MOLECULAR PHARMACOLOGY, 7, 81-86

Enhanced Synthesis of Adrenal Dopamine β-Hydroxylase Induced by Repeated Immobilization in Rats

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(Received July 24, 1970)

SUMMARY

Repeated immobilization of rats results in a striking increase in adrenal medullary dopamine β-hydroxylase activity. After six periods of immobilization the levels are highest preceding the next immobilization, decrease during the immobilization, and increase upon termination of the immobilization. Six hours after the immobilization the activities return nearly to the preimmobilization levels. Denervation of the adrenal markedly diminishes the increase in dopamine β-hydroxylase activity that occurs with repeated immobilization. Prior treatment with hexamethonium prevents the decrease in activity during the immobilization interval, whereas the protein synthesis inhibitors actinomycin D and cycloheximide prevent the increase in enzyme activity seen after termination of immobilization. These observations support the view that dopamine β-hydroxylase is released as a result of neural stimulation during immobilization and suggest that its increase following cessation of immobilization is a consequence of accelerated synthesis of the enzyme.

INTRODUCTION

In rats, immobilization stress results in a decrease in adrenal epinephrine and an increase in urinary excretion of this catecholamine (1). Adrenal epinephrine levels remain depressed for over 24 hr after the first interval of immobilization. Repeated daily immobilization results in less epinephrine depletion and an accelerated replenishment of adrenal catecholamines. This is not a consequence of diminished release since urinary excretion of the catecholamines remains elevated (1). Since immobilization induces a marked elevation in adrenal tyrosine hydroxylase activity (2, 3), an increased rate of adrenal catecholamine biosynthesis is probably the mechanism of diminution of the changes in adrenal epinephrine after repeated immobilization. Dopamine β-hydroxylase (EC 1.14.2.1) present in the adrenal medullary storage vesicles is released with secretion of epinephrine (4). Four days after the insulin-induced release of adrenal catecholamines and dopamine β-hydroxylase, levels of the enzyme are increased (5). We have therefore examined the effects of immobilization of rats on levels of dopamine β-hydroxylase in the adrenal glands.

These studies were supported in part by the Scottish Rite Committee on Research in Schizophrenia, The National Association for Mental Health, Inc.

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METHODS

Male Sprague-Dawley rats weighing 180–250 g were immobilized daily for periods up to 2.5 hr as described previously (1, 3). The

animals were killed by decapitation immediately after the interval of immobilization or 6 or 24 hr after their release. In one experiment rats were killed either 24 hr after the end of the sixth immobilization period or 0.5, 1.0, or 2.5 hr after the start of the seventh immobilization; one group was killed 6 hr after the end of the seventh 2.5-hr period of immobilization.

The adrenal glands were removed rapidly, weighed, and homogenized in ice-cold isotonic sucrose. An aliquot (100 μl) of the homogenate was assayed for catecholamines (6), and the remaining homogenate was centrifuged at 26,000 $\times$ g for 20 min. The supernatant fraction 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 each) of the supernatant and the sediment suspended in 500 μl of cold water were also assayed for dopamine β-hydroxylase.

Dopamine β-hydroxylase was assayed in the same three fractions by the technique of Friedman and Kaufman (7), as modified by Viveros *et al.* (5). Instead of Cu^{2+} or *p*-hydroxymercuribenzoate to inactivate the inhibitors of dopamine β-hydroxylase (8), *p*-chlormercuriphenylsulfonic acid was used in the present study. Optimal enzyme activity was attained when a sufficient amount of the latter reagent was added to inactivate the inhibitors without inhibiting dopamine β-hydroxylase. Total adrenal dopamine β-hydroxylase has been calculated as the sum of the activities in the two supernatant and the sediment fractions described above, and is the quantity reported in this paper. About 30% of the enzyme activity was present in the first 26,000 $\times$ g supernatant, 10–15% in the second, and the remainder in the 26,000 $\times$ g sediment.

In some experiments, hexamethonium (20 mg/kg) was administered intraperitoneally 30 min before immobilization to prevent catecholamine release from the adrenal medulla (9). In other experiments, actinomycin D (1 mg/kg) or cycloheximide (1 mg/kg) was given subcutaneously 15 min before and 2 hr after immobilization to inhibit adrenal protein synthesis (10). The effect of adrenal denervation was examined in animals in which the left splanchnic nerve had been severed 4 days before immobilization intervals were begun (3). In one experiment, the adrenal medullas were largely separated from the cortices. The adrenal was split, and the medulla was scooped out of each half. The medullary and cortical portions were each homogenized in 1.0 ml of ice-cold sucrose and assayed for dopamine β-hydroxylase as above.

RESULTS

Effect of immobilization on rat adrenal dopamine β-hydroxylase. A single immobilization for 2.5 hr did not alter significantly levels of rat adrenal dopamine β-hydroxylase, but 6 hr after the immobilization interval levels of dopamine β-hydroxylase were significantly elevated (Fig. 1). Immediately after the seventh or 42nd interval of immobilization, levels of dopamine β-hydroxylase were significantly higher than those found in the adrenals of the control animals. Six hours after the seventh or 42nd immobilization the elevation of the enzyme was more marked. The increment in enzyme levels during the 6 hr after immobilization was greater after the 42nd immobilization than after the seventh, and greater after the seventh than after the first.

Dopamine β-hydroxylase levels in rat adrenal before, during, and after seventh immobilization. Twenty-four hours after six daily 2.5-hr intervals of immobilization, adrenal dopamine β-hydroxylase was 3 times as great as in unstressed control rats (Fig. 2). During the seventh interval of immobilization a progressive decrease in adrenal dopamine β-hydroxylase was apparent (Fig. 2). Six hours after a 2.5-hr interval of immobilization, however, the enzyme was nearly at the level found before the seventh immobilization. Prior treatment with hexamethonium prevented the decrease in both epinephrine (Table 1) and dopamine β-hydroxylase (Fig. 2) found immediately after the seventh immobilization.

Effect of inhibitors of protein synthesis on dopamine β-hydroxylase activity in rat adrenal. Neither actinomycin D nor cycloheximide, in the doses used, altered the

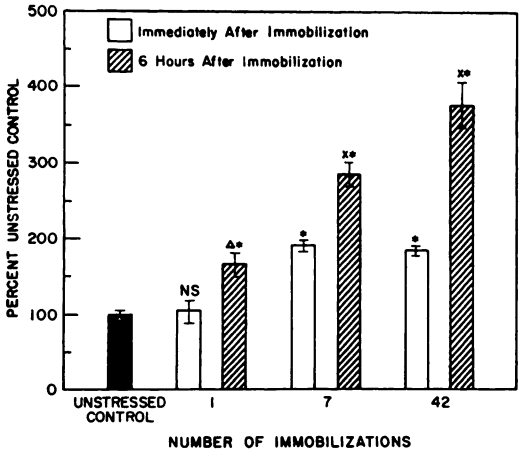

FIG. 1. *Effect of repeated immobilization on rat adrenal dopamine β-hydroxylase activity*

Rats were immobilized for 2.5 hr daily for 1, 7, or 42 days and were killed either immediately after or 6 hr after the last immobilization. Results are the mean levels ± standard errors for groups of six rats and are expressed as percentages of the dopamine β-hydroxylase activity found in the unstressed control group. The level of dopamine β-hydroxylase in the unstressed control group was 2.54 nmoles of octopamine-^{3}H formed per hour per pair of adrenals.

* $p < 0.001$ compared to unstressed controls.

△* $p < 0.01$ compared to the group killed immediately after immobilization.

×* $p < 0.001$ compared to the group killed immediately after immobilization.

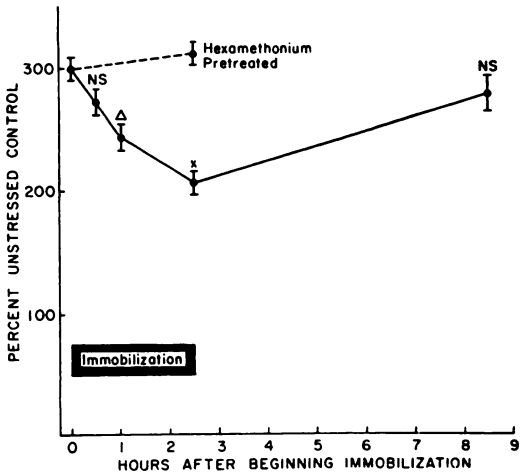

FIG. 2. *Dopamine β-hydroxylase activity before, during, and after seventh immobilization*

Rats were immobilized daily for 2.5 hr and were killed either 24 hr after the end of the sixth immobilization (zero time) or 0.5, 1.0, 2.5, or 8.5 hr after the start of the seventh immobilization. Results are the mean levels ± standard errors for groups of six rats and are expressed as percentages of the dopamine β-hydroxylase activity found in the unstressed control group. The level of dopamine β-hydroxylase in the unstressed control group was 1.32 nmoles of octopamine-^{3}H formed per hour per pair of adrenals. One group was given hexamethonium (20 mg/kg, intraperitoneally) 30 min before the start of the seventh immobilization and killed 2.5 hr after the start of that immobilization. All values are significantly increased ($p < 0.001$) when compared to the unstressed controls.

△ $p < 0.05$ compared to the group killed 24 hr after the sixth immobilization.

× $p < 0.01$ compared to the group killed 24 hr after the sixth immobilization. NS = not significantly different from the group killed 24 hr after the sixth immobilization.

levels of dopamine β-hydroxylase in the adrenal glands of unstressed animals (Fig. 3). In untreated, stressed animals dopamine β-hydroxylase was markedly increased (compared with unstressed animals) immediately prior to the seventh immobilization, decreased immediately following the seventh immobilization, and then increased to nearly prestress levels 6 hr later. Treatment of the stressed animals with actinomycin D prevented the post-stress increase in dopamine β-hydroxylase, whereas prior treatment with cycloheximide lowered the dopamine β-hydroxylase activity to levels even lower than those found immediately after immobilization.

Effect of denervation on adrenal dopamine β-hydroxylase activity. In unstressed animals the adrenal gland which had been denervated by section of the splanchnic nerve had less dopamine β-hydroxylase activity than the contralateral intact adrenal (Table 2). Denervation markedly diminished the elevation of this enzyme found in intact adrenals 6 hr after a seventh immobilization. The procedure of denervation, as described in METHODS, did not diminish the weight of the adrenals and did not result in any obvious change in circulation. The latter would not be expected, since the nerves were cut about 1 cm above the adrenal.

TABLE 1

Effect of hexamethonium on immobilization-induced alterations in adrenal epinephrine

Rats were immobilized for 2.5 hr daily and killed either 24 hr after the sixth immobilization or immediately after the seventh immobilization. One group received hexamethonium (20 mg/kg, intraperitoneally). Results are the mean levels ± standard errors for groups of six rats.

No. of immobilizations	Epinephrine
	μg/adrenal pair
0	14.8 ± 0.9
6	15.8 ± 1.5
7	9.9 ± 1.2[a]
7 plus hexamethonium	13.9 ± 1.5[b]

[a] $p < 0.02$ compared to the group killed 24 hr after the sixth immobilization.

[b] Not significantly different from the group killed 24 hr after the sixth immobilization.

Dopamine β-hydroxylase content of separated adrenal medulla and adrenal cortex. Over 95% of the total dopamine β-hydroxylase activity in the adrenal glands of both control animals and those subjected to repeated immobilization was found in the adrenal medulla (Table 3).

DISCUSSION

Immobilization of rats appears to be a stressful procedure which elicits a marked adrenal medullary response, as indicated by catecholamine excretion (1) and subsequent elevation of enzymes concerned with epinephrine synthesis (2, 3). Repeated immobilization causes marked increases in tyrosine hydroxylase (3) and dopamine β-hydroxylase (Fig. 1). As indicated above, Viveros *et al.* (4) showed that dopamine β-hydroxylase is released from the perfused adrenal medulla

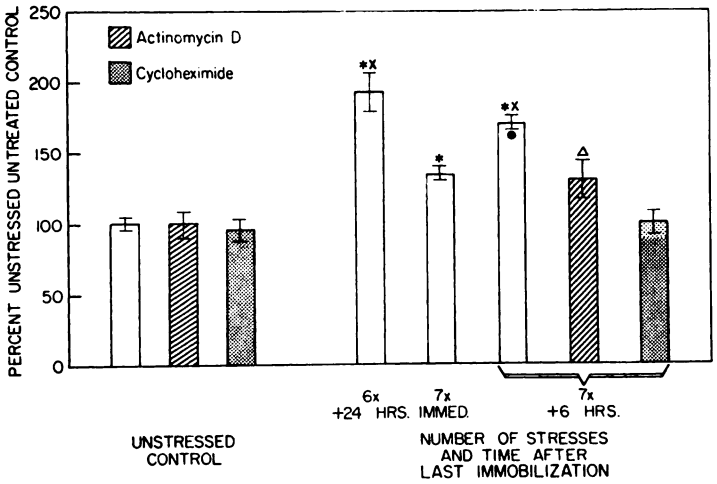

FIG. 3. *Effect of protein synthesis inhibitors on rat adrenal dopamine β-hydroxylase activity*

Rats were immobilized for 2.5 hr daily and were killed either 24 hr after the sixth immobilization, immediately after the seventh immobilization, or 6 hr after the seventh immobilization. Actinomycin D (1 mg/kg) or cycloheximide (1 mg/kg) was given subcutaneously 15 min prior to the start of the last immobilization and again 2 hr after its termination. Results are the mean levels ± standard errors for groups of 6–12 rats and are expressed as percentages of the dopamine β-hydroxylase activity found in the unstressed control group. The level of dopamine β-hydroxylase in the unstressed control group was 1.69 nmoles of octopamine-^{3}H formed per hour per pair of adrenals.

Δ $p < 0.05$ compared to the unstressed, untreated control group.

* $p < 0.001$ compared to the unstressed, untreated control group.

*× $p < 0.001$ compared to the group killed immediately after the seventh immobilization.

● $p < 0.02$ compared to the group killed 6 hr after the seventh immobilization and treated with actinomycin D.

along with catecholamines. Levels of dopamine β-hydroxylase found 6 hr after the cessation of repeated immobilization were higher than immediately after such immobilization, suggesting that the enzyme had been released *in vivo* during immobilization and replenished during the 6-hr interval after immobilization was terminated. This view is supported by the finding of a progressively decreasing enzyme level during the seventh immobilization (Fig. 2). The decrease in enzyme activity was blocked by hexamethonium (Fig. 2), which also blocked the decrease in epinephrine (Table 1). The observation that hexamethonium blocks release of catecholamines from the adrenal medulla (8) further supports the view that the decrease in dopamine β-hydroxylase is a consequence of release of this enzyme from the adrenals.

The increase in adrenal dopamine β-hydroxylase during the 6 hr after immobilization was prevented by inhibitors of protein or RNA synthesis (Fig. 3). Cycloheximide caused a decrease in activity of this enzyme to levels below those found in the adrenal immediately after repeated immobilization. It did not have this effect in unstressed animals, and did not alter catecholamine levels in either stressed or unstressed animals. These results suggest that the turnover rate of dopamine β-hydroxylase had increased during repeated immobilization. This conclusion is also supported by the observation that the increment in activity of this enzyme during the 6 hr after the first, seventh, and 42nd intervals of immobilization progressively increased (Fig. 1). Actinomycin D prevented the increase in dopamine β-hydroxylase activity to prestress levels after immobilization, but did not decrease the activity of this enzyme in unstressed animals. Although this might be a reflection of differences in relative dose, it seems more likely that after administration of actinomycin D the synthesis of dopamine β-hydroxylase continued to keep pace with its removal rate before the stress. Presumably actinomy-

TABLE 2

Effect of adrenal denervation on rat adrenal dopamine β-hydroxylase activity

The left adrenal gland was denervated by splanchnic nerve section 4 days prior to the onset of the first of seven daily 2.5-hr periods of immobilization. Results are expressed as mean values ± standard errors for groups of six rats.

Treatment	Dopamine β-hydroxylase	
	Denervated adrenal	Innervated adrenal
	nmoles octopamine-^{3}H formed/hr/gland	
Control	0.85 ± 0.09[a]	1.39 ± 0.07
Immobilized	1.20 ± 0.11[a,b]	3.75 ± 0.40[c]

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

[b] $p < 0.05$ compared to the nonimmobilized, denervated group.

[c] $p < 0.01$ compared to the nonimmobilized, innervated group.

TABLE 3

Dopamine β-hydroxylase activity in adrenal medulla and cortex of nonimmobilized and repeatedly immobilized rats

Rats were immobilized for 2.5 hr daily for 40 days and killed 6 hr after the last immobilization. Results are expressed as mean values ± standard errors for groups of six to eight rats.

Treatment	Dopamine β-hydroxylase		Activity in cortex
	Medulla	Cortex	
	nmoles octopamine-^{3}H formed/hr/pair		%
Nonimmobilized	4.09 ± 0.14	0.14 ± 0.06	3.3
Immobilized	7.94 ± 0.45[a]	0.27 ± 0.06[b]	3.3

[a] $p < 0.01$ compared to the medullas of nonimmobilized animals.

[b] $p < 0.01$ compared to the cortices of nonimmobilized animals.

cin D prevented an acceleration of synthesis of this enzyme (by blocking RNA but not enzyme protein formation) required to elevate the enzyme to its level prior to stress when release was accelerated.

The stimulus to accelerate dopamine β-hydroxylase synthesis is apparently partially mediated by nerve impulses, since denervation results in a decrease in enzyme levels and markedly diminishes the response of the adrenal which leads to elevation of this enzyme induced by immobilization (Table 2). Denervation does not greatly alter catecholamine levels or diminish adrenal weight, so that it is unlikely that this is the result of circulatory changes. The slight increase in enzyme levels observed in the denervated adrenal might be due to incomplete denervation, since the adrenal may receive a small number of nerve fibers from the first two lumbar ganglia (11). The increase in adrenal dopamine β-hydroxylase levels after repeated immobilization is a consequence of an increase of the enzyme activity in the adrenal medulla, since less than 5% of the enzyme activity was found in the cortex (Table 3). The epinephrine content of the cortical portion, which represents about 80% of the weight of the adrenal, was about 2.5 μg/pair of adrenals and about 10% of the total adrenal epinephrine (3). Thus the enzyme activity which was found in the cortex is probably a result of incomplete separation of the two parts of the adrenal.

ACKNOWLEDGMENT

We would like to thank Mrs. Darina Kvetňanská for her excellent technical assistance.

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