MOLECULAR PHARMACOLOGY, 9, 184–190

Role of the *Beta* Adrenergic Receptor in the Elevation of Adenosine Cyclic 3′,5′-Monophosphate and Induction of Serotonin *N*-Acetyltransferase in Rat Pineal Glands

TAKEO DEGUCHI[1]

Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20014

(Received September 28, 1972)

SUMMARY

DEGUCHI, TAKEO: Role of the *beta* adrenergic receptor in the elevation of adenosine cyclic 3′,5′-monophosphate and induction of serotonin *N*-acetyltransferase in rat pineal glands. *Mol. Pharmacol.* **9**, 184–190 (1973).

The adenosine cyclic-3′,5′ monophosphate level in rat pineal gland is elevated 15-fold 2 min after intravenous injection of *l*-isoproterenol and returns to the baseline level after 30 min. Pineal serotonin *N*-acetyltransferase (EC 2.3.1.5) activity rises after 1 hr of lag phase and increases 70–100-fold 3 hr after injection of *l*-isoproterenol. Prior treatment with *l*-propranolol, a *beta* adrenergic blocking agent, blocks both the elevation of cyclic AMP and the increase in *N*-acetyltransferase activity by *l*-isoproterenol. When *l*-propranolol is injected after the cyclic AMP level has returned to the baseline and before *N*-acetyltransferase activity starts to rise, the increase in enzyme activity is blocked. *l*-Propranolol injected after *N*-acetyltransferase activity has reached its maximum level causes a precipitous fall in *N*-acetyltransferase activity, to 5 % of the initial level in 15 min, whereas *d*-propranolol does not decrease enzyme activity. Other *beta* adrenergic blocking agents, practolol and pronethalol, also cause a rapid disappearance of *N*-acetyltransferase activity. Cycloheximide, an inhibitor of protein synthesis, blocks the increase in *N*-acetyltransferase activity when administered 30 min before or 1 hr after the injection of *l*-isoproterenol and before enzyme activity starts to increase. If cycloheximide is injected after the maximum level of *N*-acetyltransferase activity has been reached, there is no decrease in enzyme activity over 15 min. When rat pineal with a high level of *N*-acetyltransferase activity is incubated *in vitro*, there is no change in enzyme activity. In the presence of either *l*-propranolol or dichloroisoproterenol, however, *N*-acetyltransferase activity disappears very rapidly. These observations suggest that the maintenance of the high level of *N*-acetyltransferase activity requires continuous stimulation of the *beta* adrenergic receptor on the pineal cell, regardless of the level of cyclic AMP.

INTRODUCTION

The neurotransmitter catecholamines control a variety of metabolic processes by stimulating the formation of adenosine cyclic 3′,5′-monophosphate via a *beta* adrenergic receptor (1). Recent studies have shown that serotonin *N*-acetyltransferase (EC 2.3.1.5), the enzyme involved in the biosynthesis of the pineal hormone melatonin, is inducible by catecholamines or their precursor, L-dopa, in cultured rat

[1] Fellow of the Foundations' Fund for Research in Psychiatry (Grant 70-494).

pineal (2) or *in vivo* (3). Dibutyryladenosine cyclic 3′,5′-monophosphate and theophylline, drugs that elevate cyclic AMP levels, also increase *N*-acetyltransferase activity (2, 3). The increase in *N*-acetyltransferase activity by L-dopa is prevented by the *beta* adrenergic blocking agent but not by the *alpha* adrenergic blocking agent (3). These observations indicate that pineal *N*-acetyltransferase is regulated by catecholamines via the *beta* adrenergic receptor, which in turn activates the formation of cyclic AMP. Recently Strada *et al.* (4) reported that norepinephrine increases cyclic AMP levels and *N*-acetyltransferase activity in cultured rat pineal and that the elevation of cyclic AMP is prevented by propranolol.

This report describes the temporal relationship between the elevation of cyclic AMP and increases in *N*-acetyltransferase activity, as well as a novel function of the *beta* adrenergic receptor for the maintenance of *N*-acetyltransferase activity.

MATERIALS AND METHODS

Chemicals. Acetyl-1[^{14}C]-coenzyme A (49.8 mCi/mmole) was purchased from New England Nuclear Corporation. *l*-Propranolol, *d* propranolol, practolol, and pronethalol were kindly supplied by Ayerst Laboratories, and dichloroisoproterenol was a product of Eli Lilly and Company. Other chemicals were obtained from commercial sources.

Animals. Male Sprague-Dawley rats weighing 160–180 g were supplied by Hormone Assay Laboratories, Chicago. They were kept under diurnal lighting conditions with light on from 6:00 a.m. to 6:00 p.m. Drugs were dissolved in 0.9% NaCl and injected into the tail vein or subcutaneously, as indicated in each experiment. Rats were killed by decapitation between 2:00 and 3:00 p.m. Each group consisted of five or six rats.

Assay of N-acetyltransferase activity. A pineal was quickly removed and chilled in ice. *N*-Acetyltransferase activity was assayed by the method of Deguchi and Axelrod (5) immediately after the pineal was removed.

Assay of cyclic AMP. A pineal was quickly removed, frozen on Dry Ice, and homogenized in 0.7 ml of 5% trichloracetic acid with a 1-ml glass homogenizer. After the precipitate had been removed by centrifugation, the supernatant fraction was extracted four times with 5 ml of ether each time. The aqueous layer was evaporated to dryness, and the residue was dissolved in 0.01 M sodium acetate (pH 4.0). The cyclic AMP level was measured by the method of Gilman (6). In several experiments tissue samples were assayed for cyclic AMP after purification by the method of Krishna *et al.* (7). There was no difference in the amount of cyclic AMP between purified and unpurified tissue samples.

Recently Weiss and Strada (8) reported that the cyclic AMP level in rat pineal increases after decapitation by 3 pmoles/pineal in 30 sec. The effect of decapitation on cyclic AMP should not affect the interpretation of the observations reported here, because the pineals were frozen within 30 sec after decapitation and there was no significant difference in the baseline level of cyclic AMP between intact, propranolol-treated, and denervated pineals.

RESULTS

Elevation of cyclic AMP and N-acetyltransferase activity. When *l*-isoproterenol was injected intravenously, cyclic AMP levels immediately increased (Fig. 1). Two minutes after injection there was a 15-fold increase in cyclic AMP, which was sustained for 10 min and then fell to the initial level 30 min after injection of *l*-isoproterenol.

N-Acetyltransferase activity showed a negligible amount of increase during the first hour. After 1 hr there was a rapid rise of enzyme activity, reaching a maximal level 3 hr after injection of *l*-isoproterenol, and returning almost to the initial level after 5 hr. The maximum level of *N*-acetyltransferase activity was 70–100 times the initial level.

The dose responses of cyclic AMP and *N*-acetyltransferase activity are shown in Table 1. The maximum response of both cyclic AMP and *N*-acetyltransferase activity was observed at the dose of 5 mg/kg of *l*-isoproterenol.

Effect of l-propranolol on elevation of cyclic AMP. Treatment with *l*-propranolol did not change the basal level of cyclic AMP (Table

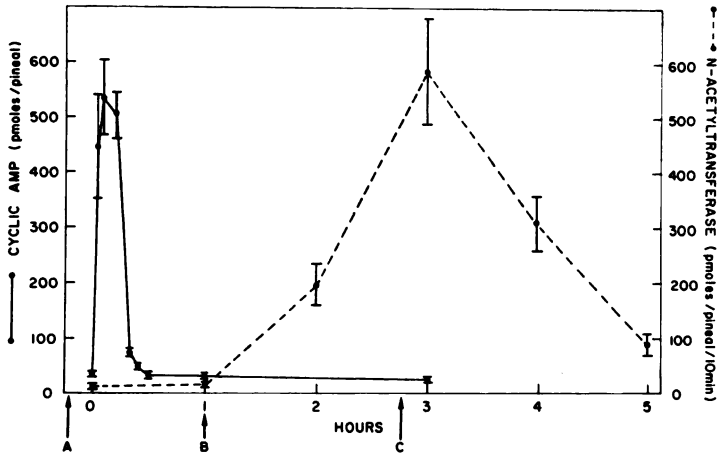

FIG. 1. *Elevation of cyclic AMP and increase in N-acetyltransferase activity by l-isoproterenol*

l-Isoproterenol HCl (5 mg/kg) was injected intravenously into rats, which were killed at the times indicated. The cyclic AMP level and *N*-acetyltransferase activity were measured as described under MATERIALS AND METHODS. Vertical bars indicate standard errors of the mean. The arrows (*A*, *B*, and *C*) indicate the times when *l*-propranolol or cycloheximide was injected into rats in the experiments shown in Table 3.

TABLE 1

Dose responses of cyclic AMP and N-acetyltransferase activity to l-isoproterenol

l-Isoproterenol HCl was injected into the tail veins of rats at the doses indicated. The cyclic AMP level was measured 5 min after the injection of *l*-isoproterenol, and *N*-acetyltransferase activity assayed 3 hr after injection. The results are expressed as means ± standard errors (n = 5).

Dose of isoproterenol	Cyclic AMP	*N*-Acetyltransferase
mg/kg	*pmoles/pineal*	*pmoles/pineal/10 min*
0	35 ± 4	8 ± 2
0.5	179 ± 31	122 ± 36
2.0	202 ± 58	175 ± 30
5.0	435 ± 65	753 ± 129
10.0	558 ± 104	883 ± 86

2). Prior treatment of rats with *l*-propranolol almost completely blocked the elevation of cyclic AMP at the dose of 2 mg/kg of *l*-isoproterenol and partially blocked it at the dose of 5 mg/kg of *l*-isoproterenol.

Effect of l-propranolol and cycloheximide on increase of N-acetyltransferase activity. *l*-Propranolol injected 30 min prior to injection of *l*-isoproterenol (point *A* in Fig. 1) blocked the increase in *N*-acetyltransferase activity (Table 3). When *l*-propranolol was injected 60 min after injection of *l*-isoproterenol, by which time cyclic AMP had returned to the initial level (*B* in Fig. 1), the increase in *N*-acetyltransferase activity was prevented. When *l*-propranolol was injected 15 min before the rats were killed, when *N*-acetyltransferase activity had already reached its maximum level (*C* in Fig. 1), *N*-acetyltransferase activity rapidly disappeared, decreasing to less than 5% of the level in rats given *l*-isoproterenol only.

Cycloheximide, administered 30 min prior

TABLE 2

Effect of l-propranolol on elevation of cyclic AMP

l-Propranolol HCl (20 mg/kg) was injected subcutaneously into rats 30 min prior to intravenous injection of *l*-isoproterenol HCl at the doses indicated. Cyclic AMP was measured 5 min after the injection of *l*-isoproterenol. Results are expressed as means ± standard errors (n = 6).

Dose of isoproterenol	Cyclic AMP	
	Untreated	Propranolol
mg/kg	*pmoles/pineal*	
0	39 ± 4	34 ± 6
2	148 ± 26	45 ± 3
5	500 ± 51	136 ± 30

to or 60 min after injection of *l*-isoproterenol, when cyclic AMP levels had returned to baseline level but *N*-acetyltransferase activity had not yet started to increase (*B* in Fig. 1), prevented the increase in *N*-acetyltransferase activity (Table 3). When cycloheximide was injected after *N*-acetyltransferase activity reached its peak (15 min prior to killing) (*C* in Fig. 1), there was almost no decrease in *N*-acetyltransferase activity.

The rapid disappearance of *N*-acetyltransferase activity after *l*-propranolol administration could be due to the formation of some inhibitor of the enzyme. To study this possibility, the pineal homogenates of two groups of rats, which had received *l*-isoproterenol alone or *l*-isoproterenol plus *l*-propranolol, were mixed. No inhibitory effect on *N*-acetyltransferase activity was found in the homogenate of pineals treated with *l*-isoproterenol and *l*-propranolol (Table 4). *l*-Propranolol added to the reaction mixture neither inhibits nor activates *N*-acetyltransferase activity.

Various *beta* adrenergic blocking agents and their stereoisomers were injected into rats during the time when *N*-acetyltransferase activity was high (Table 5). *l*-Propranolol rapidly decreased *N*-acetyltransferase activity to 10% of the initial level in 15 min, whereas a 4-fold higher dose of *d*-propranolol did not change *N*-acetyltransferase activity. Two other *beta* adrenergic blocking agents, *dl*-practolol and *dl*-pronethalol, also caused a rapid fall in enzyme activity. Pronethalol, a weak *beta* adrenergic

TABLE 3

Effect of l-propranolol or cycloheximide on increase in N-acetyltransferase activity

l-Isoproterenol HCl (5 mg/kg) was injected into the tail veins of rats 3 hr before they were killed. Either *l*-propranolol (20 mg/kg) or cycloheximide (20 mg/kg) was injected subcutaneously at the times indicated. Results are expressed as means ± standard errors (n = 5).

l-Propranolol or cycloheximide injection	*N*-Acetyltransferase	
	Propranolol	Cycloheximide
	pmoles/pineal/10 min	
30 min before isoproterenol (*A* in Fig. 1)	48 ± 12	14 ± 4
60 min after isoproterenol (*B* in Fig. 1)	34 ± 6	56 ± 8
15 min before death (*C* in Fig. 1)	33 ± 6	590 ± 132
Isoproterenol only	733 ± 83	

TABLE 4

Effect of mixing pineal homogenates

Three rats received an intravenous injection of *l*-isoproterenol HCl (10 mg/kg) 3 hr before they were killed. Another group of three rats received an intravenous injection of *l*-isoproterenol (10 mg/kg) 3 hr, and a subcutaneous injection of *l*-propranolol HCl (20 mg/kg) 15 min, before they were killed. Three pineals were homogenized in 0.25 ml of 0.05 M potassium phosphate (pH 6.5), and 50 µl of the homogenate were added to each reaction mixture. The values have been calculated from the results of duplicates.

Pineal homogenate	*N*-Acetyltransferase
	pmoles/pineal/10 min
Isoproterenol injected	765
Isoproterenol + propranolol injected	29
Combination of both homogenates	838

TABLE 5

Effect of beta adrenergic blocking agents on N-acetyltransferase activity

l-Isoproterenol (10 mg/kg) was injected intravenously 3 hr, and a *beta* adrenergic blocking agent 15 min, before the rats were killed. Results are expressed as means ± standard errors (n = 5).

Beta blocking agent	*N*-Acetyltransferase
	pmoles/pineal/10 min
None	647 ± 46
l-Propranolol (0.5 mg/kg)	460 ± 97
l-Propranolol (2.5 mg/kg)	81 ± 18[a]
l-Propranolol (10 mg/kg)	62 ± 7[a]
d-Propranolol (10 mg/kg)	666 ± 65
dl-Practolol (20 mg/kg)	61 ± 14[a]
dl-Pronethalol (10 mg/kg)	215 ± 46[a]

[a] $p < 0.01$ compared to rats without beta adrenergic blocking agent.

blocking agent, was less effective than *l*-propranolol in decreasing *N*-acetyltransferase activity.

Effect of beta adrenergic blocking agents on disappearance of N-acetyltransferase activity in vitro. To study the effect of *beta* adrenergic blocking agents more directly, the rat pineals were removed 3 hr after injection of *l*-isoproterenol and incubated *in vitro* (Table 6). The pineals incubated for 15 min showed no decrease in enzyme activity. In the presence of *l*-propranolol, however, *N*-acetyltransferase activity rapidly disappeared, decreasing to less than 10% of the initial level after 15 min. Another *beta* adrenergic blocking agent, dichloroisoproterenol, also caused a rapid disappearance of *N*-acetyltransferase activity.

Inhibitors of protein synthesis, cycloheximide and puromycin, added to the incubation medium did not result in any change of *N*-acetyltransferase activity in 15 min.

These observations indicate that *beta* adrenergic blocking agents not only block the induction of *N*-acetyltransferase activity but also immediately decrease *N*-acetyltransferase activity *in vivo* and *in vitro*.

TABLE 6

Effect of beta adrenergic blocking agents on N-acetyltransferase activity in vitro

Rats received intravenous injections of *l*-isoproterenol HCl (5 mg/kg) 3 hr before they were killed. The pineals were quickly removed and incubated in 0.7 ml of Krebs-Henseleit-bicarbonate buffer containing 0.1% glucose and 0.1% bovine serum albumin (fraction V) in a 10-ml beaker under 95% O_2-5% CO_2. Drugs were added to the incubation medium as indicated. After 15 min at 37°C, *N*-acetyltransferase activity was assayed. Each value was obtained from five or six samples and is expressed as mean ± standard error.

Addition	Incubation time	*N*-Acetyltransferase
	min	*pmoles/pineal/10 min*
None	0	400 ± 35
None	15	459 ± 33
Propranolol (2 mM)	15	24 ± 3
Dichloroisoproterenol (2 mM)	15	30 ± 6
Cycloheximide (100 μg/ml)	15	480 ± 40
Puromycin (200 μg/ml)	15	528 ± 54

DISCUSSION

Serotonin *N*-acetyltransferase in rat pineal is a unique enzyme in that it is rapidly and remarkably inducible by catecholamines by their precursor, L-dopa, by monoamine oxidase inhibitors, by dibutyryladenosine cyclic 3′,5′-monophosphate, or by theophylline in cultured rat pineals (2) or *in vivo* (3). *N*-Acetyltransferase shows a marked circadian change, with a 15–40-fold increase of enzyme activity in darkness, controlled by sympathetic nerves (9), and a rapid decrease under light (10, 11) or on administration of a *beta* adrenergic blocking agent in darkness (11). Electrical stimulation of sympathetic nerves causes an increase in *N*-acetyltransferase activity (12). These observations have suggested that the induction of *N*-acetyltransferase in rat pineal is probably mediated by the adenyl cyclase system on the pineal cell, which is responsive to catecholamines (13).

The present study demonstrates that *l*-isoproterenol, a synthetic catecholamine, elevates cyclic AMP levels and increases *N*-acetyltransferase activity in rat pineal *in vivo*. An immediate elevation of cyclic AMP is followed by a delayed, slow increase in *N*-acetyltransferase activity. *l*-Isoproterenol elevates the cyclic AMP level and increases *N*-acetyltransferase activity in pineal at a dose of 0.5 mg/kg. The responses of both cyclic AMP and *N*-acetyltransferase activity reach their maximal levels at a dose of *l*-isoproterenol between 2 and 5 mg/kg. If we assume that *l*-isoproterenol is distributed evenly in the whole body, its concentration in tissues would be 2 μM at a dose of 0.5 mg/kg and 20 μM at a dose of 5 mg/kg. Injected *l*-isoproterenol, however, could rapidly be inactivated by catechol *O*-methyltransferase in the liver. Thus the tissue concentration of *l*-isoproterenol in the pineal would be much lower. Strada *et al.* (4) have shown that the cyclic AMP level did not increase in a cultured rat pineal at a concentration of 1 μM norepinephrine but reached almost the maximal level at 10 μM norepinephrine. We have observed that 1 μM *l*-isoproterenol increases

N-acetyltransferase activity and 10 μM *l*-isoproterenol causes a maximal increase in *N*-acetyltransferase activity in a cultured rat pineal.[2] The responses *in vivo* of both cyclic AMP and *N*-acetyltransferase activity reported here are comparable to those observed in a cultured rat pineal.

When the elevation of cyclic AMP is blocked by previous treatment with *l*-propranolol, a *beta* adrenergic blocking agent, *N*-acetyltransferase activity does not increase, suggesting a causal relationship between cyclic AMP and the synthesis of *N*-acetyltransferase. Cycloheximide blocks the increase in *N*-acetyltransferase activity when injected before *N*-acetyltransferase activity starts to increase, thus indicating that cyclic AMP induces the synthesis of new enzyme molecules. Cyclic AMP probably acts on the translation process, because actinomycin D has no effect on the induction of *N*-acetyltransferase (3). When *l*-propranolol is injected after cyclic AMP has returned to its initial level (1 hr after injection of *l*-isoproterenol), *N*-acetyltransferase activity shows little increase. *l*-Propranolol injected after *N*-acetyltransferase activity has reached its maximum level decreases the enzyme activity rapidly, whereas the stereoisomer *d*-propranolol did not decrease *N*-acetyltransferase activity. Other *beta* blocking agents, practolol and pronethalol, also caused a rapid fall of enzyme activity, indicating the specificity of *beta* adrenergic blocking agents for the pineal *N*-acetyltransferase. When the pineal is removed at high *N*-acetyltransferase activity and incubated with *l*-propranolol or dichloroisoproterenol, enzyme activity disappears very rapidly. Neither cycloheximide nor puromycin affects *N*-acetyltransferase activity in this system.

It has been previously shown that cycloheximide injected in darkness decreases *N*-acetyltransferase activity with a half-time of 60 min, indicating that the turnover of *N*-acetyltransferase molecules is quite slow (11). The rapid disappearance of *N*-acetyltransferase activity caused by *beta* adrenergic blocking agents would be a different process from the classical degradation of enzyme molecules. Maintenance of the high level of *N*-acetyltransferase activity seems to require continuous stimulation of the *beta* adrenergic receptor on the pineal cell; once the receptor has been blocked, regardless of the level of cyclic AMP, *N*-acetyltransferase activity disappears immediately. The rapid inactivation of *N*-acetyltransferase by *beta* adrenergic blocking agents could be due either to rapid conversion of the active to an inactive form of the enzyme or to disaggregation of the enzyme molecule to its subunits.

It is still questionable whether the precipitous disappearance of *N*-acetyltransferase activity is linked to the cyclic AMP system. Although cyclic AMP returned to its baseline level 30 min after *l*-isoproterenol (Fig. 1) and *l*-propranolol did not change the basal level of cyclic AMP (Table 2), it is possible that a functionally active pool of cyclic AMP is very small and its change is undetectable. It is also possible that the ratio of the active to the inactive form of protein kinase might have been changed by *beta* adrenergic blocking agents, although the total amount of cyclic AMP was unaffected by these drugs. It would be of special interest to know how quickly the active protein kinase (cyclic AMP-independent form) is converted to the inactive form (cyclic AMP-dependent form) *in vivo*, and how rapidly blockade of the *beta* adrenergic receptor affects the conversion of the two forms of protein kinase. The observations reported here suggest that cyclic AMP may have two functions for pineal *N*-acetyltransferase: induction of the enzyme, which requires a high concentration of cyclic AMP, and maintenance of *N*-acetyltransferase in the active form, for which a low level of cyclic AMP might suffice. An alternative explanation for the rapid inactivation of *N*-acetyltransferase by *beta* adrenergic blocking agents could be an effect on processes other than the adenyl cyclase system that regulate inactivation of *N*-acetyltransferase in pineals.

ACKNOWLEDGMENT

The author thanks Dr. Julius Axelrod for his helpful suggestions and encouragement throughout this study.

[2] T. Deguchi and J. Axelrod, in preparation

REFERENCES

1. G. A. Robison, R. W. Butcher, and E. W. Sutherland, "Cyclic AMP," pp. 145–231. Academic Press, New York, 1971.
2. D. C. Klein, G. R. Berg, and J. Weller, *Science* **168**, 979–980 (1970).
3. T. Deguchi and J. Axelrod, *Proc. Nat. Acad. Sci. U. S. A.* **69**, 2208–2211 (1972).
4. S. J. Strada, D. C. Klein, J. Weller, and B. Weiss, *Endocrinology* **90**, 1470–1475 (1972).
5. T. Deguchi and J. Axelrod, *Anal. Biochem.* **50**, 174–179 (1972).
6. A. G. Gilman, *Proc. Nat. Acad. Sci. U. S. A.* **67**, 305–312 (1970).
7. G. Krishna, B. Weiss, and B. B. Brodie, *J. Pharmacol. Exp. Ther.* **163**, 379–385 (1968).
8. B. Weiss and S. J. Strada, *in* "Advances in Cyclic Nucleotide Research" (P. Greengard and G. A. Robison, eds.), Vol. 1, pp. 357–374. Raven Press, New York, 1972.
9. D. C. Klein, J. L. Weller, and R. Y. Moore, *Proc. Nat. Acad. Sci. U. S. A.* **68**, 3107–3110 (1971).
10. D. C. Klein and J. L. Weller, *Science* **177**, 532–533 (1972).
11. T. Deguchi and J. Axelrod, *Proc. Nat. Acad. Sci. U. S. A.* **69**, 2547–2550 (1972).
12. P. H. Volkman and A. Heller, *Science* **173**, 839–840 (1971).
13. B. Weiss, *J. Pharmacol. Exp. Ther.* **166**, 330–338 (1969).