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Induction and Superinduction of Serotonin *N*-Acetyltransferase by Adrenergic Drugs and Denervation in Rat Pineal Organ

(neuronal regulation/supersensitivity)

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ABSTRACT **Activity of serotonin *N*-acetyltransferase (EC 2.3.1.5) in rat pineal organ is rapidly and markedly elevated *in vivo* after administration of β-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA), norepinephrine, epinephrine, isoproterenol, monoamine oxidase inhibitors, or theophylline. Serotonin or 5-hydroxytryptophan has no effect on the increase in activity of this enzyme. Inhibitors of protein synthesis or propranolol, a β-adrenergic blocking agent completely inhibit(s) the increase in activity of serotonin *N*-acetyltransferase induced by drugs, indicating that new enzyme molecules are formed via stimulation of β-receptors of pineal cells and adenosine 3′:5′-cyclic monophosphate. When rat pineal organ is denervated by ganglionectomy, β-(3,4-dihydroxyphenyl)-L-alanine induces much more serotonin *N*-acetyltransferase than in the innervated gland. This superinduction by denervation appears to be due to changes of the postsynaptic site, probably the β-adrenergic receptor on the pineal cell.**

Serotonin *N*-acetyltransferase (EC 2.3.1.5.) catalyzes the conversion of serotonin to *N*-acetylserotonin, the precursor of melatonin, a pineal hormone (1). *N*-Acetyltransferase activity in rat pineal organ has been shown to exhibit a circadian change with a 15- to 40-fold increase in the dark (2). The nocturnal rise was completely blocked by ganglionectomy or by decentralization of the superior cervical ganglion (3). Electrical stimulation of the sympathetic nerve that innervates the pineal organ resulted in a more than 3-fold elevation of *N*-acetyltransferase activity (4). *N*-Acetyltransferase activity increased in response to norepinephrine or dibutyryl adenosine 3′:5′-cyclic monophosphate in cultured rat pineal organ (5). It has also been demonstrated that formation of melatonin from tryptophan or serotonin was stimulated by norepinephrine (6) and blocked by propranolol (7) in a culture of rat pineal organ. These observations suggested that the concentration of *N*-acetyltransferase in rat pineal organ is regulated by norepinephrine released from adrenergic nerve endings by way of a β-receptor and adenosine 3′:5′-cyclic monophosphate (cyclic AMP). There is, however, no conclusive evidence as to whether or not this mechanism is operating in the pineal organ *in vivo*.

In this study, the effect of drugs on *N*-acetyltransferase activity was examined in rat pineal organs *in vivo*. We demonstrated that catecholamines, their precursor β-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA), inhibitors of monoamine oxidase (EC 1.4.3.4.), or theophylline cause a marked induction of pineal *N*-acetyltransferase that is blocked by propranolol or cycloheximide. In addition, a superinduction of *N*-acetyltransferase activity (100-fold increase) is observed with L-DOPA or inhibitors of monoamine oxidase in denervated rat pineal organ.

Abbreviation: L-DOPA, β-(3,4-dihydroxyphenyl)-L-alanine.

MATERIALS AND METHODS

Chemicals. [^{14}C]Acetyl coenzyme A (59.2 Ci/mol) was purchased from New England Nuclear Corp. (Boston, Mass.). For generous supplies of drugs, the authors are indebted to the following companies: *l*-propranolol, Ayerst Laboratories (New York, N.Y.), β-isopropylphenylhydrazine (Catron), Lakeside Laboratories (Milwaukee, Wisc.); pargyline, Abbott Laboratories (Chicago, Ill.); phenoxybenzamine, Smith Kline and French Laboratories (Philadelphia, Pa.); MK-486 (L-α-hydrazinomethyldihydroxyphenylalanine), Merck, Sharp and Dohme Research Laboratories (West Point, Pa.). Other chemicals were obtained from commercial sources.

Animals. Sprague–Dawley male rats weighing 160–180 g were supplied from Hormone Assay Laboratories, Chicago,

TABLE 1. *Effect of various drugs on N-acetyltransferase activity*

Drug	Dose, mg/kg of body weight	*N*-Acetyltransferase pmol per pineal per 10 min
Saline		5 ± 1
L-DOPA	150	202 ± 33*
MK-486 plus L-DOPA	150	40 ± 14†
L-Epinephrine	1.5	46 ± 5*
L-Norepinephrine	1.5	19 ± 3*
Dopamine	5	6 ± 1
L-Isoproterenol	10	333 ± 72*
L-5-Hydroxytryptophan	100	4 ± 1
Serotonin creatinine sulfate	25	11 ± 3
Catron	10	211 ± 32*
Pargyline	75	105 ± 8*
Dibutyryl cyclic AMP	60	6 ± 1
Theophylline	75	42 ± 7*

Drugs were injected subcutaneously in rats 2 and 4 hr before they were killed at 2:00 p.m. MK-486 (150 mg/kg) was injected in rats 30 min before injection of L-DOPA. The results are expressed as mean ± standard error of the mean.

* $P < 0.01$ compared to rats injected with saline.

† Differs from rats injected with saline at $P < 0.05$.

Ill. The rats were kept under diurnal lighting conditions with light on from 6:00 a.m. to 6:00 p.m. for at least 3 days. Bilateral ganglionectomy or decentralization of superior cervical ganglion was performed under ether anesthesia. Ptosis was used to monitor the success of the operation. Rats were used 5 or 6 days after surgery.

All drugs were dissolved in 0.9% NaCl and injected subcutaneously unless indicated. L-DOPA was injected as suspension (30 mg/ml) in 0.9% NaCl. The rats were killed by decapitation between 2:00 and 3:00 p.m.

Assay of N-Acetyltransferase Activity. A pineal organ was quickly removed, chilled, and homogenized in 70 μl of a reaction mixture containing 2.5 μmol of potassium phosphate (pH 6.5), 0.1 μmol of tryptamine, and 3.4 nmol of [^{14}C]acetyl coenzyme A in a 1-ml glass homogenizer. The reaction (37° for 10 min) was stopped by the addition of 0.5 ml of 0.5 M borate buffer (pH 10.0). The reaction mixture was transferred by a Pasteur pipette into a glass-stoppered test tube containing 6 ml of toluene–isoamyl alcohol (97:3) and stirred for 30 sec in a Vortex mixer. After centrifugation at 3500 rpm (750 × *g*) for 10 min, 2 ml of the organic phase was transferred into a scintillation vial containing 10 ml of Bray's solution (8), and radioactivity was measured. The details of the assay will be published (9). Each group consisted of 5 or 6 rats, and the experiments were repeated at least twice.

RESULTS

Effect of drugs on pineal *N*-acetyltransferase activity

N-Acetyltransferase activity in rat pineal organ is lowest during daytime (2). Various drugs affecting biogenic amines or cyclic AMP were tested for their capabilities of increasing *N*-acetyltransferase activity during daytime (Table 1). L-DOPA was most effective; it caused a 40-fold increase in enzyme activity. Prior treatment with MK-486, an inhibitor of aromatic L-amino-acid decarboxylase (EC 4.1.1.26), prevented induction of the enzyme by L-DOPA indicating that L-DOPA is converted to a catecholamine to induce *N*-acetyltransferase. Epinephrine or norepinephrine increased *N*-acetyltransferase activity 10- or 4-fold, respectively. A synthetic catecholamine, L-isoproterenol, caused a 60-fold increase in the enzyme activity. The small amount of increase by catecholamines is presumably due to the rapid inactivation of these compounds. Inhibitors of monoamine oxidase, pargyline or Catron, increased *N*-acetyltransferase activity 20- or 40-fold, respectively. Theophylline also caused elevation of enzyme activity considerably, whereas dibutyryl cyclic AMP had no effect at the dose used. Serotonin or its precursor, 5-hydroxytryptophan, failed to change *N*-acetyltransferase activity.

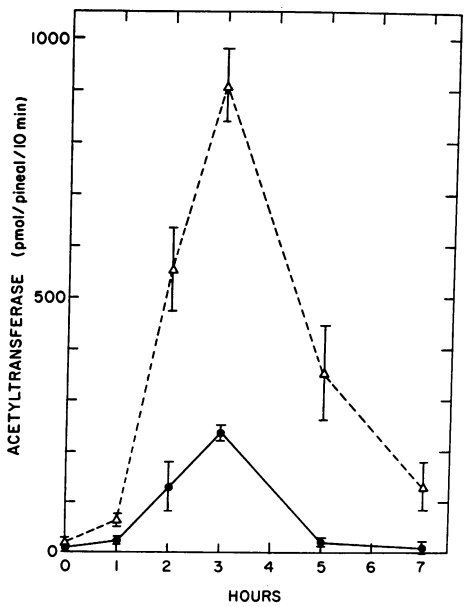

FIG. 1. Rate of increase of *N*-acetyltransferase activity after injection of L-DOPA. L-DOPA (300 mg/kg of body weight) was injected into rats between 8:00 a.m. and 1:00 p.m., and rats were killed between 2:00 and 3:00 p.m. at various time intervals after injection of L-DOPA. *Vertical bars* indicate standard error of the mean. *Solid line* represents innervated pineal organ, and *dotted line* represents denervated pineal.

TABLE 2. *Effect of adrenergic blockers on pineal N-acetyltransferase*

Prior treatment	Saline	Catron	L-DOPA
None	5 ± 1	193 ± 36	170 ± 27
l-Propranolol	8 ± 1	12 ± 2	15 ± 2
Phenoxybenzamine	57 ± 13	318 ± 72	168 ± 27

l-Propranolol (20 mg/kg of body weight) or phenoxybenzamine (20 mg/kg) was injected 30 min before injection of either saline, Catron, or L-DOPA. 3 hr after injection of either saline, Catron (20 mg/kg), or L-DOPA (300 mg/kg), *N*-acetyltransferase activity was measured. The values are expressed as pmol per pineal per 10 min.

Rate of increase in *N*-acetyltransferase activity by L-DOPA is shown in Fig. 1. In the first hour, there was a negligible change in enzyme activity. After 1 hr, there was a rapid increase in enzyme activity, reaching maximum concentrations 3 hr after injection of L-DOPA, and returning to the initial concentration after 5–7 hr.

Effect of adrenergic blocking agents

l-Propranolol, a β-adrenergic blocking agent, completely prevented the increase in *N*-acetyltransferase activity by Catron or L-DOPA (Table 2). On the other hand, phenoxybenzamine, an α-adrenergic blocker, did not block the increase in *N*-acetyltransferase activity by Catron or L-DOPA. Only phenoxybenzamine increased *N*-acetyltransferase activity.

Superinduction after ganglionectomy

The question arose as to whether intact innervation was necessary for increase of *N*-acetyltransferase activity by Catron or L-DOPA. When the pineal organ was denervated by ganglionectomy, the administration of Catron or L-DOPA increased *N*-acetyltransferase activity 100-fold as compared to a 20- to 30-fold increase in the innervated pineal organ (Fig. 2). Decentralized pineal organs showed a similar increase in enzyme activity as intact pineal organs. There was no superinduction up to 12 days after decentralization of the organ.

The effect of destruction of sympathetic nerve terminals by 6-hydroxydopamine (10) was examined. Chemical sympathectomy by 6-hydroxydopamine also caused a superinduction by L-DOPA (Fig. 2).

Rate of superinduction in the ganglionectomized pineal is shown in Fig. 1. Maximal elevation was attained 3 hr

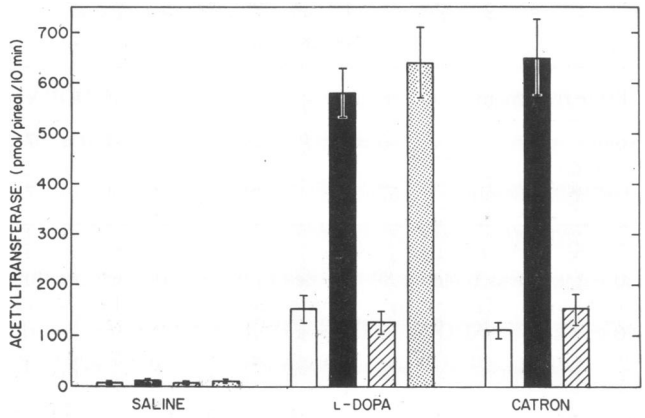

FIG. 2. Superinduction of *N*-acetyltransferase after denervation. Rats were ganglionectomized (■) or decentralized (▨) 5 days before the experiment. 6-Hydroxydopamine (▨; 150 mg/kg of body weight, dissolved in 0.1% ascorbic acid) was injected into the tail vein 6 and 1 day before the experiment. L-DOPA (300 mg/kg) or Catron (20 mg/kg) was injected subcutaneously in rats 3 hr before they were killed. *Vertical bars* indicate standard error of the mean. □, Intact pineal organ.

after injection of L-DOPA, returning to the initial level after 7 hr, a similar pattern to that detected in the innervated pineal organ. The superinduction was apparent 24 hr, but not 12 hr, after ganglionectomy (Table 3).

l-Propranolol again blocked superinduction of *N*-acetyltransferase by L-DOPA, indicating that this increase in enzyme activity was also mediated by β-adrenergic receptor (Table 4). Phenoxybenzamine significantly enhanced the increase of *N*-acetyltransferase activity by L-DOPA, resulting in 125-fold rise in enzyme activity in 3 hr.

Effect of cycloheximide or actinomycin D

Cycloheximide, an inhibitor of protein synthesis, completely prevented the increase of *N*-acetyltransferase activity in both intact and ganglionectomized pineal organs (Table 5), indicating the synthesis of new enzyme protein. Actinomycin D, a compound that inhibits RNA synthesis, did not block the increase of *N*-acetyltransferase activity.

TABLE 3. *Superinduction of pineal N-acetyltransferase after ganglionectomy*

Time after ganglionectomy (hr)	*N*-Acetyltransferase pmol per pineal per 10 min
0	227 ± 35
4	200 ± 26
12	176 ± 33
24	831 ± 67*
48	575 ± 32*
120	651 ± 61*
216	654 ± 48*

N-Acetyltransferase activity was assayed 3 hr after injection of L-DOPA (300 mg/kg of body weight).

* $P < 0.01$ compared to intact pineal organ.

Effect of drugs that inhibit uptake and storage of catecholamines

The superinduction of *N*-acetyltransferase after denervation could be due to the inability of the pineal organ to inactivate the catecholamines by reuptake into the sympathetic nerve endings (11), thus increasing the available norepinephrine at the receptor site. For examination of this possibility, rats were pretreated with drugs that block uptake of catecholamine into nerve endings (12) before administration of L-DOPA. Neither cocaine nor desmethylimipramine caused a superinduction of *N*-acetyltransferase by L-DOPA (Table 6). Reserpine, a compound that prevents the storage of catecholamines, did not affect the induction of *N*-acetyltransferase by L-DOPA.

It was also possible that superinduction is due to increased delivery of the catecholamines to the ganglionectomized pineal organ. [^{3}H]Norepinephrine, therefore, was injected into intact and ganglionectomized rats, and the concentration of [^{3}H]norepinephrine in the pineal organ was measured. The concentration of [^{3}H]norepinephrine in ganglionectomized pineal organs was less than 10% that in intact organs.

DISCUSSION

Previous work has demonstrated that the sympathetic nerves innervating the rat pineal are necessary for *in vivo* changes in concentration of serotonin in light and darkness (13), hydroxyindole-*O*-methyltransferase (14, 15), 5-hydroxytryptophan decarboxylase (16), and serotonin *N*-acetyltransferase (3). It has also been shown that catecholamines (6) or dibutyryl cyclic AMP (7) stimulate the formation of [^{14}C]-melatonin from [^{14}C]tryptophan and increase serotonin *N*-acetyltransferase activity (5) in pineal-organ culture. The results described here demonstrate that L-DOPA or catecholamines, but not serotonin, cause a marked induction of serotonin *N*-acetyltransferase in rat pineal organ *in vivo*. Inhibitors of monoamine oxidase, compounds that cause elevation of the concentration of tissue catecholamines, also induce a marked increase in the concentration of *N*-acetyltransferase. Theophylline, a compound that inhibits the degradation of cyclic AMP, induces an increase in *N*-acetyltransferase activity, suggesting that the elevation of the enzyme is probably mediated by cyclic AMP. Inhibition of protein synthesis or prior treatment with *l*-propranolol blocks induction of the enzyme *in vivo*. These observations indicate that the concentration of *N*-acetyltransferase is regulated by catecholamines *in vivo* via a β-receptor on the pineal cell that in turn activates the formation of cyclic AMP.

TABLE 4. *Effect of adrenergic blockers on superinduction of N-acetyltransferase*

Treatment	*N*-Acetyltransferase pmol per pineal per 10 min
None	8 ± 2
L-DOPA	605 ± 98
l-Propranolol plus L-DOPA	72 ± 20
Phenoxybenzamine plus L-DOPA	1006 ± 121

l-Propranolol (20 mg/kg of body weight) or phenoxybenzamine (20 mg/kg) was injected into ganglionectomized rats 30 min before injection of L-DOPA (300 mg/kg). 3 hr after injection of L-DOPA, *N*-acetyltransferase activity was measured. Each group differs from the other at $P < 0.05$.

TABLE 5. *Effect of cycloheximide and actinomycin D*

Treatment	N-Acetyltransferase pmol per pineal per 10 min	
	Intact pineal	Ganglionectomized
None	9 ± 1	12 ± 2
L-DOPA	140 ± 24	551 ± 24
Actinomycin D plus L-DOPA	153 ± 25	399 ± 78
Cycloheximide plus L-DOPA	5 ± 1	10 ± 1

Actinomycin D (1 mg/kg of body weight) or cycloheximide (20 mg/kg) was injected into rats 30 min before injection of L-DOPA. *N*-acetyltransferase activity was assayed 3 hr after injection of L-DOPA (300 mg/kg).

In denervated pineal organs, L-DOPA or Catron, the inhibitor of monoamine oxidase, induces *N*-acetyltransferase activity much more than in innervated pineal organs. This superinduction could be explained by the reduced inactivation by uptake of catecholamines into nerve endings or by increased delivery of circulating catecholamine to the pineal organ. The lack of effect of agents that block uptake and reduced concentration of [^{3}H]norepinephrine in ganglionectomized pineal organs indicate that neither of these mechanisms for superinduction is operating. The experiments reported here demonstrate that β-receptors are involved in superinduction of *N*-acetyltransferase in denervated pineal organs. Denervation of muscle or nictitating membrane causes supersensitivity of endorgans to neutrotransmitters (17). Several drugs also induce supersensitivity in nictitating membrane (18). Although several possible mechanisms have been proposed for supersensitivity (18), the superinduction reported here seems to be specific to the denervation process and is probably related to the changes of postsynaptic sites. Denervation might cause an increased responsiveness of existing β-adrenergic receptors of pineal cells or an increase in the number of active receptor sites available to the neurotransmitter. Recently, it has been shown that, in the denervated muscle, the area of binding of α-bungarotoxin, a compound that binds irreversibly to acetylcholine receptors, is increased (19, 20). Although other possibilities have not been excluded, our observations would suggest that a similar process takes place on the β-adrenergic receptors on pineal organs after denervation. Weiss (21) has reported that adenylate cyclase in rat pineal organ becomes more sensitive to norepinephrine 4 weeks after denervation. The delayed appearance of supersensitivity of adenylate cyclase to norepinephrine, however, does not explain our observation of superinduction of *N*-acetyltransferase after denervation.

The inhibitors of monoamine oxidase, Catron and pargyline, also cause superinduction in ganglionectomized pineal organs. Since there is almost no norepinephrine in the denervated pineal organ (22), it is possible that inhibitors of monoamine oxidase might elevate the concentration of circulating norepinephrine or might act directly on the receptor site itself. Axelrod *et al.* (6) showed that Catron stimulated formation of melatonin from tryptophan, and Klein and Weller (23) have found that harmine, another inhibitor of monoamine oxidase, increased the concentration of *N*-acetyltransferase in cultured rat pineal organs. It might thus be possible that some of the inhibitors of monoamine oxidase stimulate the adrenergic receptor directly.

TABLE 6. *Effect of prior treatment with drugs on induction of N-acetyltransferase by* L-*DOPA*

Prior treatment	N-Acetyltransferase pmol per pineal per 10 min
None	229 ± 30
Cocaine	160 ± 29
Desmethylimipramine	151 ± 23
Reserpine	229 ± 41

Cocaine HCl (5 mg/kg of body weight) was injected into tail vein 30 min before injection of L-DOPA. Desmethylimipramine (20 mg/kg) was injected subcutaneously 24 hr and 30 min before injection of L-DOPA. Reserpine (2.5 mg/kg) was injected subcutaneously 24 hr and 30 min before injection of L-DOPA. *N*-acetyltransferase activity was assayed 3 hr after injection of L-DOPA (300 mg/kg).

Serotonin *N*-acetyltransferase of the pineal organ is a special enzyme in that it is rapidly and markedly responsive to adrenergic neurotransmitter in cultured rat pineal organs and in innervated pineal organs. *N*-acetyltransferase, thus, offers one of the best models for biochemical and pharmacological studies of neuronal control of endorgans, nerve conduction, neurotransmitter release, β-receptor sites, adenylate cyclase action, and synthesis of enzyme molecules.

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