6. Specimens of *A. afarensis* with wear on the distal edge of the lower canine include L.H.-14 (where there is more apical wear than on M. 18773), A.L. 128-23, A.L. 198-1, A.L. 333w-58, and A.L. 400-1a.
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12. I thank the following people for permission to examine fossils, for discussion, and for assistance in preparing this report: P. Andrews, R. Clarke, J. Cronin, E. Delson, D. Gantt, P. Gingerich, R. Hay, A. Hill, F. C. Howell, D. Johanson, P. Jones, B. Kimbel, B. Latimer, M. Leakey, R. Moore, A. A. Mturi, B. A. Ogot, D. R. Pilbeam, Y. Rak, V. Sarich, C. Stringer, P. V. Tobias, and E. Vrba. L. Dmytryk did the artwork and J. Aicher did the photography. Supported by National Science Foundation grant BNS 79-06849.

7 February 1980; revised 30 October 1980

Biopterin Cofactor Biosynthesis: Independent Regulation of GTP Cyclohydrolase in Adrenal Medulla and Cortex

Abstract. *Guanosine triphosphate cyclohydrolase, the enzyme that is apparently rate-limiting in biopterin biosynthesis, is increased in adrenal cortex and medulla of rats treated with insulin or reserpine. Denervation and hypophysectomy block the increase in medullary and cortical enzyme activity, respectively, whereas cycloheximide prevents the increase in both tissues. These results provide evidence for induction and regulation of guanosine triphosphate cyclohydrolase.*

L-Erythro-5,6,7,8-tetrahydrobiopterin (BH_4) is the putative cofactor for mixed function oxygenases participating in the synthesis of tyrosine, catecholamines, and serotonin (*1*) and in lipid metabolism (*2*). The low concentration of tissue BH_4 (*3*) relative to the Michaelis constants (K_m's) of tyrosine, tryptophan, and phenylalanine hydroxylases for BH_4 (*1*) indicates that availability of this cofactor could regulate the activity of these enzymes. Recently, large increases in the BH_4 content of rat adrenal medulla and cortex were observed after the administration of insulin and reserpine, and these increases were blocked by cycloheximide (*4*). These results suggested that the increase in cofactor concentration might be due to the induction of synthesis of one or more of the enzymes in the biopterin biosynthetic pathway. Reserpine and insulin have already been shown to induce the synthesis of adrenomedullary tyrosine hydroxylase and dopamine β-hydroxylase (*5*, *6*).

Reserpine acts on the adrenal medulla by blocking the uptake of catecholamines into the storage vesicles and indirectly, like insulin, by increasing splanchnic nerve discharge (*5*). Both drugs are also known to stimulate the adrenal cortex by enhancing the secretion of adrenocorticotropic hormone (ACTH) from the pituitary (*7*).

These observations led to the examination of the effect of these drugs in adrenal medullary and cortical guanosine triphosphate (GTP) cyclohydrolase activity (E.C. 3.5.4.16; D-erythrodihydroneopterin triphosphate synthetase), the first, and probably the rate-limiting, enzyme in the pathway leading from GTP to BH_4 (*8*). In this report, we describe a cycloheximide-sensitive increase in GTP cyclohydrolase activity of adrenal medulla and cortex following insulin or reserpine administration.

The specific activity of GTP cyclohydrolase is sixfold higher in the adrenal medulla than in the cortex (Table 1). Because of the larger mass of the cortex, the total enzyme activity is approximately the same in the two tissues. Significant increase in the activity of the enzyme in both tissues occurred 12 hours after administration of reserpine (Table 1) or 20 hours after administration of insulin (Fig. 1), the times at which maximum increases in BH_4 in medulla and cortex were observed (*4*). Reserpine treatment did not modify the apparent K_m's of the cortical or medullary enzyme for GTP. To test whether the increase in maximum velocity (V_{max}) after reserpine administration was due to induction of synthesis of the enzyme, we injected reserpine simultaneously with cycloheximide at doses previously shown to block the induction of synthesis of tyrosine hydroxylase and opioid peptides in the adrenal medulla (*5*, *6*, *9*). The protein synthesis inhibitor did not significantly affect the tissue mass of cortex or medulla but it decreased the specific activity of GTP cyclohydrolase in the medulla. Furthermore, cycloheximide completely prevented the reserpine-mediated increase in enzyme activity in both tissues. Reserpine and insulin are known to enhance secretion of ACTH from the pituitary (*7*), and hypophysectomy suppresses the increase in cortical, but not in medullary, BH_4 produced by these drugs (*4*). To determine if the increase in GTP cyclohydrolase was also hormonally mediated, we administered reserpine to hypophysectomized animals. The adrenal medulla showed no change, whereas the cortex showed an apparent increase in the specific activity of GTP cyclohydrolase after removal of the pituitary, presumably resulting from a faster loss of total tissue mass than loss of the activity of this enzyme (Table 1). Although reserpine produced its usual effect on the medulla of hypophysectomized animals, there was no effect on the cortex.

Both insulin-induced hypoglycemia

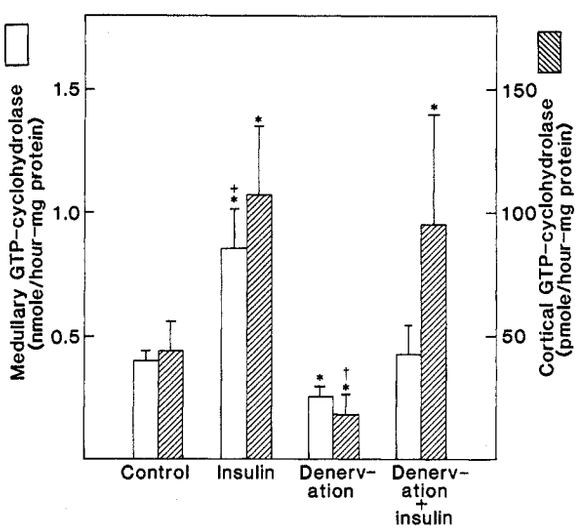

Fig. 1. Effect of denervation on the response of adrenal medullary and cortical GTP cyclohydrolase to insulin hypoglycemia. Increased splanchnic activation was obtained by injection of 10 units of insulin subcutaneously to fasted rats. After 4 hours the hypoglycemic shock was interrupted by administration of 1.0 ml of 40 percent sucrose by means of gastric tube and free access to food. The animals were killed 20 hours later. Splanchnic nerves were transected bilaterally between the diaphragm and coeliac ganglia 14 days before the experiment. Enzyme activity was measured as indicated in Table 1. Values are means ± standard error; $N = 5$ except for the group indicated by denervation plus insulin, where $N = 3$. $^*P < .05$ when compared to control groups. $^\dagger P < .005$ when compared to denervation plus insulin.

0036-8075/81/0717-0349$00.50/0

and reserpine treatment indirectly increase splanchnic nerve discharge to the adrenal medulla (*5*). Splanchnic transection, which blocks the increase in medullary but not in cortical BH_4 (*4*), decreased the basal enzyme activity and prevented the effect of insulin in the adrenal medulla (Fig. 1). Adrenal denervation produced a decrease in cortical GTP cyclohydrolase but did not modify the magnitude of the response to insulin. The selective effects of hypophysectomy and adrenal denervation indicate a differential control of the GTP cyclohydrolase induction in the adrenal cortex and medulla.

These results provide, to our knowledge, the first demonstration of regulation of GTP cyclohydrolase in vivo. Although other interpretations are possible, the cycloheximide-sensitive increase in enzyme activity with no change in affinity for GTP support the suggestion that there is induction of enzyme synthesis. The differential control of GTP cyclohydrolase by transsynaptic stimulation of the medulla and through the effect of the pituitary hormones on the cortex indicates that these increases are probably associated with the specific synthetic and secretory function of these two tissues, rather than being a nonspecific response to pharmacological stress. The activity of medullary and cortical dihydrofolate reductase and dihydropteridine reductase, two other enzymes apparently involved in the synthesis and maintenance of the cofactor in reduced form, was also examined. Neither enzyme showed an increase in activity 24 hours after reserpine administration.

These results suggest a causal relation between the increases in the biopterin cofactor concentration (*4*) and the regulation of the enzyme. In the medulla, the increase in GTP cyclohydrolase and its final product BH_4 (*4*) probably contributes to the enhanced tyrosine hydroxylation that follows the drug-induced increase in splanchnic nerve discharge (*10*). The induction in GTP cyclohydrolase would thereby provide for an increased turnover of the reduced cofactor and allow tyrosine hydroxylase to function under more optimal conditions of elevated cofactor concentration. It is noteworthy that the increased splanchnic discharge or depletion of the vesicular catecholamine pool initiates a complex series of mutually complementary regulatory events in the adrenal medulla: induction of tyrosine hydroxylase, dopamine β-hydroxylase, opiate peptides, and chromaffin vesicle synthesis, along with increased uptake and storage of catecholamines (*5*, *6*, *9*) and increased levels of BH_4 (*4*) and GTP cyclohydrolase. All of these changes are the expression of the ability of the chromaffin cell to adapt biochemically to increased functional demands (*11*). The function of BH_4 in the adrenal cortex and the reason for this regulatory mechanism to control GTP cyclohydrolase are unknown. The dependence of the increases in cortical GTP cyclohydrolase and BH_4 on the presence of the hypophysis suggest a relation with the synthesis and secretion of corticosteroids.

While BH_4 appears to be the essential cofactor for phenylalanine, tyrosine, and tryptophan hydroxylases and for the O-alkyl-glycerolipid cleavage enzyme (*1*, *2*), its widespread tissue distribution suggests a participation in other, unknown biochemical reactions. Thus, regulation of GTP cyclohydrolase under situations of increased demand for BH_4 may not be limited to the adrenal medulla and cortex but may also occur in other nonneuronal tissues as well as in peripheral and central catecholamine- and serotonin-containing neurons.

Table 1. The activity of GTP cyclohydrolase in adrenal medulla and cortex. Groups of five male Sprague-Dawley rats (175 to 200 g; Charles River) were injected intraperitoneally with reserpine (5 mg/kg), cycloheximide (1 mg/kg), or both, 12 hours before they were killed. Hypophysectomy was done 12 days before the experiments. Medullas were separated from cortices under a dissecting microscope and the tissues homogenized in 0.5 ml of incubation buffer (0.1*M* tris-HCl, *p*H 7.8, 0.3*M* KCl, 2.5 m*M* EDTA, and 10 percent glycerol). After centrifugation, the supernatant was applied to a small Sephadex G-25 column to separate the enzyme from small molecular weight material. Portions of the void volume eluate (200 μl) were incubated in the dark for 90 minutes with 2 m*M* GTP (the GTP concentration is at least 250 times higher than the apparent K_m of the medullary or cortical enzyme). The reaction was stopped and the resulting reduced pteridines oxidized by the addition of 25 μl of 1 percent I_2 and 2 percent KI in 1*N* HCl. Protein precipitates were removed by centrifugation and 25 μl of ascorbic acid added to the supernatant. After neutralization with NaOH the reaction product, neopterin triphosphate, was dephosphorylated by incubation with alkaline phosphatase (1 unit per incubation). The resulting neopterin was separated and quantified fluorometrically as described (*3*). Values are means ± standard error; for the statistical analysis we used Student's *t*-test.

Treatment	Medulla (pmole/hour)		Cortex (pmole/hour)	
	Per milligram of protein	Per gland	Per milligram of protein	Per gland
Control	313 ± 17	50 ± 4	50 ± 8	33 ± 7
Reserpine	722 ± 67*	124 ± 15*	147 ± 26*	137 ± 29*
Cycloheximide	207 ± 37*	32 ± 6*	45 ± 8	50 ± 16
Reserpine plus cycloheximide	255 ± 35	38 ± 6	63 ± 15	48 ± 13
Hypophysectomy	385 ± 88	20 ± 5*	86 ± 8*	24 ± 3
Hypophysectomy plus reserpine	646 ± 86*†	33 ± 6*	92 ± 17*	34 ± 12

*$P < .05$ compared to control group. †$P < .05$ compared to hypophysectomy alone.

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12. We thank E. J. Diliberto, Jr., and D. Duch, Department of Medicinal Biochemistry, Wellcome Research Laboratories, for letting us report on their unpublished measurements of dihydropteridine and dihydrofolate reductase. Reserpine (Sandril) was generously supplied by Eli Lilly & Co. Indianapolis.

18 December 1980; revised 6 March 1981