bohydrate, including about 31 percent *N*-acetylhexosamines and 10 percent *N*-acetylneuraminic acid; the remainder is peptide (*5*). Figure 1 shows that the far-ultraviolet curve of Ca 851 has a minimum around 216 m$\mu$, thus it probably is largely disordered. It is devoid of $\alpha$-helix but might have a low content of the $\beta$ conformation (*10*). The nonhelical structure of this glycoprotein is confirmed by circular dichroism measurements (Fig. 2) and by its $b_0$ value, which was zero. A blood-group H(O) specific ovarian cyst glycoprotein (Ca 1047, $S_{20,w} = 7.6S$ at a concentration of 0.63 percent) which contains about 35 percent *N*-acetylhexosamine yielded a dispersion curve similar to that of Ca 851; its $b_0$ value was + 63, and thus it is also a largely disordered chain. Featureless rotatory dispersion and circular dichroism curves running near base lines were observed also with a blood-group substances and virus recep- from *Sassafras albidum* which is free of peptide and hexosamine and has a molecular weight of 250,000.

The findings on the two substances from human secretions are in general agreement with those by Beychok and Kabat (*1*). Our observations on the blood-group substances and virus receptors from cell membranes indicate, however, that they possess ordered structures to a significant degree, thus they differ from the glycoproteins in secretions. These findings are also important in considerations of cell membrane structure. Ours appears to be the first observation of the $\alpha$ and $\beta$ conformations in blood-group glycoproteins, and the conformation can be attributed to the peptide parts of the molecules. Also, these studies tempt one to conclude that biological activities of the important cell-membrane glycoproteins depend not only on their terminal carbohydrates but in part on the conformation of their peptide constituents, since the disaggregation product Ca 990-Ki shows not only a lesser conformational order but also considerably lower blood-group and antiviral activities than do Ca 1014 and Ca 989, the much larger aggregates from which it is derived (*5, 11*).

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## Hydroxyindole-O-Methyl-Transferase Activity: Effect of Sympathetic Nerve Stimulation

Abstract. *Stimulation of the preganglionic nerve trunk to the superior cervical ganglion causes a reduction in pineal hydroxyindole-O-methyl-transferase levels which is time-dependent. The results provide direct evidence for a role of afferent input in the control of pineal enzymatic activity.*

There is considerable evidence to support the concept that the level of pineal hydroxyindole-*O*-methyl-transferase (HIOMT), as well as the levels of other pineal constituents, is under neural control (*1*). A circadian rhythm in rat pineal HIOMT has been described in which enzyme activity falls during hours of light and rises during hours of darkness. Blinding by bilateral orbital enucleation or placing the animals in constant light or darkness interrupts rhythms of all pineal constituents studied to date with the exception of serotonin, the rhythm of which apparently persists in blinded animals or animals kept in darkness. Moreover, in animals exposed to continuous light for 7 to 30 days, the levels of HIOMT in the pineal are low, while in those rats which have been kept in darkness or blinded there is an elevated level of the enzyme. This light-dark difference can be abolished by lesions producing bilateral degeneration of the inferior accessory optic tract (*2*) or by superior cervical ganglionectomy. The postganglionic sympathetic fibers arising from the superior cervical ganglion are thought to supply the pineal gland in the rat its sole source of innervation (*3*). Therefore, neural control of HIOMT is undoubtedly mediated via these fibers. In order to obtain direct evidence for such control of pineal enzyme activity, we have studied the effect of stimulating the preganglionic cervical sympathetic fibers on the HIOMT activity in the rat pineal gland.

We assayed HIOMT activity at optimum substrate concentrations, using a modification of the procedure of Axelrod *et al.* (*1*). Individual pineals were homogenized in 0.5 ml of 0.05*M* phosphate buffer (*p*H 7.9). The entire homogenate or an aliquot of it was transferred to a 15-ml glass-stoppered centrifuge tube containing 687 m$\mu$mole of *N*-acetyl-serotonin, 44 m$\mu$mole of purified *S*-adenosyl-methionine (*4*), and 4 m$\mu$mole of methyl-$C^{14}$-*S*-adenosyl-methionine (50 $\mu$c/$\mu$mole, New England Nuclear). The final volume was 850 $\mu$l. After 30 minutes' incubation, at 37°C, 1 ml of 0.2*M* borate buffer, *p*H 10, and 10 ml of chloroform were added. The tube was shaken for 5 minutes, the aqueous phase removed, and the chloroform washed with 1 ml of buffer. Five milliliters of the chloroform extract was transferred to a counting vial, the chloroform evaporated, the residue taken up in 1 ml of ethanol, and the radioactivity measured after the addition of 20 ml of phosphor. A control incubation in which 0.5 ml of *p*H 7.9 buffer was substituted for the enzyme was run concurrently. These blanks were found to be equivalent to those which contained boiled pineal homogenate. The data are expressed as micromicromoles of melatonin formed per pineal per hour.

Rats (90-day-old males of the Holtzman strain) were anesthetized with ether, and bilateral orbital enucleations

Table 1. Effect of stimulation of preganglionic fibers to the superior cervical ganglion. Stimulation was carried out for 4 hours; controls were killed 4 hours postoperatively; $P$ values were obtained by using a paired sample $t$-test. S.E.M., standard error of the mean; N.S., not significant.

| Blinded animals (No.) | Treatment | Hydroxyindole-*O*-methyl-transferase activity ($\mu\mu$mole of melatonin formed per gland, per hour, $\pm$ S.E.M.) | Change (%) from control | $P$ |
|---|---|---|---|---|
| 8 | Stimulated | $305 \pm 16.4$ | $-18$ | $< .01$ |
| 8 | Decentralized | $370 \pm 20.2$ | Control | |
| 8 | Sham operated | $371 \pm 32.6$ | 0 | N.S. |

were performed. Ten to 20 days after surgery animals were placed in one of three groups. Members of these groups were anesthetized with ether and treated as follows: group 1, bilateral decentralization (severing of the preganglionic nerve fibers between the spinal cord and the ganglion) of the superior cervical ganglia; group 2, bilateral decentralization of the superior cervical ganglia followed by attachment on the end of one preganglionic nerve trunk of a bipolar nichrome electrode; group 3, sham operation in which a midline neck incision was made and the preganglionic nerves to the superior cervical ganglion were exposed bilaterally. Those rats in the second group were stimulated with square pulses by using a constant-current stimulator (9 seconds out of each minute, 10 cycle/sec, 10 msec pulse duration, 3 to 5 ma) for 4 hours. Animals were run in sets of three. At any time that a rat was being stimulated, two other rats were in the cage with it, one decentralized and one sham-operated; the rats recovered from the effects of anesthesia approximately 20 minutes postoperatively and were free to run about the cage. The rats were killed with ether and their pineals were removed and placed in dry ice prior to assay. Those stimulated animals in which the electrode was not found to be attached to the nerve or in which the nerve between the ganglion and the electrode had broken were excluded from the experimental series.

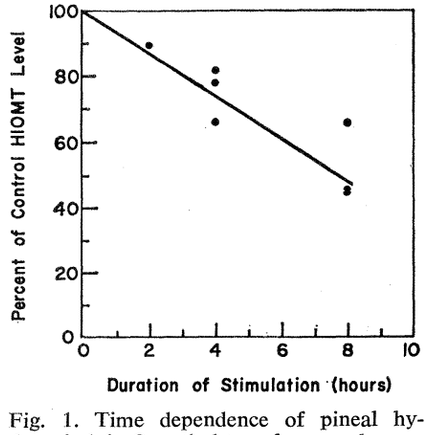


Fig. 1. Time dependence of pineal hydroxyindole-*O*-methyl-transferase changes accompanying preganglionic stimulation of the superior cervical ganglion of animals with bilateral inferior accessory optic tract lesions.

An additional experiment was performed using animals in which unilateral inferior accessory optic tract lesions were made, followed by ipsilateral orbital enucleations (*2*). The animals subsequently suffered bilateral decentralization of their superior cervical ganglia and unilateral stimulation for periods of 2, 4, or 8 hours. In these rats the remaining eye became markedly endophthalmic after the ganglion was decentralized. The endophthalmos could be reversed by stimulation and this reversal served as a convenient measure of the effects of stimulation. The HIOMT levels of the stimulated animals were compared with those of the acutely decentralized controls that were killed after the same period of time as their mates in each case. Brains from stimulated and control animals were examined for lesion localization. All members of the experimental series had large unilateral lesions of the lateral hypothalamus.

Stimulation of preganglionics to the superior cervical ganglion in blinded animals produced a significant reduction in HIOMT of 18 percent, $P < .01$ (Table 1). The fall in HIOMT from levels measured in decentralized controls was linearly dependent on the duration of the stimulation in animals which had had bilateral inferior accessory optic tract lesions (Fig. 1). Decentralization resulted in no significant change in enzyme, by 4 or 8 hours, as compared with sham-operated controls. The effect of stimulation cannot be attributed to the spread of current into neighboring muscle; pineal HIOMT of a group of blind, decentralized rats, the neck muscles of which were stimulated for 4 hours, was unchanged as compared with that of unstimulated controls. The HIOMT activity was additive when homogenized pineals from stimulated animals were incubated together with those from controls. Furthermore, no change in activity was observed with sequential dilution of homogenates of pineals from stimulated rats. Thus, the reduction in enzyme levels cannot be explained in terms of the elaboration of an inhibitory substance.

These studies demonstrate that the level of pineal HIOMT can be modified by stimulating preganglionic nerves to the superior cervical ganglion. This finding is consistent with the view that the changes in enzyme levels observed with changes in environmental lighting are mediated via postganglionic fibers from the superior cervical ganglion. It is of interest, in this regard, that the rate of decline of HIOMT activity between midnight and noon in the intact animal placed in diurnal lighting conditions (*1*) is not dissimilar from that produced by nerve stimulation in blinded animals or animals with bilateral visual accessory tract lesions. Whether or not the changes in enzyme levels which accompany differences in environmental lighting are indeed related to alterations in sympathetic firing rate can be determined only by the monitoring of sympathetic neuronal activity. Our experiments do, however, provide direct evidence that modification of afferent input by nerve stimulation can affect enzyme activity of the innervated structure.

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