THE PHARMACOLOGY OF THE PINEAL GLAND

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INTRODUCTION

Only recently have a sufficient number of publications been available to legitimize a review of the pharmacology of the mammalian pineal organ. Two decades ago Kitay & Altschule reviewed the world literature on pineal physiology, which comprises several thousand papers, and concluded only that removal of the pineal, or administration of pineal extracts, somehow affected pigmentation in lower vertebrates and gonadal function in mammals (1). As the studies described below demonstrate, much more information is now available concerning the pharmacology of the pineal. This review subdivides present knowledge into two areas: (*a*) the effects on mammals of administering pineal extracts or pure synthetic or natural pineal constituents and (*b*) the effects of drugs and hormones on the pineal itself.

As might be anticipated, the bulk of studies cited in both categories deals with the pineal hormone, melatonin. Melatonin was first isolated from bovine pineal extracts in 1958 by Lerner and his colleagues (2), who used as a marker the capacity of the hormone to aggregate the pigment granules in amphibian melanophores around the cell nucleus. Five years later, Wurtman et al (3) showed that melatonin affected a physiological function in mammals, that is, the size and secretion of the ovary, and subsequent studies have demonstrated that melatonin administration also modifies the growth, composition, and functional activities of numerous other organs. Only recently an assay was developed that allows quantification of the melatonin in human urine (4). The concentrations of the compound vary with a characteristic daily rhythm, peaking at night. The pineal's apparent role as the sole or major source of melatonin, the presence of melatonin in urine, and the demonstration that physiologic effects follow a pinealectomy or the administration of melatonin seem to justify labeling it a pineal hormone.

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Melatonin synthesis and pineal biosynthetic activity are generally controlled by the sympathetic nerves of this organ (5, 6). Therefore, it should not be surprising that drugs known to modify the synthesis, release, or metabolism of norepinephrine in peripheral organs also affect pineal function. Melatonin is itself a derivative of another biogenic amine, serotonin, whose metabolism and actions are also affected by numerous drugs. Indeed, the pineal has often provided an apt tool for examining monoaminergic mechanisms for pharmacologists not specifically concerned with its particular functional properties.

EFFECTS OF MAMMALIAN PINEAL EXTRACTS ON GONADAL FUNCTION

The ability of mammalian pineal organ constituents to modify gonadal function has been recognized for at least four decades. Initial reports (7) were interpreted as showing that pineal extracts stimulated the gonads. However, O. Fischer (8), and then Engel (9), and E. Fischer (10) showed that the administration of pineal extracts to rats or mice slowed gonadal maturation (i.e. it delayed the spontaneous rupture of the vaginal membrane). In 1954, Kitay & Altschule (11) produced ovarian atrophy by giving young rats daily injections of a bovine pineal extract. This effect was confirmed in 1959 by Wurtman et al (12) who used a protein-free extract, and it was subsequently shown (13, 14) that pineal extracts, as well as media in which rat pineals have been incubated (15), can suppress the compensatory hypertrophy of the remaining ovary after unilateral gonadectomy.

In 1960, Fiske et al (16) demonstrated that maintaining rats in a continuously illuminated environment caused pineal weight to decrease. [It had been previously shown (17–19) that this treatment also caused persistence of estrous cytology in rat vaginal smears.] Suspecting that the ovarian effects of light might be related to an inhibition of the synthesis of an antigonadal factor in the pineal, Wurtman et al (20) in 1961, administered bovine pineal extracts to light-treated rats, and found that such extracts blocked the ovarian hypertrophy. In 1962, Ifft (21) provided further evidence that light suppresses the synthesis of antigonadal factors in the pineal by showing that treatment with pineal extracts interrupted the persistent vaginal estrus in light-treated animals. The ability of pineal extracts to block light-induced changes in ovarian function provided the experimental basis for the original evidence that melatonin was the—or at least one of the—antigonadal factor(s) in pineal extracts (3). Once this evidence was obtained, most investigators stopped using crude or partially purified pineal extracts to explore pineal function in favor of melatonin and other pure compounds that had been identified in these extracts.

In 1963, Reiss and his co-workers (22) reported that while pineal extracts decreased gonadal weight in mature animals, they stimulated gonad growth in immature mice, rats, and rabbits. (A similar age-dependence was observed in the effects of pineal extracts on pituitary and thyroid weights.) In 1961, Meyer et al (23) observed that the same bovine pineal extracts that reversed the persistent vaginal estrus caused by exposure to continuous illumination also corrected the spontaneous persistent estrus exhibited by "middle-aged" (18-month-old) rats.

Evidence has been adduced that a constituent of pineal extracts directly inhibits gonadal responses to gonadotropins. Thus, Soffer et al (24) in 1965, and Hipkin (25) in 1970, observed that the concurrent administration of pineal extracts suppressed the increase in uterine weight caused by giving human chorionic gonadotropin (HCG) to immature mice. Extracts of human pineals failed to modify this response to HCG (26). Davis (27), in 1971, noted that bovine pineal extracts potentiated the direct effects of another pituitary hormone, pitocin, on contractions of the perfused rat uterus.

Several investigators have prepared pineal extracts that allegedly lack melatonin and that produced antigonadal effects in rats and mice. Benson and his associates reported that (*a*) a "melatonin-free" extract, prepared by subjecting aqueous extracts of human pineals to centrifugation and ultrafiltration, partially blocked compensatory ovarian hypertrophy (28); (*b*) an aqueous extract of bovine pineals, allegedly containing only 0.0035 μg per dose of melatonin, caused as much inhibition of compensatory ovarian hypertrophy as 30 μg of melatonin in pure solution (29); (*c*) an isobutanol extract of pineals subjected to ultrafiltration also suppressed this phenomenon in 6- to 10-week-old mice to a greater extent than could be attributed to its melatonin content (30); and (*d*) aqueous extracts subjected to ultrafiltration decreased the postcastration rise in serum luteinizing hormone (LH) in rats (31). A similar suppression of compensatory ovarian hypertrophy was noted by Vaughan & Reiter (32) in 1972, and by Bensinger et al (33) in 1973, working in Reiter's laboratory. In contrast, Debeljuk (34) could find no effects of a melatonin-free pineal extract on the weight of the gonads, or on pituitary LH concentration. Studies reporting antigonadal effects of pineal homogenates that have been treated to remove their melatonin are certainly of some significance, if only because they encourage exploration for additional antigonadal pineal constituents, which might be hormones. However, their interpretation is complicated by a number of problems: (*a*) quantitation of the melatonin concentrations in the extracts, before and after organic extraction or other treatment, has been crude at best; and (*b*) it seems likely that the extraction procedure removes other substituents of the pineal homogenates in addition to melatonin, and contaminates the homogenates with small amounts of organic solvents which could modify biologic responses to authentic pineal constituents in test animals. Positive findings using such extracts have, so far, been obtained by only two laboratories.

Moszkowska and her co-workers have utilized Sephadex G-25 gel filtration to separate various peptide fractions from pineal extracts. When given to rats or added to brain cultures, their F3 fraction reportedly increases the concentrations of the releasing factor(s) for follicle-stimulating hormone (FSH) and LH in the hypothalamus (35). Its administration, or the administration of an ultrafiltrate (36), also decreases gonadal weight, presumably by suppressing the release of hypothalamic-releasing factors and, thereby, of pituitary gonadotropins (37). The addition of whole pineals to hypothalamus-adenohypophysis tissue culture also reportedly decreases LH content (38).

Pineal extracts have also been reported to inhibit adrenocortical function in rats (39) and electric-shock-induced seizures in cats (40). Their administration sup-

presses compensatory adrenal hypertrophy following unilateral adrenalectomy and decreases the thickness of the zona fasciculata and the plasma corticosterone concentration in intact animals (39, 41).

EFFECTS OF EXOGENOUS MELATONIN ON MAMMALS

Gonadal Function

In 1963, evidence was first presented that melatonin administration affected gonad function in experimental animals (3). It was shown that the injection of relatively low doses of the pineal compound (1 μg per day, subcutaneously) could slow the growth of a maturing rat ovary and decrease the incidence of estrous vaginal smears in animals exposed to continuous illumination. During the next few years, additional studies revealed that environmental light, acting via the eyes, brain, and sympathetic nervous system, decreases the activity of the pineal enzyme, hydroxyindole-O-methyltransferase (HIOMT), that catalyzes the terminal step in melatonin biosynthesis (5, 42). On the basis of these observations, a widely accepted theory of pineal function was formulated, according to which the mammalian pineal functions as a *neuroendocrine transducer,* synthesizing and secreting melatonin—and perhaps other methoxyindole hormones—in response to the release of norepinephrine from its sympathetic nerve terminals (6, 43, 44). The rate at which impulses traverse the pineal sympathetic nerves is, in turn, controlled inversely by environmental lighting: light acts indirectly to *decrease* impulse flow to the pineal, thereby slowing melatonin synthesis. Once released, melatonin acts on neuroendocrine centers in the brain to suppress the secretion of LH and to produce other effects, which are described below.

Numerous other investigators have subsequently examined the antigonadal effects of melatonin in rats and other mammals. In 1965, Moszkowska (45) confirmed that melatonin administration reduces ovarian weight and showed that its repeated administration also delayed vaginal opening. Chu et al (46), and then McIsaac et al (47), demonstrated that exogenous melatonin decreased the incidence of estrous vaginal smears in rats and mice exposed to normal lighting regimens, and thus probably interfered with ovulation. Collu et al (48) in 1971, and then Ying & Greep (49) in 1973, showed directly that it blocked spontaneous ovulation. Most recently, Thorpe & Herbert (50) demonstrated that melatonin administration causes premature termination of estrus in ferrets exposed to constant light. Melatonin inhibits the compensatory ovarian hypertrophy that follows unilateral oophorectomy in mice, when administered on the day of birth (51) or the day of surgery (52), but not when administered 40 hr after unilateral oophorectomy (53). It inhibits the increase in rat ovarian, but not uterine, weight caused by the administration of HCG (54, 55) and, in large doses, decreases the incidence of induced ovulations in immature mice treated with pregnant mares' serum gonadotropin (PMSG) (56–58). Mature monkeys given 10 mg melatonin per day for seven days exhibited anovulation and a shortened luteal phase (59).

Exogenous melatonin also inhibits the growth and functional activity of the male gonad: it causes testicular weight to regress in weasels (60), suppresses the light-induced increase in testicular (and ovarian) weight in rats (61), inhibits the testicular recrudescence in hamsters that normally follows exposure to a long photoperiod (62), decreases the weights of the rat seminal vesicle and ventral prostate (63–67), and lowers the concentration of testosterone in testicular venous blood (68). That melatonin may act directly on the testes, as well as indirectly, via the brain, is suggested by evidence that it suppresses testosterone synthesis in vitro (69) and that it accelerates testicular and prostatic regression in hypophysectomized animals (70). Melatonin may also indirectly affect testicular function by modifying the metabolism of testosterone in the liver and hypothalamus (71, 72).

It has also been reported (73, 74) that melatonin implanted subcutaneously in golden hamsters blocks the antigonadotropic effects of exposure to short periods of light, that is, the decreases in testicular and ovarian weight and in pituitary LH and prolactin levels. This observation has been taken as evidence that melatonin is not the antigonadotropic substance of the pineal body in golden hamsters. Unfortunately, no data on plasma melatonin levels were presented in this study; hence, it is not possible to rule out such alternative explanations for its paradoxical effects as the induction of melatonin-metabolizing enzymes by the constant high levels of this compound in the plasma.

The doses of melatonin reportedly needed to inhibit sexual maturation and spontaneous gonadal growth have generally been well under 1 mg/kg per day; those required to block spontaneous or hormone-induced ovulation are greater and probably vary according to when they are administered in relation to the Critical Period (49). It should be noted that Ebels & Prop in 1965 and DeProspo & Hurley in 1971 failed to detect any effect of melatonin or pineal extracts on the weight of the rat ovary (75–77), while Thieblot et al (78) in 1966, reported that large doses of melatonin stimulated gonadal growth and increased the luteinization of the ovaries. Melatonin also reportedly increases the synthesis of progesterone by perfused corpora lutea taken from humans on the twenty-first day of the menstrual cycle (79).

Pituitary Gland and Gonadotropin Secretion

Adams and his colleagues (80) reported in 1965 that doses of melatonin that delayed puberty and decreased ovarian weight also increased the content of LH in the pituitary. They interpreted this finding as suggesting that melatonin suppresses LH secretion. A number of investigators have subsequently used the increase in LH secretion following castration to amplify melatonin's effects on the pituitary. Thus, Clementi et al (81, 82), Fraschini et al (83, 84), and Debeljuk et al (85) reported that melatonin implants placed in particular brain regions—the median eminence and the "reticular area" of the brain stem—or injected daily for 33 days suppressed the postcastration rise in pituitary LH content. Melatonin's parallel suppression of the castration-induced rise in serum LH was observed by Roche et al (86) in 1970, and Fraschini et al (87) in 1971, but not by Talbot & Reiter (88) in 1973. Some investigators have observed melatonin-induced decreases in pituitary weight among un-

treated (89, 90), light-exposed (91), or castrated (85) rats; others (76) have failed to detect such changes. Melatonin administration has also been reported to decrease serum FSH (92), to block the preovulatory surge in serum LH (49), and to increase serum prolactin (93, 94). The pineal hormone may act by decreasing the synthesis or release of gonadotropin-releasing factors in the hypothalamus (35).

Other Glands

A decrease in thyroid weight following melatonin administration was described as early as 1963 by Baschieri and co-workers (95) and confirmed by Turner and associates (96, 97), who correlated it with a decrease in the pituitary concentration of thyroid-stimulating hormone (TSH). In doses of 10–150 μg per animal per day, melatonin also was found to decrease the uptake of ^{131}I by the thyroid (95, 98–100) and to decrease oxygen consumption (101). Using the rate at which ^{131}I disappears from the thyroid as an index, Turner and his associates have concluded that melatonin also slows the secretion of iodinated thyroid hormones (89, 102–104).

Some investigators have observed in vivo decreases in adrenal corticosterone production in rats given large doses of melatonin (50–200 μg/100 g body weight) (105). Others, using smaller doses, have failed to observe changes in either the synthesis of corticosterone or the plasma or pituitary levels of ACTH (1 μg/kg body weight) (106). Melatonin may decrease the formation of Δ^4-3-ketonic corticosteroids in vitro (107) and may suppress the compensatory adrenal hypertrophy that follows unilateral adrenalectomy (51, 108).

Melatonin reportedly suppresses the secretion of insulin in vitro from islets of Langerhans perfused with a glucose-containing medium (109), but does not affect basal insulin secretion (110).

While melatonin apparently does not affect its own synthesis—from ^{14}C-tryptophan in cultured rat pineals (111)—it does influence pineal metabolism in vivo. Thus, Fiske & Huppert (112) found that melatonin administration extinguished the daily rhythm in rat pineal serotonin content, and Cady & Dillman (113) reported that melatonin increased the uptake of thyroxine in bovine pineal slices. Pavel (114) observed a 50% decrease in the arginine-vasotocin content of the pineal, as well as a decrease in the antidiuretic activity of the cerebrospinal fluid following melatonin administration to cats.

High doses of melatonin (80 mg/kg) reportedly suppress the secretion of growth hormone (GH) (115, 116); this action may be related to the effect of melatonin on brain serotonin levels (117). It has also been reported that melatonin decreases the somatomedin-like activity of rat plasma (118). Gram quantities of melatonin also reduce the rise in plasma GH among humans made hypoglycemic with insulin (119). One report suggests that melatonin stimulates the synthesis of GH by the pituitary (120).

Effects on the Brain

That melatonin might directly influence the central nervous system was first suggested by the demonstrations that ^{3}H-melatonin passes freely from the bloodstream into the brain (121), and that melatonin implants placed within the median emi-

nence or the brain stem "reticular formation," but not within the pituitary, could block the castration-induced rise in pituitary LH content (81, 83, 84). In 1968, Anton-Tay and his collaborators (117) demonstrated that the systemic administration of melatonin modified brain serotonin levels: the concentration of the neurotransmitter initially fell within the cerebral cortex, a brain region containing only the axons and terminals of serotoninergic neurons, while it rose within the brain stem, presumably reflecting changes within cell bodies. Increases in the serotonin content of particular brain regions after melatonin administration were also subsequently observed by Piezzi & Wurtman (122).

The mechanism responsible for melatonin's effects on brain serotonin remains to be characterized. It could represent direct hormonal action on processes occurring within serotoninergic neurons—e.g. the synthesis, storage, release, or intraneuronal metabolism of the amine—or, perhaps, a transsynaptic effect resulting from a primary action of other neurons. The observation (123) that melatonin administration also increases pyridoxal kinase activity in the rat brain would seem to suggest that the pineal hormone accelerates serotonin synthesis, if, as appears unlikely, decarboxylation is the rate-limiting step in this process. Melatonin could affect brain serotonin by acting peripherally to modify the plasma amino acid pattern and, thereby, brain tryptophan levels (124). Parenthetically, drugs and lesions that affect serotonin-containing brain neurons have, like melatonin administration, been found to affect the secretion of gonadotropins and growth hormone from the pituitary gland (125, 126). In a recent study, intraarterial injections of melatonin (250 μg/kg) were reported to increase brain dopamine and decrease brain norepinephrine in rats, while a lower dose (40 μg/kg), injected intracisternally, raised the levels of both monoamines (127). Effects of melatonin on brain neurotransmitter metabolism may be related to the behavioral effects discussed below.

Relatively low doses of melatonin (under 50 μg/kg per day, for five days) were found to enhance the incorporation of ^{3}H-leucine into brain proteins (128), especially within the hypothalamus (129). In contrast, much larger doses (1–3 mg/kg) were needed to change brain serotonin levels. The determination of whether a particular dose of melatonin is physiologic, i.e. that it presents its site of action with melatonin concentrations that might occur in untreated animals as a result of its endogenous secretion, is rendered impossible by the paucity of information about the locus and rate of melatonin secretion: it is not known whether the pineal hormone normally is secreted into the bloodstream or into the cerebrospinal fluid. If the latter, then a several hundredfold greater fraction of endogenous melatonin enters the brain than the fraction of exogenous melatonin injected systemically (130); hence a 1-mg dose would be physiologically equivalent to 2–5 μg secreted from the pineal organ. Moreover, until very recently (4), no quantitative data were available concerning even that small fraction (131) of endogenous melatonin that passes, unchanged, into the urine.

That melatonin acts directly on the brain is also suggested by numerous reports describing its effects on sleep and other forms of behavior. Melatonin has been shown to decrease both wheel-running activity in rats (132, 133) and the incidence of adventitious movements in mice receiving large doses of L-DOPA (134). [Its

administration apparently failed to affect total activity in rats (135)]. It induces sedation, and sometimes sleep, in cats (136) and humans (137–139), potentiates hexobarbital sleeping time in mice (140), and causes electroencephalographic patterns consistent with sleep stages in cats (141). Its administration also reportedly blocks ouabain-induced seizures in rats (142).

Other Effects

In concentrations of 1.25–300 X 10^{-6} M, melatonin inhibits spontaneous contractions by the isolated, perfused duodenum (143) and uterus (144); higher concentrations also suppress pitocin-induced concentrations by perfused uterine horns (145). The induction of bronchoconstriction in serotonin-treated dogs can be inhibited by large doses (6 X 10^{-5} mol/kg) of melatonin (146).

Melatonin administration has been reported to decrease urinary estrogens in women with advanced breast cancer (147), but to increase the incidence of mammary adenocarcinomas among rats treated with 9,10-dimethyl-1,2-benzanthracene (148), as well as tumor growth in mice (149); implants of melatonin reportedly suppress bile production in rabbits (150). The hormone allegedly decreases blood glucose in rats (151, 152), increases the amount of brown adipose tissue present in hamsters (153), and is as effective as serotonin in raising cyclic GMP (3',5'-guanosine monophosphate) levels in human monocytes (154).

EFFECTS OF OTHER PINEAL CONSTITUENTS ON MAMMALS

Mammalian pineals can deaminate serotonin to form an aldehyde, which is then rapidly oxidized to 5-hydroxyindoleacetic acid (5-HIAA), or reduced to form 5-hydroxytryptophol; these compounds can then be O-methylated, yielding 5-methoxyindoleacetic acid (5-MIAA) or 5-methoxytryptophol (155). This latter methoxyindole has been reported to have biological activities similar to those of melatonin. Its administration, via implants or intraperitoneally (20 μg/day), decreases ovarian weight and the proportion of daily vaginal smears exhibiting estrous cytology (47), delays spontaneous vaginal opening (156), suppresses HCG-induced uterine weight gain (55), and decreases pituitary LH content (83); however, it reportedly fails to suppress the increases in pituitary and plasma LH levels resulting from castration (88). Very large doses of methoxytryptophol (300–700 mg/kg) also reportedly induce sleep in mice (157), while lower doses (100 μg) block compensatory adrenal hypertrophy following unilateral adrenalectomy (108). DeProspo & Hurley reported that the administration of 100 μg of 5-methoxytryptophol, but not of serotonin or 5-hydroxytryptophan, for 10 days increased adrenal weight (76).

In 1962, Milcu, Pavel & Neascu (158) identified an oxytocic principle in bovine pineal extracts; this compound was a peptide closely related to, but differing from, arginine vasopressin, lysine vasopressin, or oxytocin. A similar agent was found in pig pineals three years later (159). In 1966, synthetic arginine vasotocin was found to have biological activities and chromatographic properties similar to those of the pineal compound (160), and in 1970, Cheesman & Fariss (161) confirmed the structure of this pineal principle as 8-arginine vasotocin. Very low doses of this

compound inhibit the compensatory ovarian hypertrophy that follows unilateral ovariectomy (162, 163), the stimulation of ovarian and uterine growth caused by PMSG (160), and the compensatory adrenal hypertrophy that follows unilateral adrenalectomy (164). The incubation of mouse pituitaries with arginine vasotocin decreases the levels of a gonad-stimulating constituent (165), while the administration of 1 μg of arginine vasotocin daily for three days reportedly decreases the weights of the ovary, testicle, and accessory sex organs in rats (166, 167). Crude extracts of ovine, bovine, and porcine pineals were recently found to contain high levels of gonadotropin-releasing hormones (168). Lipolytic peptides were found by Rudman et al (169) to increase the tissue responsiveness to lipolytic agents of pineal extracts from animals fasted for 48 hr.

EFFECTS OF DRUGS AND HORMONES ON THE MAMMALIAN PINEAL

The biosynthetic activity of the mammalian pineal is primarily controlled by the release of norepinephrine from its postganglionic sympathetic nerves. One major consequence of norepinephrine release is an acceleration in the synthesis of the biogenic amine, serotonin, and its derivative hormone, melatonin. Inasmuch as most of the drugs known to affect the mammalian pineal do so by acting either on noradrenergic synapses or at steps in the conversion of tryptophan to serotonin and melatonin, a brief description is provided of these synapses and of pineal indole biosynthesis.

Sympathetic Nervous Control of Pineal Indole Biosynthesis

The mammalian pineal organ receives most or all of its innervation via postganglionic sympathetic neurons, which originate in the superior cervical ganglia (170) and terminate near pineal parenchymal cells and blood vessels (171); the density of noradrenergic nerve terminals within the pineal is unusually great. Pineal sympathetic terminals contain relatively large quantities of serotonin (172) in addition to their "true" neurotransmitter, norepinephrine; this phenomenon probably reflects competition for norepinephrine storage sites between the catecholamine and serotonin molecules, which are present in very high concentrations within neighboring pineal parenchymal cells (173). Rat pineal "gliocyte" cells can concentrate another presumed neurotransmitter, aminobutyric acid (GABA), in vitro (174).

The effects of norepinephrine on pineal metabolism have been most thoroughly studied within a rat organ culture system in which individual rat pineals are incubated for up to 48 hr with synthetic media containing isotopically labeled tryptophan (175, 176). The pineals readily take up the tryptophan and convert it to protein (177) or to 5-hydroxy- and 5-methoxyindoles (111). Norepinephrine accelerates the syntheses of serotonin and melatonin in cultured pineals by a process involving β-adrenergic receptors (178, 179), and possibly, adenylate cyclase and cyclic AMP (3',5'-adenosine monophosphate). The evidence that cyclic AMP participates in the control of melatonin synthesis is threefold: (*a*) norepinephrine activates adenylate cyclase in pineal homogenates (180–186); (*b*) dibutyryl cyclic AMP, but not cyclic

AMP itself, accelerates melatonin synthesis from ^{14}C-tryptophan within cultured pineal organs (176, 178, 187–189); and (*c*) dibutyryl cyclic AMP was found in some studies (179, 190) to enhance the activity of serotonin-N-acetyltransferase—an enzyme involved in melatonin biosynthesis—when added to pineal organ cultures. This evidence is, of course, only indirect; at present there seems to be a paucity of plausible theories to explain just how cyclic AMP might control the enzymatic steps in melatonin biosynthesis.

The first step in melatonin biosynthesis involves the hydroxylation of tryptophan to form 5-hydroxytryptophan; this process is catalyzed by a tryptophan hydroxylase enzyme, which may differ from the tryptophan hydroxylase in the brain (191), and which is probably not saturated with its amino acid substrate in vivo (192). The 5-hydroxytryptophan is then converted to a serotonin (5-hydroxytryptamine) through the action of the pyridoxal-dependent enzyme, aromatic L-amino acid decarboxylase. Some of the serotonin formed is inactivated by oxidative deamination, which is catalyzed by monoamine oxidase, an enzyme present in both sympathetic terminals and parenchymal cells in the rat pineal (193). The remainder (and larger fraction) is converted first to N-acetylserotonin, through the action of serotonin-N-acetyltransferase (SNAT), and then to melatonin, through the action of hydroxyindole-O-methyltransferase, which catalyzes the transfer of a methyl group from S-adenosylmethionine.

The mammalian pineal synthesizes and contains relatively enormous amounts of serotonin (172, 194). During daylight hours the rat pineal probably stores most of this amine. With the onset of darkness, 80–90% of it is released and probably converted to melatonin, causing marked daily rhythms in the pineal concentrations of both serotonin (195) and melatonin (196). The onset of darkness, by activating the sympathetic nerves to the pineal (197), also causes major increases in the activities of SNAT and HIOMT, the two enzymes that catalyze the conversion of serotonin to melatonin (5, 198). Pineal sympathetic nerves can also be activated by stress, hypoglycemia (199), and, possibly, endogenous factors. Thus, some rhythmicity in melatonin biosynthesis appears to persist when animals are deprived of light-dark cycles by being placed in a continuously dark environment (200). [Weekly rhythms in melatonin synthesis may also exist (201).] Obviously, in examining the effects of any drug on pineal indole metabolism, great care should be taken to include adequate types of controls for the time-dependent changes that normally characterize melatonin biosynthesis.

Almost all of the melatonin synthesized by cultured pineal organs is found in the culture medium, and not within the pineal itself (202). This observation, plus the chemical structure of melatonin—a highly lipophilic compound that is uncharged at physiologic pH—suggests that melatonin secretion from the pineal, like corticosterone secretion from the adrenal cortex, is not an active process, but depends simply on the gradient between intracellular and extracellular melatonin concentrations. As lamented above, it is not presently known whether melatonin is secreted into the blood or into the cerebrospinal fluid. In any event, sympathetic stimulation, by accelerating melatonin biosynthesis, should also accelerate the secretion of the hormone.

Effects of Drugs on Pineal Indole Synthesis

SEROTONIN The rate at which cultured rat pineals synthesize serotonin can be increased by adding 5-hydroxytryptophan to, or elevating the tryptophan concentration of, the medium (192, 202), although it has been reported that high tryptophan concentrations can inhibit total tryptophan hydroxylation in cultured pineal organs (203). L-norepinephrine lowers the serotonin content of cultured pineals, possibly by increasing the conversion of the indole to N-acetylserotonin (204); this effect is blocked by propranolol. Mescaline increases the conversion of ^{14}C-tryptophan to ^{14}C-serotonin by cultured pineals, an effect that is not mimicked by LSD or psilocybin (205).

In vivo, pineal serotonin levels are increased by injecting tryptophan (206–208) or 5-hydroxytryptophan (206), and decreased by *p*-chlorophenylalanine (pCPA) (208), an inhibitor of both tryptophan hydroxylase and catechol synthesis (209), or by RO4-4602 (210), an inhibitor of aromatic L-amino acid decarboxylase. The daily rhythm in pineal serotonin content is disrupted by giving rats reserpine or the monoamine oxidase inhibitor, β-phenylisopropylhydrazine (206, 211), but not by bretylium or guanethidine (206). As a result, pineal serotonin levels fail to decline nocturnally. N-methyl-3-piperidyl benzoate, an anticholinergic agent, exerts similar effects (212). The administration of norepinephrine, dopamine (207), or actinomycin D (211) blocks the daytime increase in pineal serotonin. Presumably, the catecholamines do so by accelerating the N-acetylation of the serotonin; the mechanism by which actinomycin acts in this situation is not established. An enzyme in the rat pineal, presumably the tryptophan hydroxylase, is capable of converting phenylalanine to tyrosine; this conversion is inhibited in vivo by the administration of pCPA or tryptophan (213).

MELATONIN The rate at which cultured rat pineals synthesize ^{14}C-melatonin from ^{14}C-tryptophan is increased by a number of compounds structurally related to L-norepinephrine—i.e., D-norepinephrine, L-epinephrine, dopamine, tyramine, octopamine, tryptamine (111)—as well as by amphetamine (214), and the monoamine oxidase inhibitors Catron® (111) and harmine (215). It is unaffected by morphine (216) and decreased by cyclohexamide (111). The increase in melatonin synthesis caused by adding norepinephrine to the culture medium can be blocked by propranolol, but not by phenoxybenzamine (178); the increase caused by dibutyryl cyclic AMP is unaffected by either receptor blocking agent (178), but is blocked by cyclohexamide or actinomycin D (189).

ENZYMES A number of compounds have been shown to inhibit HIOMT activity in vitro, but apparently none of them blocks melatonin synthesis in vivo. Such inhibitors include substituted N-benzoyltryptamines and N-phenylacetyltryptamines (217), haloperidol (218, 219), fluphenazine, GABA (218), and oxypertine (219). L-Norepinephrine in concentrations of 10^{-5} M increases the HIOMT activity of rat pineals maintained in organ culture (175), possibly by accelerating formation of the enzyme. The addition of dimethyltryptamine (DMT) to pineal homogenates accelerates the O-methylation of N-acetylserotonin as well (220).

Pineal tryptophan hydroxylase is inhibited by pCPA in vivo, in vitro, or when added to organ cultures (203, 221, 222).

The administration of L-DOPA to rats causes a rapid and major increase in pineal melatonin content in vivo (223); it also increases the activities of the melatonin-forming enzymes, SNAT (179) and HIOMT (223). Presumably, the mechanism by which L-DOPA acts involves either the release of norepinephrine from pineal sympathetic nerve terminals, or the intravascular conversion of the catechol amino acid to dopamine, which acts directly on pineal parenchymal cells to stimulate melatonin synthesis (111). The latter is more consistent with the finding that pineal sympathetic denervation potentiates the DOPA-induced increase in pineal melatonin (223). Now that an assay is available for measuring the melatonin excreted in human urine, the increase in pineal melatonin synthesis after L-DOPA administration might provide the basis for an in vivo "pineal function test" in humans.

The SNAT activity of cultured rat pineals is increased by the addition of norepinephrine (179, 186, 188, 190, 198, 224–226); this effect may or may not be potentiated by prior pineal denervation (224, 225) and blocked by adding cyclohexamide or propranolol to the media (227). One group of investigators observed a similar change in SNAT after adding dibutyryl cyclic AMP to cultures (190); another did not (179). SNAT activity in cultured pineal organs is also increased by epinephrine, L-DOPA, octopamine (198), isoproterenol, theophylline, and the monoamine oxidase inhibitors pargyline and Catron (179), but not by serotonin or 5-hydroxytryptophan (179, 198). The magnitude of the increase in SNAT activity caused by isoproterenol depends upon the time of day that the pineal organ was taken from the donor animal (228). Cocaine and procaine also increase SNAT activity in cultured rat pineals (229). That this effect is mediated by the release of norepinephrine from surviving sympathetic nerve terminals is indicated by its failure to occur in pineals taken from animals previously subjected to bilateral superior cervical ganglionectomy, and its blockade by propranolol and phentolamine.

The nocturnal rise in pineal SNAT activity is suppressed in rats treated with reserpine, propranolol, or cyclohexamide, but not by phenoxybenzamine, pCPA, or, paradoxically, actinomycin D (227). Isoproterenol administration elevates SNAT activity in vivo; this effect is blocked by propranolol, but not by phenoxybenzamine (179) or phentolamine (230). The increase in SNAT activity is associated with a fall in pineal serotonin content, which can be blocked by giving rats pargyline, a monoamine oxidase inhibitor (230), or by adding cyclohexamide or propranolol to the media (227). Isoproterenol raises N-acetylserotonin levels in vivo; this effect is reversed by propranolol but not by phentolamine (231). Pargyline also first decreases, then increases, SNAT activity in vivo, if given during the light period of a diurnal cycle (232).

Pineal SNAT activity increases rapidly in rats under stress from immobilization, or made hypoglycemic with insulin (199). These increases are mediated by the release of catecholamines from three loci: sympathetic nerve terminals in the pineal, sympathetic nerve terminals in other organs that are not susceptible to damage by 6-hydroxydopamine, and the adrenal medulla. They are blocked by propranolol.

Effects of Drugs on Pineal Cyclic AMP and Adenylate Cyclase

L-Norepinephrine activates the adenylate cyclase in pineal homogenates (180) and accelerates the synthesis of cyclic AMP in both homogenates (181) and cultures (182) of rat pineals. Cyclic AMP levels in the pineal are also elevated by D-norepinephrine, L-epinephrine, and isoproterenol (181). The effect of L-norepinephrine is blocked by propranolol and dichloroisoproterenol and partially inhibited by trifluoperazine and chlorpromazine, but it is not influenced by phenoxybenzamine or phentolamine (181, 184). It is potentiated by exposing donor animals to continuous light for three days prior to taking the pineal—probably because this treatment decreases the occupancy of pineal receptors by endogenous norepinephrine (183)—or by adding theophylline, a phosphodiesterase inhibitor, to the medium (185). Deguchi (233) found that isoproterenol administration produced an elevation in pineal cyclic AMP content and, 1 hr later, in SNAT activity. Pretreatment with propranolol blocked both increases; administration of the β-blocker subsequent to isoproterenol after cyclic AMP had returned to baseline blocked the increase in SNAT activity. Pretreatment with cyclohexamide blocked only the rise in SNAT activity.

Effects of Drugs on Pineal Phospholipids

In concentrations of $1\text{–}300 \times 10^{-6}$ M, L-norepinephrine increases the rate at which cultured pineal organs incorporate inorganic ^{32}P into phosphatidyl inositol (PI) (234), monophosphoinositide (235), and phosphatidyl glycerol (PG) (236), and decreases the synthesis of phosphatidylethanolamine (235). The synthesis of pineal PI and PG are also accelerated by D-norepinephrine, L-epinephrine, dopamine, tyramine, and octopamine (236), as well as phenylephrine and propranolol (237), while monophosphoinositide synthesis is accelerated by acetylcholine plus eserine or by serotonin. An α-receptor blocker, phenoxybenzamine, has also been reported to block the phospholipid stimulatory effects of norepinephrine and phenylephrine, but not of propranolol (237), suggesting two distinct components to phospholipid stimulation in the pineal. Local anesthetics such as dibucaine accelerate the degradation of phospholipids in the pineal (238).

Effects of Drugs on Pineal Morphology

Using electron microscopy and autoradiography to localize the ^{3}H-norepinephrine taken up and retained within rat pineal organs, Wolfe and his colleagues showed in 1962 (239) that the pineal norepinephrine is stored in granulated vesicles of postganglionic sympathetic neurons. This granularity is decreased by reserpine and increased by iproniazid administration (240). The cellular localization of pineal serotonin was examined using histochemical fluorescence technology; about half of this amine was found within pineal parenchymal cells, and half within sympathetic nerve endings (241). Recently, pCPA has been shown to decrease the number of yellow-fluorescing pineal cells thought to contain this amine (242). Parenchymal serotonin is depleted by α-methyltyrosine; neural serotonin is depleted when tryptophan hydroxylase is inhibited by α-propyldopacetamide (243). In general, drugs

that modify the storage of norepinephrine within sympathetic nerve terminals elsewhere in the body act similarly in the pineal, for example, metaraminol (241), desmethylimipramine (244), 6-hydroxydopamine (245), tyramine (246), and anti-nerve-growth factor (247), and both norepinephrine and dibutyryl cyclic AMP cause morphologic changes in pineals compatible with increased biosynthetic activity (248).

Effects of Hormones on the Pineal

Estradiol administration may (249) or may not (250) affect HIOMT activity. Paradoxically, estradiol increases pineal protein content and protein synthesis (251). Progesterone also reportedly decreases HIOMT activity, but without affecting pineal weight (249). In castrated rats, estradiol decreases pineal HIOMT, while norepinephrine partially reverses this decrease (252). Testosterone also decreases HIOMT activity, and norepinephrine has also been demonstrated to partially reverse this effect (253, 254). Testosterone also increases pineal protein synthesis in castrated male rats. Superior cervical ganglionectomy blocks this effect (255) and decreases pineal uptake of both testosterone and estradiol (256), suggesting a possible role of sympathetic transmission in the effects of these hormones on the pineal. Thyroxine and cortisol allegedly stimulate the Krebs and Embden-Meyerhof enzymes in vivo, as well as pineal aryl sulfatase (257). Epinephrine and melatonin increase the uptake of thyroxine by the pineal in culture, while TSH and aldosterone are without effect (113). Estradiol administration (500 mg/kg for three days) decreases pineal adenylate cyclase activity assayed in vitro; testosterone and progesterone are without effect in this system (258).

SUMMARY

Considerable evidence is now available that melatonin, a methoxyindole synthesized in, and secreted from, the mammalian pineal organ, is responsible for many, if not all, of the biological activities attributed to this gland. The best-studied effects of melatonin are those involving actions on the brain (and possibly elsewhere) to suppress the maturation and functional activity of the gonads. Melatonin synthesis exhibits a 24-hr rhythmicity, and melatonin levels in human urine are correspondingly greatest during the hours of darkness. The synthesis of melatonin in vivo and in vitro is stimulated by drugs that enhance the interactions of norepinephrine with pineal β-receptors, and suppressed by β-receptor blocking agents. Pineal extracts may contain additional biologically active compounds besides melatonin; these include peptides and other methoxyindoles. It seems likely that a number of experimental and, perhaps, clinical uses will be found for pineal constituents.

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