AMP, such as iodide metabolism, hormone synthesis, phospholipid metabolism, and proteolysis (*7*). These functions do not appear to be dependent on alteration in the cell nucleus, and our histochemical findings are consistent with this conclusion. However, we cannot exclude an increase in nuclear cyclic AMP which may not have been detected by this staining method.

The presence of cyclic GMP in intimate proximity to the plasma membrane was rather surprising for several reasons. First, unlike membrane bound adenylate cyclase, guanylate cyclase has been reported to be a soluble enzyme in most tissues examined (*8*). Finding the reaction product of this nucleotide in close association with the plasma membrane suggests that guanylate cyclase might be membrane bound and may become dissociated from plasma membrane during the process of tissue homogenization. Alternatively, cyclic GMP may have a high affinity for some membrane protein of the thyroid follicular cell.

These observations suggest that patterns of cyclic nucleotide localization as demonstrated by immunocytochemistry may be helpful in elucidating the roles of the cyclic nucleotides in cell function. The localization of cyclic GMP in close proximity to the thyroid follicular cell membrane adjacent to the follicular colloid raises the possibility that cyclic GMP may have a role in moderating thyroglobulin iodination since thyroglobulin iodination is thought to occur adjacent to the follicular cell membrane (*9*).

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Pineal β-Adrenergic Receptor: Diurnal Variation in Sensitivity

Abstract. *The responsiveness of the pineal β-adrenergic receptor that regulates serotonin-*N*-acetyltransferase activity is nearly ten times greater at the end of the light period (0600 to 1800 hours) than at the end of the dark period (1800 to 0600 hours). These changes in sensitivity of the postsynaptic β-adrenergic receptor are related to diurnal changes in the release of noradrenaline from sympathetic nerves innervating the pineal. Supersensitivity of the receptor appears to result from decreased release of the neurotransmitter during daytime, and subsensitivity from increased release at night.*

Supersensitivity in cholinergic and in adrenergic nervous systems occurs after denervation, decentralization, or treatment with certain drugs. Subsensitivity is most frequently manifest as a result of a repetitive stimulus or repeated administration of drugs. A distinction is frequently made between presynaptic and postsynaptic changes in sensitivity. Presynaptic (or prejunctional) changes in sensitivity result from treatment with agents which change the action of nerve terminals on the release, uptake, or local metabolism of the neurotransmitter. Postsynaptic (or postjunctional) changes are changes in the responsivity of the receptor or of the end organ itself to a given stimulus (*1*).

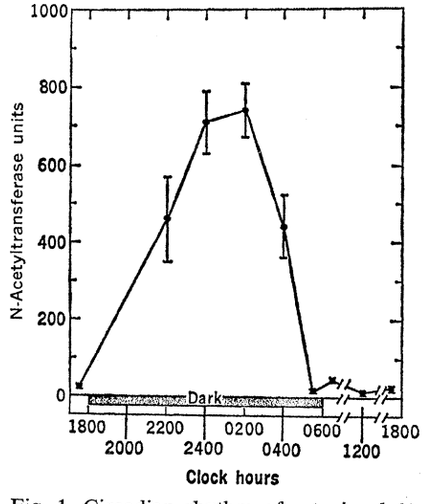

Fig. 1. Circadian rhythm of rat pineal *N*-acetyltransferase activity showing decline beginning 4 hours before the onset of light. Each point is the mean ± the standard error of six pineal glands; the results are expressed as picomoles of ^{3}H-labeled *N*-acetyltryptamine per pineal gland per 10 minutes.

Our laboratory has recently reported the phenomenon of supersensitivity and subsensitivity in the pineal gland (*2*). A β-adrenergic receptor in the pineal cells regulates the synthesis of serotonin-*N*-acetyltransferase (E.C. 2.3.1.5), an enzyme involved in the synthesis of the hormone melatonin (*3*). Removal of endogenous neurotransmitter noradrenaline by preliminary treatment of rats with reserpine, by denervation, or exposure to continuous light results in increased responsiveness of the enzyme to induction by the β-adrenergic agonist isoproterenol (*2*). Repeated administration of isoproterenol to normal animals without any surgical or other pharmacological intervention causes subsensitivity and abolishes the supersensitivity resulting from denervation or reserpine administration. Hence, the prior degree of interaction of neurotransmitter with the receptor determines the subsequent responsiveness of the organ to further stimulation (*1, 2*).

There is a marked circadian rhythm in the activity of serotonin-*N*-acetyltransferase, which increases 30- to 50-fold during the night as compared to daytime values (*4*). This rhythm persists in constant darkness and is abruptly abolished by light (*4, 5*) or by blockade of β-adrenergic receptors (*5*), which cause precipitous falls in enzyme activity.

The circadian rhythm of *N*-acetyltransferase appears to be driven by diurnal changes in the turnover (and presumably release) of noradrenaline from sympathetic fibers innervating the

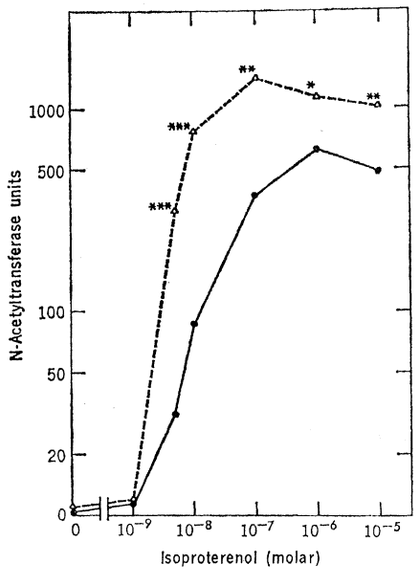

Fig. 2. Diurnal change in sensitivity of rat pineal *N*-acetyltransferase (picomoles per gland per 10 minutes) to activation by isoproterenol in organ culture. Dose-response curves for pineal glands obtained early (1830 hours) in dark period (△- - -△) and late (0530 hours) in dark period (●—●). Points are geometric means for five to six determinations. Data were analyzed for significance by Student's *t*-test performed on mean $\log_{10}$ (± 95 percent confidence intervals) for each point: * < .05; ** < .01; *** < .001.

pineal gland (*6*). The turnover of noradrenaline in rat pineals is accelerated during the night (*6*).

In a study of the activity of *N*-acetyltransferase throughout the night we found that enzyme activity begins to fall about 4 hours before the onset of daylight. This early fall in enzyme activity and the remarkable amplitude of the nighttime peak of *N*-acetyltransferase activity, when considered in the context of increased turnover of noradrenaline during the night, led us to study whether changes in sensitivity of the β-adrenergic receptor occurred during a 24-hour period.

Female Osborne Mendel rats (NIH strain, weighing 180 to 220 g), given free access to food and water, were kept under diurnal lighting conditions with the lights on between 0600 and 1800 hours, for at least 5 days before the experiments. Rats were killed by decapitation (at night, with the help of dim red light), and the pineal glands were removed within 30 seconds. Some glands were immediately assayed for *N*-acetyltransferase, and other glands were placed in organ culture at 35°C in BGJb culture medium (334 milliosmolar, Grand Island Biological Co.), supplemented with streptomycin (100 μg/ml), penicillin (100 unit/ml), and glutamate (20 m*M*). Four pineals were incubated in 2.5 ml of culture medium in Falcon plastic petri dishes (60 mm in diameter) continuously flushed with 95 percent O_2 and 5 percent CO_2. After 10 hours of incubation, in the presence of various concentrations of isoproterenol, the glands were assayed for *N*-acetyltransferase activity, which was assayed as described (*7*), with the use of 20 nmole of acetyl coenzyme A.

N-Acetyltransferase activity was monitored throughout the 24-hour period (Fig. 1). Our experiments confirmed and extended previous observations (*2*, *4*). *N*-Acetyltransferase activity remained low throughout the day; with the onset of darkness enzyme activity increased 30- to 40-fold over a period of 4 to 6 hours. Peak enzyme activity occurred between midnight and 0200 hours. After 0200 hours, enzyme activity rapidly fell to low daytime levels.

There were several possible mechanisms that could explain this early fall in enzyme activity: (i) development of subsensitivity to neurotransmitter; (ii) decreased release of endogenous neurotransmitter; (iii) onset of an inhibitory or modulatory signal; or (iv) the exhaustion of a precursor essential for the synthesis or activation of the enzyme.

To examine whether or not there were changes in sensitivity, pineals were removed at the beginning (1830 hours) and at the end (0530 hours) of the dark cycle and cultured in vitro in the presence of various concentrations of isoproterenol as described above. *N*-Acetyltransferase activity was then measured after 10 hours in organ culture. Response to isoproterenol (10^{-8} *M*) early in the dark period was eight to ten times greater than at the end of the dark period (Fig. 2). The early morning (0530 hours) curve is to the right of the evening (1830 hours) curve. There is also a decrease in the slope of the steep portion of the dose-response curve, as well as a decreased maximal response.

Since isoproterenol is not subject to presynaptic reuptake mechanisms (*8*), the changes in responsiveness we describe are postjunctional. Of greater significance is the fact that these changes are endogenous changes occurring as a function of circadian variation in neuronal activity (*6*). With decreased sympathetic activity during the day, the pineal becomes more sensitive; with increased activity at night, it becomes less sensitive.

In contrast to most reports of supersensitivity induced by pharmacological or surgical intervention (*1*), the changes described are rapid, occurring within less than 12 hours. Although previous reports in the cholinergic system of the cat iris had described super- and subsensitivity in response to physiologic stimuli (light intensity) (*9*) we now report diurnal changes in sensitivity in response to endogenous events.

The 40-fold rise in enzyme activity after the onset of darkness can then be accounted for by a small increase in the concentration of neurotransmitter at the receptor sites at a time when these receptors are maximally sensitive. In addition the fall in enzyme activity late at night could similarly result from a decrease in sensitivity of the receptors, coupled with an early slowing in the noradrenaline turnover. Thus, concurrent changes in receptor sensitivity and signal intensity would serve as a unique amplification and dampening system whereby small changes in catecholamine concentration at the receptor site produce 30- to 40-fold changes in enzyme activity.

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