from the tRNA of sweet corn, garden peas, and spinach as the *cis* isomer (*11*), the assignment being based primarily on the melting point of the natural material, which was different from that reported (*19*) for the synthetic product. The isolation of zeatin and zeatin riboside from plant tissue, however, has been reported to afford the *trans* isomer (*19, 22*).

The presence of a second $N^6$-isopentenyl-2-methylthioadenosine in tRNA may obscure the identity of naturally occurring materials thought to be identical to **3** on the basis of uptake of labeled sulfate-$^{35}S$ and [*methyl*-$^{14}C$] methionine or on the basis of ultraviolet spectra. In view of the fact that natural systems may not be limited to two $N^6$-alkyl-2-methylthioadenosines, it would seem judicious to base structural assignments on relatively unambiguous data, for example, mass spectral determination or comparison of physical properties with those of a well-defined synthetic sample.

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### References and Notes

1. Abbreviations used in this paper include: tRNA, transfer ribonucleic acid; *m/e*, mass-to-charge ratio; O.D.$_{260}$, optical density at 260 nm; su$^+_{III}$, mutant strain of *E. coli*; CTAB, cetyltrimethylammonium bromide; tris, tris (hydroxymethyl) aminomethane; DEAE, diethylaminoethyl.
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## Corticosterone Regulation of Tryptophan Hydroxylase in Midbrain of the Rat

Abstract. *The tryptophan hydroxylase activity in the rat midbrain decreases after adrenalectomy and is restored by treatment with corticosterone. Cycloheximide, administered intracisternally, prevents the restoration of the enzyme activity by corticosterone. Cycloheximide administration to adrenalectomized rats results in a further decrease in the enzyme activity, an indication that the enzyme has a rapid turnover even in the absence of corticosterone.*

Tryptophan hydroxylase is the first of two enzymes responsible for the biosynthesis of serotonin from tryptophan. It is found in the regions of the brain where, as judged by specific histofluorescence, the cell bodies and endings of neurons containing this amine are located (*1*). Serotonin may function as a chemical synaptic transmitter (*2*), and regulatory mechanisms for serotonin formation and utilization are being investigated. Possible effects of adrenal steroids on serotonin concentration in the brain have been studied (*3*), but the results are inconclusive. Therefore, we investigated the role of corticosterone, principal adrenal glucocorticosteroid in the rat (*4*), in controlling the activity of tryptophan hydroxylase in rat brain. We report the effects of adrenalectomy and replacement by corticosterone on the production of this enzyme in the midbrain of the rat, where the cell bodies and therefore the cell nuclei of neurons containing serotonin are located.

Albino male rats (Sprague-Dawley strain, Charles River) (300 to 400 g) were subjected to bilateral adrenalectomies. The animals were maintained in group cages for 1 to 2 weeks on 0.8 percent NaCl and were given free access to standard laboratory chow before use. Corticosterone was administered intraperitoneally in 50 to 200 $\mu$l of ethanol; control animals received the same amount of ethanol alone. The rats were decapitated in the early afternoon, and the brains were rapidly removed and chilled. The midbrain was dissected according to the following anatomical landmarks: the anterior boundary was the anterior end of the superior colliculus and the posterior end of the mammillary body; the posterior boundary was the posterior end of the inferior colliculus and the anterior ridge of the pons (isthmus) (*5*).

Tryptophan hydroxylase was assayed by the method of Ichiyama *et al.* (*6*). L-Tryptophan-1-$C^{14}$ (New England Nuclear; specific activity 9 c/mole) was used as substrate, and $^{14}C$-$CO_2$ liberated by the action of L-amino acid decarboxylase on 5-hydroxytryptophan, was measured as the product. Initial experiments established that the amount of labeled $CO_2$ produced is linearly related to the amount of midbrain homogenate added to the reaction vessel. We also conducted experiments which ruled out that direct inhibition or activation of the enzyme could explain the differences in enzyme activi-

ties between the various treatments used. These experiments consisted of mixing together midbrain homogenates from normal and adrenalectomized rats or of adding substances such as cycloheximide, corticosterone, and adrenocorticotropin to midbrain homogenates. In all of these experiments, tryptophan hydroxylase in the homogenates was neither inhibited nor activated. To reduce the background level of radioactive $CO_2$ in the labeled tryptophan, the isotope was dissolved in 0.01*N* HCl and a portion (20 $\mu$l) was added to the buffered medium for each enzyme assay.

A study of the distribution of the enzyme in various brain regions (*7*) revealed a pattern of tryptophan hydroxylase activity which is similar to that described for the cat brain (*8*) and different in several important respects from that reported for the hamster (*6*). In our study, the parts of the brain regions with highest enzyme activity were, in descending order, septum, hypothalamus, midbrain, and thalamus.

We determined the effects of bilateral adrenalectomy on the tryptophan hydroxylase in the midbrain. Adrenalectomy resulted in a 75 percent decrease in enzyme activity, compared to normal rats (Fig. 1). To determine whether absence of corticosteroids was responsible for this decrease, we administered corticosterone, the principal glucocorticosteroid in the rat, to adrenalectomized animals. We observed partial but never complete restoration of the enzyme activity in the midbrain; the increase was dependent on the dosage and length of time after hormone injection. Seventeen hours before the rat was killed, 0.4 mg of corticosterone produced a 43 percent increase in enzyme activity compared to that in untreated adrenalectomized rats; and a dose of 2 mg, produced an increase of 71 percent. Repeated daily doses of 1 mg for 5 days resulted in a 107 percent increase in activity over that in adrenalectomized rats.

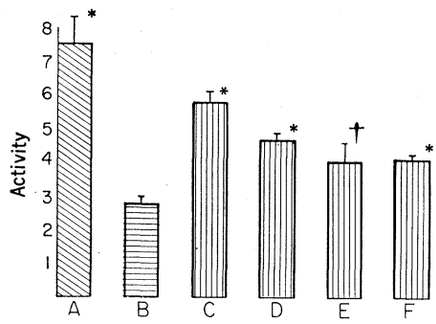


Fig. 1. Tryptophan hydroxylase activity in the midbrain of rats subjected to different hormonal conditions. The enzyme activities are expressed as counts per minute of labeled $CO_2$ evolved per gram of tissue per 1-hour incubation period. (A) Normal. (B) Adrenalectomy. (C) Adrenalectomy; rat was given 1 mg of corticosterone each day for 5 days before being killed. (D) Adrenalectomy; rat was given 2 mg of corticosterone 17 hours before being killed. (E) Adrenalectomy; rat was given 0.4 mg of corticosterone 17 hours before being killed. (F) Adrenalectomy; rat was given 3 mg of corticosterone 4 hours before being killed. Asterisk, $P < .005$; dagger, $P < .05$ compared to adrenalectomy (Student's *t*-test).

We detected a significant increase in tryptophan hydroxylase in midbrain 4 hours after a 3-mg dose of corticosterone (Fig. 1 and Table 1A). This increase gave us the opportunity to determine whether *de novo* protein synthesis was responsible for the increase in the enzyme activity induced by corticosterone. Cycloheximide (350 $\mu$g), an inhibitor of protein synthesis in higher organisms (*9*), was administered into the cisterna magna to suppress brain protein synthesis. Control experiments, with radioactive leucine injected intraperitoneally 30 minutes after the cycloheximide injection, showed that protein synthesis in the midbrain and brainstem was inhibited by about 80 percent. Injection of cycloheximide alone into adrenalectomized rats resulted in a decrease of 38 percent in tryptophan hydroxylase activity in the midbrain within 4 hours (Table 1B). A similar decrease was detected in normal rats 4 hours after cycloheximide injection. These results indicate a rapid turnover of the enzyme in the normal and the adrenalectomized rat.

The ability of corticosterone to cause an increase in tryptophan hydroxylase activity in the midbrain of an adrenalectomized rat within 4 hours after injection was completely inhibited by the presence of 350 $\mu$g of cycloheximide. Two groups of adrenalectomized rats were injected with 3 mg of corticosterone (Table 1C). One group received at the same time an intracisternal injection of cycloheximide in saline; the other group received saline alone. Four hours later, the midbrains of the rats injected with corticosterone and saline had 175 percent higher activities of tryptophan hydroxylase than the midbrains of the rats injected with corticosterone and cycloheximide. If the results of three separate experiments are normalized to the enzyme activties in the adrenalectomized animals, the activity in the group injected with corticosterone and cycloheximide is somewhat below that of the adrenalectomized rats which received cycloheximide alone. Cycloheximide prevented the induction by corticosterone of tryptophan hydroxylase, and led instead to the decrease of enzyme activity observed in adrenalectomized rats treated with cycloheximide alone.

Table 1. Prevention of corticosterone effect on tryptophan hydroxylase by cycloheximide. The tryptophan hydroxylase activity in midbrain is expressed as counts per minute of labeled $CO_2$ evolved per gram of tissue per 1-hour incubation period (mean ± S.E.M.). The statistical significance was determined by means of Student's *t*-test. Differences between enzyme activities in A, B, and C are due to the fact that these experiments were run at different times with different batches of isotope. Normalization of the activities to those in adrenalectomized animals permits a comparison of the three experiments. Abbreviations are: ADX, adrenalectomy; cort., corticosterone; and cyclo., cycloheximide.

| Treatment of ADX | Animals (No.) | Activity | Normalized to ADX |
|---|---|---|---|
| | | *Part A* | |
| None | 4 | 14,376.1 ± 184.76 $P < .005$ | 1.00 |
| 3 mg cort. | 4 | 21,122.7 ± 90.1 | 1.47 |
| | | *Part B* | |
| None | 8 | 4,705.5 ± 283 $P < .05$ | 1.00 |
| Cyclo. | 10 | 3,406.6 ± 326 | .74 |
| | | *Part C* | |
| 3 mg cort. | 4 | 15,943.7 ± 145.7 $P < .005$ | 1.47 |
| 3 mg cort. + cyclo. | 4 | 5,790 ± 120.9 | .54 |

The mechanism by which corticosterone increases tryptophan hydroxylase activity in the midbrain of adrenalectomized rats remains to be elucidated. It may occur through a translational level control of enzyme synthesis (*10*), or it may be mediated by action of corticosterone on the cell nucleus, since retention of this hormone by cell nuclei of rat brain has been observed (*11*).

Tryptophan hydroxylase has a rapid rate of turnover in the midbrain, and one of the important regulatory influences on this enzyme may be the concentration of corticosterone circulating in the blood. It is important to consider how the enzyme concentration could influence the rate of turnover of serotonin. Serotonin concentrations in the brain do not vary greatly in different physiological states even though large differences in the rate of turnover of serotonin are observed (*12, 13*). For example, in the development of tolerance to morphine, no change in sero-

tonin concentration was observed in spite of a several-fold increase in the rate of turnover (*12*). At a constant concentration of serotonin, the rates of biosynthesis and utilization of this substance must be equal. Therefore, a change in the concentration of tryptophan hydroxylase might indicate a corresponding change in the rate of serotonin turnover. Data in support of this relationship have been obtained by Fuxe (*14*), who has found by histofluorescence a decrease in serotonin turnover as a result of adrenalectomy in rats. One can envision a homeostatic mechanism in the brain whereby a stressful situation leads to a high concentration of corticosterone in the blood, and this in turn increases the activity of tryptophan hydroxylase in the midbrain. The increased activity of this enzyme permits the increased synthesis of serotonin, which is then available in increased quantity to supply the increased demand at the synaptic endings which results from continuing stress. It is particularly important that the relevance of these findings to the severe emotional instability often associated with adrenal cortical hormone excess or insufficiency (*15*) be explored.

*Note added in proof*: In recent experiments we have obtained smaller differences in midbrain tryptophan hydroxylase activities between normal and adrenalectomized rats than are reported here. They range from 16 to 40 percent, with a mean of 24 percent ($N = 29$). This may be due to a recent change in housing conditions for the rats, from a stressful to a nonstressful environment, for we have also observed that subjecting normal but not adrenalectomized rats to various types of stress leads to increased tryptophan hydroxylase activity in the midbrain.

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## Acetyl Coenzyme A Carboxylase: Filamentous Nature of the Animal Enzymes

Abstract. *Acetyl coenzyme A carboxylases purified from several animal tissues exist as enzymatically active polymeric filaments of high molecular weight and have similar electron microscopic, hydrodynamic, and catalytic properties. These filaments reversibly dissociate into inactive protomers of uniform size. Their reassembly into catalytically active filaments is promoted by the presence of an allosteric activator.*

Acetyl coenzyme A carboxylase (E.C. 6.4.1.2) catalyzes the regulatory and committed step in fatty acid biosynthesis *de novo* in animal tissues (*1*, *2*). Citrate acts as a "feed forward" allosteric activator of the acetyl CoA carboxylases from these tissues (*2*) but has no activating effect on those from plant (*3*) or microbial sources (*4*, *5*); activators of the latter enzymes are still unknown. Under conditions of carboxylase assay, the activation of the avian liver enzyme by citrate or isocitrate occurs concomitantly with ag-

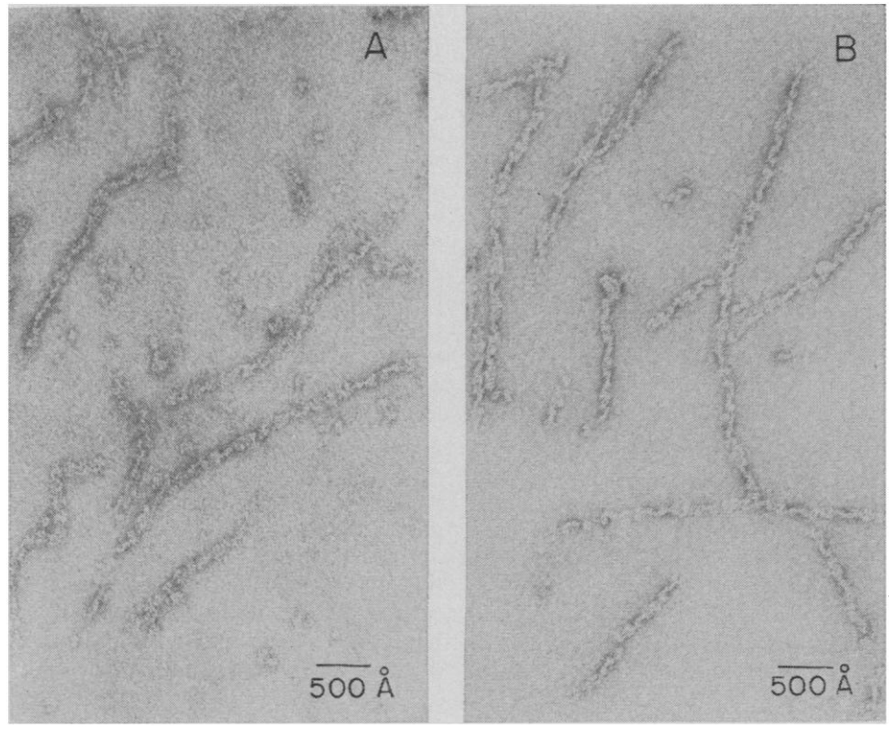


Fig. 1. Filamentous forms of acetyl CoA carboxylases from avian liver (A) and bovine adipose tissue (B), in the presence of citrate. Dilute solutions (20 μg/ml) of chicken liver (*8*) or bovine perirenal adipose tissue (*12*) carboxylase in 50 m*M* tris ($Cl^-$) buffer containing 10 m*M* potassium citrate, 5 m*M* 2-mercaptoethanol, and 0.1*M* ethylenediaminetetraacetate at *p*H 7.5 were applied as droplets to carbon-collodion support films. After the preparations were stained with 4 percent aqueous uranyl acetate and the collodion was removed by heating at 180°C for 10 minutes (*16*), they were photographed through a Siemens Elmiskop IA electron microscope.