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Effect of Hypophysectomy on Adrenal Dopamine β-Hydroxylase Activity in the Rat

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SUMMARY

Dopamine β-hydroxylase activity in the adrenal medulla is decreased 1 week after hypophysectomy. This activity can be restored by treatment with either adrenocorticotropin or dexamethasone. Adrenal denervation also decreases adrenal dopamine β-hydroxylase activity and prevents elevation of this enzyme in response to immobilization. Intact pituitary-adrenal and neuronal systems are required to maintain normal adrenal dopamine β-hydroxylase levels in nonimmobilized rats and to raise the levels in immobilized rats.

INTRODUCTION

Dopamine β-hydroxylase, the enzyme present in the adrenal medullary storage vesicles which catalyzes conversion of dopamine to norepinephrine, appears to be released during the stress induced by the immobilization of rats and to be replenished by new synthesis during the 6-hr interval following immobilization (1). Intact innervation of the adrenal gland is required to maintain dopamine β-hydroxylase levels in nonimmobilized animals and to elevate dopamine β-hydroxylase levels after immobilization (1). Both tyrosine hydroxylase (2) and phenylethanolamine *N*-methyltransferase (3) activities are influenced by hypophysectomy. A decrease in adrenal dopamine β-hydroxylase activity after hypophysectomy has been noted (1), and the hormonal influences on changes of levels of this enzyme during stress have been examined.

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METHODS

Hypophysectomized, thyroidectomized, and appropriately sham-operated male rats weighing 180–250 g were purchased from Hormone Assay Laboratories, Inc., Chicago. The animals were killed by decapitation; the adrenal glands were rapidly removed, cleaned, weighed, and homogenized in 1.0 ml of ice-cold 0.25 M sucrose. An aliquot (100 μl) of the homogenate was assayed for catecholamines (4), and the remaining homogenate was centrifuged at 26,000 × *g* for 20 min. The supernatant fraction (S_1) was removed, and a 50-μl aliquot was assayed for dopamine β-hydroxylase. The sediment was resuspended in 500 μl of cold water and centrifuged as above. Aliquots (50 μl) of the supernatant fraction (S_2) and of the sediment (P_2) suspended in 500 μl

of cold water were also assayed for dopamine β-hydroxylase.

Dopamine β-hydroxylase was assayed in the S_1, S_2, and P_2 fractions by the technique of Friedman and Kaufman (5), as modified by Viveros *et al.* (6), using *p*-chloromercuriphenylsulfonic acid instead of Cu^{++} or *p*-hydroxymercuribenzoate to inactivate the natural inhibitors of dopamine β-hydroxylase. To determine the variability of the inhibition of the enzyme when adrenal weights varied from 14 mg/pair (as in the hypophysectomized rats) to 60 mg/pair (as in the adrenocorticotropin-treated rats), purified dopamine β-hydroxylase was added to representative samples from the various groups, which were assayed with *p*-hydroxymercuribenzoate. In the sucrose supernatant fraction (S_1) inhibition varied from 10 to 20%, but in the particulate fraction (P_2) it was about 30–45%. The distribution of the enzyme did not appear to vary between groups. The degree of inhibition was similar in all groups except for the hypophysectomized animals, the adrenals of which displayed less inhibition. Since adrenals of hypophysectomized rats had less than normal amounts of dopamine β-hydroxylase activity, correction for inhibition would have increased the already significant differences, and such corrections therefore were not made.

In some experiments each animal was treated with ACTH (5 international units, subcutaneously; ACTHar-Gel, Armour Pharmaceutical Company, Berkeley Heights, N. J.), dexamethasone 21-phosphate (9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione 21-phosphate) (1 mg, subcutaneously; Decadron, Merck Sharp & Dohme, West Point, Pa.), L-thyroxine (10 mg, subcutaneously), or 0.9% sodium chloride (0.25 ml, subcutaneously) daily for 6 or 7 days, as indicated. Denervation of the left adrenal was performed by section of the splanchnic nerve. The splanchnic nerves were exposed through a midline incision; the left side was cut, and the right side was exposed but not severed. The right adrenal served as a control for the denervated left. The denervation procedure required only 2–3 min, but Pentothal anesthesia lasted for a total of 1 hr. The operation was performed 2–3 days after hypophysectomy. The animals were killed 15 days after hypophysectomy.

In other experiments animals were immobilized in a prone position for 2.5 hr daily by inserting their heads through two parallel concentric steel wire loops fixed on a metal plate and fastening their limbs with adhesive tape to four specially mounted metal strips. The animals were killed either 24 hr after the sixth immobilization or 6 hr after the seventh immobilization.

RESULTS

Effect of hypophysectomy on adrenal dopamine β-hydroxylase activity. By 7 days after hypophysectomy, dopamine β-hydroxylase activity was decreased by 25–30% over control levels (Table 1). The enzyme activity remained depressed for up to 180 days after hypophysectomy. However, 2 or 4 days after hypophysectomy, dopamine β-hydroxylase levels were not yet significantly different from control levels.

TABLE 1

Effect of hypophysectomy on adrenal dopamine β-hydroxylase activity

Enzyme activity is the sum of the dopamine β-hydroxylase found in the S_1, S_2, and P_2 fractions. Control levels ranged from a mean ± standard error of 1.91 ± 0.14 units (nanomoles of octopamine-^{3}H formed per hour per pair of adrenals) in rats sham-hypophysectomized 2 days previously (body weight, about 200 g) to 3.18 ± 0.19 units in rats sham-hypophysectomized 180 days previously (body weight, about 400 g). Results are therefore expressed as percentage change from the dopamine β-hydroxylase level found in rats sham-hypophysectomized on the same day. Each group contained at least six rats.

Days after hypophysectomy	Change in dopamine β-hydroxylase activity	*p*
	%	
2	−11.8 ± 9.1	NS[a]
4	+4.0 ± 9.3	NS
7	−26.6 ± 9.5	<0.02
21	−23.3 ± 8.9	<0.05
28	−29.7 ± 6.7	<0.01
40	−35.9 ± 8.0	<0.01
180	−42.5 ± 7.6	<0.01

[a] Not significant

Effect of ACTH, dexamethasone, or thyroxine treatment on adrenal dopamine β-hydroxylase activity in hypophysectomized rats. Treatment with ACTH for 7 days significantly increased the adrenal dopamine β-hydroxylase activity of hypophysectomized rats (Table 2). Treatment with dexamethasone starting 8–10 days after hypophysectomy also significantly elevated adrenal dopamine β-hydroxylase activity, but no increase in this enzyme was found in thyroxine-treated, hypophysectomized rats.

Effect of repeated immobilization on adrenal dopamine β-hydroxylase activity in hypophysectomized rats and hypophysectomized rats treated with ACTH or dexamethasone. After seven daily periods of immobilization there was a marked increase in adrenal dopamine β-hydroxylase levels in sham-hypophysectomized animals (Table 3). In hypophysectomized rats significant increases in adrenal dopamine β-hydroxylase were also found after immobilization, but the levels were much lower than those found in the immobilized sham-hypophysectomized

TABLE 2

Effect of treatment of hypophysectomized rats with ACTH, dexamethasone, or thyroxine on adrenal dopamine β-hydroxylase activity

Results are expressed as means ± standard errors and are the sum of the dopamine β-hydroxylase activities in the S_1, S_2, and P_2 fractions. Each group contained at least six rats. Daily injections of ACTH, dexamethasone, or thyroxine were begun either 8 or 40 days after hypophysectomy and continued for 6 days.

Days after hypophysectomy	Treatment	Dopamine β-hydroxylase activity
		mμmoles octopamine-^{3}H formed/hr/adrenal pair
8	Sham-hypophysectomized control	2.99 ± 0.11
	Hypophysectomized control	2.14 ± 0.11[a]
	Hypophysectomized (ACTH)	2.57 ± 0.14[b]
	Hypophysectomized (dexamethasone)	3.06 ± 0.16[c]
	Hypophysectomized (thyroxine)	2.34 ± 0.09[d]
40	Sham-hypophysectomized control	3.40 ± 0.26
	Hypophysectomized control	2.18 ± 0.08[a]
	Hypophysectomized (ACTH)	3.41 ± 0.22[c]

[a] $p < 0.01$ compared to sham-hypophysectomized control.

[b] $p < 0.05$ compared to hypophysectomized control.

[c] $p < 0.01$ compared to hypophysectomized control.

[d] Not significantly different from hypophysectomized control.

TABLE 3

Effect of repeated immobilization on dopamine β-hydroxylase levels in hypophysectomized rats

Results are expressed as means ± standard errors and are the sum of the dopamine β-hydroxylase activities in the S_1, S_2, and P_2 fractions. Each group contained at least six rats. Rats were killed 6 hr after the seventh daily immobilization. ACTH or dexamethasone was administered 1 hr prior to the onset of each period of immobilization. Rats were hypophysectomized 30 days prior to the first immobilization.

Treatment	Dopamine β-hydroxylase activity
	mμmoles octopamine-^{3}H formed/hr/adrenal pair
Sham-hypophysectomized control	1.59 ± 0.11
Hypophysectomized control	1.22 ± 0.11[a]
Sham-hypophysectomized, immobilized 7 times	3.37 ± 0.15[b]
Hypophysectomized, immobilized 7 times	2.23 ± 0.12[c]
Hypophysectomized, immobilized 7 times (ACTH)	3.62 ± 0.31[d]
Hypophysectomized, immobilized 7 times (dexamethasone)	2.97 ± 0.23[d]

[a] $p < 0.05$ compared to sham-hypophysectomized control.

[b] $p < 0.01$ compared to sham-hypophysectomized control.

[c] $p < 0.01$ compared to hypophysectomized control.

[d] $p < 0.01$ compared to hypophysectomized-immobilized 7 times.

animals. In hypophysectomized rats given ACTH 1 hr prior to each immobilization, adrenal dopamine β-hydroxylase levels reached those found in the control immobilized rats. Dexamethasone was not so effective as ACTH in restoring the effect of immobilization on adrenal dopamine β-hydroxylase.

Effect of denervation on adrenal dopamine β-hydroxylase activity in hypophysectomized rats. Following denervation there was a significant decrease in dopamine β-hydroxylase activity in sham-hypophysectomized rats (Table 4). The difference in levels on the two sides was not due to an increase on the innervated side, since levels in the innervated adrenals were normal (Table 2). In hypophysectomized rats the decrease in dopamine β-hydroxylase was more marked in the denervated adrenal than in the innervated one. After immobilization there was only a small increase in dopamine β-hydroxylase activity in the denervated adrenals of sham-hypophysectomized rats and no increase in the denervated adrenals of hypophysectomized rats. Enzyme activity increased in the innervated glands of both groups.

TABLE 4

Effect of denervation of adrenal gland on adrenal dopamine β-hydroxylase levels in hypophysectomized rats

Results are expressed as means ± standard errors and are the sum of dopamine β-hydroxylase activities in the S_1, S_2, and P_2 fractions. Each group contained at least six rats. The innervated adrenal gland (right) of each rat served as a control for the denervated gland. Rats were denervated 5 days after hypophysectomy and were killed 15 days after hypophysectomy.

Treatment	Dopamine β-hydroxylase activity	
	Denervated	Innervated
	mμmoles octopamine-³H formed/hr/adrenal pair	
Sham-hypophysectomized control	0.85 ± 0.09[a]	1.39 ± 0.07
Hypophysectomized	0.13 ± 0.03[a,b]	0.34 ± 0.03[c]
Sham-hypophysectomized, immobilized 7 times	1.20 ± 0.11[a,d]	3.75 ± 0.40[c]
Hypophysectomized, immobilized 7 times	0.17 ± 0.02[a]	0.52 ± 0.07

[a] $p < 0.01$ compared to innervated gland of same group.

[b] $p < 0.01$ compared to denervated sham-hypophysectomized control.

[c] $p < 0.01$ compared to innervated sham-hypophysectomized control.

[d] $p < 0.05$ compared to denervated sham-hypophysectomized control.

TABLE 5

Effect of thyroidectomy on adrenal dopamine β-hydroxylase levels

Results are expressed as means ± standard errors and are the sum of the dopamine β-hydroxylase activities in the S_1, S_2, and P_2 fractions. Each group contained at least six rats. Rats were killed 24 hr after the sixth daily immobilization. The rats were 21 days post-thyroidectomy at the time of killing.

Treatment	Dopamine β-hydroxylase activity
	mμmoles octopamine-³H formed/hr/adrenal pair
Sham-thyroidectomized	2.86 ± 0.16
Thyroidectomized	3.38 ± 0.24[a]
Sham-thyroidectomized, immobilized 6 times	4.78 ± 0.24
Thyroidectomized, immobilized 6 times	5.07 ± 0.35[b]

[a] Not significantly different from sham-thyroidectomized animals.

[b] Not significantly different from rats sham-thyroidectomized and immobilized six times.

In hypophysectomized rats the decrease in dopamine β-hydroxylase was more marked in the denervated adrenal than in the innervated one. After immobilization there was only a small increase in dopamine β-hydroxylase activity in the denervated adrenals of sham-hypophysectomized rats and no increase in the denervated adrenals of hypophysectomized rats. Enzyme activity increased in the innervated glands of both groups.

Effect of thyroidectomy on adrenal dopamine β-hydroxylase activity. There were no changes in adrenal dopamine β-hydroxylase activity in rats thyroidectomized 3 weeks previously (Table 5). Similar increases in dopamine β-hydroxylase levels were induced by six daily immobilizations in thyroidectomized rats and their controls.

DISCUSSION

After hypophysectomy there is a decrease in adrenal catecholamines (3, 7), phenyl-

ethanolamine *N*-methyltransferase activity (3, 8), and tyrosine hydroxylase activity (2, 8). Seven days after hypophysectomy dopamine β-hydroxylase activity is also decreased (Table 1). As indicated under METHODS, the adrenals from hypophysectomized animals contained less dopamine β-hydroxylase inhibitor than adrenals from control or repeatedly immobilized rats. The true decrease in enzyme activity in hypophysectomized rats is therefore greater than is apparent from the uncorrected values shown in Table 1. If corrections are made for this difference, the adrenals of hypophysectomized animals have only about one-half the normal dopamine β-hydroxylase activity. The minimal time required for the decrease in dopamine β-hydroxylase levels observed after hypophysectomy is difficult to determine because of the possible effect of the poorly controlled stress of surgery and transportation of the rats, which may tend to elevate levels of this enzyme. Thus the decrease in dopamine β-hydroxylase levels might actually begin earlier than 1 week after hypophysectomy. The decrease in adrenal dopamine β-hydroxylase activity after hypophysectomy is a consequence of decreased enzyme activity in the medulla, since less than 5% of dopamine β-hydroxylase activity is found in the cortex of normal rats (7).

In hypophysectomized rats treatment with ACTH or dexamethasone for 6 days increases adrenal levels of dopamine β-hydroxylase, but thyroxine is ineffective in restoring levels of this enzyme (Table 2). Immobilization of rats appears to be a stressful procedure during which the adrenal medulla releases dopamine β-hydroxylase (1, 7) as well as epinephrine (9). Adrenal levels of phenylethanolamine *N*-methyltransferase and tyrosine hydroxylase (1, 10) and of dopamine β-hydroxylase (1, 7) are increased after repeated immobilization. Phenylethanolamine *N*-methyltransferase appears to be primarily under the control of adrenal cortical hormones. This conclusion is the result of the following observations. (a) Denervation results in only a small decrease in adrenal levels of this enzyme (10, 11). (b) Hypophysectomy markedly lowers phenylethanolamine *N*-methyltransferase levels (3, 8) and prevents the elevation of this enzyme induced by repeated immobilization (1, 8). (c) In hypophysectomized rats, ACTH or dexamethasone restores to normal the levels of adrenal phenylethanolamine *N*-methyltransferase (3) and its elevation during repeated immobilization (1, 8).

Adrenal tyrosine hydroxylase activity is decreased by hypophysectomy (2, 8) and is increased by repeated immobilization (10), even in hypophysectomized animals (8, 12), but not in denervated adrenals (10, 12). Thus both neuronal and hormonal influences control adrenal tyrosine hydroxylase activity. Although denervation does not lower adrenal tyrosine hydroxylase levels (9, 13, 14), dopamine β-hydroxylase activity is lower in the denervated adrenal gland than in the intact gland of both sham-hypophysectomized and hypophysectomized rats (Table 4). The levels of dopamine β-hydroxylase, however, are much lower in either adrenal of hypophysectomized rats. Denervation depresses the levels of dopamine β-hydroxylase in normal animals and produces an even more striking change in the levels of the enzyme in hypophysectomized animals (Table 4). It also prevents the increase in adrenal dopamine β-hydroxylase activity seen in intact glands after repeated immobilization (Table 4). Thus dopamine β-hydroxylase, as well as tyrosine hydroxylase, appears to be under both neuronal and humoral control.

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