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Supersensitivity and Subsensitivity of the β-Adrenergic Receptor in Pineal Gland Regulated by Catecholamine Transmitter

(sympathetic nerve/norepinephrine/isoproterenol/cell responsiveness/tolerance)

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ABSTRACT **Depletion of neural norepinephrine by reserpine treatment or by denervation resulted in a greater induction of serotonin *N*-acetyltransferase (EC 2.3.1.5) and a higher elevation of cyclic AMP in postsynaptic pineal cell to small amount of isoproterenol. This increase in responsiveness occurs rapidly within 24 hr after treatment with reserpine. Repeated administration of isoproterenol to the denervated or reserpine-treated rats not only suppressed the superinduction, but also caused a decreased response to isoproterenol in cultured pineal cells. Cultured pineal cells from denervated or reserpine-treated rats were about 10 times more responsive to small amounts of isoproterenol. The response of cultured pineal cells of rats which were repeatedly injected with isoproterenol was markedly reduced after exposure to submaximal amounts of catecholamines. The maximal increase in *N*-acetyltransferase was the same in denervated, reserpine-treated, isoproterenol-treated, and untreated pineal cells. Exposure of rats to continuous lighting (a procedure that reduces sympathetic nerve activity) resulted in a superinduction of pineal *N*-acetyltransferase by isoproterenol. These observations indicate that the responsiveness of the postsynaptic β-adrenergic receptor is conditioned by prior exposure to its agonist, norepinephrine. Decreased norepinephrine results in supersensitivity, and repeated exposure to large amounts of catecholamines causes subsensitivity (tolerance).**

Norepinephrine released from sympathetic nerves interacts with the β-adrenergic receptor on the postsynaptic cell membrane which in turn stimulates adenylate cyclase system and then regulates the metabolism in the end organ (1). The intensity of the effect in the end organ is related to the amount of neurotransmitter released and to the responsiveness of the receptor to its agonist. Recently, it has been shown that after injection of 3,4-dihydroxy-L-phenylalanine (L-DOPA), the precursor of catecholamines, there was a much higher increase in serotonin *N*-acetyltransferase activity (superinduction) in denervated rat pineals than innervated pineals (2). Denervation superinduction of *N*-acetyltransferase was also observed with the catecholamines norepinephrine or isoproterenol *in vivo* and in cultured rat pineals (Deguchi and Axelrod, submitted). There was a greater increase in adenosine 3′:5′-cyclic monophosphate (cyclic AMP) in denervated pineals than innervated pineals after injection of norepinephrine or isoproterenol. These observations indicate that denervation causes a rapid change at the postsynaptic site on the pineal cell which is reflected in the enhanced elevation of cyclic AMP and superinduction of *N*-acetyltransferase.

Abbreviation: L-DOPA, 3,4-dihydroxy-L-phenylalanine.

The present study shows that after depletion of neurotransmitter from innervated pineals, there is an increased responsiveness of β-adrenergic receptor with respect to the elevation of cyclic AMP and induction of *N*-acetyltransferase. Conversely, the continuous occupation of the receptor sites by agonist results in a decreased response of *N*-acetyltransferase in rat pineals.

MATERIALS AND METHODS

Animals. Sprague–Dawley male rats weighing 180–200 g were supplied by Hormone Assay Laboratories, Chicago, Ill. Rats were kept under diurnal lighting conditions with light on from 6:00 a.m. to 6:00 p.m. When indicated, rats were continuously exposed to fluorescent light of 324–540 lux for 7 days. Drugs were dissolved in 0.9% NaCl and injected subcutaneously into rats. When indicated, isoproterenol was subcutaneously injected in 0.5 ml of 15% gelatin to assure the long-lasting effect. Reserpine solution obtained from CIBA Pharmaceutical Co. was subcutaneously injected into rats. The rats were killed between 10:00 and 11:00 a.m. Bilateral ganglionectomy or presynaptic decentralization of superior cervical ganglion was performed under ether anesthesia, and ptosis was used to monitor the success of the operation. Bilateral enucleation was performed under ether anesthesia, and rats were kept under diurnal lighting conditions.

Pineal Culture. After rats were decapitated, pineals were quickly and aseptically removed and cultured by a modification of Klein and Weller's procedure (3). Four to six pineals for each group were placed in a sterile plastic dish (5-cm diameter) with 2.5 ml of BGJb medium (Grand Island Biological Co.) containing ascorbic acid (0.1 mg/ml), glutamine (2 mM), streptomycin (100 μg/ml), and penicillin (100 U/ml). Pineals were cultured for 10 hr at 37° under 95% and 5% CO_2.

Assay of Serotonin N-Acetyltransferase Activity. A pineal was quickly removed and *N*-acetyltransferase activity was assayed by the method described (4) with 20 nmol of acetyl-[1-^{14}C]coenzyme A (6.6 Ci/mol, Amersham-Searle Corp.).

Assay of Cyclic AMP. Cyclic AMP in pineals was measured by the method of Gilman (5) as described (6).

RESULTS

Supersensitivity after norepinephrine depletion

We have shown that administration of L-DOPA, a precursor of norepinephrine, caused a much greater increase in *N*-acetyltransferase activity in a denervated rat pineal than

TABLE 1. *Superinduction of N-acetyltransferase by isoproterenol in vivo*

Dose of L-isoproterenol (mg/kg)	N-Acetyltransferase (pmol per pineal per 10 min)		
	Innervated	Denervated	Reserpine treated
0	19 ± 3	23 ± 4	9 ± 2
0.25	132 ± 18	1620 ± 264*	1320 ± 317*
2.0	1760 ± 147	1400 ± 167	1310 ± 172

Denervated rats were bilaterally ganglionectomized 7 days before the experiment. Reserpine (1.5 mg/kg) was subcutaneously injected into rats 24 hr before they were killed. L-Isoproterenol·HCl was subcutaneously injected into rats 3 hr before they were killed and pineal N-acetyltransferase activity was assayed. Five rats were used in each group.

* $P < 0.01$.

in an innervated pineal (2). This superinduction of N-acetyltransferase was prevented by a β-adrenergic blocking agent or inhibitor of protein synthesis, indicating that a β-adrenergic receptor is involved and that new protein synthesis takes place. To examine whether supersensitivity of pineal cells to catecholamines is due to the absence of the catecholamine neurotransmitter or the nerve itself, rats were treated with reserpine to deplete norepinephrine from the sympathetic nerve. Administration of low amounts of isoproterenol caused a 10-fold greater increase in N-acetyltransferase activity in both the reserpine-treated and denervated pineals compared to innervated pineals (Table 1). When high doses of isoproterenol were injected, there was no difference in the increase of pineal N-acetyltransferase activity in innervated, denervated, or reserpine-treated pineal gland.

To examine whether supersensitivity to catecholamines could be demonstrated in pineals *in vitro*, pineals were cultured in the presence of submaximal concentration of isoproterenol. Denervated, decentralized, or reserpine-treated pineals showed a 3- to 4-times greater induction of N-acetyltransferase activity (Table 2). After decentralization, superinduction was apparent after a longer time (14 days) than denervation (2 days) or reserpine treatment (24 hr). When pineals were depleted of their serotonin content by treatment with p-chlorophenylalanine, pineals in organ culture did not show an increased response to isoproterenol, indicating that the effect of reserpine is due to depletion of catecholamines and not serotonin.

TABLE 2. *Superinduction of N-acetyltransferase by isoproterenol in cultured pineals*

Treatment	N-Acetyltransferase (pmol per pineal per 10 min)
None	327 ± 41
Denervated	1330 ± 208*
Decentralized	1090 ± 131*
Reserpine	917 ± 97*
p-Chlorophenylalanine	365 ± 63

Rats were bilaterally ganglionectomized 7 days (denervated) or bilaterally decentralized 14 days (nerve cut preganglionically) before they were killed. Innervated rats received subcutaneous injection of either reserpine (1.5 mg/kg) or p-chlorophenylalanine (300 mg/kg) 24 hr before they were killed. The pineals were cultured for 10 hr in the presence of 5 nM L-isoproterenol.

* $P < 0.01$ compared to untreated rats.

Pineals from innervated and denervated rats were cultured in the presence of various concentrations of isoproterenol (Table 3). In denervated pineals 5 nM isoproterenol caused a maximal increase in N-acetyltransferase activity, whereas innervated pineals showed a maximal increase in N-acetyltransferase activity at 100 nM. The maximal response of enzyme activity in both the innervated and denervated pineals was essentially the same (Table 3). These observations indicated that in the absence of the neurotransmitter caused by denervation or by reserpine treatment, the postsynaptic β-adrenergic receptor is considerably more responsive to small amounts of catecholamines, but the maximum response remains unchanged.

Subsensitivity after repeated catecholamine administration

To see whether supersensitivity could be prevented after catecholamine depletion, rats that had been pretreated with reserpine or whose pineals had been denervated were given isoproterenol repeatedly. Repeated administration of isoproterenol not only suppressed superinduction of N-acetyltransferase in reserpine-treated pineals (Table 4) or denervated pineals (Table 5), but also caused a markedly reduced response when pineals were exposed to isoproterenol in organ culture. Intact rats received repeated injections of isoproterenol, and their pineals were cultured in the presence of various concentrations of isoproterenol (Table 3). The response of N-acetyltransferase to isoproterenol was much lower in the isoproterenol-treated rat pineals compared to the untreated pineals. The maximum response at high concentration of isoproterenol was essentially the same in both isoproterenol-treated and untreated rats. These observations indicate that the continuous presence of catecholamine agonist results in a reduced responsiveness of the postsynaptic β-adrenergic receptor.

TABLE 3. *Super- and subsensitivity to isoproterenol in cultured pineals*

Concentration of isoproterenol (nM)	N-Acetyltransferase (pmol per pineal per 10 min)		
	Denervated	Intact	Isoproterenol treated
1	329 ± 90*	13 ± 2	—
5	1330 ± 208*	327 ± 41	26 ± 7*
20	2190 ± 333*	681 ± 155	69 ± 24*
100	1180 ± 146	943 ± 77	319 ± 52*
1000	1490 ± 173	1720 ± 176	1380 ± 108

Denervated rats were bilaterally ganglionectomized 7 days before they were killed. Isoproterenol-treated rats received L-isoproterenol·HCl (2.0 mg/kg, dissolved in 0.5 ml of 15% gelatin) 8, 16, and 24 hr before they were killed. Pineals were cultured for 10 hr in the presence of the L-isoproterenol concentration indicated.

* $P < 0.01$ compared to intact rats at the same isoproterenol concentration.

TABLE 4. *Suppression of reserpine-induced superinduction by isoproterenol*

Treatment	*N*-Acetyltransferase (pmol per pineal per 10 min)
None	612 ± 96
Reserpine	1030 ± 112*
Reserpine + gelatin	1260 ± 112†
Reserpine + isoproterenol	136 ± 21†

Reserpine (1.5 mg/kg) was subcutaneously injected into rats 24 hr before they were killed. One group of reserpine-treated rats received L-isoproterenol·HCl (1.5 mg/kg) dissolved in 0.5 ml of 15% gelatin 8 and 16 hr before they were killed. Another group of reserpine-treated rats received 0.5 ml of 15% gelatin 8 and 16 hr before they were killed. All pineals were cultured for 10 hr in the presence of 20 nM L-isoproterenol.

* $P < 0.05$ compared to untreated rats; † $P < 0.01$.

Supersensitivity and lighting

Pineal *N*-acetyltransferase activity undergoes a marked circadian rhythm with highest enzyme activity occurring at night (7). The nocturnal rise of *N*-acetyltransferase activity could be abolished when rats were kept in continuous light (8). This finding suggested that lighting suppresses release of norepinephrine from sympathetic nerves. To establish whether the reduced release of norepinephrine caused by light would result in a supersensitivity in pineals, rats were exposed to light for 7 days and their pineals were then cultured in the presence of isoproterenol. The cultured pineals of rats exposed to continuous lighting were 4-times more responsive with respect to *N*-acetyltransferase than pineals from rats under diurnal lighting conditions (Table 6). Pineals from blinded rats showed a lower increase in *N*-acetyltransferase activity. The very marked nocturnal rise in pineal *N*-acetyltransferase (7) might be explained by the development of supersensitivity of the pineal β-adrenergic receptor due to the absence or reduced release of norepinephrine caused by light during daytime.

Increased response of pineal cyclic AMP

The elevation of cyclic AMP in response to isoproterenol was greater in denervated pineals than in the intact pineal gland

TABLE 5. *Suppression of denervation superinduction of N-acetyltransferase by isoproterenol*

	N-Acetyltransferase (pmol per pineal per 10 min)
Innervated	108 ± 10
Denervated	494 ± 117*
Denervated + isoproterenol	26 ± 2*

Rats were bilaterally ganglionectomized 48 hr before they were killed. A group of denervated rats received 0.5 mg/kg of L-isoproterenol·HCl (dissolved in 0.5 ml of 15% gelatin) 8, 16, 24, and 32 hr before they were killed. Pineals were cultured for 10 hr in 5 nM L-isoproterenol.

* $P < 0.01$ compared to intact rats.

TABLE 6. *Effect of blinding or continuous lighting on induction of N-acetyltransferase in pineal*

	N-Acetyltransferase (pmol per pineal per 10 min)
Diurnal	381 ± 24
Blinded	209 ± 21*
Continuous light	1350 ± 210*

A group of rats was exposed to light for 7 days. Another group of rats was bilaterally enucleated 7 days before they were killed. The pineals were cultured for 10 hr in 5 nM L-isoproterenol.

* $P < 0.01$ compared to the rats exposed to diurnal lighting conditions.

(Deguchi and Axelrod, submitted). The elevation of cyclic AMP was also much greater in reserpine-treated pineals than untreated pineals *in vivo* (Table 7) and in culture (Table 8). Treatment with a protein-synthesis inhibitor, cycloheximide, did not block the supersensitivity of cyclic AMP elevation in reserpine-treated pineals (Table 9). Incorporation of [^{3}H]-leucine into pineal protein was reduced by 35% 10 hr after exposure to cycloheximide. Shortly after administration of cycloheximide, protein synthesis is completely blocked (9) and gradually recovered to about 65% of the control value during 10 hr. These results indicate that superinduction of *N*-acetyltransferase in denervated or reserpine-treated pineals is mediated by enhanced elevation of concentrations of pineal cyclic AMP. The new synthesis of receptor or adenylate cyclase system does not appear to be involved in the supersensitivity of β-adrenergic receptor by depletion of catecholamines.

DISCUSSION

Our results demonstrate that the responsiveness of a pineal cell is dependent on the previous exposure of the β-adrenergic receptor of the cell to the neurotransmitter, norepinephrine. When the norepinephrine liberated from the sympathetic nerve is abolished by denervation or reduced by reserpine, decentralization, or continuous lighting, the sensitivity of β-adrenergic receptor of the pineal cell with respect to the response of cyclic AMP or *N*-acetyltransferase is greatly enhanced. The same magnitude of induction of *N*-acetyltransferase can be achieved by 10% of the concentration of isoproterenol as compared to the pineal that was exposed to normal amounts of the neurotransmitter. The dose–response curve of *N*-acetyltransferase induction and cyclic AMP elevation shifts to the left by depletion of neurotransmitter. The

TABLE 7. *Elevation of cyclic AMP with isoproterenol in vivo*

Time after isoproterenol (min)	Cyclic AMP (pmol per pineal)	
	Untreated	Reserpine treated
0	16 ± 4	12 ± 2
10	120 ± 18	261 ± 33*
20	62 ± 8	171 ± 7*

Reserpine (1.5 mg/kg) was subcutaneously injected into rats 24 hr before they were killed. L-Isoproterenol·HCl (0.25 mg/kg) was subcutaneously injected into rats 10 or 20 min before they were killed. Each group contained seven animals.

* $P < 0.01$ compared to untreated rats.

TABLE 8. *Elevation of cyclic AMP with isoproterenol in cultured pineals*

Concentration of isoproterenol	Cyclic AMP (pmol per pineal)	
	Untreated	Reserpine treated
0	12 ± 3	7 ± 1
0.3 μM	57 ± 8	151 ± 20*
3 μM	73 ± 14	155 ± 15*

Reserpine (1.5 mg/kg) was subcutaneously injected into rats 24 hr before they were killed. Pineals were cultured in culture medium without isoproterenol for 60 min and then transferred into the culture medium containing the L-isoproterenol indicated. After 10 min of incubation, the content of cyclic AMP was measured. Each group contained five rats.

* $P < 0.01$ compared to untreated rats.

TABLE 9. *Effect of cycloheximide on the enhanced elevation of cyclic AMP in reserpine-treated pineals*

Treatment	Cyclic AMP (pmol per pineal)
None	121 ± 11
Reserpine	271 ± 12*
Reserpine + cycloheximide	239 ± 14*
Cycloheximide	124 ± 7

Reserpine (1.5 mg/kg) was subcutaneously injected into rats 24 hr before they were killed. Cycloheximide (1.5 mg/kg) was subcutaneously injected 10 and 20 hr before they were killed. Cyclic AMP content was measured 10 min after subcutaneous injection of L-isoproterenol·HCl (0.25 mg/kg). Each group had seven animals.

* $P < 0.01$ compared to untreated rats.

maximum responsiveness, however, does not change. In kinetic terms this suggests that in the absence of its agonist, the K_m of the β-adrenergic receptor is reduced but the V_{max} remains the same. Such a phenomenon implies that the conformation or availability of the β-adrenergic receptor has changed to make it more reactive to catecholamines.

Conversely, if the number of catecholamine molecules reacting with the β-adrenergic receptor is increased for a period of time, the pineal cell becomes less responsive to its agonist. That is, larger amounts of catecholamines are necessary to produce the same degree of increase in *N*-acetyltransferase activity. The dose–response curve of *N*-acetyltransferase induction shifts to the right by a repeated administration of agonist, but the maximum induction of the enzyme remains unchanged. Thus, the continuous exposure of the β-adrenergic receptor to large amounts of its agonists changes its conformation or availability to make it less responsive. This phenomenon is essentially the same as tolerance.

Supersensitivity in the absence of the neurotransmitter or subsensitivity caused by the repeated administration of isoproterenol occurs relatively rapidly within 24 hr. This finding together with the unchanged maximum response and the lack of the effect of protein-synthesis inhibitor suggests a rapid change in conformation or availability of receptor rather than the new synthesis of receptor sites. It appears that supersensitivity and tolerance are part, but at opposite poles, of the same phenomenon.

Increased sensitivity of smooth muscles to sympathomimetic amines has been observed after denervation, decentralization, and treatment with certain drugs (10). The biochemical aspects of the supersensitivity have remained unclear. It has been reported that chronic denervation (4 weeks) or continuous lighting increased sensitivity of adenylate cyclase to norepinephrine and sodium fluoride in rat pineals (11). Previously it has been proposed on the basis of enzymatic studies that the tolerance to narcotic drugs is due to unavailability of opiate receptor sites (12). A decrease in the number of insulin receptors has been shown in fat cells of obese rats with high plasma insulin level (13). A recent report has also shown that adenylate cyclase in adrenal cortex becomes more responsive to adrenocorticotropic hormone in hypophysectomized rats (14). Perhaps other physiologic and pharmacologic response might be due to the changes in conformation or availability of receptor sites imposed by the absence or excess of physiologic agents of drugs. Such a phenomenon would be a rapid and parsimonious adaptive mechanism for responsive cells.

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