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PHOTIC AND NEURAL CONTROL OF THE 24-HOUR NOREPINEPHRINE RHYTHM IN THE RAT PINEAL GLAND

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ABSTRACT

WURTMAN, RICHARD J., JULIUS AXELROD, GÖRAN SEDVALL AND ROBERT Y. MOORE: Photic and neural control of the 24-hour norepinephrine rhythm in the rat pineal gland. J. Pharmacol. Exp. Therap. **157**: 487–492, 1967. The norepinephrine content of the rat pineal gland varies 3-fold during each 24-hr day. Norepinephrine levels are highest at the end of the dark period and fall during the light period. This rhythmic variation in pineal norepinephrine is abolished when animals are blinded or are kept in continuous light or darkness. It appears to be generated by nerve impulses which are initiated by photic stimulation of the retina. These impulses are carried to the brain by the inferior accessory optic tract and reach the pineal by a pathway which includes the preganglionic sympathetic trunk to the superior cervical ganglion.

The rat pineal gland is unusually rich in such biogenic amines as serotonin (Quay, 1963), histamine (Machado *et al.*, 1965), dopamine and norepinephrine (Pellegrino de Iraldi and Zieher, 1966), as well as in enzymes which synthesize and metabolize these compounds (Wurtman *et al.*, 1963; Lovenberg *et al.*, 1967; McGeer and McGeer, 1966; Snyder *et al.*, 1965a). In this species, pineal serotonin is stored in both the parenchymal cells and the sympathetic nerve endings (Bertler *et al.*, 1963), while the norepinephrine is highly localized to the nerve endings (Pellegrino de Iraldi and Zieher, 1966). Pineal sympathetic denervation (by bilateral destruction of the superior cervical ganglia) has been shown to deplete the gland of almost all of its norepinephrine (Pellegrino de Iraldi and Zieher, 1966).

The amounts of serotonin present in the rat (Quay, 1963) and monkey (Quay, 1966) pineal gland are not constant, but vary in a predictable manner during each 24-hr day. Serotonin content is highest in the middle of the daylight period, and falls with the onset of darkness (Quay, 1963). The activity of the pineal enzyme, hydroxy-indole-O-methyl transferase (HIOMT), which converts N-acetylserotonin to melatonin, also varies with a 24-hr period. It is lowest at the end of the day and rises with darkness (Axelrod *et al.*, 1965). Both of these rhythms are controlled by the sympathetic nerves to the pineal gland (Axelrod *et al.*, 1965; Fiske, 1964). This report describes a 24-hr rhythm in the norepinephrine content of the pineal gland and defines some neural pathways responsible for its control.

METHODS. Adult female Sprague-Dawley rats weighing 160 to 200 g were used in all experiments. The rats were maintained under controlled lighting conditions for at least 1 week prior to use. Light was provided by cool white

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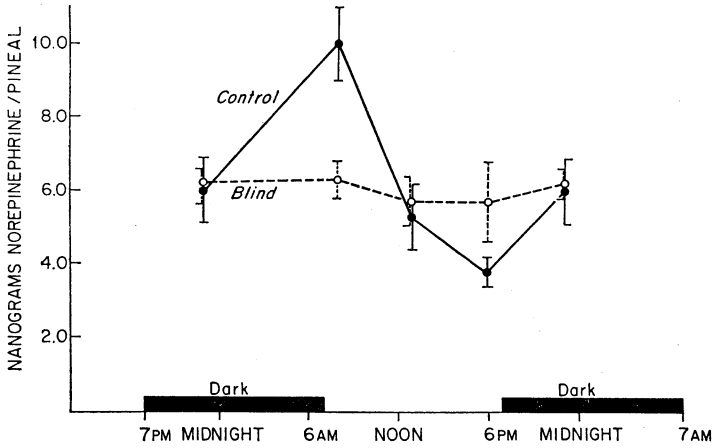

FIG. 1. Twenty-four-hour variation in pineal norepinephrine levels and the effect of blinding. Rats were blinded and maintained with sham-operated controls under diurnal lighting for 5 days. (Results in all figures are shown as mean ± S.E.)

fluorescent lamps; each animal was exposed to about 25 to 50 footcandles of illumination. In "diurnal lighting" experiments lights were on from 7 A.M. to 7 P.M. Animals were blinded by bilateral orbital enucleation (performed under light ether anesthesia), or the preganglionic fibers to their superior cervical ganglia were transected as described before (Snyder *et al.*, 1965b). Bilateral transection of the inferior accessory optic tract was performed by unilateral enucleation and ipsilateral section of the medial forebrain bundle (Moore *et al.*, 1967); the location of each medial forebrain bundle lesion was identified by histologic examination.

The norepinephrine content of the pineal gland at any particular time was determined using glands from groups of 12 to 20 rats. Two or three organs were pooled for each assay. The pineals were homogenized in 5 ml of perchloric acid (0.4 N). After centrifugation, the supernatant was transferred to a 25-ml beaker containing 200 mg of acid-washed alumina, 5 ml of 2 N EDTA and 15 mg of sodium bisulfite. The pH was adjusted to 8.6 with concentrated solutions of potassium hydroxide, and the contents were poured over a column containing an additional 200 mg of alumina which had been brought to pH 8.6. The column was then washed with 0.2 N sodium acetate and glass-distilled water, and the norepinephrine was eluted with three 1-ml portions of 0.2 N acetic acid. Using this procedure, the recovery of catecholamine averaged 70 ± 2%. The norepinephrine was assayed spectrophotofluorometrically (Euler and Lishajko, 1961).

RESULTS. *Twenty-four-hour variation in pineal norepinephrine content and effect of blinding.* Previous experiments had shown that the content of norepinephrine in the rat pineal gland varies markedly during a 24-hr period (Wurtman and Axelrod, 1966). When rats were kept under alternating 12-hr periods of light and darkness, pineal norepinephrine levels were greatest at the end of the dark period and fell continuously during the light period (fig. 1).

To examine the influence of environmental lighting on the rhythms, rats were blinded by bilateral orbital enucleation and kept with sham-operated animals under diurnal lighting conditions for 5 days. They were then killed, and their pineals were analyzed for norepinephrine content. In contrast to control animals, blinded rats showed no alteration in pineal norepinephrine levels during the 24-hr day (fig. 1). At each time point studied, the pineal norepinephrine content in the blinded animals was approximately intermediate between the maximum and minimum values observed in sham-operated rats.

Effect of continuous light or darkness on pineal norepinephrine rhythm. The abolition of the 24-hr changes in pineal norepinephrine levels by blinding suggested that this rhythm was generated by diurnal changes in environmental lighting, perceived by the retinas. If the rhythm were dependent upon cyclic lighting cues, it might be expected to disappear in rats deprived of such cues by maintenance under constant lighting conditions.

Groups of rats were kept under diurnal

lighting or continuous light or darkness for 7 days. Animals were killed at times corresponding to the expected peak and trough in pineal norepinephrine levels (7 A.M. and 7 P.M.), and their glands were assayed for the catecholamine. Among rats kept under diurnal lighting, the pineal norepinephrine content at 7 A.M. was 3 times higher than at 7 P.M. However, the norepinephrine content of glands taken from rats kept in either continuous light or darkness was low and was similar at both times of day (fig. 2).

In another experiment the dependence of the norepinephrine rhythm on light was examined by exposing animals to a reversed lighting schedule (lights on from 7 P.M. to 7 A.M.) for 8 days. The rats were then killed at 4 P.M. (after 9 hr of darkness), and their pineal norepinephrine content was compared with that of control animals killed at 5 P.M. (after 10 hr of light). Although both groups of rats were killed at about the same time of day, animals that were then in darkness had about 3 times as much norepinephrine in their pineal glands as rats which were then in normal diurnal lighting (7.10 ± 0.65 *vs.* 2.33 ± 0.35 ng/gland; mean ± S.E.).

Neural pathway controlling the norepinephrine rhythm in the pineal. It had previously been shown that biochemical rhythms in the pineal gland were abolished following its sympathetic denervation (Axelrod *et al.*, 1965; Fiske, 1964; Snyder *et al.*, 1965b). Because most or all of the norepinephrine in this organ is present within sympathetic nerve endings,

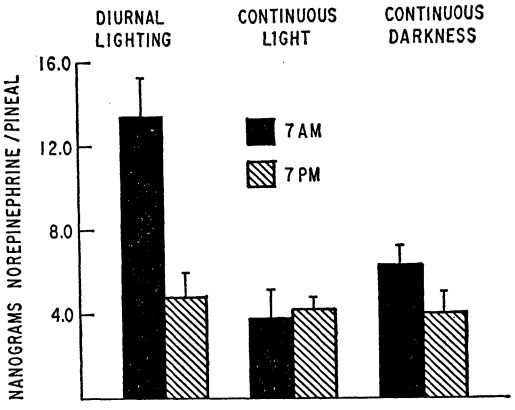

FIG. 2. Pineal norepinephrine levels in continuous light or darkness. Rats were kept under diurnal lighting or continuous light or darkness for 7 days.

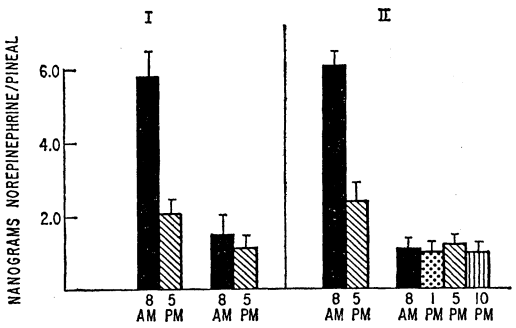

FIG. 3. Pineal norepinephrine levels following preganglionic decentralization of the superior cervical ganglia. Animals were kept under diurnal lighting for 14 days (experiment I) or 21 days (experiment II).

it was not feasible to study the effect of ganglionectomy on the rhythmic changes in pineal norepinephrine levels. Instead, the flow of impulses from the central nervous system to the pineal sympathetic nerves was interrupted by cutting the preganglionic fibers to the superior cervical ganglia. Rats prepared in this fashion and control animals were kept under diurnal lighting for 2 weeks; they were then killed at 8 A.M. or 5 P.M., and their pineals were assayed for norepinephrine. The expected daytime fall in norepinephrine content was observed in pineals from control animals. However, the catecholamine level in the decentralized organs was very low, and was similar at both times studied (fig. 3, part I).

It was possible that the norepinephrine rhythm had persisted in the decentralized gland, but that its period was no longer exactly 24 hr. The times of day corresponding to maximum and minimum norepinephrine levels might then have shifted, thereby obscuring the rhythm. For this reason, another experiment was performed in which rats kept under diurnal lighting were killed at four times of day, 3 weeks after surgery. Again, norepinephrine levels were low and approximately equal in the decentralized rats at all times studied (fig. 3, part II), whereas normal animals showed the usual diurnal variation at 8 A.M. and 5 P.M. These observations indicated that the nervous pathway by which light impulses produce the norepinephrine rhythm includes the brain and the sympathetic nerves to the pineal.

To study the central nervous pathways which

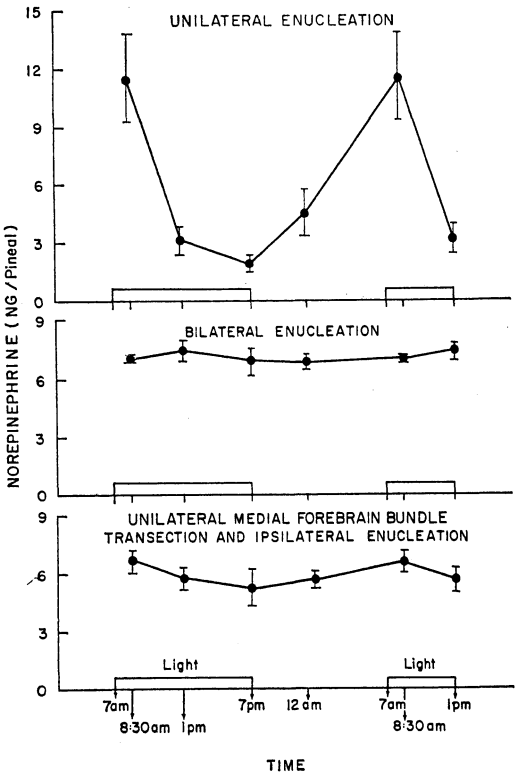

FIG. 4. Twenty-four-hour rhythm in pineal norepinephrine content after blinding or transection of the inferior accessory optic tract (at the level of the medial forebrain bundle). Animals were placed under diurnal lighting 28 days after surgery and killed 14 days later.

mediate this light effect, advantage was taken of the recent observation that the photic control of HIOMT is lost when the medial forebrain bundles are transected (Axelrod *et al.*, 1966). This is because at the site of its transection (the caudal lateral hypothalamus), the medial forebrain bundles contain the fibers of the inferior accessory optic tract (Hayhow *et al.*, 1960). Since this tract is entirely crossed in the rat, the effect of light on HIOMT can also be blocked by cutting the medial forebrain bundle unilaterally and removing the eye on the same (ipsilateral) side of the head (Moore *et al.*, 1967). To determine whether the inferior accessory optic tract also mediates the photic control of the pineal norepinephrine rhythm, animals were prepared in this manner with bilateral section of the inferior accessory optic tracts. Control animals were subjected to a unilateral or bilateral enucleation, but no brain lesion. Four weeks after surgery, all groups were placed under diurnal lighting for 14 days; they were then killed at various times. As expected, the norepinephrine rhythm persisted in animals without one eye, but was abolished following bilateral enucleation. Rats subjected to section of the inferior accessory optic tracts also failed to show a 24-hr variation in their pineal norepinephrine levels (fig. 4).

DISCUSSION. Many biologic functions have been shown to vary with a 24-hr period (Aschoff, 1965). Most of these rhythms appear to be endogenous. They persist when an animal is deprived of external lighting cues (*i.e.*, after being blinded or being kept under continuous darkness). Under these conditions, the rhythmic functions can sometimes be shown to "free-run"; *i.e.*, their period changes from exactly 24 hr to something more or less depending upon the species (Aschoff, 1960). Such rhythms have been called "circadian" (Halberg, 1959).

The mammalian pineal gland appears to be unusual in that at least two of its biochemical functions vary with a 24-hr rhythm which is not endogenous but is generated by the external light input. The daily rise and fall in HIOMT activity can be abolished if rats are blinded or are maintained in darkness (Axelrod, 1965). The studies described in this report indicate that a similar exogenous mechanism generates the 24-hr rhythm in pineal norepinephrine content. Evidence for this hypothesis may be summarized as follows: 1) the rhythm is lost in blind animals, even though they are maintained under diurnal lighting (fig. 1); 2) pineal norepinephrine levels are the same at 7 A.M. as at 7 P.M. in rats kept in continuous darkness (fig. 2); 3) cutting the inferior accessory optic tract abolishes the norepinephrine rhythm in the pineal even though the animals are kept under diurnal lighting and can perceive visual cues through the remaining eye and the primary optic tracts (fig. 4). The experiments described here indicate that the photic information which generates the norepinephrine rhythm reaches the rat pineal gland *via* the following route. Light impinges upon the retina, generating nerve impulses. These travel *via* the inferior accessory optic tract to its terminal nucleus in the rostral midbrain

tegmentum (Hayhow *et al.*, 1960; Moore *et al.*, 1967). From this point they are carried *via* an unknown pathway through the brainstem and spinal cord to the neurons of the intermediolateral cell column, which supply the preganglionic innervation of the superior cervical ganglia. They then traverse the postganglionic sympathetic axons of the nervi conarii to the parenchymal cells of the pineal gland.

Several possible mechanisms could operate at the cellular level to produce the 24-hr rhythm in pineal norepinephrine content. First, more norepinephrine could be synthesized at night. This is suggested by the observation that tyrosine hydroxylase activity in the rat pineal varies over a 24-hr period in the same manner as norepinephrine content (McGeer and McGeer, 1966). Since this enzyme is thought to control the rate of norepinephrine synthesis *in vivo* (Levitt *et al.*, 1965), increased tyrosine hydroxylase activity may represent a basis for increased norepinephrine synthesis. Second, less norepinephrine might be released from the pineal sympathetic nerves at night while synthesis continued at a steady rate. A similar mechanism has been suggested as the basis for the rhythm in pineal serotonin content (Snyder and Axelrod, 1965). Third, more norepinephrine might be metabolized *in situ* by monoamine oxidase or catechol-O-methyl transferase during the daytime. There is no evidence to support this, however, and *in vitro* studies have failed to demonstrate any changes in monoamine oxidase activity during the 24-hr day (Snyder and Axelrod, 1965).

The synthesis of norepinephrine in sympathetically innervated organs appears to depend upon the rate at which impulses reach the nerve endings. Ganglionic decentralization depresses the rate at which isotopically labeled tyrosine is converted to norepinephrine in the rat salivary gland (Musacchio and Weise, 1965); ganglionic stimulation has an opposite effect (Sedvall and Kopin, 1967). Similarly, pineal norepinephrine levels fall following decentralization of the superior cervical ganglion (fig. 3; Pellegrino de Iraldi and Zieher, 1966). Thus, it is possible that the nocturnal increase in pineal norepinephrine levels results from enhanced sympathetic nervous activity, perhaps in response to darkness. Further evaluation of this hypothesis would require direct monitoring of sympathetic electrical activity under conditions of light and darkness in nocturnal species.

The observations reported here are consistent with the hypothesis that a cyclic variation in the release of norepinephrine from sympathetic nerve endings controls some or all of the other 24-hr rhythms in pineal biochemical function. A similar rhythm in norepinephrine content has been demonstrated in the salivary gland (Wurtman and Axelrod, 1966). It would seem possible that such cyclic variations in tissue norepinephrine content and release might result in parallel alterations in the physiologic responses to drugs which act by releasing catecholamines.

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