56° prior to intraperitoneal injection. Uniformly labeled L-[^14C]leucine, 280 Ci/mol, was obtained from New England Nuclear, diluted with 154 mM NaCl and administered intravenously, 2 μCi per animal. L-[1^-14C] Ornithine monohydrochloride, 58 Ci/mol, NCS tissue solubilizer, and Spectrafluor concentrated liquid scintillator were obtained from Amersham/Searle. Male Holtzman rats, 180–200 g, were used in all studies. The rats were maintained in a quiet room with 14 hr light–10 hr dark (2000–0600 hr) cycles for 3–4 days prior to study.

All injections were given between 0800 and 0900 hr, and the animals were sacrificed at appropriate times thereafter by decapitation. Alternate right and left kidneys were removed from animals of each group and trimmed of fat. The capsule was stripped, the organ was bisected along the long axis, and the calyceal system was removed. A 150–200 mg wedge of tissue was removed from each kidney, weighed, and placed in 1 ml of chilled 67 mM Sorensen’s phosphate buffer, pH
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Animals received 154 mM NaCl. Five minutes before each interval indicated on the abscissa, the animals received 2 μCi of L-[14C]-leucine intravenously. They were sacrificed by decapitation 10 min after labeled leucine administration. (X - - - X) = saline-treated controls at 0 time and 5 hr after the experiment was started, (O - - - O) = 125 μg of cycloheximide, (O - - - O) = 250 μg of cycloheximide, (O - - - O) = 1000 μg of cycloheximide.
Each point represents the mean of four values; the brackets indicate the SEM.

RESULTS

Effect of Cycloheximide on Renal Ornithine Decarboxylase Activity. Intact rats were injected with cycloheximide, trunk blood was collected for plasma corticosterone levels at the time of sacrifice, and the kidneys were removed for assay of ornithine decarboxylase activity. During the first hour following cycloheximide treatment, decarboxylase activity fell to about 30% of control levels and remained there for the next hour. Thereafter, a progressive rise in activity occurred, reaching a peak approximately six times higher than control levels 8 hr after cycloheximide, followed by a return to control levels by 16 hr. Plasma corticosterone levels also rose rapidly, reaching a peak 2 hr after cycloheximide administration and then falling toward control levels by 8 hr (Fig. 1), indicating the prompt and sustained release of pituitary adrenocorticotropic hormone (ACTH). An apparent secondary rise in plasma corticosterone levels coincided in time with the normal diurnal peak observed in untreated animals.

Effect of Increasing Doses of Cycloheximide on Renal Ornithine Decarboxylase Activity. Various doses of cycloheximide were administered to intact rats, which were sacrificed either 1 hr or 8 hr after treatment. With doses ranging from 62.5 μg to 4000 μg, little difference in the fall of decarboxylase activity at 1 hr was observed. However, plasma corticosterone values at this time were much higher with cycloheximide doses of 250 μg or less than with higher dose levels (Fig. 2), suggesting that larger doses of the drug inhibited adrenal response to the pituitary ACTH released. The magnitude of the 8 hr peak in decarboxylase activity paralleled that of the earlier steroid response, with greatest enzyme activity after the 250 μg dose of cycloheximide. With larger doses, the
Renal Ornithine Decarboxylase after Cycloheximide

**Fig. 4.** Effect of cycloheximide on renal ornithine decarboxylase activity in the adrenalectomized rat. Animals were adrenalectomized 15 hr prior to intraperitoneal injection of 154 mM NaCl (controls), 125 μg of cycloheximide, or 125 μg of cycloheximide plus 500 μg of cortisol. They were sacrificed by decapitation 8 hr later for determination of renal decarboxylase activity. Each bar represents the mean of several values; the brackets indicate the SEM. The number of animals in each group is indicated in parentheses.

The decarboxylase response was blunted, and animals receiving 4000 μg did not survive the full 8 hr.

**Effect of Cycloheximide on Renal Protein Synthesis in the Intact Rat.** Renal protein synthesis was monitored by determining the incorporation of [14C]leucine into trichloroacetic acid-precipitable renal protein at the end of a 30 min interval. As shown in Fig. 3, baseline incorporation of labeled leucine was markedly reduced within 30 min by cycloheximide administration. The effect was dose-related, with 62.5, 250, and 1000 μg of cycloheximide reducing incorporation to 32%, 10%, and 6% of preinjection control, respectively. Inhibition of protein synthesis was still essentially complete 8 hr after 1000 μg of cycloheximide was administered. Within 2 hr of injection of the lower doses of antimetabolite, however, incorporation of [14C]leucine had begun to recover, and it increased steadily over the next 6 hr. The recovery from 62.5 μg paralleled and preceded that from 250 μg of cycloheximide. Incorporation of [14C]leucine 8 hr after 62.5 μg and 250 μg of cycloheximide were administered had returned to approximately 85% and 60% of preinjection control, respectively.

**Effect of Cycloheximide on Renal Ornithine Decarboxylase Activity in the Adrenalectomized Rat.** Since glucocorticoids are reported to induce renal decarboxylase activity (10, 17, 18) and since there was a substantial early rise in plasma corticosterone after cycloheximide administration, it seemed possible that the subsequent rise in enzyme activity was due, not to cycloheximide itself, but to corticosterone released in response to the stress caused by administration of the drug. To test this hypothesis, we adrenalectomized rats 16 hr before injecting cycloheximide. Adrenalectomy did not significantly affect either the unstimulated level of renal decarboxylase activity or the magnitude of the increase in activity after cycloheximide (Fig. 4). Furthermore, administration of 500 μg of cortisol intraperitoneally at the same time as cycloheximide did not potentiate the decarboxylase response.

Effect of Cycloheximide on Renal Protein Synthesis and Ornithine Decarboxylase Activity in the Hypophysectomized Rat. The considerable release of ACTH, as indicated by the sustained rise in plasma corticosterone, together with the fact that growth hormone has been shown to stimulate renal ornithine decarboxylase activity (17, 19) suggested that the pituitary gland was involved in the increase in renal enzyme activity seen after cycloheximide treatment. The effect of cycloheximide on renal protein synthesis in the hypophysectomized rat is shown in Fig. 5. The same dose of cycloheximide caused greater inhibition of [14C]leucine incorporation than in the intact rat, both in terms of its degree and its duration. Protein synthesis was almost completely abolished for 8 hr after 250 μg of cycloheximide, but the recovery pattern after 125 μg was indistinguishable from that observed with 250 μg in the intact rat.

Since the hypophysectomized animal exhibited an apparent increased sensitivity to cycloheximide, the 125 μg dose was selected to study renal decarboxylase activity response in the hypophysectomized rat. As shown in Fig. 6, unstimulated renal decarboxylase activity was decreased 9 hr after hypophysectomy. Renal decarboxylase activity was significantly greater at 4 and 8 hr following administration of 125 μg of cycloheximide than at 9 hr in the hypophysectomized controls (P < 0.05), but this increase represented only a small fraction of that seen in intact animals. In another experiment, animals given 125 μg of the drug showed a gradual decrease in decarboxylase activity to the level of the hypophysectomized controls by 24 hr, indicating that the enzyme response to cycloheximide was actually impaired, and not simply delayed, in the hypophysectomized animal.

Effect of Exogenous Pituitary Factors on Renal Ornithine Decarboxylase Activity in the Hypophysectomized Rat Treated with Cycloheximide. The apparent increased sensitivity to cycloheximide and the minimal increase in renal decarboxylase activity after administration of the drug to hypophysec-
tissue.

ACTH One of the roles of ACTH during the 8 hr after the injection is the increased activity in the enzyme, which was observed in the intact animal and sustained pituitary response. This activity was unimpaired, even though renal protein synthesis was temporarily interrupted with cycloheximide.

**DISCUSSION**

In this study, we have examined the nature of the renal ornithine decarboxylase activity response after administration of cycloheximide, an inhibitor of protein synthesis, to the rat. Within 30 min, 250 μg of cycloheximide essentially abolished renal protein synthesis. Within the first hour, there was also a significant decrease in decarboxylase activity, suggesting that synthesis of new enzyme was inhibited and that the half-life of renal decarboxylase is brief, as it appears to be in other tissues (3, 20, 21). By 2 hr, renal protein synthesis had begun to recover toward normal. By 4 hr, renal decarboxylase activity was already twice the preinjection level and rose to levels four to six times greater than control at 8 hr. Hepatic ornithine decarboxylase activity has been reported to increase after administration of puromycin, another inhibitor of protein synthesis, to intact rats (12, 13), but an early fall in decarboxylase activity was not observed in these studies. However, basal levels in the liver are so low that they essentially preclude reliable detection of a fall in enzyme activity. The maximum hepatic ornithine decarboxylase response was observed only 3.5 hr after puromycin administration, whereas it required 8 hr to reach the peak observed in the kidney. There are a number of possible explanations for this apparent discrepancy. The drugs themselves may have different time courses of action and of metabolic clearance. Data derived from experiments involving inhibitors of protein synthesis must be interpreted with caution, since these compounds may affect both synthetic and degradative pathways and may also produce effects unrelated to protein synthesis (21). It is possible that there are isoenzymes of ornithine decarboxylase in various tissues, variably sensitive to inhibitors of protein synthesis. The finding of a single species of this enzyme in the liver of rats treated with thioacetamide (22) does not preclude the possibility that there are tissue-specific ornithine decarboxylase isoenzymes.

Two distinct ornithine decarboxylases, one a biosynthetic enzyme and the other an inducible catabolic enzyme, have been identified in *Escherichia coli* (23).

The initial fall in renal decarboxylase activity was similar after all doses of cycloheximide administered. This was not an artifact of the assay system, since still lower levels can readily be measured. This finding suggests that the rate of fall was maximal when protein synthesis was inhibited to 32% or less of baseline, and was dependent upon the metabolic half-life of decarboxylase activity. The lowest level of activity after cycloheximide (12 ± 1.5 nmol 14CO2/g) is still significantly higher than the levels observed in the rat 9 hr after hypophysectomy (3.6 ± 0.6) (P < 0.05). Thus, it is possible that cycloheximide also inhibits the system that metabolically degrades ornithine decarboxylase, "stabilizing" the activity at a reduced level. The subsequent rise in decarboxylase activity, in contrast, was directly related to doses of cycloheximide up to 250 μg per animal, as was the corticosterone response. Larger doses appeared to be toxic, completely inhibiting protein synthesis for as long as 8 hr and resulting in lower responses of renal decarboxylase activity and adrenal corticosterone secretion and in high mortality in the animals to which they were given.

The rat responds to cycloheximide as to a stress, releasing pituitary ACTH and stimulating adrenal steroidogenesis un-
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