Pineal N-Acetyltransferase Activity: Effect of Sympathetic Stimulation

Abstract. *Stimulation of preganglionic sympathetic fibers to the superior cervical ganglia elevates the activity of pineal* N-*acetyltransferase. After the stimulation-induced rise in enzyme activity, a return toward baseline levels occurs whether or not nerve stimulation is continued. The ability of pineal* N-*acetyltransferase activity to fall in the presence of stimulation may account for the persistence of its rhythm in blinded animals.*

The mammalian pineal contains a number of enzymes essential to the biosynthesis of melatonin (*1*). Pineal *N*-acetyltransferase catalyzes the conversion of serotonin to its *N*-acetyl derivative (*2*), which is the immediate precursor of melatonin. *N*-acetyltransferase activity in darkness is 15 times greater than in light. A rhythm in the activity of this enzyme has been demonstrated in diurnal lighting; this rhythm is abolished by exposure of the animals to continuous light, but persists in continuous darkness or after blinding (*3, 4*). We report that stimulation of preganglionic cervical sympathetic fibers in rats during the light phase of the diurnal lighting cycle results in increased activity of pineal *N*-acetyltransferase. This provides direct evidence that *N*-acetyltransferase is under neuronal control mediated by postganglionic sympathetic fibers to the pineal arising from the superior cervical ganglion, as we have previously demonstrated for hydroxyindole *O*-methyltransferase (HIOMT) (*5*).

Male Holtzman rats (220 to 350 g) were used. Environmental lighting conditions were controlled 24 hours prior to operation; the animals were placed in the dark for 8 to 12 hours and then exposed to light (intensity 450 to 650 lu/m^2, GE Chroma 70 fluorescent lamps) for 8 to 12 hours. During this 24-hour period the rats were housed individually. In each experiment, the following operations were included: bilateral decentralization of the superior cervical ganglia; bilateral decentralization of the superior cervical ganglia, followed by the attachment of a bipolar, nichrome electrode to the end of one preganglionic trunk; sham-operated rats in which a midline neck incision was made and the preganglionic nerves to the superior cervical ganglia were exposed bilaterally. The preganglionic cervical sympathetic trunk was stimulated with square wave pulses by a constant-current stimulator (9 seconds out of each minute, 10 pulses per second, 10-msec pulse duration) as previously described (*5*). All operations were performed with ether anesthesia. Bilateral ganglionic decentralization produced endophthalmos. A prominent exophthalmos was seen ipsilateral to the stimulated cervical sympathetic trunk during each 9 seconds of stimulation. The exophthalmic response is caused by contraction of musculus orbitalis (Müller's muscle), a sympathetically innervated strip of smooth muscle bridging the inferior orbital fissure (*6*). This response was used to determine the threshold for electrical stimulation (0.23 ± 0.02 ma for this series of animals). Twice the threshold amperage was used. Amperage was increased whenever necessary to maintain a constant degree of exophthalmic response during stimulation. Four groups of stimulated animals were examined, including stimulation for 1, 2, or 3 hours followed by immediate decapitation, and stimulation for 2 hours followed by an additional hour without stimulation prior to decapitation. Within 2 minutes after decapitation, the pineals were dissected out, and the assay for *N*-acetyltransferase was begun (*4*). The experiments were performed over a period of 3 months; animals for two or three time points were usually included in each run (Table 1).

Animals whose preganglionic cervical sympathetic nerves were stimulated showed an increase in pineal *N*-acetyltransferase activity when compared to decentralized animals (*7*). One hour of stimulation led to a 164 percent increase ($P < .005$); 2 hours resulted in a 214 percent increase ($P < .005$); 3 hours resulted in a 131 percent rise ($P < .005$); 2 hours of stimulation with an additional hour without stimulation led to a 57 percent rise ($P < .02$). The differences in enzyme activity between decentralized and sham-operated animals were not statistically significant. Rats stimulated for 3 hours exhibited 50 percent lower enzyme activity than rats stimulated for 2 hours ($P < .025$). Rats stimulated for 2 hours and killed after an additional hour were also lower in enzyme activity than the 2-hour stimulated animals (− 66 percent, $P < .05$). The differences in *N*-acetyltransferase activity between the two groups of stimulated animals killed after 3 hours was not statistically significant.

The rise in pineal *N*-acetyltransferase activity brought about by increased afferent input to the pineal via its sympathetic innervation shows that this enzyme, as well as HIOMT, is under neural control. Whereas HIOMT exhibits a linear decrease in activity with increased duration of nerve stimulation (*5*), pineal *N*-acetyltransferase does not show a linear response to duration of stimulation. After the initial increase in *N*-acetyltransferase activity during the first 2 hours of stimulation, enzyme activity falls toward basal levels whether or not stimulation is continued. This reduction of *N*-acetyltransferase activity is not due to the effect of light input in animals with high pineal enzyme activity. These animals have undergone bilateral decentralization of their superior cervical ganglia, a procedure that interrupts the only known pathway through which environmental lighting information reaches the pineal (*8*). The lower enzyme activity found after 3 hours of stimulation could be the result of failure

Table 1. Effect of cervical sympathetic stimulation on pineal *N*-acetyltransferase activity. Enzyme activity is expressed as number of picomoles of *N*-acetyl[^{14}C]serotonin formed per gland per hour ± standard error. The numbers in parentheses indicate the size of each group. The *P* values were obtained by means of a two-tailed *t*-test.

Treatment	Activity (picomole per gland per hour)			
	1 hour	2 hours	3 hours	2 hours + 1 hour without stimulation
Stimulated	107.1 ± 20.4* (11)	173.0 ± 29.9* (16)	86.6 ± 13.4* ‡ (13)	59.0 ± 8.1† § (7)
Decentralized	40.6 ± 9.3 (14)	55.1 ± 9.6 (19)	37.5 ± 2.7 (20)	
Sham-operated	30.4 ± 4.3 (12)	31.4 ± 1.9 (9)	32.8 ± 5.6 (9)	

* Greater than controls, $P < .005$. † Greater than 3-hour controls, $P < .02$. ‡ Less than 2-hour stimulated group, $P < .025$. § Less than 2-hour stimulated group, $P < .05$.

of neuronal transmission to the pineal, possibly secondary to an exhaustion of norepinephrine from nerve endings in the gland. This explanation seems unlikely for the following reasons. (i) The sympathetically mediated, exophthalmic response was maintained during the 3 hours of stimulation, and (ii) with the use of this preparation a linear fall in HIOMT activity over an 8-hour period of electrical stimulation was demonstrated (*5*). If neuronal transmission indeed remains intact, then the reduction in *N*-acetyltransferase activity after the stimulation-induced increase may be due to a process intrinsic to the pineal and not dependent on continued nerve stimulation. The response of pineal *N*-acetyltransferase to electrical stimulation may be relevant to the observation that a rhythm in *N*-acetyltransferase activity persists in blinded rats and in rats kept in constant darkness (*3*). The physiological response of pineal *N*-acetyltransferase to darkness and to electrical stimulation appears similar; after the initial rise in activity that occurs in both cases, enzymatic activity returns toward basal levels without any apparent change in afferent input to the pineal.

In vitro studies of pineal function (*3, 4*) have demonstrated that norepinephrine and dibutyryl cyclic adenosine monophosphate cause an increase in *N*-acetyltransferase activity. Norepinephrine also elevates pineal adenyl cyclase activity in vitro (*9*). However, serotonin, which is present in pineal nerves (*10*), is ineffective in raising *N*-acetyltransferase activity in vitro (*3*). Stimulation of *N*-acetyltransferase activity produced by norepinephrine and dibutyryl cyclic adenosine monophosphate can be blocked by cycloheximide (*4, 11*), an inhibitor of protein synthesis. Therefore, the rise in *N*-acetyltransferase activity in vitro is presumably mediated by a norepinephrine-stimulated adenyl cyclase–cyclic adenosine monophosphate mechanism that is dependent on protein synthesis (*11*). Our in vivo results are consistent with this hypothesis. The postganglionic sympathetic fibers to the pineal undoubtedly contain norepinephrine (*12*). Excitation of these nerve fibers by preganglionic sympathetic stimulation presumably releases norepinephrine from pineal nerve endings and, as predicted, increases activity of pineal *N*-acetyltransferase.

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7. The increase in *N*-acetylated product formed can be attributed primarily to increased *N*-acetyltransferase activity and not to increased HIOMT activity. Although there were significantly greater amounts of [^{14}C]melatonin formed, *N*-acetyl[^{14}C]serotonin accounted for the major portion of the increase in *N*-acetylated product. For example, comparing 2-hour stimulated animals to decentralized controls, the amount of [^{14}C]melatonin formed showed a 36 percent increase ($P < .02$), but the amount of *N*-acetyl[^{14}C]serotonin formed showed a 249 percent increase ($P < .001$), and the ratio of *N*-acetyl[^{14}C]serotonin to [^{14}C]melatonin formed showed a 123 percent increase ($P < .01$). A decrease in HIOMT activity could also have accounted for the increase in *N*-acetyl[^{14}C]serotonin, but HIOMT activity was certainly not decreased since there was an increase in [^{14}C]melatonin formed.
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Subacute Sclerosing Panencephalitis: Isolation of Suppressed Measles Virus from Lymph Node Biopsies

Abstract. *Measles virus was isolated in mixed cultures of lymph node cells and HeLa cells. The agent was isolated by cocultivation from biopsy specimens of two of five patients with subacute sclerosing panencephalitis. The virus was identified by hemagglutination-inhibition, immunofluorescent, and neutralization tests. Biopsies from controls did not show evidence of measles virus.*

Subacute sclerosing panencephalitis (SSPE), a fatal disease of children and young adults, has been shown to be associated with a suppressed measles virus infection of the central nervous system (*1–4*). One characteristic laboratory finding in SSPE is an extremely high concentration of measles antibodies in the serum and spinal fluid of virtually all known cases of this chronic neurological disease (*5, 6*). Despite the presence of circulating antibodies, the disease inexorably progresses to death.

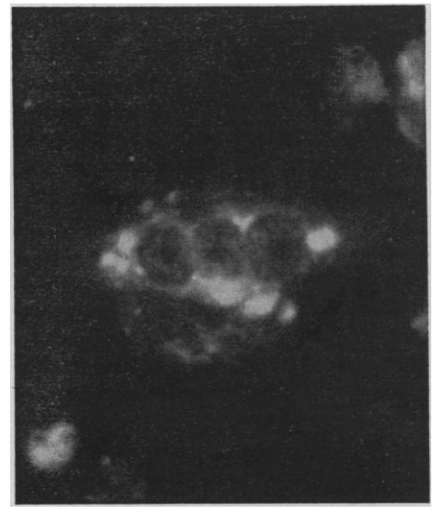

Fig. 1. Cocultivation of lymph node cells and HeLa cells demonstrating measles antigen by immunofluorescence (× 540).

The present report demonstrates that the infection is not limited to the central nervous system and suggests that SSPE may be associated with a specific defect of cellular immunity.

Lymph node biopsies from five patients with SSPE and five controls were included in this study (*7*). Although these specimens were obtained at different times, they were handled and processed in a similar fashion. Clinical and laboratory data supported the diagnosis of SSPE.

A part of each node was washed in Hanks balanced salt solution and minced into small fragments which were then suspended in Eagle's minimum essential medium (EMEM) enriched with 20 percent fetal bovine serum (FBS). After 3 days the cultures showed fibroblasts growing out of the edge of the explants and nonattaching cells which were presumed to be lymphocytes dispersed in the medium. Both types of cells appeared free of