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Light and Propranolol Suppress the Nocturnal Elevation of Serotonin in the Cerebrospinal Fluid of Rhesus Monkeys

Abstract. *Markedly elevated nighttime concentrations of serotonin in rhesus monkey cerebrospinal fluid were reduced to daytime levels by exposing the monkeys to continuous light or to the β-adrenergic antagonist propranolol. Nighttime elevations of melatonin in cerebrospinal fluid were also suppressed by propranolol and light. Serotonin released in large quantities at night appears to be regulated like melatonin, and may act as a cerebroventricular hormone to influence brain and pituitary function at night.*

Concentrations of serotonin in rhesus monkey cerebrospinal fluid (CSF) reach nocturnal peaks that average 20 ± 7 times the mean daytime values and range up to 70 times higher (*1*). This large diurnal rhythm for serotonin went undetected until the development in 1982 of a sensitive gas-chromatographic mass-spectrometric assay that permitted quantitation of the amine in CSF (*1*). Diurnal rhythms in brain serotonin content have been found in many studies; however, these rhythms vary in phase in different brain regions and nuclei and in different species and are of relatively small amplitude (all less than twofold) (*2*). A diurnal rhythm in another neurotransmitter amine, norepinephrine, has been described in rhesus monkey CSF; peak elevations average 1.7 times higher than the nighttime troughs and occur at midday in coincidence with activity and temperature maxima (*3*).

The origin of CSF serotonin and the basis for its nighttime release are not known. A temporal relation of serotonin to the circadian variation in melatonin concentrations observed in one monkey (*1*) suggested that serotonin, the precursor of melatonin biosynthesis in the pineal gland, is released from the pineal directly into the cerebroventricular system. This seemed possible because the pineal in primates comprises a portion of the roof of the third ventricle and because the pineal contains very high concentrations of serotonin that decrease 80 percent at night in the rhesus monkey (*4*, *5*). Other evidence, however, indicates that melatonin from the pineal in sheep, rodents, and man is released into the blood and reaches the CSF compartment secondarily (*6*); such a route would be unlikely for pineal serotonin since serotonin crosses the blood-brain barrier poorly and since there is only slight evidence for diurnal variations in blood serotonin content (*7*).

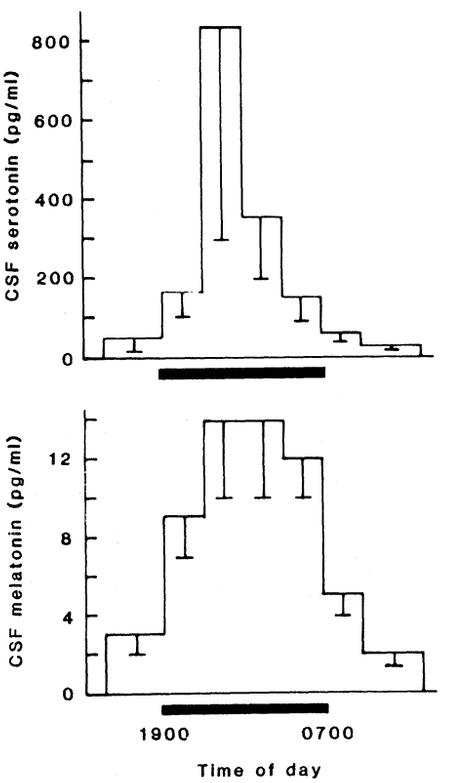


Fig. 1. Diurnal variations in CSF serotonin and melatonin in six monkeys. Lights (approximately 500 lux at eye level) were on from 0700 to 1900. Water was always available and food (Ralston Purina Monkey Chow No. 5038) was provided at 0900 and 1600 and was not withdrawn. CSF samples were collected at 90-minute intervals and pooled for analysis. The following time points are represented, left to right: 1500 to 1930, 1930 to 2230, 2230 to 0130, 0130 to 0430, 0430 to 0730, 0730 to 1030, and 1030 to 1500. Values are means ± standard errors.

To determine whether the diurnal rhythm for CSF serotonin is regulated like the diurnal rhythm for melatonin, we studied rhesus monkeys during normal light-dark cycles and under conditions that alter the release of pineal melatonin. Adult male *Macaca mulatta* were maintained on a 12-hour light-dark cycle and adapted to chair restraint. Under anesthesia a cannula was inserted between the lumbar vertebrae and advanced to the cervical subarachnoid space. After a 48-hour recovery period CSF was collected in 90-minute samples through the cannula, which was encased in a water jacket cooled to 10°C and which led into a fraction collector housed in a freezer at −40°C (*8*). Melatonin was quantified by radioimmunoassay (*9*) and serotonin by capillary gas chomatography and negative chemical ionization mass spectrometry after derivatization with pentafluoropropionic anhydride (*1*).

Serotonin and melatonin rhythms were temporally coincident in all six monkeys studied, with concentrations of both substances remaining low during the day, rising at the beginning of the dark period, and falling to baseline at the onset of light (Fig. 1). The peak concentration of serotonin at nighttime was 841 ± 541 pg/ml (from 2230 to 0130), while the mean concentrations during the entire dark and entire light periods were 378 ± 146 and 46 ± 10 pg/ml, respectively.

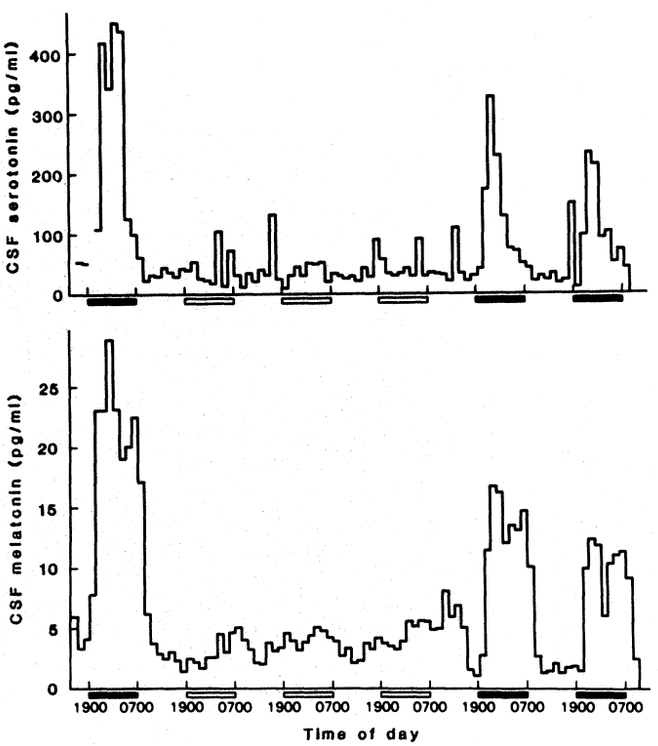
Since light suppresses nocturnal secretion of melatonin in rodents and monkeys (*10*), we exposed one monkey to three consecutive 24-hour periods of continuous light, followed by 2 days of 12-hour light-dark cycles. The large nocturnal elevations in CSF serotonin and melatonin were abolished by exposure to constant light (Fig. 2). Returning the animal to a light-dark cycle immediately reestablished the nocturnal rise in serotonin and melatonin. Similar responses to light suppression were seen in other animals (Fig. 3). The marked nocturnal

increase in serotonin exhibited peaks from 300 to 3500 pg/ml in four monkeys, while in constant light the mean concentration of serotonin in these monkeys was reduced to 53 ± 18 pg/ml.

The suppression by light of the nocturnal increase in serotonin favors the suggestion that diurnal rhythms for serotonin and melatonin are regulated in a similar manner. The lack of a nocturnal elevation of serotonin in two monkeys who, for unknown reasons, also lacked a diurnal melatonin rhythm in our pilot study (*1*) offers additional support for this suggestion. In a second experiment we sought to determine whether propranolol, which suppresses melatonin formation in rodents (*11–13*), might also alter serotonin levels in monkey CSF. Propranolol's properties as a β-adrenergic antagonist are thought to be responsible for blocking the nighttime signal from the suprachiasmatic nucleus to the pineal gland via noradrenergic ganglionic fibers (*14*), and it seemed that propranolol might act like light to prevent the usual nocturnal rise in serotonin.

Propranolol markedly reduced the melatonin and serotonin elevations in monkeys kept on a light-dark schedule but given the drug 1 hour before darkness (Fig. 3). This finding is congruent with the light suppression data, but would not have been predicted from the model of pineal melatonin generation developed on the basis of rodent studies. According to this model, production of adenosine 3′,5′-monophosphate resulting from the stimulation by norepinephrine of β-adrenergic receptors on pinealocyte membranes leads to a marked increase in the activity of *N*-acetyltransferase, the rate-limiting enzyme in the pathway for melatonin synthesis from serotonin (*14*). While it is known that pineal serotonin concentrations decrease at night in rodents and monkeys, this change has been interpreted as being primarily a consequence of substrate utilization during melatonin production (*12, 15*). However, our observation of an effect of propranolol on serotonin release suggests that the mobilization and release of serotonin is an adrenergically mediated event occurring before or during melatonin generation. The similar suppressive effects of light and propranolol on nocturnal elevations of serotonin and melatonin indicate similar regulatory influences on these two indoleamines and indicate a possible pineal origin of the serotonin in CSF. In fact, other data on primates and rodents indicate that serotonin synthesis, turnover, and nocturnal release in pinealocytes may be sufficient to account for the serotonin concentrations found in the CSF at night (*16*). Other possible sources of CSF serotonin are the supra- and subependymal plexuses of serotonin-containing axons that line the brain ventricles of rhesus monkeys, humans, and rats, and whose terminals extend directly into the ventricles (*17*). It is not known whether the serotonin content of these neurons varies diurnally like that of the pineal gland or whether propranolol might affect them. There is, however, evidence for noradrenergic and serotonergic interactions elsewhere, including α- and β-adrenergic modulation of serotonin release (*18*).

A nocturnal, light-regulated flooding of serotonin through the cerebroventricular system may play a role in other diurnal physiological alterations, including components of the sleep-wake cycle and certain neuroendocrine rhythms such as those of growth hormone, prolactin, and follicle-stimulating hormone (*19*). The nighttime increase in the concentration of prolactin in plasma and CSF can be induced in daylight hours by giving serotonin intraventricularly or by peripheral administration of the serotonin precursors L-tryptophan and 5-hydroxytryptophan (*20*). Release of follicle-stimulating hormone is suppressed equally as well by intraventricular serotonin as by melatonin (*20*). Although it is not known whether the target sites for melatonin's multiple effects on reproduc-



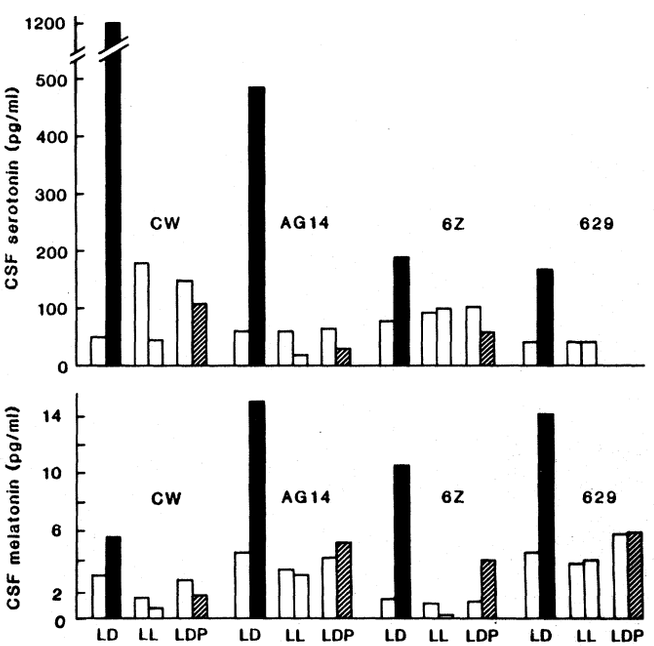


Fig. 2 (left). Changes in serotonin and melatonin concentrations in CSF collected continuously for 90-minute periods in one monkey exposed to 12 hours of light and 12 hours of darkness, 3 days of constant light, and 2 days of 12-hour light-dark cycles. Fig. 3 (right). Effects of exposing individual monkeys to light-dark (*LD*), light-light (*LL*), and light-dark plus propranolol (*LDP*) on mean concentrations of serotonin and melatonin in CSF. Lights were on from 0700 to 1900 for LD and LDP animals. The latter received 20 mg of DL-propranolol hydrochloride per kilogram (subcutaneously). In a pilot study of two animals, DL-propranolol (20 mg/kg) given orally was also effective in suppressing nighttime serotonin and melatonin elevations, which resumed on the subsequent night.

tion are central or peripheral (*21*), the concentration of CSF serotonin at night exceeds that of melatonin 50-fold and might contribute to some of the effects attributed to melatonin. Serotonin and related indoleamines (*21*) may thus contribute to diurnal alterations in physiological functions, a suggestion in keeping with the possible role of the cerebroventricular system as a conduit for neurohormonal integration of brain function (*22*).

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Endogenous Inhibitors of Monoamine Oxidase Present in Human Cerebrospinal Fluid

Abstract. *Inhibitory activity against the enzyme monoamine oxidase is present in low molecular weight fractions (less than 100,000) of human cerebrospinal fluid. These endogenous substances of different molecular weights (3000 to more than 35,000) act like monoamine oxidase inhibitor drugs to inhibit both type A and type B monoamine oxidase.*

There has been intense interest in monoamine oxidase (MAO) activity since the reports by Murphy *et al.* and others, which linked low platelet MAO activity to schizophrenia and schizophrenic symptoms (*1*). Inhibitors of MAO have been identified in blood (*2*) and urine (*3*). We report the isolation from human cerebrospinal fluid (CSF) of low molecular weight substances that inhibit both type A and type B MAO. The pharmacological effects of these endogenous inhibitors and of the MAO inhibitor drugs are similar: both reduce the activity of MAO toward its substrates. The presence of these inhibitors in CSF indicates that there may be a naturally occurring endogenous mechanism for the regulation of MAO activity in the brain.

The oxidation of monoamine neurotransmitters by MAO is one of the brain's major metabolic mechanisms for the degradation and inactivation of these neurotransmitters (*4*). In the brain, MAO exists in two forms, type A and type B (*5*). Both type A MAO, which deaminates dopamine, norepinephrine, and serotonin, and type B MAO, which also deaminates dopamine and norepinephrine but not serotonin, can be inhibited by antidepressant drugs of the MAO inhibitor type (*6*). The MAO inhibitor drugs are psychotogenic in schizophrenia, presumably as a result of increased dopamine concentrations after MAO inhibition. Schizophrenia has been related to increased brain concentrations of dopamine (*7*). Conversely, MAO inhibitor drugs are therapeutically effective against depressive diseases; these diseases have been linked to decreased brain concentrations of norepinephrine, serotonin, and dopamine (*8*). The MAO inhibitor drugs increase brain concentrations of these neurotransmitters by decreasing their inactivation by MAO (*9*). The therapeutic effects of these drugs have been postulated to derive from the increased neurotransmitter concentrations.

There may be an association between low concentrations of plasma MAO inhibitors and depressive symptoms that are responsive to MAO inhibitor drugs. Persons with high concentrations of platelet type B MAO activity have been shown to have lower concentrations of endogenous MAO inhibitors in their plasma than persons with midrange or low concentrations of platelet type B MAO activity (*2*). High concentrations of platelet type B MAO activity have