

A PROTECTIVE ROLE OF NERVE ENDINGS IN THE STRESS-STIMULATED INCREASE IN PINEAL N-ACETYLTRANSFERASE ACTIVITY

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Introduction

Catecholamines serve as both neurotransmitters and adrenal hormones. This dual role raises interesting questions as to whether a metabolic process which is transsynaptically regulated by an adrenergic mechanism is influenced by circulating adrenal and neuronal catecholamines released in response to stress, or whether it is protected against such a stress-induced increase in circulating catecholamines. The activity of pineal N-acetyltransferase (acetyl CoA:serotonin N-acetyltransferase EC 2.3.1.5) is normally transsynaptically regulated by norepinephrine (1,2). It can also be affected by circulating adrenergic drugs (1,2) and thus presents a good model to study these questions (3).

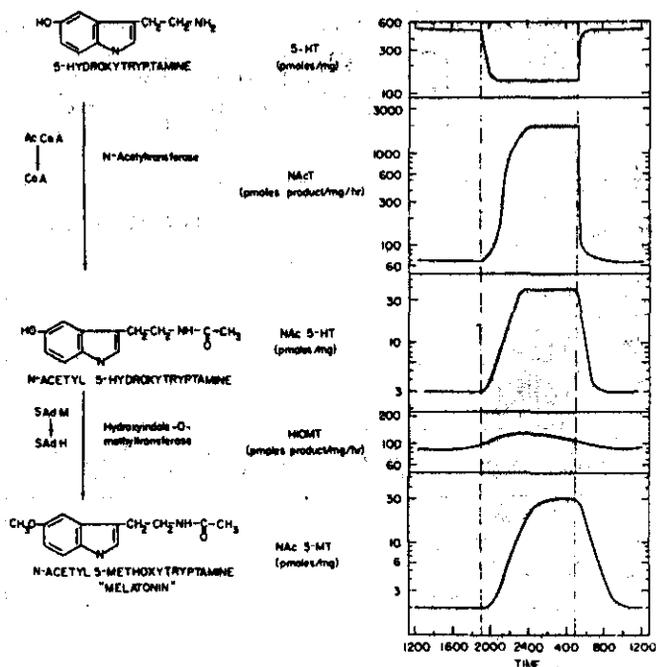


Figure 1 Rhythms in indole metabolism in the rat pineal gland. The metabolic pathway from 5-hydroxytryptamine to melatonin is on the left. The daily variations in the concentrations of metabolites and activities of enzymes are on the right. The shaded portion indicates the dark period of the lighting cycle. AcCoA, acetyl coenzyme A; CoA, coenzyme A; SAdM, S-adenosyl methionine; SAdH, S-adenosyl homocysteine; 5-HT, 5-hydroxytryptamine, serotonin; NAc5-HT, N-acetyl-5-hydroxytryptamine; HIOMT hydroxyindole-O-methyltransferase; NAc 5-MT, N-acetyl 5-methoxytryptamine, melatonin. From Klein (1).

N-Acetyltransferase activity controls large daily changes in the amount of serotonin and the amount and rate of production of N-acetylserotonin and melatonin in the pineal gland (Fig. 1). In this manuscript we describe our studies which suggest that sympathetic nerve endings in the pineal gland may play a protective role in stress. Perhaps they act by removing circulating catecholamines from extracellular spaces, thus preventing an increase in N-acetyltransferase activity during stress.

The transsynaptic regulation of pineal N-acetyltransferase activity

N-Acetyltransferase converts serotonin (5-hydroxytryptamine) to N-acetylserotonin (N-acetyl-5-hydroxytryptamine). The activity of N-acetyltransferase increases 30- to 100-fold each night. The rate of conversion of N-acetylserotonin to melatonin is controlled by the amount of N-acetylserotonin available for O-methylation by hydroxyindole-O-methyltransferase. The maximum activity of this enzyme changes little at night when the rate of melatonin production increases 10-fold; the O-methylation reaction is regulated by mass action. In contrast, the acetylation reaction is regulated by the activity of N-acetyltransferase and this in turn is controlled by a transsynaptic mechanism (1,2). The transsynaptic stimulation of pineal N-acetyltransferase (Fig. 2) is initiated by the release of norepinephrine from nerve endings in the pineal gland. Norepinephrine interacts

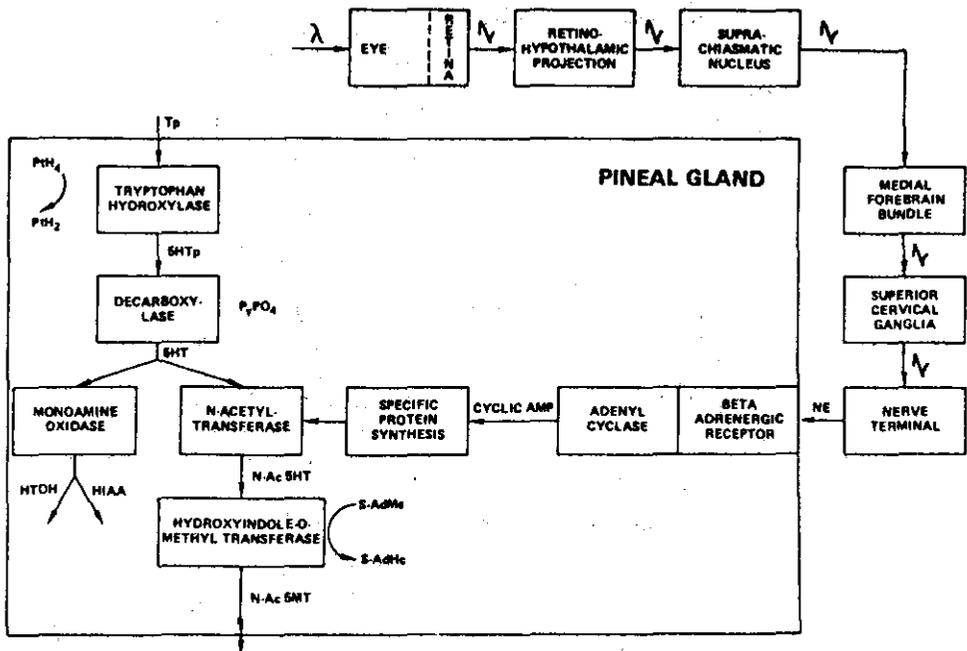


Figure 2 A model of the β -adrenergic regulation of indole metabolism in the pineal gland. Cyclic AMP, adenosine 3',5'-monophosphate; Tp, tryptophan; Pth₄, tetrahydropteridine; Pth₂, dihydropteridine; 5HTP, 5-hydroxytryptophan; P₅PO₄, pyridoxal phosphate; 5HT, 5-hydroxytryptamine, serotonin; HTOH, hydroxytryptophol; HIAA, hydroxyindole acetic acid; N-Ac 5HT, N-acetyl 5-hydroxytryptamine, N-acetyl serotonin; AcCoA, acetyl coenzyme A; S-AdMe, S-adenosyl methionine; S-AdHc, S-adenosylhomocysteine; N-Ac 5-MT, N-acetyl 5-methoxytryptamine, melatonin. The symbol λ represents neuronal transmission. From Klein and Yowler (4).

with a β -adrenergic receptor on postsynaptic structures resulting in an increase in the production of cyclic AMP (5-6). Acting through a mechanism which appears to depend upon protein synthesis and require membrane hyperpolarization, cyclic AMP causes an increase in the activity of pineal N-acetyltransferase (7-9). This mechanism controls daily changes in pineal N-acetyltransferase activity (1,2).

Sympathetic nerve endings in the pineal gland have their cell bodies in the superior cervical ganglia; when sympathetic nerves in the pineal gland degenerate after animals are superior cervical ganglionectomized (SCGX), the daily rhythm in N-acetyltransferase ceases (10). The rhythm also ceases if the superior cervical ganglia are decentralized (10). This procedure leaves the sympathetic innervation to the pineal gland intact, but blocks input from the central structures which carry signals from the suprachiasmatic nuclei. These hypothalamic nuclei appear to drive the circadian rhythm in the activity of pineal N-acetyltransferase (11). Light, acting via specific monosynaptic retino-hypothalamic projections terminating in the suprachiasmatic nuclei (11), synchronizes this rhythm generating system with environmental photoperiods.

Stress stimulation of pineal N-acetyltransferase activity

Using intact Sprague-Dawley rats fed ad libitum and maintained on an L:D 14:10 lighting schedule, we found that swimming stress caused an increase in N-acetyltransferase activity (Table 1).

TABLE 1

Effect of swimming stress on pineal N-Acetyltransferase activity in intact and SCGX animals

Treatment	Surgical Group	N-Acetyltransferase Activity (nmoles/gland/hr)
None	Intact	0.15 \pm 0.02 *
	SCGX	0.44 \pm 0.06 **
Swimming stress	Intact	0.66 \pm 0.15
	SCGX	7.78 \pm 1.02 **
Isoproterenol	Intact	9.17 \pm 0.46
	SCGX	10.62 \pm 1.74

Rats were stressed for 2.5 hrs between 1200 and 1600 hrs. The pineal glands were then quickly removed and stored at -75°C prior to assay by a radiochemical procedure (8). The water temperature used for swimming stress fell from 31.3°C to 29.7°C during the 2.5 hrs. Isoproterenol (20mg/kg) was injected subcutaneously in 0.1 ml 0.85% NaCl. *Significantly smaller than all other groups, $P < .01$. **Significantly greater than intact animals receiving the same treatment $P < .01$. Data is based on 6 animals and is presented as the mean + S.E. Intact and surgically prepared rats were obtained from the Zivic Miller Co. (Allison Park, Pennsylvania). Surgically prepared animals were 10 days post-operative.

This is in agreement with earlier studies which showed that immobilization stress and hypoglycemia cause a small increase in N-acetyltransferase activity and pineal melatonin in starved

animals kept in a L:D 24:0 lighting schedule for 48 hours (3). The increase in N-acetyltransferase activity we observed in intact animals was only 4 times greater than control values and less than 5% of that seen in unstressed animals given a large dose of isoproterenol.

In SCGX rats, however, the activity of N-acetyltransferase after swimming stress was 50 times greater than controls and not significantly smaller than that seen following isoproterenol treatment (Table 1). Partial chemical (6-OH-dopamine) sympathectomy also increases the N-acetyltransferase response to stress (3). It was postulated that this increase resulted from either an increased release of neuronal or adrenal catecholamines into the circulation in chemically sympathectomized animals, or from supersensitivity resulting from sympathectomy. Of these two possibilities, only the latter has applicability in our experiments because we used a specific surgical sympathectomy.

We have confirmed previous findings (3) that the increase in N-acetyltransferase caused by stress is blocked by a β -adrenergic blocking agent. After 2.5 hrs of swimming the activity of pineal N-acetyltransferase in pineal glands of SCGX animals treated with propranolol was identical to that in unstressed animals. This indicates that swimming stress increased N-acetyltransferase via β -adrenergic stimulation of the pineal gland.

TABLE 2

Effect of injection of D,L-propranolol on the response of SCGX rats to swimming stress

Treatment	N-Acetyltransferase Activity (nmoles/gland/hr)
None	0.39 \pm 0.12
Swimming stress	7.19 \pm 0.52
Swimming stress + D,L-propranolol	0.37 \pm 0.11

Rats were subjected to swimming stress for 2.5 hrs beginning at 1330 hrs. The water temperature fell from 31.2°C to 29.4°C during this time. D,L-Propranolol (20 mg/kg) was injected in 0.1 ml of 0.85% NaCl immediately before the animals were stressed. For other details see the legend of Table 1.

The effects of stress on "supersensitive" pineal glands.

In unpublished studies we have examined whether supersensitive pineal glands exhibited a greater response to stress. Supersensitivity was induced by subjecting intact animals to up to 22 days of light (12). The response to swimming stress was not substantially increased. In addition,

only a small increase in the response to stress was observed at night after 19 hours of light (Table 3), when pineal glands should be more sensitive than they are at noon (13,14). This increase was quite small compared to that seen in isoproterenol-treated animals or in animals in the dark at night. The failure of these supersensitive glands to show a larger response suggests that supersensitivity alone may not be responsible for the increased response in SOGX animals.

TABLE 3
Influence of the time of day on the response to swimming
stress in intact rats

Treatment	Time of Treatment	N-Acetyltransferase Activity (nmoles/gland/hr)
Light	1200-1430	0.17 ± 0.04
	2400-0230	0.21 ± 0.11
Swimming stress	1200-1430	0.66 ± 0.15
	2400-0230	1.07 ± 0.19
Darkness	1200-1430	0.26 ± 0.11
	2400-0230	6.27 ± 0.21
Isoproterenol	1200-1430	16.34 ± 0.83
	2400-0230	21.40 ± 1.30

Water temperature during the 1200-1430 swimming stress fell from 31.3°C to 28.9°C, and from 31.5°C to 29.2°C during the 2400-0230 stress. Rats stressed from 2400-0230 hrs were maintained in constant light from 0500 hrs of the same day. Isoproterenol (20 mg/kg) was injected subcutaneously in 0.1 ml 0.85% NaCl. For other details see the legend of Table 1.

The effects of stress on pineal N-acetyltransferase activity in animals with
decentralized superior cervical ganglia

The failure of pineal glands which had been made supersensitive by prolonged exposure to constant light to show an increased stress response led us to examine the possibility that nerve endings in the pineal gland were playing a protective role, perhaps by taking up circulating catecholamines released during stress. To test this we determined if pineal glands deprived of sympathetic stimulation but retaining nerve endings, as a result of decentralization of the superior cervical ganglia, responded to stress differently than those from SOGX animals. Both types of glands should be equally supersensitive (15). Following swimming stress, pineal N-acetyltransferase activity in animals with decentralized superior cervical ganglia was about 25 percent of that seen in identically stressed SOGX animals (Table 4). This suggested to us that nerve endings reduced the response to stress.

TABLE 4

Comparison of the effect of SOGX and decentralization on the response to swimming stress

Surgical Group	Treatment	N-Acetyltransferase Activity (nmoles/gland/hr)
Intact	None	0.44 ± 0.06
	Swimming stress	1.01 ± 0.22
Decentralized	None	0.41 ± 0.06
	Swimming stress	4.35 ± 0.81 *
SOGX	None	0.73 ± 0.19
	Swimming stress	12.35 ± 2.11

Rats were stressed for 2.5 hrs beginning at 1330 hrs. During this time the water temperature dropped from 31.3°C to 29.7°C. Both the decentralized and SOGX rats were 10 days post-operative. *Significantly different from all other values, $P < .01$. For other details see the legend for Table 1.

It seems reasonable to assume that pineal glands taken from decentralized rats were at least as supersensitive as pineal glands taken from SOGX animals because both types of glands are deprived of sympathetic stimulation and exhibit a supersensitive cyclic AMP response to norepinephrine (15). It would thus appear that the decreased response to stress of pineal glands taken from animals with decentralized superior cervical ganglia as compared to that from SOGX animals may be due primarily to the presence of nerve endings, and not supersensitivity. The response of pineal glands from decentralized animals may have been larger than that seen in intact glands, both of which have nerve endings, primarily because of the supersensitivity of the former group resulting from the chronic decrease in sympathetic tone.

Another approach to the question of whether the sympathetic neuron, specifically the neuronal uptake of catecholamines (uptake₁) (16), was preventing a large pineal N-acetyltransferase response of intact animals to stress was to treat these animals with drugs which are known to block uptake₁. Treatment with phentolamine, the adrenergic blocking agent which also inhibits uptake₁ (17), increased the pineal N-acetyltransferase response to swimming stress from 3.7 to 18 times control values (Table 5). These results are in agreement with those of experiments in which we used the tricyclic antidepressant desipramine, one of the most potent inhibitors of uptake₁ (16). Desipramine treatment (10-20 mg/kg) of intact animals resulted in a 50-fold increase in N-acetyltransferase activity following 2.5 hours of swimming stress (unpublished results).

TABLE 5

The effect of phentolamine on the response of intact rats to swimming stress

Treatment	N-Acetyltransferase Activity (nmoles/gland/hr)
None	0.14 ± 0.03
Phentolamine	0.17 ± 0.02
Swimming stress	0.52 ± 0.29
Swimming stress + phentolamine	2.51 ± 0.55 *

Rats were stressed for 2.5 hrs beginning at 1330 hrs. During this time the water temperature fell from 31.2°C to 29.6°C. Phentolamine (20mg/kg) was injected subcutaneously in 0.5 ml 0.85% NaCl. *Significantly greater than all other values, $P < 0.01$. For other details see legend to Table 1.

General implications

The mechanism through which catecholamines are taken up by nerve endings has been well characterized (16). It functions physiologically to terminate transsynaptic transmission by removing active neurotransmitter from extracellular spaces. It would appear from the above studies that this mechanism also plays an important protective role in stress. Apparently, circulating catecholamines, released due to stress, pass through blood vessels into the extracellular space of the pineal gland. In intact animals this space contains sympathetic nerve fibers which could remove circulating adrenal and neuronal catecholamines via neuronal uptake mechanism. In this manner stimulation of the highly sensitive adrenergic-cyclic AMP system which regulates pineal N-acetyltransferase activity would be prevented.

It would seem that the relative degree to which a tissue is innervated by sympathetic fibers would determine the degree to which adrenergically regulated processes in the tissue respond to stress. Those tissues like the liver (the source of glucose during stress) which receives less sympathetic innervation, would respond more than other tissues, such as the pineal gland, in which innervation is extensive. The potential importance of a neuronal uptake process as a protective mechanism in the pineal gland is obvious: it prevents spurious changes in melatonin synthesis during times of stress. The general importance of this mechanism in stress remains to be determined.

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DISCUSSION

Dr. Illnerova asked for an explanation of the small increase observed by Dr. Klein after immobilization as contrasted to the large increase reported from Wurtman's laboratory. Dr. Klein thought those large increases might be due to the insensitivity of the assay system used at MIT; control values were obtained at just a few counts above background. Dr. Illnerova also mentioned that she had obtained similar results to Dr. Klein after administration of desmethylimipramine; however, she used in place of stressed animals, animals which were transferred to light at night after being kept in the dark. Under these conditions, Dr. Klein had reported a very rapid fall in N-acetyltransferase. She concluded that her results indicated that uptake is quite important.

Dr. Telegdy commented that his laboratory had run into similar problems as described by Dr. Klein when they tried to correlate hypothalamic serotonin levels and pineal serotonin level. Difficulty was introduced because the circadian rhythm of the pineal gland and the hypothalamus were not parallel; this led to the question as to whether pineal serotonin was influencing the hypothalamic serotonin level. To determine the effect of the pineal on the stress mechanism, the gland was extirpated, but this was ineffective. However, when the cervical ganglia were removed, there was a facilitated stress response. He wondered if the mechanism for this is action on the hypothalamus indirectly by the pineal. Perhaps the cervical ganglia have a special innervation to the hypothalamus which might have some regulatory function.

Dr. Kvetnansky wondered if there was some in vivo function for epinephrine in the pineal; he asked if Dr. Klein had measured pineal epinephrine levels. Dr. Klein replied that he doubted that there is a physiological role for epinephrine as a transmitter and that no one has presented evidence that is synthesized in the pineal.

Dr. Ganong asked Dr. Klein to what extent he equated changes in pineal N-methyltransferase with release of melatonin from the gland. Dr. Klein said his only observations were in organ culture. When N-acetyltransferase goes up, the rate of melatonin formation goes up. The rate of release also correlates well; i.e., when melatonin production stops, release stops. Since the pineal does not store melatonin, levels can change in 12 hours from undetectable to about 50 picamoles. The pineal can make tremendous amounts of melatonin (2,000 picamoles in a 12 hour period), but it stores very little. Dr. Ganong then commented that Dr. Klein's data would indicate that under stress there should be a sharp rise in melatonin levels and when a ganglionic blocking drug was given or the subject was denervated, there should be a high rise in melatonin with every stress. Dr. Klein did not know if this would be true for ganglionic blocking agents but believed it would hold for propranolol or DMI. He felt that the nerve endings protect against stress increase; the stress will produce huge increases in melatonin only when the protective mechanism is blocked.

Dr. Reis inquired whether the gangliectomy was bilateral or unilateral. When informed that it was unilateral, he asked if unilateral gangliectomy would produce half the rise in level, but Dr.

Klein said that he had no basis for prediction. Dr. Reis then discussed some results with 6-OHDA. Animals so treated have their peripheral adrenergic innervation destroyed. In these animals, blood pressure is maintained, but if they are adrenalectomized the blood pressure drops to a lethal level even though adrenalectomy of control animals produces no effect on blood pressure. The 6-OHDA treated animals also become extremely sensitive to perfused pressor agents; this is evidence that the nerve endings are of importance to the circulatory system. Thus, even though one can not normally detect a role of adrenal epinephrine on the circulation, this is because the tests are run in the presence of nerve terminals. However, after selective denervation, any activity may be important. Dr. Reis concluded that patients under drug therapy for hypertension or depression may be driving up their melatonin levels; this may be related to the behavioral response.