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Effect of Stimulation on the Levels of Tyrosine Hydroxylase, Dopamine β-Hydroxylase, and Catecholamines in Intact and Denervated Rat Adrenal Glands

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SUMMARY

Rats were treated with insulin (5 international units/kg) alone and in combination with actinomycin D (1 mg/kg) to determine the effects of neural stimulation and the requirement for protein synthesis upon rat adrenal tyrosine hydroxylase, dopamine β-hydroxylase (EC 1.14.2.1), and catecholamine levels. Four hours after insulin administration, the catecholamine levels were 80% depleted and required 4 days to recover to the control values. Dopamine β-hydroxylase activity was decreased at 24 hr, recovered to control values at 48 hr, and increased above control levels at 96 hr. Tyrosine hydroxylase activity was unchanged at 4 hr, increased at 24 hr, and remained high for at least 96 hr. Treatment of rats with actinomycin D (1 mg/kg) 1 hr before insulin administration completely abolished the rise in tyrosine hydroxylase normally seen after 24 hr, even though appreciable depletion of catecholamines still occurred. Actinomycin D given 3 and 6 hr following treatment with insulin also blocked the rise in tyrosine hydroxylase activity observed at 24 hr, but if actinomycin D administration was delayed until 12 hr after insulin injection the rise in tyrosine hydroxylase activity was no longer blocked.

In rats with the nerve supply to the left adrenal gland cut, only the intact right gland responded to insulin, with a loss of catecholamines at 4 hr and an increase in tyrosine hydroxylase at 24 hr. Rats treated with acetylcholine (20 mg/kg) and eserine (0.1 mg/kg) showed increases in tyrosine hydroxylase activity not only in the intact right gland but also in the denervated left gland. These observations indicate that an intact nervous supply is not absolutely required to bring about a rise in tyrosine hydroxylase activity, and that exposure to the neurotransmitter itself (in this case acetylcholine) is sufficient. Whether acetylcholine is directly involved in the increased levels of tyrosine hydroxylase, or whether a secretory response by the gland is the essential link, has yet to be determined.

Rats that received actinomycin D (0.5 mg/kg) 1 hr before insulin showed no rise in tyrosine hydroxylase at 24 hr, but at 48 hr the enzyme activity was twice that of the controls. In rats with the left gland denervated, only the intact right gland had an increase in tyrosine hydroxylase 48 hr after treatment with acetylcholine and actinomycin D. The evidence suggests that this rise in tyrosine hydroxylase resulted from additional neural stimulation of the gland secondary to the physiological stress produced by actinomycin D, rather than from the expression of a long-lived signal.

INTRODUCTION

The activity of tyrosine hydroxylase has been shown to increase in response to various conditions or treatments that increase sympathetic nervous activity. These include sinoaortic denervation (1), insulin-induced hypoglycemia (2), administration of reserpine or 6-hydroxydopamine (3, 4), and immobilization stress (5). Thoenen, Mueller, and Axel-

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rod (6, 7) demonstrated that the increase in tyrosine hydroxylase activity could be prevented by treatment with inhibitors of protein or nucleic acid synthesis, such as actinomycin D and cycloheximide, or by interrupting the nerve supply to the gland.

The present studies were initiated to discover some of the parameters affecting the increases in enzyme activity and the recovery of catecholamine content following stimulation of the adrenal gland, and to determine whether some factor or factors liberated by the nerve supply, other than the neurotransmitter acetylcholine, are required to induce[1] the synthesis of tyrosine hydroxylase.

METHODS

Male Sprague-Dawley rats (200–275 g) were used in all studies. Rats with denervated left adrenal glands were obtained from Zivic-Miller Laboratories, Allison Park, Pa. The animals receiving insulin were fasted for 24 hr and then treated by injection with insulin, 5 international units/kg of body weight, via the tail vein. Three hours later the animals either were killed or were brought out of hypoglycemic shock by the intraperitoneal injection of 1 ml of 20% glucose and killed at various times afterward. Animals receiving actinomycin D were given either 1.0 or 0.5 mg/kg in 0.9% NaCl intraperitoneally, as indicated in the tables. Doses of actinomycin D lower than 0.5 mg/kg, such as 0.1 mg/kg, were insufficient to inhibit tyrosine hydroxylase induction after insulin administration.

Rats treated with acetylcholine first received an intraperitoneal injection of atropine sulfate, 0.5 mg/kg, to protect them against the muscarinic effects of acetylcholine. The animals then were given acetylcholine chloride, 20 mg/kg, in 0.15 M sodium chloride, together with eserine, 0.1 mg/kg, via the tail vein. Three injections were given, 30 min apart, followed by a second intraperitoneal injection of atropine sulfate, 0.5 mg/kg. The dosage schedule was completed with two additional injections of acetylcholine and eserine as before, again 30 min apart. Between the drug injections, the animals were kept in separate cages and were returned briefly to restraining cages for drug treatment.

Preparation of homogenates. The rats were killed between 10 a.m. and noon by a blow on the base of the skull and bled at the neck, and the adrenal glands were removed and placed in ice-cold 0.3 M sucrose. The glands were cleaned of fat and connective tissue, blotted dry, weighed, and homogenized in ice-cold 0.3 M sucrose in Potter-Elvehjem all-glass homogenizers. Pooled glands from the same animal were homogenized in 5.0 ml of sucrose; 2.5 ml of buffer were used for glands homogenized individually. Aliquots were removed for catecholamine and dopamine β-hydroxylase determinations, and the remainder of the homogenate was centrifuged at 26,000 × *g* for 20 min. The supernatant fraction was assayed for tyrosine hydroxylase activity. No tyrosine hydroxylase activity was found in resuspended pellets when homogenization was carried out in either 0.3 M sucrose or distilled water, but approximately 50% of the activity was recovered in the pellet when homogenization was carried out in 0.01 M sodium phosphate buffer, pH 6.0.

Assay of dopamine β-hydroxylase. Dopamine β-hydroxylase was assayed as described previously (8). The reaction mixture contained potassium phosphate buffer, pH 5.8, 200 mM; fumarate, pH 5.8, 120 mM; ascorbate, 2.0 mM; ATP, 0.1 mM; tranylcypromine, 1 mM; ^{3}H-tyramine, generally labeled (7.3 Ci/mmole), 0.01 mM; catalase, 600 units; Triton X-100, 0.5%; and 0.4 ml of the sucrose homogenate in a final volume of 1.0 ml. In addition, each reaction mixture contained a final concentration of 4 mM *p*-hydroxymercuribenzoate to inactivate endogenous inhibitors. The mixtures were incubated in air at 37° for 30 min, and the reactions were stopped by the addition of 1 ml of 7% perchloric acid. After centrifugation, 1 ml of the supernatant fluid was assayed by the periodate oxidation method of Friedman

[1] The term "induction" is used in a broad sense, i.e., to indicate a rise in enzyme activity with formation of increased amounts of new enzyme molecules, viewed as a likely cause even though this still remains to be conclusively demonstrated for the enzymes discussed here.

and Kaufman (9) for the amount of octopamine formed. Five per cent glycerol was used to stop the periodate oxidation instead of 10% $NaHSO_3$, as the latter compound caused considerable quenching in the liquid scintillation spectrometer. Under these conditions dopamine β-hydroxylase activity was linear with tissue concentration.

Tyrosine hydroxylase. Tyrosine hydroxylase activity was assayed as described by Nagatsu, Levitt, and Udenfriend (10). The reaction mixture contained sodium acetate buffer, pH 6.0, 200 mM; ferrous ammonium sulfate, 0.5 mM; tranylcypromine, 0.1 mM; 2 - amino - 6,7 - dimethyl - 4 - hydroxy - 4,6,7,8-tetrahydropteridine hydrochloride, 2 mM; 2-mercaptoethanol, 20 mM; 3,5-^{3}H-tyrosine, 0.2 mM (specific activity 31.6 Ci/mmole); and 0.4 ml of the 26,000 × *g* sucrose supernatant fraction in a final volume of 1 ml. The reaction mixtures were incubated in air at 37° for 15 min, and the reaction was stopped by the addition of 0.05 ml of glacial acetic acid.

Catecholamines. Aliquots of the adrenal fractions (0.1 ml) were added to 2 ml of 3.5% perchloric acid. The mixture was centrifuged, and the supernatant fluid was decanted, diluted, and assayed for catecholamine content without further treatment, as described previously (8).

Statistical methods. Results are expressed on a per gland basis. Student's *t*-test was used to determine statistical significance.

Materials. ^{3}H-Tyramine and ^{3}H-tyrosine were obtained from New England Nuclear Corporation. Insulin was obtained from Squibb, nicotine sulfate (40% solution) from Pfaltz and Bauer, acetylcholine chloride from Calbiochem, eserine sulfate (physostigmine) from Mann Research Laboratories, atropine sulfate from Eli Lilly and Company, and actinomycin D from Merck Sharp & Dohme Research Laboratories. Angiotensin II (Hypertensin powder) was a gift of Ciba Pharmaceutical Company.

RESULTS

Effect of insulin treatment. The effects of insulin treatment on rat adrenal tyrosine hydroxylase, dopamine β-hydroxylase, and catecholamine levels are shown in Fig. 1. Catecholamine levels were 80% depleted 4 hr after insulin treatment, and returned to control levels 72–96 hr after insulin administration. Dopamine β-hydroxylase activity was decreased at 24 hr, recovered to control levels at 48 hr, and was higher than control values at 96 hr. Tyrosine hydroxylase activity was unchanged at 4 hr but increased at 24 hr, and remained high for at least 96 hr. These findings are similar to those reported previously for the rabbit, except that increase in tyrosine hydroxylase activity in this animal were not observed until 48 hr after insulin administration (2).

Effect of actinomycin D on insulin-induced changes in rat adrenals. To obtain further information on the requirement of macromolecular synthesis *de novo* for the insulin-induced increases in tyrosine hydroxylase activity, actinomycin D (1.0 mg/kg intraperitoneally) was given at various times before and after insulin administration, and the animals were killed 24 hr after receiving the insulin. As can be seen in Fig. 2, actinomycin D itself had no effect on tyrosine hydroxylase activity 24 hr after administration, but when given 1 hr before insulin it completely blocked the rise in tyrosine hydroxylase normally observed 24 hr after insulin administration, even though appreciable depletion of catecholamines had still occurred. When actinomycin D was given 3 and even 6 hr after insulin, it still blocked the rise in tyrosine hydroxylase normally observed at 24 hr, but when given 12 hr after insulin administration it no longer blocked the increase in tyrosine hydroxylase that occurred at 24 hr. This suggests that the RNA synthesis necessary for the induction of tyrosine hydroxylase is completed 6–12 hr after insulin administration.

Drug-induced changes in denervated rats. The experiments presented in Table 1 were carried out to determine whether the insulin-induced increase in tyrosine hydroxylase activity is dependent upon an intact nerve supply or whether stimulation of the gland by the neurotransmitter alone is sufficient. In rats with the nerve supply to the left adrenal gland cut, only the intact right gland responded to insulin, with a loss of catecholamines at 4 hr and an increase in

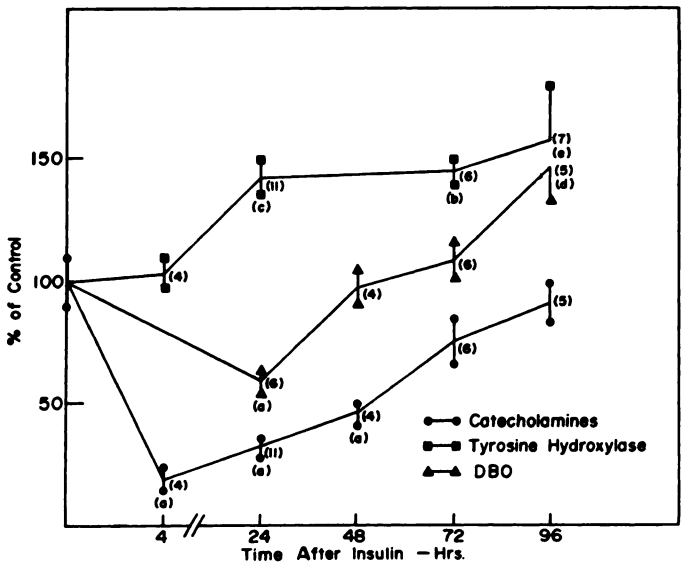

FIG. 1. *Effect of insulin administration on rat adrenal catecholamines, tyrosine hydroxylase, and dopamine β-hydroxylase levels*

The number of animals in each group is shown in parentheses. The letters in parentheses refer to the following significant p values (Student's t-test): (*a*) $p < 0.001$; (*b*) $p < 0.002$; (c) $p < 0.005$; (*d*) $p < 0.02$; (*e*) $p < 0.05$. The means ± standard errors per adrenal gland for the controls (nine animals) are: catecholamines, 10.7 ± 1.1 μg; tyrosine hydroxylase, 13.5 ± 1.1 mμmoles of dopa formed per hour; dopamine β-hydroxylase (DBO), 1.18 ± 0.07 mμmoles of octopamine formed per hour.

tyrosine hydroxylase activity at 24 hr. However, repeated injections of acetylcholine and eserine brought about a rise in tyrosine hydroxylase activity not only in the intact right gland, but also in the denervated left gland. This response required acetylcholine, since a similar dose schedule employing choline instead of acetylcholine caused a rise only in the intact right gland (Table 1). The rise (170% of controls) in tyrosine hydroxylase activity in the right gland of rats treated with both choline and eserine was not accompanied by a significant decrease in catecholamines after 24 hr. When repeated injections of acetylcholine and eserine were given for 3 consecutive days, the tyrosine hydroxylase activity in the denervated left gland 24 hr after the last injection[2] was more than twice that of untreated animals.

Attempts to obtain increases in tyrosine hydroxylase activity in denervated glands 24 hr after the administration of nicotine or angiotensin II have not been successful. Table 2 shows the results of these experiments, as well as an additional experiment, using acetylcholine, performed concurrently. One group of animals was given five injections of nicotine, 2 mg/kg, via the tail vein at 20-min intervals, and examined 24 hr later. Although there was no change in the catecholamine levels in either the denervated gland or the intact right gland, the tyrosine hydroxylase activity was significantly elevated (170% of the controls) in the intact gland but unchanged in the left gland. After similar treatment with angiotensin II (five injections via tail vein, 3 μg/kg, at 20-min intervals), there were no changes in catecholamine levels or tyrosine hydroxylase activity in either the intact or denervated glands.

Long-term effect of actinomycin D on insulin-treated rats. Since the increased activity of tyrosine hydroxylase 24 hr after insulin administration appeared to be related to the recovery of the gland, it was of interest to determine whether actinomycin D would

[2] R. L. Patrick and N. Kirshner, manuscript in preparation.

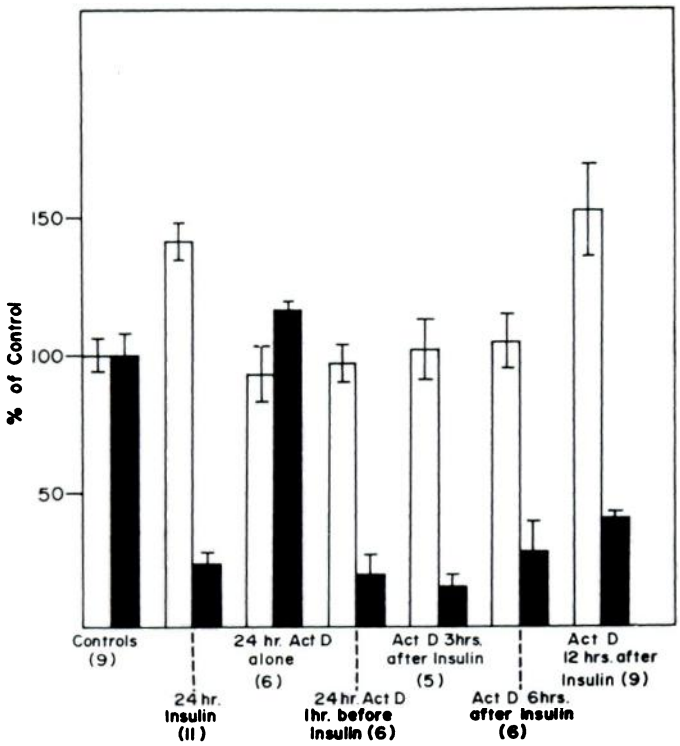

FIG. 2. *Effect of actinomycin D treatment on insulin-induced changes in rat adrenal tyrosine hydroxylase* (□) *and catecholamines* (■)

The number of animals in each group is shown in parentheses. Actinomycin D (Act D) (1.0 mg/kg) was given alone, 1 hr before insulin (5 international units/kg), and 3, 6, and 12 hr after insulin administration. Animals were killed 24 hr after insulin injection. The means ± standard errors per adrenal gland for the controls are: tyrosine hydroxylase, 13.5 ± 1.1 mμmoles of dopa formed per hour; catecholamines, 10.7 ± 1.1 μg. Significant increases in tyrosine hydroxylase were seen after 24 hr in rats treated with insulin alone ($p < 0.005$) and in animals treated with actinomycin D 12 hr after insulin ($p < 0.02$).

inhibit or delay recovery of the catecholamine content. Animals were given actinomycin D (0.5 mg/kg) 1 hr prior to the administration of insulin and examined for tyrosine hydroxylase activity 24, 48, and 72 hr after insulin treatment. It was intended to follow the animals for longer periods of time, but none survived as long as 96 hr. The unexpected results shown in Table 3 were obtained. Although prior treatment with actinomycin D prevented the rise in tyrosine hydroxylase normally observed 24 hr after insulin treatment, the enzyme activity at 48 hr was twice that of the control values. The fact that treatment with actinomycin D alone caused only a small, nonsignificant increase in tyrosine hydroxylase activity suggested two possible explanations: either the signal for induction of this enzyme was long-lived and could persist sufficiently to be expressed once the ability of actinomycin D to inhibit RNA synthesis wore off, or treatment with actinomycin D resulted in additional neural stimulation of the adrenals as a result of generalized stress to the animals. This neural stimulation, following the insulin-induced stimulation, could provide a new signal that provoked an increase in tyrosine hydroxylase activity.

In these experiments, treatment with actinomycin D appeared to have no effect on the recovery of the catecholamine content at 48 hr. However, since the tyrosine hydroxylase activity of those animals treated with actinomycin plus insulin was higher at 48 hr than that of the animals treated with insulin

TABLE 1

Drug-induced changes in adrenal tyrosine hydroxylase, dopamine β-hydroxylase, and catecholamines in rats with intact right and denervated left adrenal glands

The number of animals in each group is shown in parentheses. Rats with the nerve supply to the left adrenal gland cut 1–2 weeks previously were treated with insulin, 5 international units/kg, via the tail vein, or with acetylcholine chloride, 20 mg/kg, plus eserine, 0.1 mg/kg, intravenously, as described in METHODS. Values are means ± standard errors. Significant p values refer to the experimental gland compared to the respective control gland.

Treatment	Tyrosine hydroxylase[a]	Dopamine β-hydroxylase[a]	Catecholamines[b]
Controls (8)			
Left	10.1 ± 1.1	0.58 ± 0.08	7.6 ± 0.4
Right	10.6 ± 1.0	0.78 ± 0.13	10.1 ± 0.7
Insulin, 4 hr (6)			
Left	102% ± 8		112% ± 10
Right	97% ± 8		26% ± 3[c]
Insulin, 24 hr (8)			
Left	111% ± 10		88% ± 9
Right	150% ± 13[d]		45% ± 8[c]
Acetylcholine + eserine, 24 hr (8)			
Left	166% ± 12[e]	105% ± 7	82% ± 7
Right	153% ± 15[f]	67% ± 6	42% ± 6[c]
Choline + eserine, 24 hr (7)			
Left	95% ± 10		85% ± 8
Right	170% ± 8[c]		88% ± 15

[a] Control values are expressed as nanomoles per hour per gland; remaining values are percentages of controls.

[b] Control values are expressed as micrograms per gland; remaining values are percentages of controls.

[c] $P < 0.001$.

[d] $P < 0.05$.

[e] $P < 0.002$.

[f] $P < 0.02$.

alone, the results cannot be easily interpreted. At 72 hr the adrenal catecholamine content of the animals treated with both actinomycin D and insulin was significantly lower than that of the rats treated with insulin alone, but this may have been due to the additional adrenal stimulation resulting from the general physiological deterioration of the animals.

Evidence against a long-lived signal for tyrosine hydroxylase induction. The fact that tyrosine hydroxylase activity could be increased in denervated adrenal glands by repeated injections of acetylcholine (Table 1) suggested an experimental approach to distinguish between the two possibilities suggested above for the increase in tyrosine hydroxylase activity observed 48 hr after treatment with both actinomycin D and insulin. Rats with the left adrenal gland denervated and the right adrenal gland intact were treated with actinomycin D, 0.5 mg/kg, 1 hr before repeated injections of acetylcholine, as described in Table 1. As shown in Table 4, both the intact and denervated glands exhibited only a small, nonsignificant increase in enzyme activity 24 hr after the combined treatment, even though the right gland was markedly stimulated, as indicated by the low levels of epinephrine. At 48 hr the tyrosine hydroxylase activity in the right glands of treated animals was significantly elevated ($p < 0.01$) as compared to the control right glands, but was still not significantly greater in the denervated glands than in the controls. These results are consistent with the hypothesis that the increase in tyrosine hydroxylase activity seen in animals 48 hr after treatment with both actinomycin D

TABLE 2

Effects of acetylcholine, nicotine, and angiotensin II on intact and denervated rat adrenal glands

The number of animals in each group is shown in parentheses. Rats with the nerve supply to the left adrenal gland cut 1–2 weeks previously were treated with acetylcholine chloride, 20 mg/kg, plus eserine, 0.1 mg/kg, intravenously, as described in METHODS, with nicotine sulfate, 2.0 mg/kg intravenously every 20 min (total of five injections), or with angiotensin II, 3 μg/kg intravenously every 20 min (total of five injections). Values are means ± standard errors. Significant p values refer to the experimental gland compared to the respective control gland.

Treatment	Tyrosine hydroxylase[a]	Dopamine β-hydroxylase[a]	Catecholamines[b]
Controls (6)			
Left	13.7 ± 0.6	0.43 ± 0.04	10.7 ± 0.8
Right	11.4 ± 1.7	0.52 ± 0.08	11.4 ± 0.5
Acetylcholine + eserine, 24 hr (4)			
Left	148% ± 6[c]	94% ± 6	94% ± 2.0
Right	154% ± 10[d]	81% ± 9	60% ± 7[c]
Nicotine, 24 hr (4)			
Left	118% ± 8	88% ± 8	112% ± 6
Right	170% ± 16[e]	103% ± 17	96% ± 15
Angiotensin II, 24 hr (4)			
Left	126% ± 12	120% ± 15	106% ± 6
Right	108% ± 16	120% ± 11	114% ± 8

[a] Control values are expressed as nanomoles per hour per gland; remaining values are percentages of controls.

[b] Control values are expressed as micrograms per gland; remaining values are percentages of controls.

[c] $P < 0.001$.

[d] $P < 0.05$.

[e] $P < 0.02$.

TABLE 3

Effect of actinomycin D and insulin on rat adrenal tyrosine hydroxylase and catecholamine levels

The number of animals in each group is shown in parentheses. Normal, unoperated rats were given actinomycin D, 0.5 mg/kg intraperitoneally, 1 hr before insulin, 5 international units/kg intravenously, and were killed 24, 48, and 72 hr after insulin administration. Values for animals treated with insulin alone at the 24-, 48-, and 72-hr intervals are included for reference. Values are means ± standard errors. Significant p values refer to experimental vs. control results.

Treatment	Tyrosine hydroxylase[a]	Catecholamines[b]
Controls (6)	13.0 ± 0.5	20.6 ± 0.9
Insulin, 24 hr (11)	142% ± 8[c]	33% ± 4[d]
Actinomycin D + insulin, 24 hr (4)	97% ± 19	27% ± 9[d]
Actinomycin D, 48 hr (8)	126% ± 11	94% ± 6
Insulin, 48 hr (4)		46% ± 5[d]
Actinomycin D + insulin, 48 hr (10)	190% ± 10[d]	54% ± 8[d]
Insulin, 72 hr (6)	144% ± 6[e]	76% ± 10
Actinomycin D + insulin, 72 hr (4)	204% ± 23[d]	51% ± 11[e]

[a] Control values are expressed as nanomoles per hour per gland; remaining values are percentages of controls.

[b] Control values are expressed as micrograms per gland; remaining values are percentages of controls.

[c] $P < 0.005$.

[d] $P < 0.001$.

[e] $P < 0.002$.

TABLE 4

Effect of acetylcholine treatment on denervated rats previously treated with actinomycin D

The number of animals in each group is shown in parentheses. Rats with the nerve supply to the left adrenal gland cut 1–2 weeks previously were treated with actinomycin D, 0.5 mg/kg intraperitoneally, 1 hr before the administration of acetylcholine chloride, 20 mg/kg, plus eserine, 0.1 mg/kg, as described in METHODS. Rats were killed 24 and 48 hr after acetylcholine treatment. Values are means ± standard errors. Significant p values refer to the experimental gland compared to the respective control gland.

Treatment	Tyrosine hydroxylase	Dopamine β-hydroxylase	Catecholamines
	nmoles/hr/gland		*μg/gland*
Controls (6)			
Left	13.7 ± 0.6	0.43 ± 0.04	10.7 ± 0.8
Right	11.4 ± 1.7	0.52 ± 0.08	11.4 ± 0.5
Actinomycin D, acetylcholine, eserine, 24 hr (3)			
Left	17.4 ± 2.4[a]	0.38 ± 0.02	9.6 ± 0.6
Right	16.2 ± 3.2[a]	0.44 ± 0.04	2.3 ± 0.9[b]
Actinomycin D, acetylcholine, eserine, 48 hr (5)			
Left	17.0 ± 2.6[a]	0.49 ± 0.07	10.4 ± 1.3
Right	19.4 ± 1.2[c]	0.57 ± 0.06	3.6 ± 1.4[b]

[a] Not significantly different from controls.
[b] $P < 0.001$.
[c] $P < 0.01$.

and insulin was due to neural stimulation resulting from secondary effects of actinomycin D.

DISCUSSION

Work from several laboratories has established that secretion from the adrenal medulla occurs by exocytosis—a process in which the entire soluble contents of the catecholamine storage vesicles, including catecholamines, adenine nucleotides, chromogranins, and soluble dopamine β-hydroxylase, are expelled directly from the cell, leaving the vesicle membranes remaining within the cell (11–17). The ultimate fate of the vesicle membrane is not known, nor have the steps leading to the replenishment of the catecholamine stores been defined. Presumably this would require repair of the old membranes or synthesis of new membranes, but, in any event, it would require at least the synthesis of protein to replace that released during secretion. Reports from several laboratories have now established that neurogenic stimulation of the adrenal gland also results in increased tyrosine hydroxylase activity, and that this increased activity is most likely due to the synthesis of new enzyme (1–7). These phenomena—the synthesis of proteins associated with the storage of catecholamines, the synthesis of tyrosine hydroxylase, one of the key enzymes involved in the synthesis of norepinephrine, and the recovery of the catecholamine content—would appear to be genetically controlled.

The data of Fig. 1 indicate that maximal tyrosine hydroxylase activities are reached within 24 hr after a single injection of insulin in the rat, and that these levels are maintained for at least 4 days. Additional experiments in our laboratory indicate that the tyrosine hydroxylase levels return to normal limits within 2 weeks after insulin administration. Figure 2 shows that 6–12 hr are necessary to complete the synthesis of RNA required for the subsequent formation of tyrosine hydroxylase. The findings reported in Table 4 indicate that this messenger is not long-lived. The resynthesis of dopamine β-hydroxylase and the recovery of the catecholamine content parallel each other and lag behind the synthesis of tyrosine hydroxylase, possibly because the recovery of dopamine β-hydroxylase and catecholamines is dependent upon the synthesis of new storage vesicles.

Previous reports had shown that an in-

tact nerve supply was necessary for the induction of adrenal tyrosine hydroxylase following reserpine treatment, even though the catecholamine content of the denervated gland was severely depleted (6). These observations suggested that lowering the catecholamine content itself was not sufficient to induce the formation of tyrosine hydroxylase, and raised the possibility that a factor or factors released by the nerve, other than the neural transmitter itself, may be required to initiate protein synthesis. Our experiments (Tables 1 and 2) indicate that the normal transmitter, acetylcholine, is itself sufficient to bring about induction of protein synthesis in the rat adrenal gland. The results also show that it is acetylcholine and not one of its breakdown products, choline, that gives rise to increased enzyme activity. The increase in tyrosine hydroxylase activity in the intact right gland following treatment with choline, atropine, and eserine is most likely due to increased neural stimulation resulting from the treatment.

The relationship between the degree of stimulation and the increase in tyrosine hydroxylase activity has not been established. It would appear that a series of moderate stimuli can bring about a greater rise in tyrosine hydroxylase than can prolonged, massive stimulation, and that there is no correlation between the increase in tyrosine hydroxylase activity and the depletion of catecholamine content. For example, insulin causes an 80% depletion of the adrenal catecholamine content in 4 hr and gives rise to a 40% increase in tyrosine hydroxylase activity at 24 hr. Treatment with acetylcholine and eserine causes only a 20% decrease in the catecholamine content of the denervated left gland at 4 hr but causes a 50–60% increase in tyrosine hydroxylase activity at 24 hr. Thoenen, Mueller, and Axelrod (3) found that treatment with 6-hydroxydopamine caused no decrease in catecholamines but led to a 94% increase in tyrosine hydroxylase. The results of Table 1 also show that after treatment with choline and eserine there was no significant decrease in the catecholamine content but a marked increase in tyrosine hydroxylase in the intact right gland. The correlation found by Weiner and Mosimann (18) between loss of catecholamines and increase in tyrosine hydroxylase in cat adrenals following insulin treatment may well be the case for that particular situation, but does not appear to suggest a general rule.

While the sequence of events leading to the induction of tyrosine hydroxylase and the recovery of the dopamine β-hydroxylase and catecholamine contents of the adrenal gland still remain to be elucidated at the molecular level, the results suggest that one step in the chain of events is exposure to acetylcholine, and perhaps to other secretory agents as well, but that a significant depletion in catecholamine content is not necessary; furthermore, catecholamine depletion is not a sufficient condition to cause tyrosine hydroxylase induction. Exposure to acetylcholine does not necessarily result in a secretory response by the gland (19). Whether acetylcholine itself is directly involved in the additional steps leading to the induction of tyrosine hydroxylase, or whether the secretory response of the gland is a necessary link, has yet to be determined.

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