THE UNIVERSITY OF MANITOBA

NEUROCHEMICAL AND NEUROENDOCRINE EFFECTS OF MELATONIN ADMINISTRATION IN GONADECTOMIZED FEMALE SYRIAN HAMSTERS

by

Nancy Anna Marie Alexiuk

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF SCIENCE

Department of Anatomy

Winnipeg, Manitoba

May, 1991

NEUROCHEMICAL AND NEUROENDOCRINE EFFECTS OF MELATONIN ADMINISTRATION IN GONADECTOMIZED FEMALE SYRIAN HAMSTERS

BY

NANCY ANNA MARIE ALEXIUK

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

© **1991**

Permission has been granted to the LIBRARY OF THE UNIVERSITY OF MANITOBA to lend or sell copies of this thesis, to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film, and UNIVERSITY MICROFILMS to publish an abstract of this thesis.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

TABLE OF CONTENTS

Page No.

ACKNOWLEDGEMENTS

I would like to express my appreciation to Dr. Jerry Vriend, my supervisor, for his guidance, understanding, encouragement and assistance. I wish to acknowledge Roy Simpson for preparation of the figures and photographs, as well as Carlyle Andryo for typing the tables. Finally, I would like to thank the Anatomy Department for the Anatomical Research Fund Scholarship and for the opportunity to attend the Neuroscience Meetings in St. Louis, Missouri, 1990.

ABSTRACT

Daily late afternoon administration of the indoleamine, melatonin, in gonadectomized female Syrian hamsters (Mesocricetus auratus) for ten weeks, resulted in a marked inhibition of synthesis of the catecholamines, dopamine and norepinephrine, in the median eminence. In addition to this effect within the mediobasal hypothalamus, melatonin was found to decrease norepinephrine synthesis in the amygdala. Significant effects upon serotonergic metabolism were demonstrated by the data showing increased concentrations of serotonin (5HT) and its metabolite 5-hydroxyindole acetic acid (5HIAA), in both the median eminence and caudate nucleus of melatonin-treated hamsters. Decreased 5HT synthesis was also observed in the amygdala. These significant effects of melatonin in ovariectomized hamsters supports the interpretation that the alterations in catecholaminergic and serotonergic metabolism induced by melatonin occur independently of the gonadal steroid hormones. In agreement with the work of others, melatonin administration was demonstrated to influence circulating levels of luteinizing hormone (LH), prolactin (PRL) and thyroxin (T4), in addition to the pituitary content of LH and PRL. Melatonin's dramatic antigonadotrophic and antithyroidal effects in Syrian hamsters are interpreted to be due to its ability to modify the metabolism of the neurotransmitters which modulate the release of anterior pituitary hormones. However, the hormone changes found in

the melatonin-treated hamsters in this study, cannot easily be explained in terms of a single transmitter system. The data derived from this investigation suggest that the effects of melatonin upon catecholaminergic and serotonergic metabolism are not limited to the mediobasal hypothalamus.

INTRODUCTION

The Pineal Gland and Melatonin - A Historical Perspective

Historical records suggest that since antiquity, no other structure in the mammalian body has been so greatly misunderstood as the enigmatic pineal gland. This small, unpaired brain organ or "epiphysis cerebri", has been described for centuries with an enduring curiosity - utilizing terms such as the "cervical body", the "seat of the soul", the "third eye", the vestigeal remnant of the parietal eye, to the "appendix of the brain", and even the "penis cerebri" (for review, see Bhatnagar, 1990).

Herophilas of Alexandria (325-280 B.C.), initially portrayed the human pineal as an entity which controlled the "stream of thoughts" - a sphinctor, tap or valve regulating the flow of "pneuma" or "spirits" from the third to the fourth brain ventricles, (Miles and Grey, 1988). It was Galen (129-199 A.D.), a Greek physician practising in Rome approximately 450 years later, who disagreed with the tap function of the pineal gland since it lay outside the cerebral ventricles, and suggested a function similar to lymph nodes. Galen initially designated the term "Konareion", a Latin word which described the shape of the human pineal gland as a pine cone.

Rene Descartes (1596-1650 A.D.) referred to the pineal as the dignified "seat of the soul" and "gland H". He described it as not simply a specific anatomical locale,

but rather an intangible mathematical point from which the immortal soul united with the mortal body and carried out its function. He suggested that the pineal separated particles from the blood and then released them into various fine pores in response to specific sensory stimuli, (Descartes, 1662; Chamberlain and Herman, 1990).

Many physicians of the seventeenth and eighteenth centuries, as did the ancient Greeks, considered the pineal gland to be fundamentally associated with psychotic behavior or "madness". It was not, however, until the 1880's that documented descriptions of the anatomy, histology, embryology and innervation of the mammalian pineal gland began to appear (for review, see Kappers, 1960).

In this century, the pineal has received considerable attention from both basic scientists and clinicians alike. However, its functional importance in certain species, is regarded by some non-pinealologists with unchanging skepticism. Reiter has diagnosed the problem of the endocrine pineal's non-acceptance as a "vestigeality complex" (Reiter, 1981). Increasing evidence, however, has been presented for the functional significance of the pineal product, melatonin, in numerous species across several vertebrate classes including mammalian primates, such as the human (Cassone, 1990).

Isolation of Melatonin

It was McCord and Allen in 1917, who first documented the results of experiments in which amphibians were fed bovine pineal extracts. They found that administration of these extracts resulted in the concentration of melanin granules in tadpole melanophores. However, it was not until the work of Aaron Lerner in the late 1950's, that the skin-lightening substance within the cattle pineals was first isolated and structurally identified as melatonin (N-acetyl-5-methoxytryptamine). It was also Lerner and colleagues, who derived the indoleamine's name from the Greek word "melas", meaning black and "tosos", for labor (Lerner et al., 1958, 1959).

Several years later, Wurtman and collaborators presented a "melatonin hypothesis of pineal function". They discovered and documented two fundamental pineal principles: that melatonin inhibited estrous cyclicity when injected into female rats and that the mammalian pineal synthesized and secreted this serotonin derivative, melatonin, at a rate inversely proportional to environmental lighting (Wurtman et al., 1963; Chu et al., 1964; Wurtman and Axelrod, 1965). Melatonin, suitably entitled the "darkness hormone", is produced and released in response to the absence of light.

Quay's work in 1963-64, demonstrated that the melatonin levels in the pineal glands of rats had a very distinct

twenty-four hour cycle (Quay, 1963; 1964). The pineal derived melatonin circadian rhythm is similar in plasma (Wilkinson et al., 1977) and urine in almost all mammals (Reiter, 1987). The rhythm is characterized by a marked increase in secretion during the dark phase with a rapid fall at the onset of daylight (Axelrod, 1974; Wetterburg, 1978).

Melatonin, although primarily synthesized in the pineal gland, has also been reported to be present in the retina, especially in avian species, (Wiechmann, 1986). Other extrapineal areas in which melatonin has been reported, but not confirmed, include harderian glands of rodents (Cardinali and Wurtman, 1972), peripheral nerves, human erythrocytes (Vollrath, 1981), and rabbit platelets (Launay et al., 1982).

Morphology of the Mammalian Pineal Gland

Embryologically, the mammalian pineal gland, the principal source of melatonin, arises as an outgrowth in the posterodorsal region of the roof of the diencephalon. The weight of an adult mammal's pineal can vary from approximately one milligram (mg) in the rat to almost 150 mg in the human (Vollrath, 1981; Arendt, 1988). The pineal glands of seasonally breeding animals, such as the Syrian hamster, or sheep, tend to be more prominent (Ralph, 1975). Larger, more distinct pineals are typically found in animals which inhabit higher latitudes. This is in

contrast to those living in, or adjacent to, equatorial regions, which characteristically possess small, diffuse, or reportedly absent pineals (Oksche, 1965; Bhatnagar, 1990).

In lower vertebrates, such as fish, amphibians, and some reptiles, pineal cells have common structural and functional characteristics with the cone cells of the retina. In most reptiles and birds, the pineal organ lies adjacent to the skin surface and resembles rudimentary photoreceptor cells (Collin and Oksche, 1981).

The mammalian pineal gland is a small, solid organ which projects posteriorly from the roof of the third brain ventricle, and is composed of two major cell types. Pinealocytes, or chief cells, are considered to be modified photoreceptor cells and they represent the most abundant cellular component. They have been found to comprise approximately eighty-five percent of the cells in the gland. Pinealocytes are characterized by numerous, branching cytoplasmic processes which possess club-shaped, vesicle-containing terminals (Wolfe, 1965). Surrounding these processes are an abundance of fenestrated capillaries (Kappers, 1960). The pineal gland also contains supporting cells of neuroglial origin which are similar to astrocytes. A mysterious organelle known as the "synaptic ribbon" is found in pinealocytes and the only other structure in the mammalian body where it has presently been located, is the retina (Vollrath, 1973).

There exists a very strong relationship between aging and pineal deposition of basophilic extracellular bodies called "brain sand", "pineal sand" or "acervuli" (Krstic, 1976; 1986). These calcium phosphate, magnesium phosphate, or calcium carbonate depositions are found within a glial cellular and connective tissue matrix. These prominent concretions serve as a radiological marker.

Blood Supply

The posterior choroidal arteries, derived from the posterior cerebral arteries, represent the major source of blood supply to the pineal gland (Le Gros Clark, 1939). Blood from capillaries and venules ultimately drain into the great vein of Galen and the straight sinus, en route to the heart. It is generally accepted that the major mode of transport of melatonin is via the circulatory system.

The pineal gland has a very high quantity of blood flow that is second to only the kidney (Goldman and Wurtman, 1964; Arendt, 1988). The dark period represents the time at which pineal blood flow is greatest (Quay, 1972).

Innervation

The pineal gland is innervated primarily by postganglionic sympathetic fibers with cell bodies which are located in the superior cervical ganglia (Kappers, 1960). A neuroanatomic pathway which mediates the effects of light or darkness upon mammalian melatonin production,

is believed to originate in the retina of the eye. Photoreceptors and ganglion cells found in the retina, convert photic input (light) into an electrical and neurochemical signal which, via the retinohypothalamic tract (Moore and Lenn, 1972), is relayed to the contralateral and ipsilateral suprachiasmatic nuclei (SCN) of the hypothalamus, following decussation at the optic chiasm (Hendrickson et al, 1972). The endogenous circadian pacemaker, (the SCN), then relays information to the lateral hypothalamus, the medial forebrain bundle, (Moore, 1978), the tegmentum, and finally to the intermediolateral cell column of the thoracic cord - the source of preganglionic fibers to the superior cervical ganglia. These preganglionic fibers enter the sympathetic trunk and synapse with postganglionic fibers of the superior cervical ganglia, and ultimately reach the pineal gland as the nervus conarii (Kappers, 1960). A second pathway which provides the SCN with light information is the retinogeniculohypothalamic tract (Pickard et al., 1987).

During the dark phase, action potentials originating in the SCN stimulate these peripheral sympathetic postganglionic fibers which results in the release of the catecholamine noradrenalin (norepinephrine), from the nerve terminals. The initiation of melatonin synthesis (Romero et al., 1975) depends predominantly upon the stimulation of beta 1-adrenergic receptors (Klein and Weller, 1972; Klein et al., 1981), on the pinealocyte cell membrane, although

current investigation suggests that alpha 1- adrenergic receptors are also activated by noradrenalin (Zatz, 1981; Klein, 1985; Morgan et al., 1988; Arendt, 1989; Stankov et al., 1990), during melatonin production. The "AND" gate theory contends that concurrent activation of alpha and beta adrenergic receptors are required to stimulate melatonin production (Cena et al., 1991).

In the daytime, light impinging upon the retina suppresses norepinephrine-induced melatonin secretion. Denervation of the pineal gland via the postganglionic fibers, completely prevents the secretion of melatonin. Administration of beta-blockers such as propanolol or atenolol, inhibit melatonin release as well (Vaughan, 1976). Alpha 1-adrenergic blockade or alpha 2-autoreceptor stimulation also decrease secretion of the indoleamine (Checkley and Palazidou, 1988). The mammalian pineal gland lacks a major cholinergic, parasympathetic innervation (Kappers, 1960).

Biosynthesis of Serotonin and Melatonin

Tryptophan, an essential amino acid primarily obtained from the diet, is actively taken up from the circulation by the pineal gland (Axelrod et al., 1969). Within pinealocytes, it undergoes hydroxylation at the 5 position to form 5-hydroxytryptophan (5HTP) by the rate-limiting enzyme, tryptophan hydroxylase and reduced pteridine cofactor (Sitaram and Lees, 1978; Cooper et al., 1982).

Following this, 5-hydroxytryptophan is decarboxylated to form the indoleamine, serotonin, via the enzyme aromatic amino acid decarboxylase. The pineal gland is known to contain the highest concentration of this neurotransmitter in the brain (CNS) - approximately fifty times (per gram) greater serotonin (5HT) content (Giarman and Day, 1959; Quay, 1974; Cooper et al., 1982), than any other brain structure. In the Syrian hamster, serotonin (5HT) is produced with greater abundance during the daytime (Steinlechener et al., 1983; King et al., 1984).

During darkness, noradrenergic stimulation of beta 1-adrenoceptors on pinealocyte cell membranes, induces a very rapid increase in pineal content of cyclic AMP as well as specific protein synthesis (Klein et al., 1970; Klein and Weller, 1972; Deguchi and Axelrod, 1973; Romero et al., 1975; Klein et al., 1981). This leads to the activation of the enzyme serotonin N-acetyltransferase (Romero and Axelrod, 1975), which increases its activity thirty to seventy fold in the evening (Arendt, 1989), during the dark phase of the light/dark cycle. This enzyme is believed to limit the rate of melatonin synthesis (Klein et al., 1981; Ebadi, 1984). N-acetyl transferase, converts serotonin into N-acetyl-5-hydroxytryptamine (Weissbach et al., 1960). In the final step, N-acetyl-5 hydroxytryptamine undergoes O-methylation (Axelrod and Weissbach, 1960), via activation of the enzyme 5-hydroxyindole-O-methyltransferase (5-HIOMT). This reaction requires S-adenosyl methionine as

the methyl donor (Guchhait and Grau, 1978; Ebadi, 1984). Melatonin, N-acetyl-5-methoxytryptamine, is the final product (Axelrod, 1974; Cooper et al., 1982; Klein, 1982).

Rollag and colleagues in 1980, calculated the rate of pineal melatonin synthesis in the Syrian hamster to be approximately 1.6 picograms per minute during the light of day - rising to 70.6 pg/min. in the darkness of night. The total average melatonin production over a twenty-four period was 18.6 nanograms (ng) (Rollag et al., 1980). Melatonin synthesis, it should be noted, occurs at night in both diurnal and nocturnal species (Arendt, 1989). No present evidence exists for long term melatonin storage within the pineal gland. Apparently, melatonin is secreted upon synthesis.

Melatonin is metabolized to 6-hydroxymelatonin conjugates in the liver (Kopin et al., 1961) and is then excreted by the kidneys with urine. In the brain, melatonin is converted into N-acetyl- 5-methoxykynurenamine (Hirata et al., 1974). The half-life for elimination of melatonin is approximately twenty minutes in adult rats (Gibbs and Vriend, 1981).

Light Suppression of Melatonin Secretion

The intensities of light required for the suppression of melatonin synthesis and release vary from species to species. In the albino rat, 0.0005 microwatts per centimeter squared is required (Webb et al., 1985), to

approximately 1850 microwatts per centimeter squared needed in the Richardson's ground squirrel (Reiter et al., 1983). It was Lewy and collaborators in 1980, who demonstrated that the suppression of melatonin secretion in humans required a much greater intensity of light (2500 lux) than that necessary in most laboratory animals (Lewy et al., 1980). This work in humans has led to the implementation of bright lights (phototherapy), as an efficacious somatic treatment for winter depression (Lewy et al., 1982; Rosenthal et al., 1984), that is apparently mediated through the eyes (Wehr et al., 1987).

It was initially suggested that blue light wavelengths (approximately 500-520 nanometers) were more effective at suppressing melatonin synthesis and secretion in both Syrian hamsters (Brainard et al., 1984), and humans (Brainard et al., 1985). More recently, however, the optimal wavelength in preliminary studies suggest light from a broader range (505-555 nanometers). The green wavelengths were found to be of greater efficacy than the blue wavelengths (Oren et al., 1989).

The ambient temperature appears to be important in either increasing or decreasing susceptibility of the pineal gland to the suppressive effects of light in some species (Li et al., 1987; Steinlechner et al., 1988). Hamsters subjected to cold temperatures are reported to be more sensitive to the effects of melatonin (Pevet et al., 1989). Manipulations of light and temperature are

presently gaining considerable attention, as researchers attempt to understand the environmental triggers regulating neurochemical and neuroendocrine physiology.

Effects on Reproduction in the Syrian Hamster

Many researchers have focused upon melatonin's dramatic effects upon mammalian reproductive cycles, especially in seasonal breeders such as the Syrian or golden hamster. It was Tamarkin who first discovered that melatonin injections properly timed can duplicate the effects of natural photoperiod in the hamster (Tamarkin et al., 1976). Similarities between the reproductive events that occur with short photoperiod (<12.5 hours of light), and melatonin administration late in a long photoperiod (14 hours light/ 10 hours dark), have been documented by several scientists (Tamarkin et al., 1976; Reiter, 1980). After eight to ten weeks of exposure to light deprivation, short photoperiod or late afternoon administration of melatonin, the testes of male hamsters will undergo involution - sometimes to one tenth of their size in long photoperiod. Accessory organ weights will decrease concomitantly with the weight of the testes, which would characteristically lack mature spermatozoa. The male hamster, most frequently involved in these initial experiments, was described as being in a state of reproductive quiescence (Hoffman and Reiter, 1965; Berdtson and Desjardins, 1974).

The effects of photoperiod upon reproductive status has been demonstrated to be pineal-mediated since the gonads of pinealectomized or superior cervical ganglionectomized hamsters were functionally identical to those of control hamsters maintained in long photoperiods (Reiter and Hester, 1966; Reiter, 1968). Light deprivation or blinding produces antigonadal effects similar to melatonin administration (Reiter, 1969; Vaughan et al., 1982).

Hormonal changes which accompany testes involution in the male hamster, include decreased pituitary prolactin, as well as lowered levels of plasma prolactin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (Donofrio et al., 1973; Reiter et al., 1974; Reiter and Johnson, 1974; Reiter et al., 1975). It has been suggested that the concurrent increases in hypothalamic levels of LHRH (Jackson et al., 1984), indicate a decrease in its release and hence provides one explanation for the reductions in the gonadotropin levels.

Short photoperiod or melatonin administration late in the light period, is believed to play a significant role in the changes that occur in the neuroendocrine-thyroid axis. The alterations that occur in thyroid status has also been shown to be pineal-dependent. Marked decreases in plasma triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH), associated with short photoperiod or melatonin injections, is a well-documented occurrence in both sexes of Syrian hamster (Vriend and

Reiter, 1977; Vriend et al., 1982; Vriend, 1983; Vriend et al., 1987; Vriend and Steiner, 1988). Although the mechanism involved is not known, it is suspected that melatonin influences the metabolism of the neurotransmitters that modulate the release of thyrotropin releasing hormone (TRH), but certainly other hypotheses have not been excluded. T4 is believed to influence the circadian sensitivity of the CNS to melatonin (Vriend et al., 1987).

Daily late afternoon treatment with exogenous melatonin for 8-10 weeks, results in a disruption of the four day estrous cycles that typify the reproductive status of female hamsters maintained in long photoperiod (14L/10D) (Tamarkin et al., 1976). This phenomenon appears to be identical to the effects of short photoperiod, (Reiter and Hester, 1966; Reiter, 1968; 1969; Seegal and Goldman, 1975; Trakulrungsi et al., 1979; Jorgenson and Schwartz, 1987), as well as light deprivation or blinding (Vaughan et al., 1982). The resulting anestrous condition, due to melatonin injections or short photoperiod, is characterized by a marked reduction in circulating estradiol (Vriend et al., 1987), and prolactin levels with daily LH and FSH surges (Bridges and Goldman, 1975; Jorgenson and Schwartz, 1987), rather than an LH surge only on the proestrous day of the four day cycle (Bast and Greenwald, 1974; Seegal and Goldman, 1975; Trakulrungsi et al., 1979; Donham et al., 1984). Circulating T3, T4, and TSH hormone levels are also

decreased (Vriend et al., 1982; Vaughan et al., 1982). Ovarian preantral and antral follicular development is arrested and corpora lutea formation inhibited with concomitant hypertrophy of the progesterone-secreting ovarian interstitium (Silavin and Greenwald, 1982). Uterine and vaginal tissue atrophy (Vriend et al., 1987). Daily LH surges are accompanied by, and stimulate, abnormal surges of progesterone from the hypertrophied interstitium (Norman and Greenwald, 1971; Bridges and Goldman, 1975; Silavin and Greenwald, 1982; Terranova et al., 1982; Hubbard and Greenwald, 1983; Donham et al., 1984). Like the male hamster, these physiological processes are believed to be pineal-mediated since pinealectomy and superior cervical ganglionectomy reversed or prevented these effects (Reiter and Hector, 1966).

Melatonin appears to disrupt the feedback relationships between the ovarian steroid hormones, progesterone and estradiol, and the gonadotropins, LH and FSH, as well as prolactin. The estrous cycles of melatonin-treated hamsters are halted in the diestrus II stage of the four day cycle (Vriend et al., 1987). In untreated, control hamsters maintained under long photoperiod, estradiol, in conjunction with progesterone, is believed to play a paramount role in the prevention of daily oscillations of anterior pituitary pulses of gonadotropin release and the maintenance of the four day proestrous surges (Stetson et al., 1978). It has been suggested that the periodicity of

the gonadotropin surges may be regulated by a neural "clock" timed release mechanism, possibly located in the suprachiasmatic nuclei (SCN) (Stetson et al., 1978).

Photorefractoriness

After prolonged exposure (17-22 weeks) to short photoperiod or daily melatonin administration, both sexes of Syrian hamsters will cease to respond to this inhibitory signal. In other words, the hamster brain and neuroendocrine system develop a state of refractoriness. Even with additional melatonin injections, response is not recovered. This dynamic cessation of reproductive quiescence and subsequent entry into a gonadal restoration phase, is known as recrudescence (Reiter, 1972; 1980). In the natural mileu of a long-day breeding hamster, this is coincident with the vernal (spring) equinox. Restoration of estrous cyclicity occurs as daily LH, FSH and abnormal progesterone surges cease, serum estradiol levels increase and ovarian follicles and corpora lutea resume development (Jorgenson and Schwartz, 1987). The testes of refractory male hamsters regenerate, as demonstrated by marked increases in testicular and accessory organ weights and the resumption of efficient spermatogenesis.

In order to disrupt or "break" photorefractoriness and restore sensitivity to inhibitory daylength (short photoperiod or melatonin administration), it is necessary for the animal to experience a critical duration of

approximately ten weeks of exposure to a stimulatory long photoperiod and its accompanying melatonin signal (Reiter, 1972; Bittman, 1978; Bittman and Zucker, 1981; Hastings et al., 1989). Some mechanism within the hamster is able to differentiate between varying durations of the melatonin rhythm (Stetson et al., 1983; Hastings et al., 1989). The neurochemical changes underlying this particular phenomenon have not been sufficiently examined (Steger et al., 1985).

Melatonin "Receptors" and Putative Sites of Melatonin Action

In an attempt to determine possible sites of melatonin action, developments have occurred in receptor studies in recent years. Initial pioneering work, in which a tritiated melatonin ligand was utilized, resulted in reports of specific binding in various tissues. Highest binding was observed in hypothalamus, pituitary gland, epididymus, and adrenal gland (Cohen et al., 1978; Cardinali et al., 1978; 1979; and Pickering and Niles, 1990).

Improvements in receptor technologies have led to the development of an iodinated melatonin radioligand - [125 I] iodomelatonin. This ligand has been used in a variety of binding studies. In addition to autoradiographic methods, synaptosomal and membrane procedures have been used to characterize melatonin-binding to brain tissue.

Iodinated melatonin has been utilized to study the

localizations of both high and low affinity binding sites. In both Syrian and Djungarian hamsters, as well as in domestic sheep and in the rat, high affinity binding sites were found predominately in three areas: the SCN, (Reppert et al., 1988; Krause and Dubocovich, 1990), the median eminence of the hypothalamus (Vanecek et al., 1987; 1988; Stankov and Reiter, 1989) and the pars tuberalis of the anterior pituitary (Morgan et al., 1989; Morgan and Williams, 1989). Other areas of melatonin binding include the PVN of the thalamus, the area postrema, subiculum, hippocampus, midbrain and retina (Dubocovich and Takahashi, 1987; Morgan and Williams, 1989; Pickering and Niles, 1990; Siuciak et al., 1990).

An SCN site of action for melatonin would be consistent with melatonin's role in the entrainment of circadian rhythms to the environmental light-dark cycle. It is thought to coordinate the ambient light or darkness with the circadian cyclicity of an endogenous time-keeping mechanism found within the SCN (Moore, 1983; Ralph et al., 1988; Rusak, 1989). Melatonin has been demonstrated to decrease the neurochemical and electrical activity within this nucleus (Cassone et al., 1987; Krause and Dubocovich, 1990). SCN lesions have been clearly shown to block melatonin's antigonadotropic effects (Rusak, 1980).

Reiter et al. (1976) have suggested that there exists a marked circadian rhythmicity of melatonin "receptor" response that is characterized by an early morning decrease

in receptor sensitivity, (or down-regulation). This view is supported by data showing that evening, but not morning, injections of melatonin result in gonadal involution in hamsters. At least one report of circadian variations in melatonin binding has also appeared (Anis et al., 1989).

Melatonin binding to the median eminence/pars tuberalis region, (Vanecek et al., 1987; Morgan and Williams, 1989), would be concordant with its role in regulating reproductive and neuroendocrine changes. In sheep, some researchers report that melatonin binding occurred exclusively in the pars tuberalis of the adenohypophysis (Morgan and Williams, 1989).

Melatonin has been reported to inhibit cyclic AMP in the pituitary (Carlson et al., 1989; Morgan et al., 1989; Vanecek and Vollrath, 1990). Increasing evidence is accumulating that melatonin binding sites are coupled to adenylate cyclase via GTP binding proteins of the inhibitory class (Miles, 1989; Niles, 1990; Daniolos et al., 1990).

The issue of localization of melatonin binding and receptor sites remains a very controversial one. The question of whether melatonin acts upon one, several, or many sites within the CNS and outside, is much disputed. There is also the question of whether 'binding site' studies represent genuine physiological receptors. Since melatonin's site(s) of action has not been definitively demonstrated, this has created persisting barriers to the

understanding of its mechanism of action.

Neurotransmitter Regulation of Hormones

There is a paucity of work published on the relationship between daily melatonin administration and neurotransmitter metabolism. Two groups of investigators, independently, have examined the effects of short photoperiod upon monoamine activity in the hypothalamus of male hamsters. Steger and colleagues found significant effects of short photoperiod upon catecholaminergic metabolism in the mediobasal hypothalamus. Decreased dopamine (DA) turnover was demonstrated in a number of experiments in mediobasal hypothalamus (MBH) (Steger et al., 1985; 1986) and more specifically in the median eminence (ME) (Steger et al., 1985; 1986). Significant inhibition of norepinephrine turnover was also demonstrated in medial preoptic/SCN extracts (Steger et al., 1983), MBH (Steger et al., 1983), ME/MBH (Steger et al., 1984) and ME tissue samples (Steger et al., 1985; 1986). Benson (1987), concordant with Steger, also demonstrated decreased DA and NE turnover after nine and twelve weeks of short photoperiod exposure. Benson also found significant effects of short photoperiod upon the serotonergic system in male hamsters. Increased concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in MBH was demonstrated as well as an increased 5-HIAA/5HT ratio (Benson, 1987).

It is well documented that norepinephrine is the principal neurotransmitter involved in the stimulation of LH and FSH release (Weiner and Ganong, 1978; Meites and Sonntag, 1981). NE is also considered to facilitate prolactin and TSH release (Jobin et al., 1975; Weiner and Ganong, 1978; Meites and Sonntag, 1981).

Serotonergic stimulation of prolactin release is well documented in the literature (Weiner and Ganong, 1978; Clemens et al., 1978; Meites and Sonntag, 1981; Van de Kar et al., 1989). Some researchers argue that the serotonergic system has an inhibitory effect upon gonadotropin (LH, FSH) release (Schneider and McCann, 1970; Weiner and Ganong, 1978).

There is considerable evidence for dopamine's inhibitory modulation of prolactin release (Gudelsky, 1981; Tuomisto and Mannisto, 1985). Dopamine's modulatory role in LH and FSH secretion remains a disputed one, however, some researchers have documented evidence of an inhibitory role for DA in the regulation of TSH release (Foord et al., 1980; Ben-Jonathon, 1985).

Other substances, including neurotransmitters, neuromodulators, amino acids and hormones, especially the gonadal steroids, are believed to play significant roles in the regulation of prolactin, LH, FSH and TSH release. Acetylcholine, GABA, histamine and the opioids are believed to influence hormone regulation (Weiner and Ganong, 1978; Meites and Sonntag, 1978).

In the experiments performed by Benson (1987), and Steger and coworkers (1985; 1986), the transmitter changes documented were accompanied by marked testicular atrophy and inhibition of spermatogenesis. These changes in catecholamine turnover found in male hamsters maintained in a short photoperiod, were not secondary to reductions in testosterone levels, as demonstrated by Steger and collaborators (1986).

Steger et al., (1985), suggest that daily melatonin injections produce similar neurochemical and neurendocrine effects as those elicited by exposure of hamsters to short photoperiod. The question is then raised as to whether melatonin also acts via hypothalamic neurotransmitters. Experiments performed by Benson (1987) as well as by Steger and coworkers (1986), utilized male hamsters exclusively. No experiments relating the effects of melatonin administration upon neurotransmitter turnover have presently been published. Since females may be more sensitive to seasonal changes in photoperiod (Demarest and Moore, 1981), they may also be more sensitive to melatonin injections.

Gonadal Hormones and Neurotransmitters

Both estrogen and progesterone can have effects upon neurotransmitter metabolism that are highly dependent upon the stage of the estrous cycle and the plasma concentrations in relation to each other (Ben-Jonathon,

1985). The ovarian steroids can have varied effects upon transmitter activity in different hypothalamic nuclei as well as in extrahypothalamic structures (Crowley, 1982). Estradiol administration can decrease dopamine levels in the median eminence (Gudelsky et al., 1977; Demarest et al., 1984), can directly antagonize the inhibitory action of dopamine (Raymond et al., 1978), and stimulate prolactin release (Eikenburg et al., 1977). Progesterone can increase norepinephrine turnover in the median eminence (Crowley, 1982), but not in the periventricular nucleus. Progesterone has also been shown to increase DA turnover (Cramer et al., 1979). Both estrogen and progesterone affect the activity of the enzyme tyrosine hydroxylase in the hypothalamus (Wang and Porter, 1986). The ovarian steroids significantly alter 5HT synthesis and release in serotonergic neurons projecting to the hypothalamus (James et al., 1989).

Objectives of Current Investigation

Previous investigators have suggested that daily afternoon melatonin injections in hamsters cause similar neuroendocrine and neurochemical changes as those induced by maintenance in a short photoperiod (Reiter, 1980; Steger et al., 1985). Short photoperiod induced changes in monoamine metabolism have been documented in male hamsters (Steger et al., 1986; Benson, 1987). The first objective of the present study was to determine whether melatonin

administration influenced catecholamine (DA and NE) and serotonin (5HT) metabolism in the endocrine hypothalamus of female hamsters.

A general assumption is that melatonin acts within the hypothalamus (Stankov and Reiter, 1989). A second objective of the present study was to determine whether melatonin influences monoaminergic turnover in extrahypothalamic regions of the brain, including posterior pituitary, amygdala, and striatum.

The first two objectives of the study thus were intended to provide information on four different dopaminergic systems (tuberoinfundibular, tuberohypophyseal, mesolimbic and nigrostriatal), as well as serotonergic and noradrenergic systems originating in brain stem nuclei.

Considering the established influence of the ovarian steroid hormones (estradiol and progesterone) upon neurotransmitter turnover, the development of a gonadectomized model was necessitated. A third objective was to distinguish between direct effects of melatonin administration on monoamine metabolism from indirect effects which were secondary to melatonin-induced alterations in circulating levels of gonadal steroids.

The final objective was to examine the relationship between the hormonal (LH, PRL, and T4) changes found in melatonin-treated hamsters and the melatonin-induced alterations in dopaminergic, noradrenergic, and

serotonergic metabolism.

MATERIALS AND METHODS

Animals

Sixty-four 9 week old female Syrian hamsters (strain Lak;LVG, Charles River, St. Constance, Quebec) were used in this study. They were kept under controlled lighting (14L/10D) and temperature (22 ± 2°C) conditions. Lights came on at 0400 hrs and were shut off at 1800 hrs. Light intensity at cage level was approximately 200 footcandles. The hamsters were housed 4 per cage with food (Teklad rodent diet) and water available ad libitum.

Experimental design

The hamsters were acclimatized to the laboratory conditions for one week prior to being assigned to one of the experimental groups. At this time, thirty-two (32) of the hamsters were bilaterally ovariectomized via a midline linea alba incision. An equal number of hamsters were subjected to a sham operation. Both ovariectomized and sham-operated groups of hamsters were further divided into two groups of 16. One group received daily subcutaneous injections of 0.1 ml physiological saline; the other group received daily injections of 25 micrograms of melatonin in 0.1 ml saline. All injections were administered between 1600 and 1700 hrs.

After 5 and one-half weeks of treatment vaginal smears

were taken and examined to determine whether the animals were cycling.

After 10 weeks of treatment, both the saline-injected and the melatonin-injected groups of hamsters were subdivided into two groups (of N = 8), which were killed by decapitation two hours after the subcutaneous administration of either 0.2 ml saline or 20 mg pargyline (a monoamine oxidase inhibitor) in 0.2 ml saline. All animals were killed between 1200 and 1600 hrs. Brains were removed and immediately frozen on dry ice. The anterior and posterior pituitaries were removed (separately) and frozen on dry ice.

Radioimmunoassays

Serum was collected and stored for hormone assays. Luteinizing hormone (LH) concentrations in serum and in anterior pituitary were determined using NIAMDD reagents and protocol. Prolactin (PRL) concentrations in serum and in extracts of pituitaries were determined using materials and procedure of Soares and colleagues (1983). Serum T4 levels were determined using the commercial assay of Nuclear Medical Laboratories (TETRA-TAB RIA, Dallas, Texas).

Catecholamine determination

At the time of dissection, the brains were partially thawed. A one mm deep slice of the mediobasal hypothalamus was dissected; from this slice of tissue a circular punch

of tissue (2 mm diameter) containing the median eminence and arcuate nucleus was removed. The average tissue weight (frozen) was 2.76 mg. A tissue punch of striatum (caudate n.) was taken from a coronal section of hamster brain. The average weight of this tissue was 2.65 mg. A tissue punch of amygdala was also taken from coronal sections. The average weight of these tissue punches were 3.50 mg. The tissue samples (median eminence, striatum, amygdala, and posterior pituitary) were stored frozen until they were processed for high performance liquid chromatography (HPLC). At this time the tissues were weighed, homogenized in 0.1 N perchloric acid containing the internal standard dihydroxybenzylamine (DHBA 10 ng/ml) and centrifuged at 12,000 g for 5 minutes. The supernatants were filtered with HPLC nylon filters (0.45 micron pore size) prior to injection into the HPLC system. The monoamines, norepinephrine, dopamine, and serotonin were separated and assayed by HPLC with electrochemical detection (HPLC-EC). The dopamine metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and the serotonin metabolite, 5-hydroxyindoleacetic acid (5HIAA) were assayed simultaneously.

Synthesis of monoamines was estimated as the accumulation of monoamine (per hour) after inhibition of monoamine oxidase with pargyline (11). Accumulation was calculated by subtraction of mean monoamine concentrations of hamsters not receiving pargyline from monoamine

concentrations of hamsters injected with pargyline two hours prior to sacrifice. Parygline is a non-competitive, non-selective inhibitor of monoamine oxidase A and B at high doses. Parygline is an irreversible inhibitor of monoamine oxidase; it inactivates the flavin prosthetic group of MAO following the oxidation of parygline to its reactive intermediates.

The HPLC system consisted of a Beckman solvent delivery system (Model 114M), an Altex injector (Model 210A), and a 10 cm C-18 column (Chromatography Sciences Co., Canada). The electrochemical detector (ESA Model 5100A) was equipped with a high sensitivity cell (ESA Model 5011). The detector was set at a reduction potential of -0.35 volts (with a conditioning cell at +0.40 volts). A Shimadzu integrator (Model C-R3A) was used to record and integrate peak areas, and to calculate content of monoamines and metabolites. The mobile phase consisted of 60 mM sodium acetate, 122 nM EDTA, 762 nM octane sulphonate, 7% methanol. The mobile phase was brought to a pH of 4.25 with glacial acetic acid.

Statistical analysis

All of the data were subjected to analysis of variance (anova). Three-way anova (Treatment: saline vs melatonin; Surgery: sham vs gonadectomy; Drug: saline vs pargyline) was used to analyze the hormone data and the monoamine data. Two way anova (Treatment x Surgery) was used to analyze the data on monoamine accumulation after pargyline

admnistration. The data were then subjected to Student's t-tests. Statistical significance was considered as a p value of less than 0.05. The following levels of significance, however, are distinguished: $p < .05$, $p < .01$, $p < .001$. Figures are distributed throughout the text while Tables are located in the appendix.

RESULTS

Effects of melatonin and ovariectomy on PRL, LH and T4

As in previous studies with similar protocols of melatonin administration (Trakulrungsi et al., 1979), cessation of estrous cycles were observed in melatonin-treated hamsters by 8 - 10 weeks of treatment. As expected (Reiter, 1980), pituitary and serum PRL were significantly reduced ($p < .001$, by anova and $p < .001$, via t-test), by melatonin administration (Table 1; Fig. 1). Analysis of variance indicated that injections of melatonin resulted in a significant (40%) overall increase in pituitary content of LH ($p < .05$) (Table 1). Serum LH values in intact, melatonin-treated hamsters were significantly decreased ($p < .05$, by t-test) (Fig. 2). Although not quite as dramatic as in previous studies (Vriend et al., 1982), melatonin administration resulted in significant reductions in serum T4 concentrations ($p < .05$, by anova) (Fig. 3). Pargyline treatment also decreased plasma T4 levels ($p < .05$, by anova) (Fig. 3). These data confirmed the effectiveness of melatonin in the present study.

FIGURE 1: Serum prolactin levels in female hamsters.

C - Controls (Sham-operated; Saline-injected);

P - Pargyline administered 2 hours prior to to sacrifice;

M - Melatonin administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

* $p < .05$, compared to intact controls

** $p < .01$, compared to intact controls

*** $p < .001$, compared to intact, or gonadectomized controls.

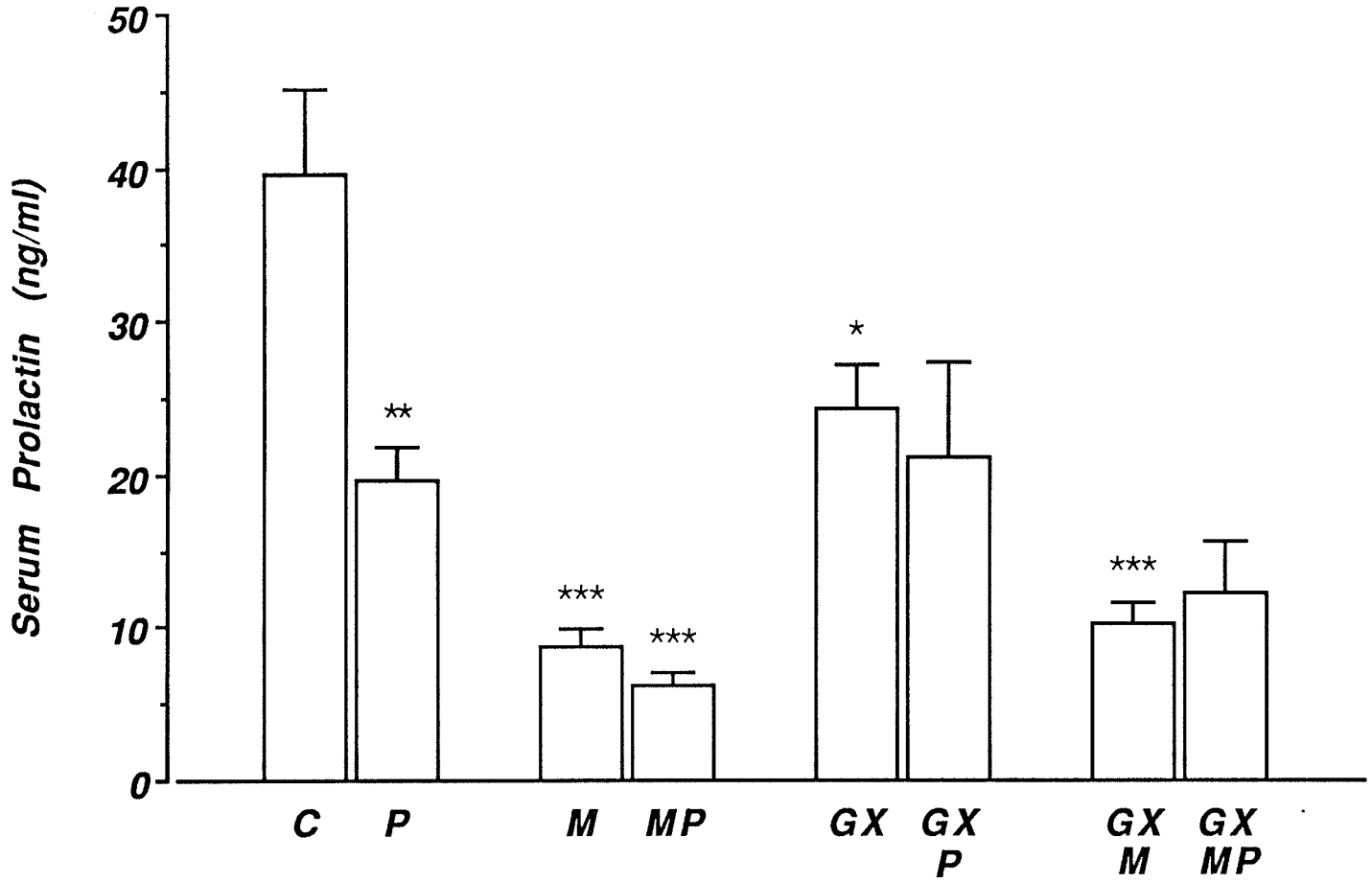

Serum Prolactin (ng/ml)
50
40
30
20
10
0
**
***
***
*
***
C
P
M
MP
GX
GX P
GX M
GX MP

FIGURE 2: Serum LH levels in female Syrian hamsters.

C - Controls (Sham-operated; Saline-injected);

P - Pargyline administered 2 hours prior to sacrifice;

M - Melatonin administered daily for 10 weeks;

S - Saline administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

* $p < .05$, compared to intact controls.

α $p < .05$, compared to intact pargyline-treated controls.

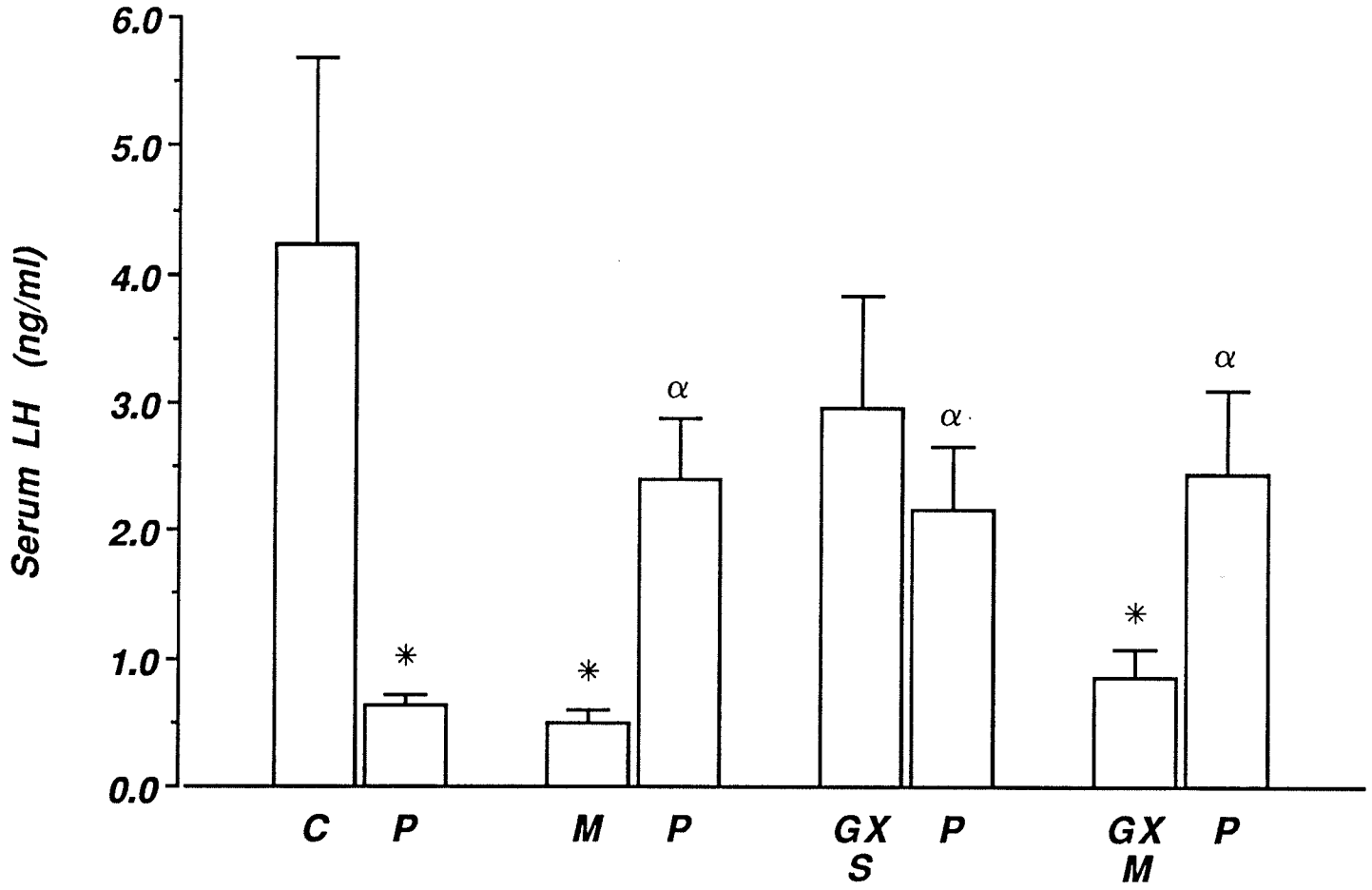

Serum LH (ng/ml)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
*
*
α
*
α
α
*
C
P
M
P
GX
S
P
GX
M
P

FIGURE 3: Serum T4 levels in female Syrian hamsters.

C - Controls (Sham-operated; Saline-injected);

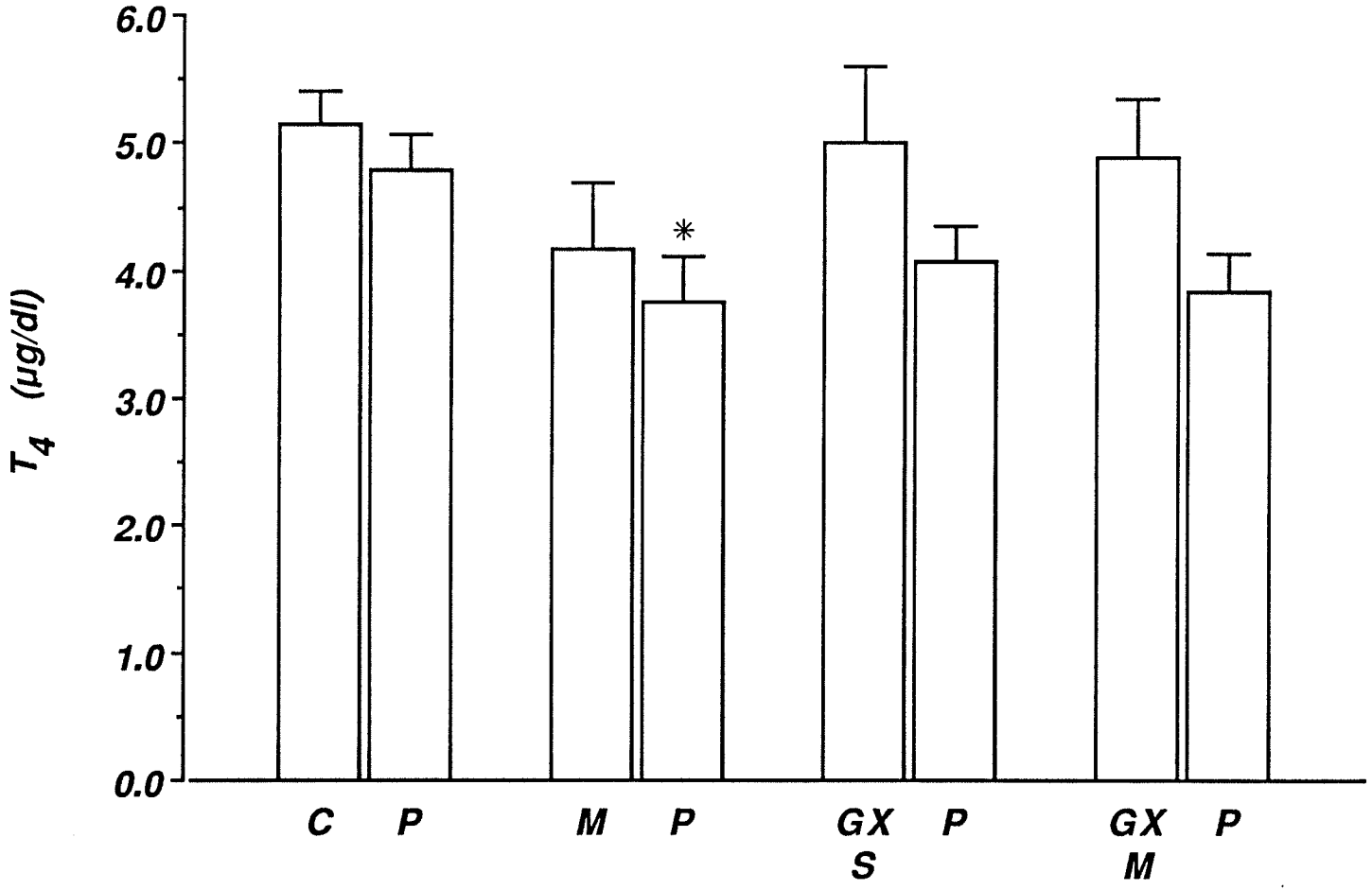
P - Pargyline administered 2 hours prior to sacrifice;

M - Melatonin administered daily for 10 weeks;

S - Saline administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

* $p < .05$, compared to pargyline-treated, intact controls.


6.0
5.0
4.0
3.0
2.0
1.0
0.0
T_4 (µg/dl)
*
C
P
M
P
GX
S
P
GX
M
P

As anticipated, ovariectomy led to a marked increase in pituitary levels of LH ($p < .001$, by anova), while pituitary PRL concentrations were reduced to a fraction of controls ($p < .001$, by anova) (Table 1). Serum levels of LH and PRL were quite variable in intact controls as would be expected in female hamsters at different stages of the estrous cycle. Serum levels of PRL, however, were significantly reduced by ovariectomy ($p < .05$, by t-test) (Fig. 1). No significant effect of ovariectomy on plasma T4 concentrations was detected (Fig. 3) by anova, and no significant interaction effects associated with ovariectomy were found.

Serotonin (5HT) metabolism in median eminence

Ovariectomized hamsters (not receiving melatonin or pargyline) had significantly lower ($p < .05$, by t-test) median eminence content of 5HT than controls (Table 2; Fig. 4). Anova indicated that the removal of the ovaries resulted in an overall decrease in 5HT content of the median eminence ($p < .05$). No significant individual differences attributable to melatonin administration could be detected by t-test. However, a small, but significant, overall increase in 5HT content of the median eminence of melatonin treated hamsters was noted by anova ($p < .01$) (Table 2; Fig. 4).

The accumulation of 5HT after pargyline administration (pg/mg/hr) was not significantly influenced by either

FIGURE 4: Effects of melatonin on serotonin (5HT) concentrations (pg/mg) in median eminence.

C - Controls (Sham-operated; Saline-injected);

P - Pargyline administered 2 hours prior to sacrifice;

M - Melatonin administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

* $p < .05$, compared to intact controls.

Anova showed an overall melatonin-induced increase in 5HT concentrations ($p < .01$), in addition to a decrease in 5HT content induced by gonadectomy ($p < .05$).

Pargyline treatment markedly increased 5HT concentrations ($p < .001$)

Pargyline	$F = 116.86$	($p < .001$)
Melatonin	$F = 7.54$	($p < .01$)
Gonadectomy	$F = 4.99$	($p < .05$)

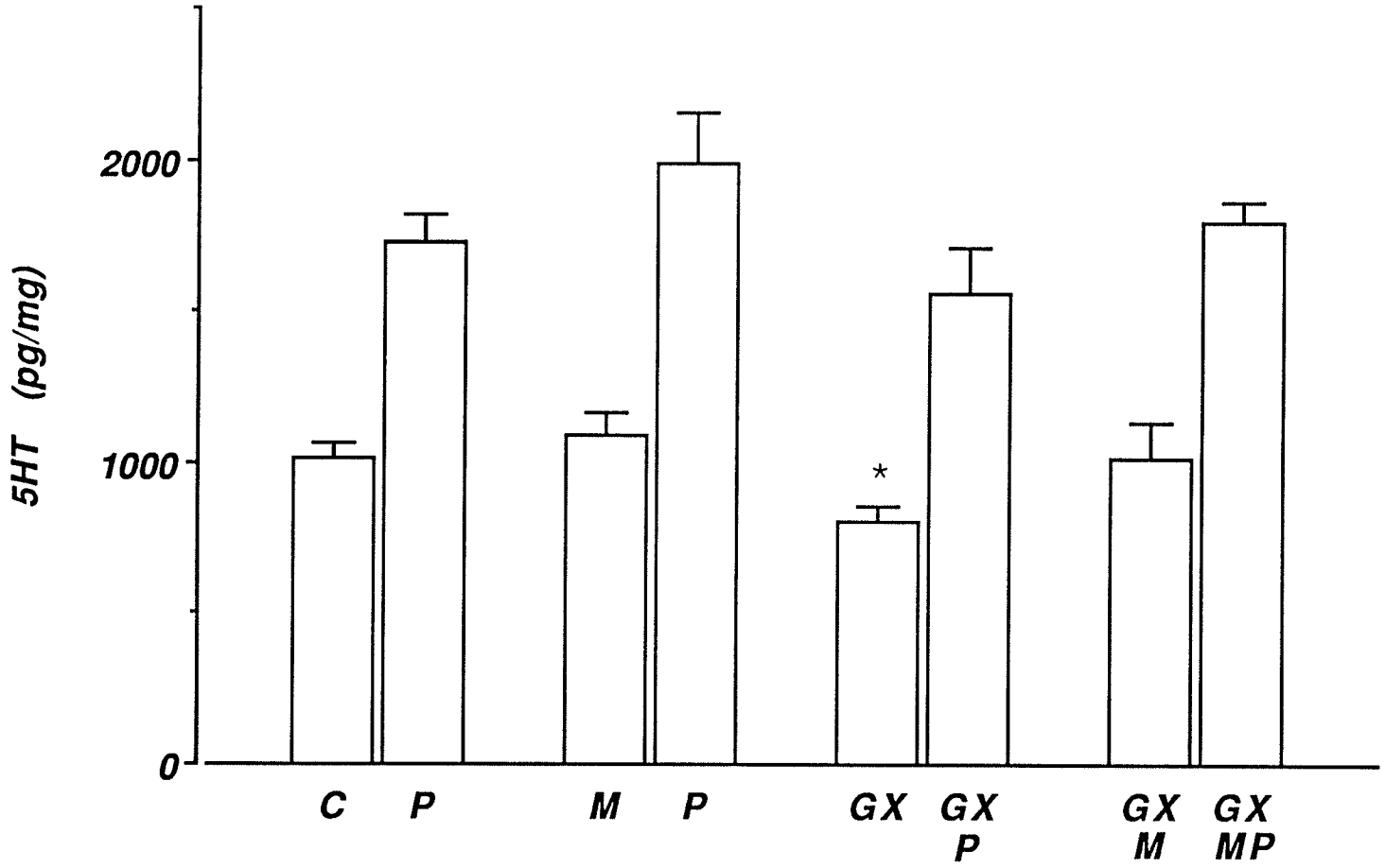

5HT (pg/mg)
2000
1000
0
*
C
P
M
P
GX
GX
P
GX
M
GX
MP

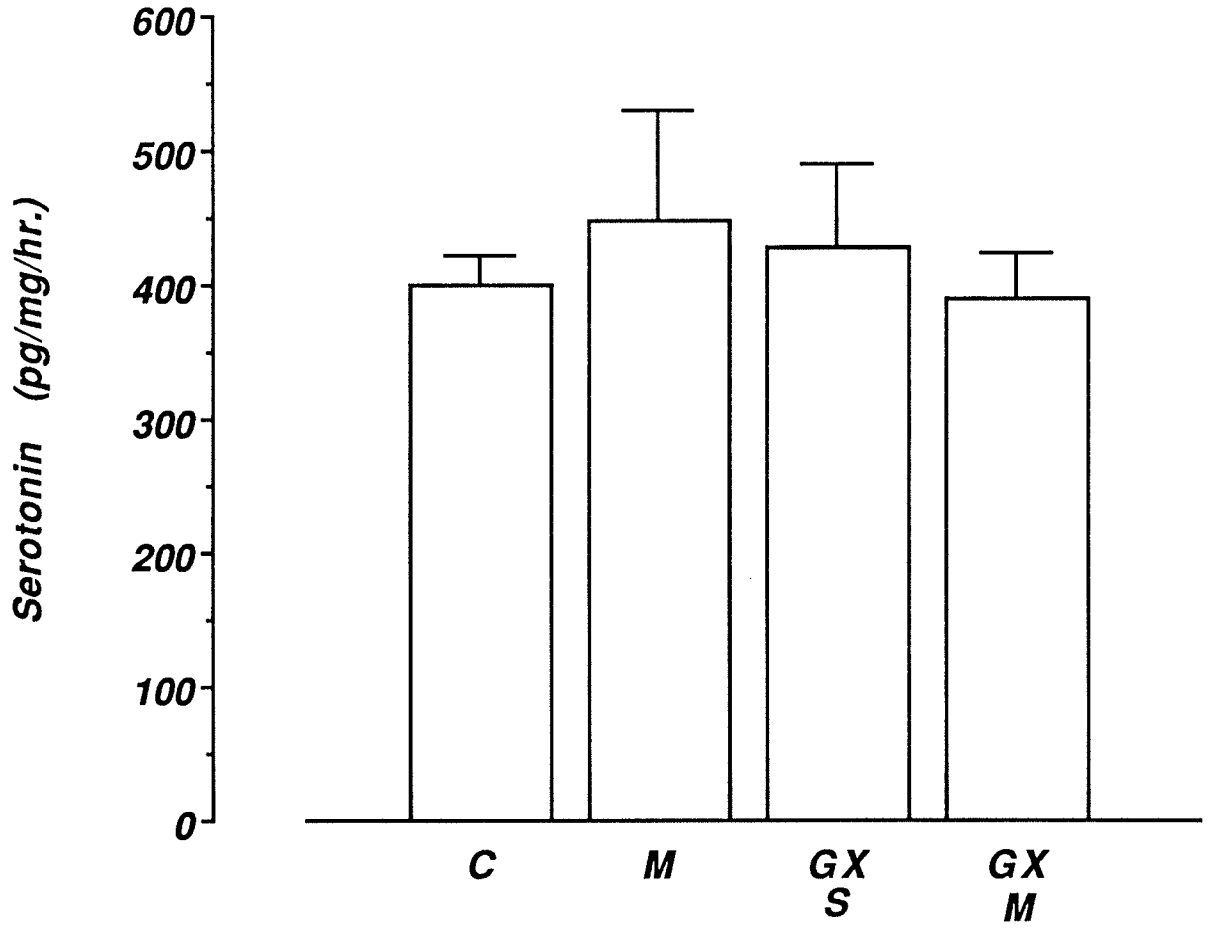
FIGURE 5: Effects of melatonin on serotonin accumulation in median eminence after administration of pargyline.

C - Controls (Sham-operated; Saline-injected);

M - Melatonin administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

S - Saline administered daily for 10 weeks.


Serotonin (pg/mg/hr.)
600
500
400
300
200
100
0
C
M
GX S
GX M

melatonin treatment or by gonadectomy (Fig. 5).

No significant effect of gonadectomy was observed on 5HIAA content or on the disappearance of 5HIAA (pg/mg/hr) after pargyline administration (Table 3). Melatonin administration resulted in a significant ($p < .05$, by anova) increase in 5HIAA content in hamsters not treated with pargyline. The disappearance of 5HIAA after administration of pargyline (Fig. 6) was also significantly greater in melatonin-treated hamsters ($p < .01$, by anova). Melatonin significantly increased 5HIAA disappearance ($p < .01$, by t-test, Fig. 6) in median eminence.

Catecholamine metabolism in median eminence

Ovariectomy resulted in a significant ($p < .01$, by t-test) increase in DA concentrations after pargyline administration (Table 2; Fig. 7).

Anova indicated a highly significant overall effect of melatonin administration ($p < .001$). An interaction effect ($p < .01$) in the anova (pargyline x melatonin) suggested that the inhibitory effect was primarily in the pargyline treated hamsters and this was confirmed by t-test. In both intact ($p < .05$) and ovariectomized ($p < .01$) hamsters significant inhibitory effects on DA content after pargyline was observed (Table 2; Fig. 7).

Calculation of the accumulation of DA after pargyline indicated a significant increase ($p < .01$) in ovariectomized hamsters (Fig. 8). Although DA accumulation

FIGURE 6: Effects of melatonin on disappearance of 5HIAA (pg/mg/hr) after administration of pargyline in median eminence and striatum (caudate nucleus). Sal. - Saline treatment for 10 weeks; (both sham-operated and gonadectomized hamsters) Mel. - Melatonin treatment for 10 weeks; (both sham-operated and gonadectomized hamsters) ** $p < .01$, compared to controls.

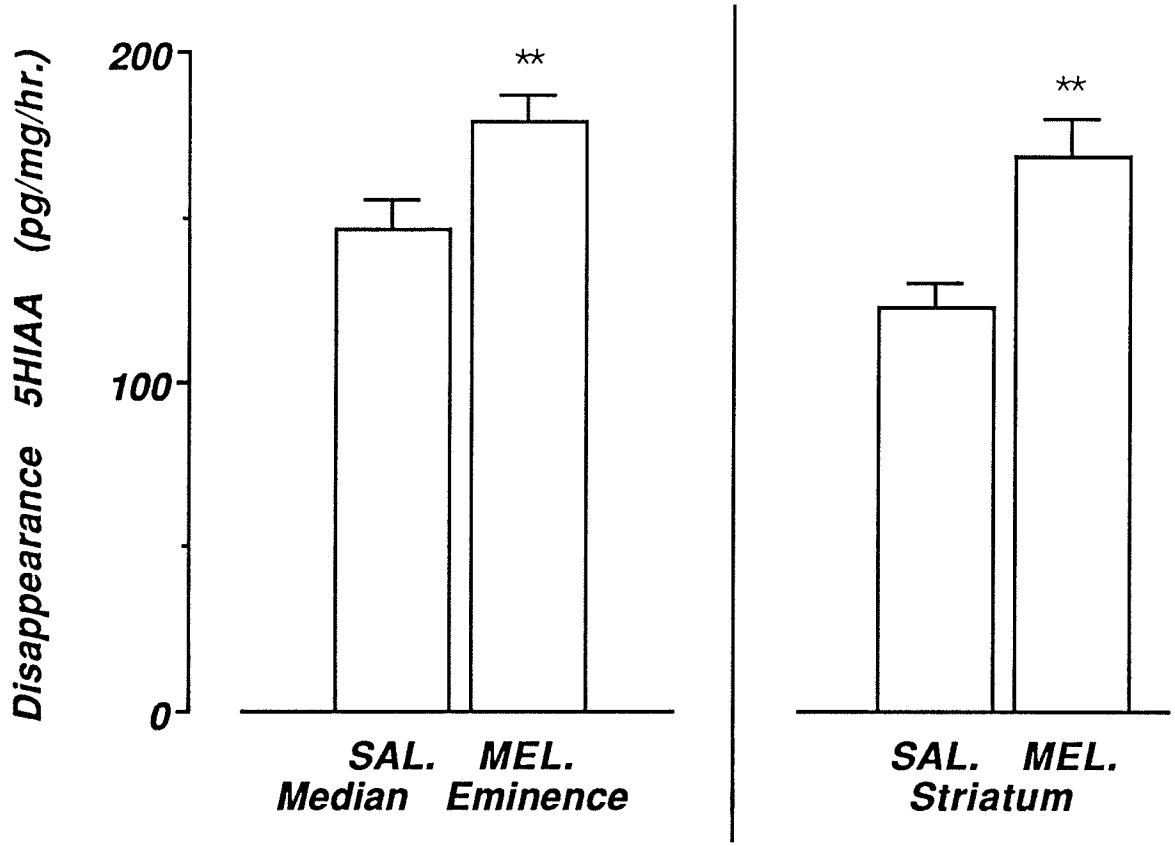

Disappearance 5HIAA (pg/mg/hr.)
200
100
0
**
**
SAL. MEL.
Median Eminence
SAL. MEL.
Striatum

FIGURE 7: Effects of melatonin on DA concentrations (pg/mg) in median eminence.

C - Controls (Sham-operated; Saline-injecetd);

P - Pargyline administered 2 hours prior to sacrifice;

M - Melatonin administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at beginning of the experiment.

* $p < .05$, compared to pargyline-treated intact controls.

** $p < .01$, compared to pargyline-treated gonadectomized controls.

α $p < .001$, compared to all other groups.

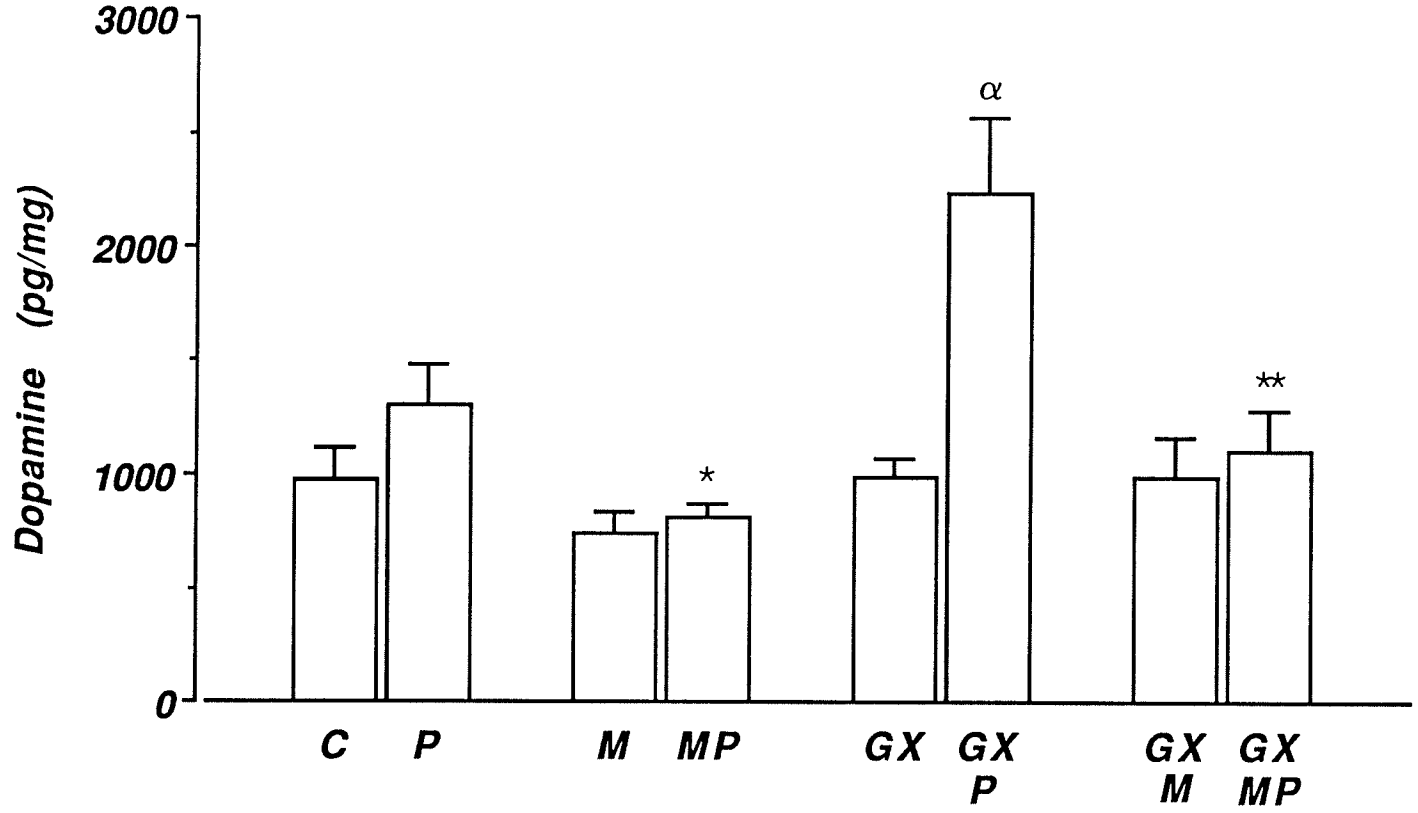

Dopamine (pg/mg)
3000
2000
1000
0
α
*
**
C
P
M
MP
GX
GX P
GX M
GX MP

FIGURE 8: Effects of melatonin on dopamine accumulation (pg/mg/hr) in median eminence after administration of pargyline.

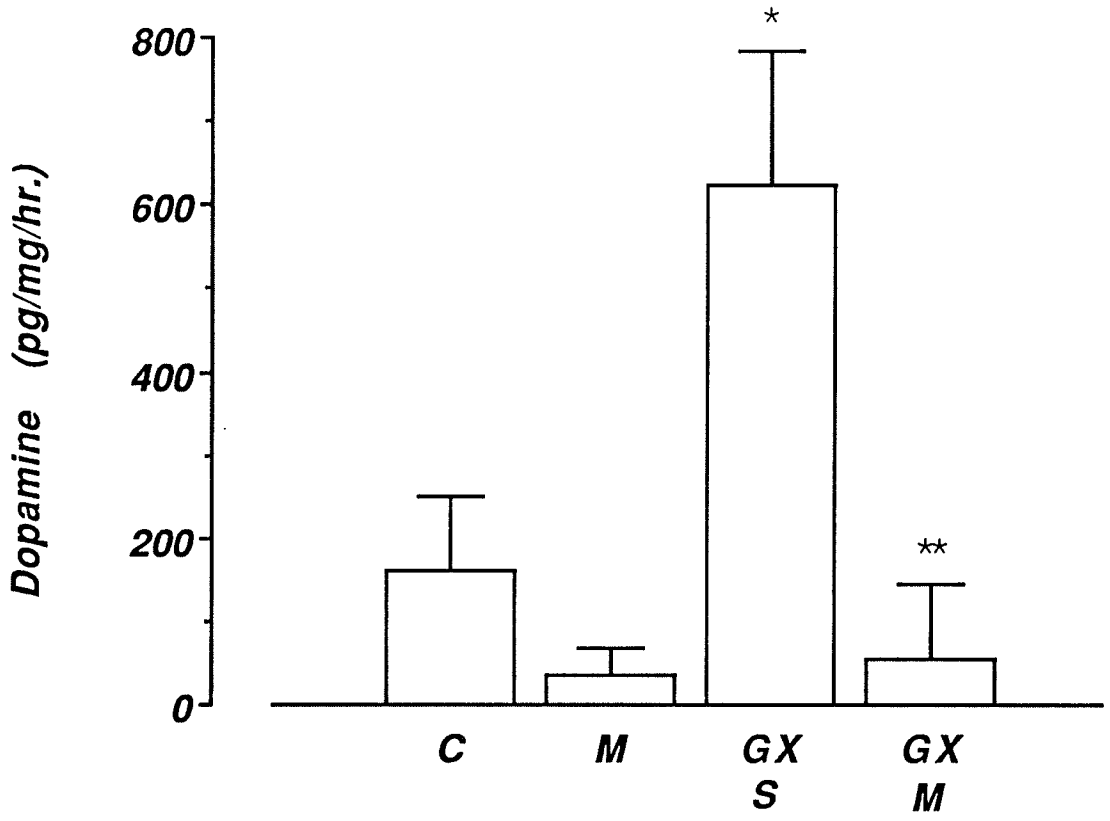
C - Controls (Sham-operated; Saline-injected);

M - Melatonin administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at beginning of experiment.

* $p < .05$, compared to intact controls.

** $p < .01$, compared to ovariectomized (GX) controls.


Dopamine (pg/mg/hr.)
800
600
400
200
0
*
**
C
M
GX S
GX M

was decreased by melatonin in both intact (to 22% of that of intact controls), and ovariectomized hamsters (to 9% of that of ovariectomized controls), this was more significant in ovariectomized animals (Fig. 8).

Concentrations of the DA metabolites, DOPAC and HVA, were not significantly influenced by either ovariectomy or melatonin administration (Table 2).

Concentrations of norepinephrine (NE) were not significantly influenced by melatonin administration or ovariectomy (Table 4). The accumulation of NE after pargyline treatment, however was significantly decreased by melatonin in ovariectomized hamsters ($p < .025$, by anova) (Fig. 9). In fact, in gonadectomized hamsters treated with melatonin, no increase in NE after pargyline was demonstrated, when compared to ovariectomized controls ($p < .05$, by t-test) (Fig. 9).

Dopamine metabolism in posterior pituitary

An overall significant inhibitory effect was demonstrated by melatonin administration on the content of DA and its metabolite, DOPAC in posterior pituitary (Table 5). DA concentrations in intact melatonin/pargyline treated hamsters were significantly decreased from respective controls ($p < .05$, by t-test). No significant effect on DA or NE accumulation after pargyline administration was detected (Table 6).

FIGURE 9: Effects of melatonin on norepinephrine (NE) accumulation (pg/mg/hr) in median eminence after administration of pargyline.

C - Controls (Sham-operated; Saline-injected);

M - Melatonin administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at beginning of experiment;

S - Saline treatment for 10 weeks.

* $p < .05$, compared to ovariectomized (GX) controls.

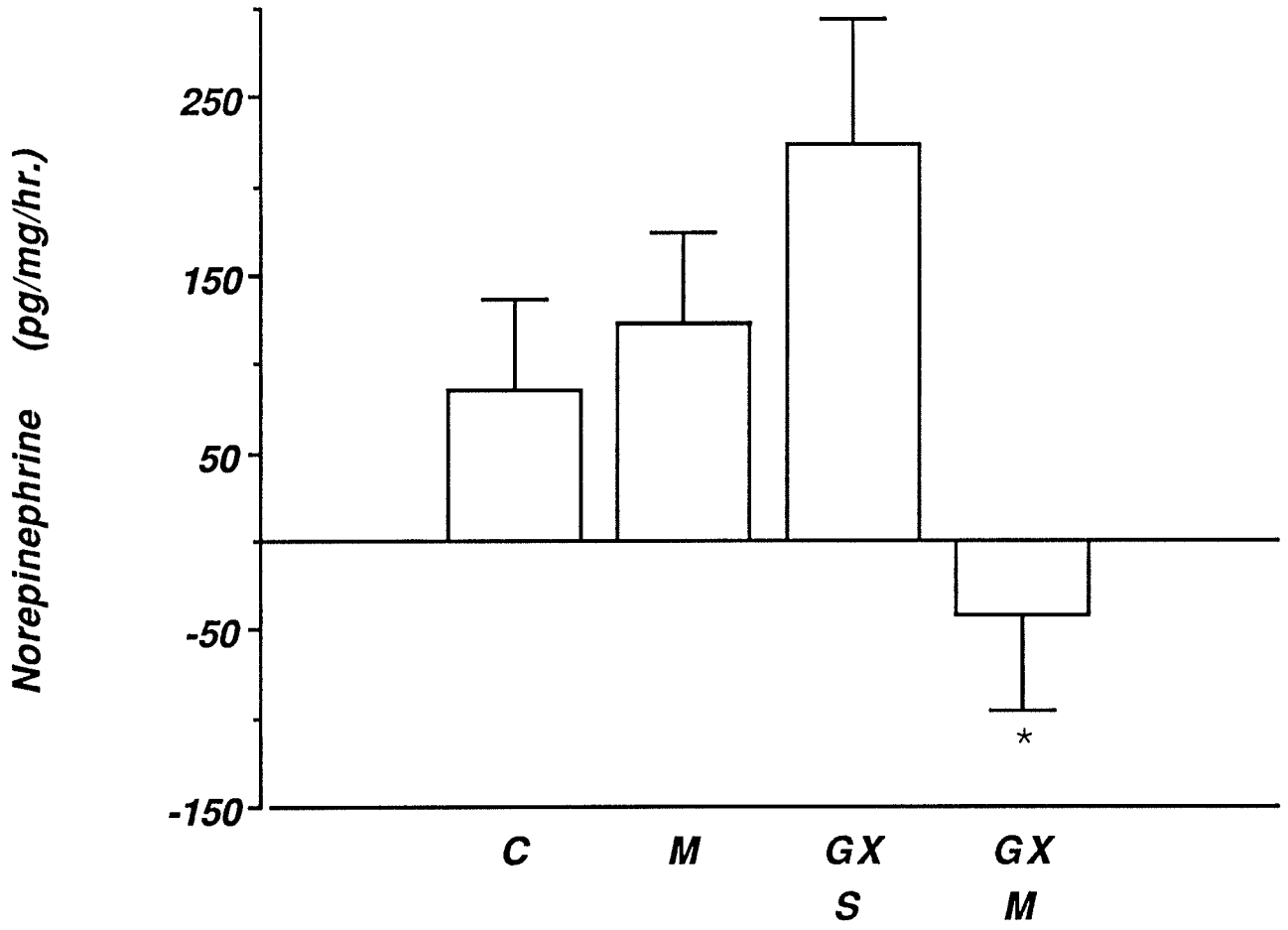

Norepinephrine (pg/mg/hr.)
250
150
50
-50
-150
*
C
M
GX S
GX M

FIGURE 10: Effects of melatonin on 5HT concentrations (pg/mg) in striatum.

C - Controls (Sham-operated; Saline-injected);

P - Pargyline administered 2 hours prior to sacrifice;

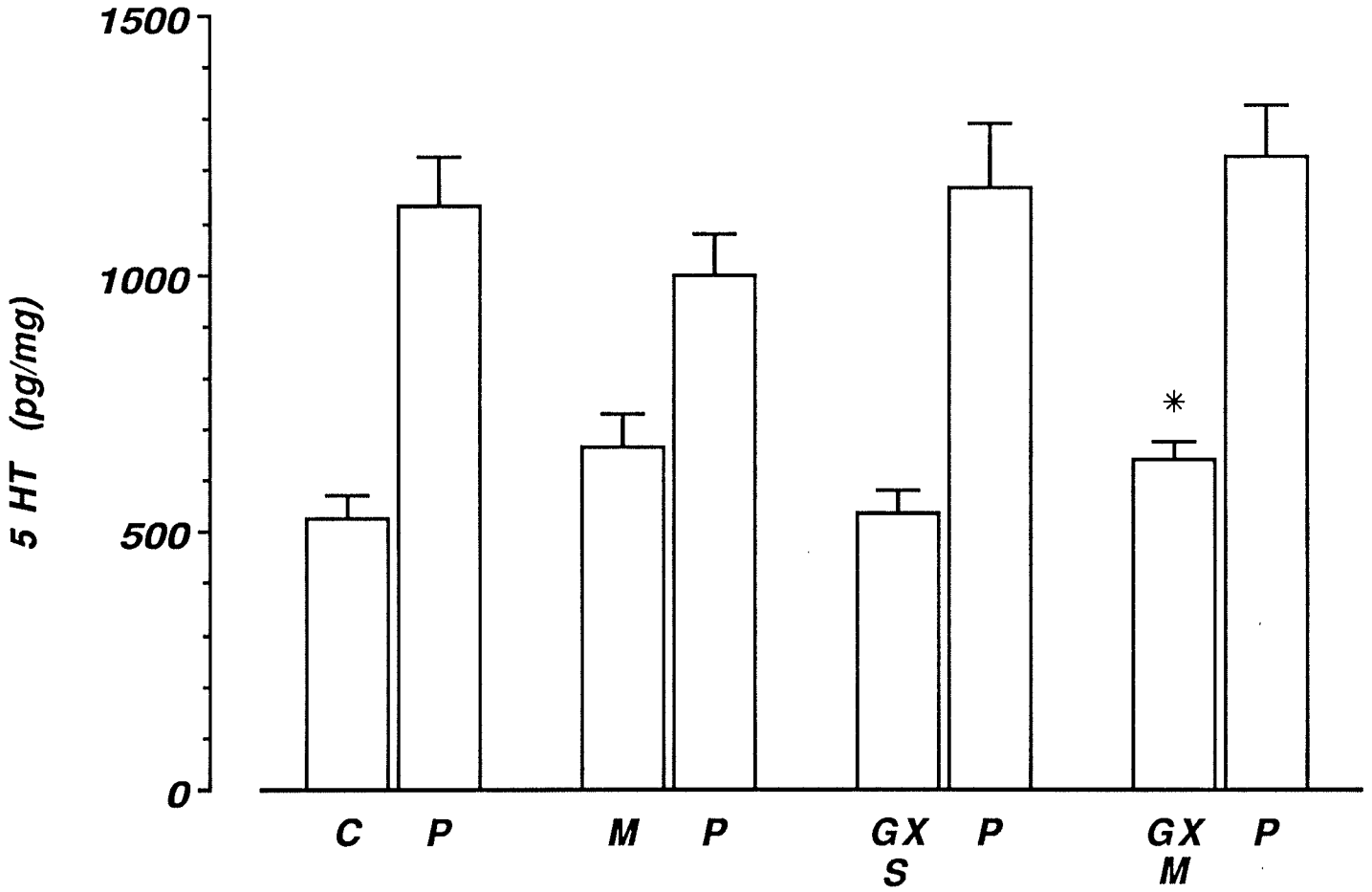
M - Melatonin administered daily for 10 weeks;

S - Saline administered for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

* $p < .05$, compared to intact controls.

Anova showed an overall increase in 5HT concentrations induced by melatonin in non-pargyline groups.


1500
1000
500
0
5 HT (pg/mg)
*
C
P
M
P
GX
S
P
GX
M
P

Monoamine metabolism in striatum

Ovariectomy did not significantly influence serotonin (5HT) concentrations in striatal tissue punches (Table 7; Fig. 10). Anova indicated increased serotonin content in striatal samples of melatonin but not in pargyline treated hamsters (interaction effect, $p < .05$). Concentrations of 5HIAA were significantly increased by melatonin treatment (interaction effect, $p < .01$, by anova) in hamsters not treated with pargyline (Table 3). Also, ovariectomized melatonin -treated hamsters had significantly higher 5HIAA concentrations than saline-treated, ovariectomized animals non-pargyline injected ($p < .05$, by t-test). The disappearance of 5HIAA after pargyline was significantly increased ($p < .01$, by anova) by administration of melatonin (Fig. 6).

No significant effects of ovariectomy or of melatonin administration could be detected in DA or NE concentrations, or in the content of the DA metabolites, DOPAC or HVA (Table 4, 7). No significant effects of either treatment was observed on the accumulation of NE, 5HT or DA after pargyline administration (Table 8; Fig 11, 12).

Monoamine metabolism in amygdala

Concentrations of NE, 5HT, and DA were not significantly different in ovariectomized or melatonin-

FIGURE 11: Effects of melatonin on 5HT accumulation (pg/mg/hr) in striatum after administration of pargyline.

C - Controls (Sham-operated; Saline-injected);

M - Melatonin administered daily for 10 weeks;

S - Saline administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

* $p < .05$, compared to intact controls.

Anova - no significance demonstrated.

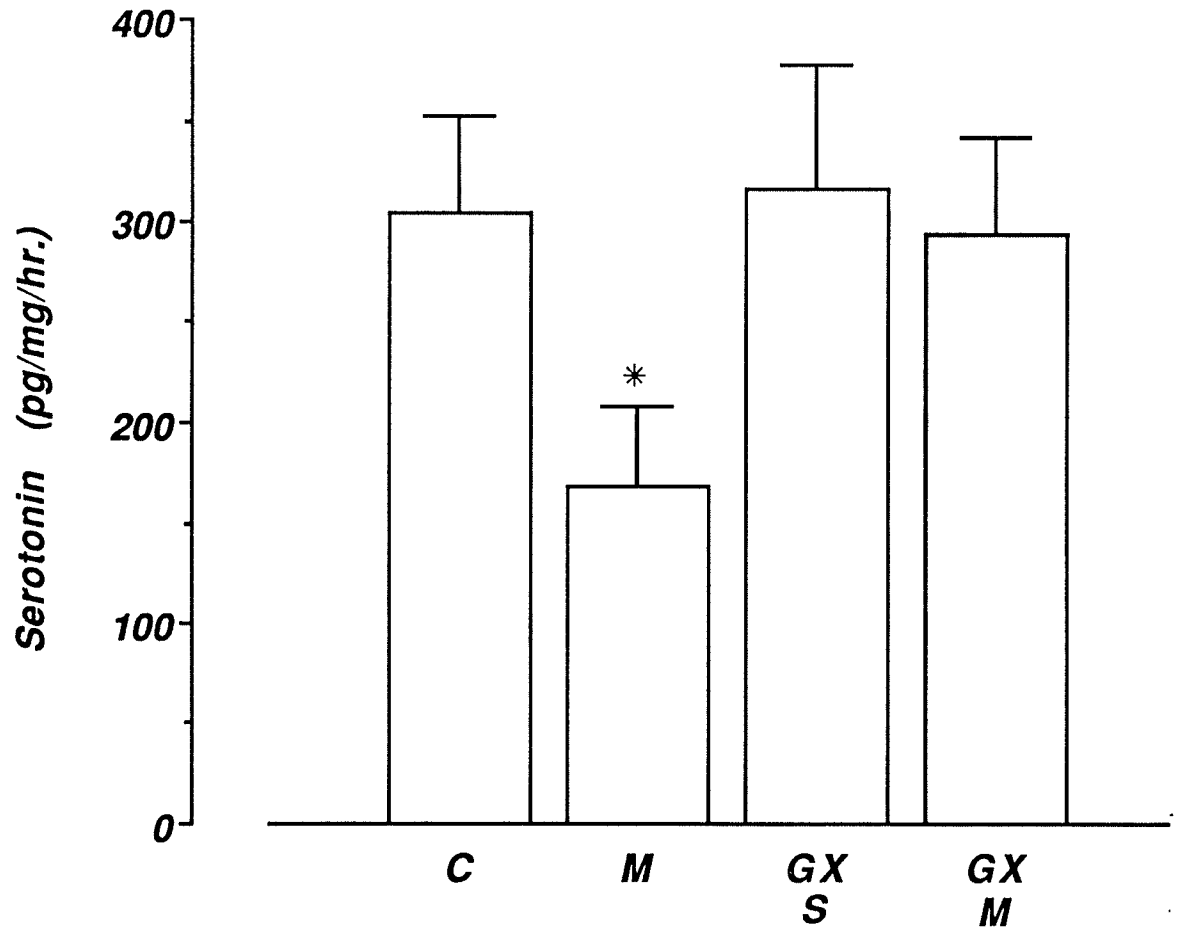

400
300
200
100
0
Serotonin (pg/mg/hr.)
*
C
M
GX S
GX M

FIGURE 12: Effects of melatonin on DA accumulation (pg/mg/hr) in striatum after administration of pargyline.

C - Controls (Sham-operated; Saline-injected);

M - Melatonin administered daily for 10 weeks;

S - Saline administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

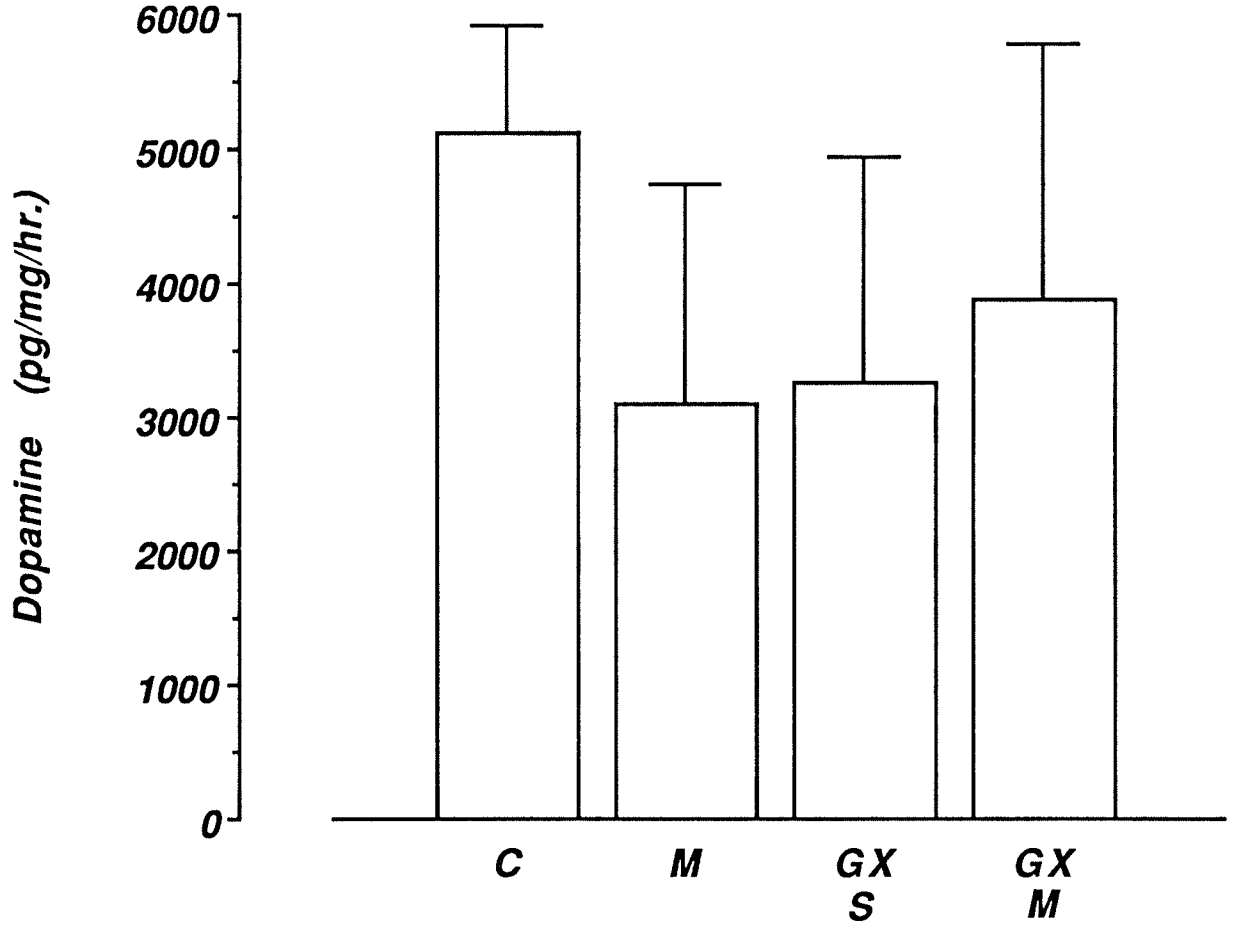

Dopamine (pg/mg/hr.)
6000
5000
4000
3000
2000
1000
0
C
M
GX S
GX M

FIGURE 13: Effects of melatonin on 5HT accumulation (pg/mg/hr) in amygdala after administration of pargyline.

C - Controls (Sham-operated; Saline-injected);

M - Melatonin administered daily for 10 weeks;

S - Saline administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

** $p < .01$, compared to ovariectomized (GX) controls.

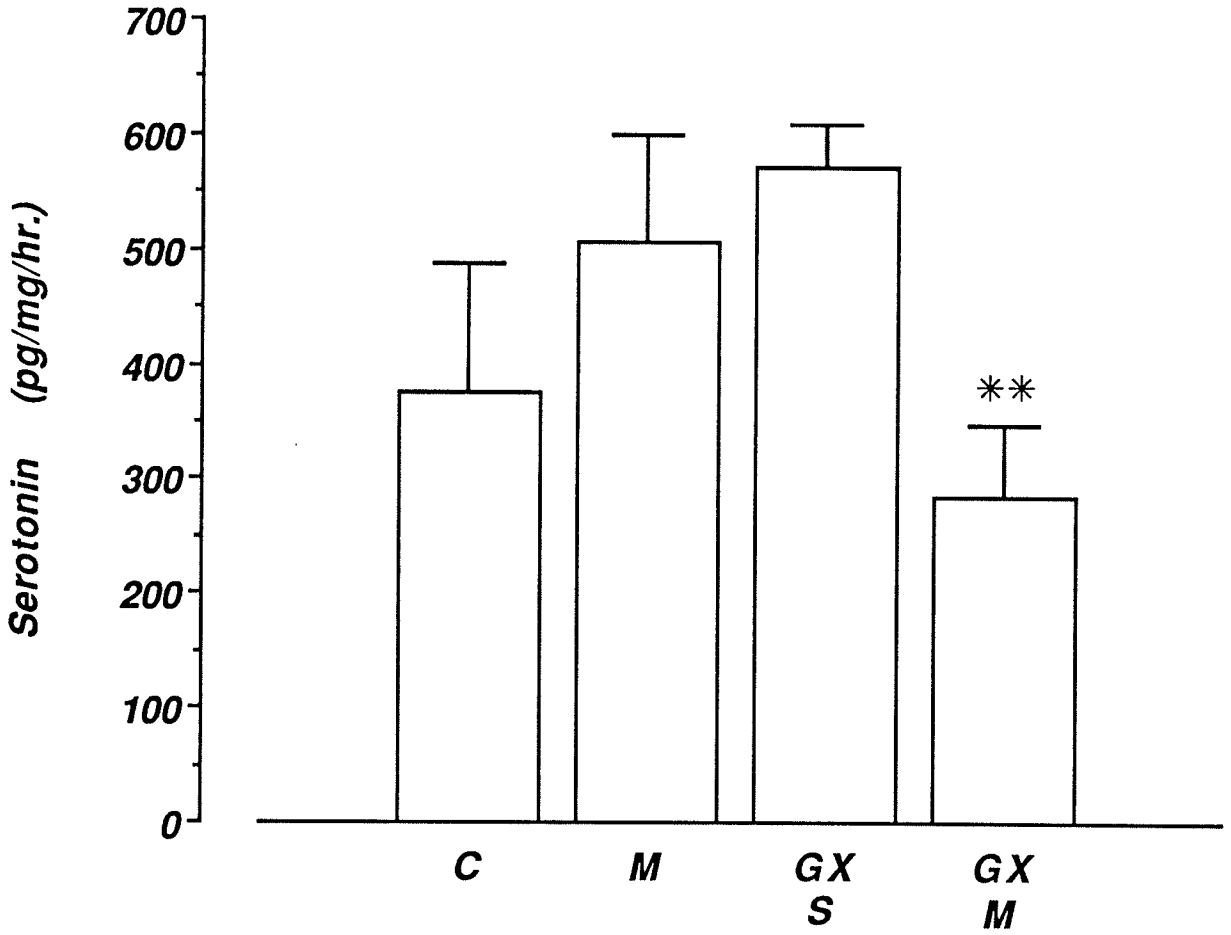

Serotonin (pg/mg/hr.)
700
600
500
400
300
200
100
0
**
C
M
GX S
GX M

FIGURE 14: Effects of melatonin on disappearance of 5HIAA (pg/mg/hr) in amygdala after administration of pargyline.

C - Controls (Sham-operated; Saline-injected);

M - Melatonin administered daily for 10 weeks;

S - Saline administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

* $p < .05$, compared to controls.

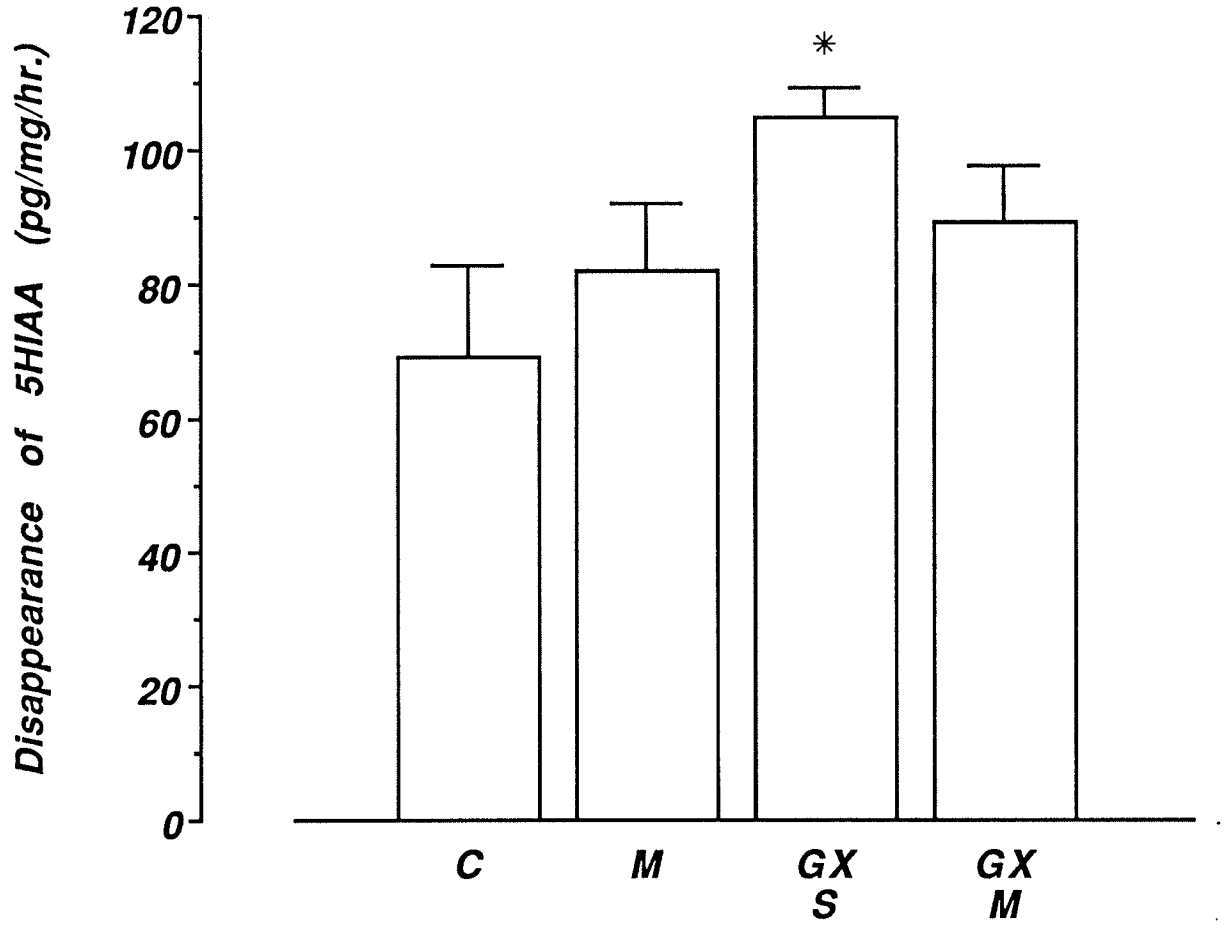

Disappearance of 5HIAA (pg/mg/hr.)
120
100
80
60
40
20
0
*
C
M
GX S
GX M

FIGURE 15: Effects of melatonin on norepinephrine (NE) accumulation (pg/mg/hr) after administration of pargyline in amygdala.

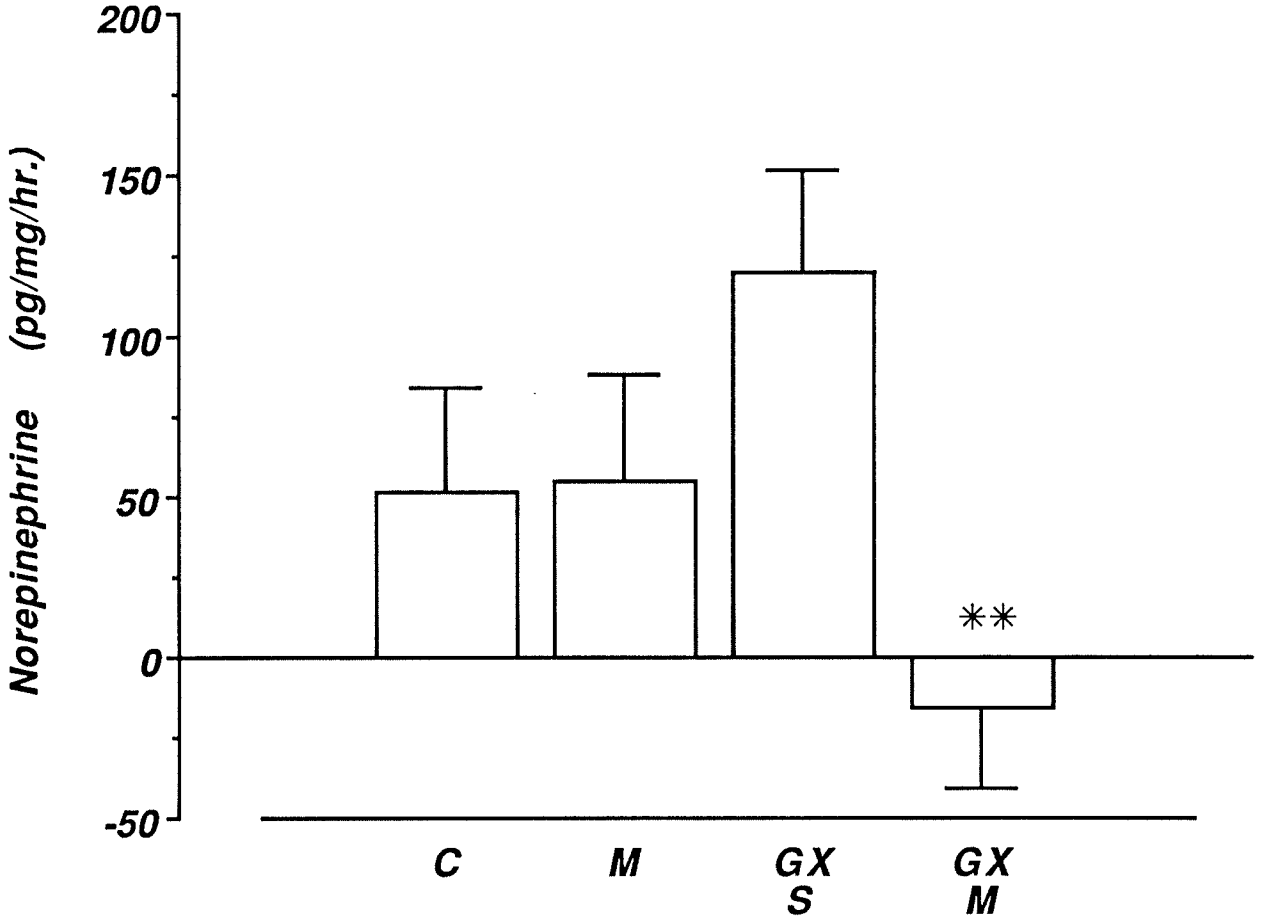
C - Controls (Sham-operated; Saline-injected);

M - Melatonin administered daily for 10 weeks;

S - Saline administered daily for 10 weeks;

GX - Gonadectomy (ovariectomy) performed at the beginning of the experiment.

** $p < .01$, compared to ovariectomized (GX) controls.


200
150
100
50
0
-50
Norepinephrine (pg/mg/hr.)
**
C
M
GX S
GX M

treated hamsters (Tables 4, 9, 10). Although no significant differences could be detected in the 5HT content in the amygdala of melatonin-treated animals, significant decreases in 5HT accumulation after pargyline was demonstrated as an interaction effect - the reductions in 5HT turnover were found in only the ovariectomized hamsters treated with melatonin ($p < .025$, by anova) (Fig. 13). There was an overall significant increase in the disappearance of 5HIAA after pargyline in ovariectomized hamsters ($p < .05$, by anova) (Fig. 14).

Although concentrations of NE in the amygdala were not significantly different (Table 4), melatonin administration resulted in a significantly decreased accumulation of NE after pargyline ($p < .05$ by anova) in ovariectomized hamsters only (melatonin/ovariectomy interaction $F=5.06$). No significant effects of melatonin administration upon DA accumulation after pargyline, however, could be detected in the amygdala tissue punches (Table 10). There were significantly increased DOPAC and HVA values in GX hamsters not treated with melatonin or pargyline. This was shown to be significant via an interaction effect in the anova ($p < .05$, Table 10).

DISCUSSION

Since the work of Tamarkin et al., (1976), demonstrating that afternoon melatonin administration induced an anestrous condition in female Syrian hamsters,

numerous investigators have studied the effects of melatonin on the secretion of pituitary hormones - particularly those involved in reproduction. The endocrine hypothalamus is hypothesized to be a putative site at which melatonin modulates the monoamine systems that are involved in the regulation of hypothalamic and pituitary hormone release. The data derived from the present study provides direct evidence that melatonin administration influences the metabolism of serotonin, dopamine and norepinephrine in the endocrine hypothalamus. Although the data also provide evidence for extrahypothalamic effects of melatonin on monoamine metabolism, the melatonin-induced changes in hypothalamic amines are considered to be involved in the regulation of secretion of pituitary hormones.

Melatonin influences serotonin metabolism;

No significant effect of melatonin administration on the rate of 5HT accumulation after pargyline administration was found in the median eminence/arcuate region of the hypothalamus (Fig. 5). This would suggest no effect of melatonin administration on 5HT synthesis in this region. However, increased 5HIAA levels (Table 3) as well as the amount that disappeared after pargyline (Fig. 6), was evidence that melatonin administration did significantly influence serotonin metabolism in the median eminence/arcuate region of the hypothalamus. Since the melatonin-induced increase in 5HIAA content of the median

eminence/ arcuate region was statistically unrelated to the presence or absence of the ovaries, the data suggest that melatonin influenced 5HIAA concentrations independently of variations in ovarian hormones. Ovariectomy, by itself, however, had a small but significant inhibitory effect on 5HT content of the median eminence/arcuate region (Table 2; Fig. 4).

Measurement of 5HIAA in tissue extracts has been interpreted by neurochemists as representing catabolism of excess intracellular 5HT by monoamine oxidase (Kuhn et al., 1986). The rate of synthesis of 5HT is not necessarily reflected in the tissue concentrations of 5HIAA. 5HT concentrations in rats and hamsters were reported to be higher during the daytime (Ferraro and Steger, 1990), while 5HIAA levels in rats may be higher during the dark phase of the light-dark photic cycle (Hery et al., 1972).

Evidence for melatonin-induced increases in the content of 5HIAA in the hypothalamus was demonstrated to occur in male hamsters (Vriend et al., in press). Similar increases in daytime 5HIAA content of the mediobasal hypothalamus were induced in male hamsters by short photoperiod or by blinding (Benson, 1987; Vriend, 1989). The present study represents the first report of the effects of melatonin administration on serotonin metabolism in female hamsters.

The hypothesis that the neuroendocrine effects of melatonin are mediated by central serotonergic neurons

(originating from brainstem raphe nuclei), was based on data demonstrating increased 5HT levels in rats after daytime melatonin administration (Anton-Tay et al., 1968). Sugden and Morris (1979) later showed that removal of the pineal gland, the major source of melatonin production, significantly reduced 5HIAA levels in the hypothalamus, midbrain and hippocampus of the rat. Decreases in mediobasal hypothalamic and brainstem 5HT and 5HIAA levels in pinealectomized rats was also demonstrated by Aldegunde and collaborators (1987).

Although no significant effects of melatonin on serotonin synthesis in the caudate nucleus was detected (via ANOVA), significant melatonin-induced increases in concentrations of 5HIAA were observed in this region (Table 3). Therefore, the data do not appear to be consistent with a single site of action for melatonin on serotonergic neurons within the median eminence.

Statistically the increased 5HIAA observed in caudate nucleus of melatonin-treated hamsters was unrelated to the presence or absence of ovaries. Therefore this effect on 5HIAA is interpreted as occurring independently of melatonin-induced changes in concentrations of gonadal steroids. Thus the effect of melatonin on 5HIAA concentrations were similar in both median eminence/ arcuate region and in caudate nucleus.

Decreased accumulation of 5HT after pargyline administration in amygdala tissue of melatonin-treated

hamsters that were ovariectomized (Fig. 13) suggested that melatonin administration significantly inhibited serotonin synthesis in the amygdala. Since no significant inhibition was observed in intact hamsters, the data suggest that the presence of ovarian hormones masked an effect of melatonin on serotonin synthesis. The inhibitory effect of melatonin in gonadectomized hamsters provides evidence for an effect of melatonin unrelated to any effects of gonadal steroids.

Although the present results demonstrate that melatonin injections significantly influence serotonergic metabolism, it is not entirely clear how this occurs. Does melatonin treatment shift the peak period of daytime serotonin synthesis? Does melatonin act directly on serotonergic axon terminals, on cell bodies of serotonergic neurons which distribute themselves to the caudate nucleus, the amygdala and the median eminence, or does melatonin act on other neurons which synapse on serotonergic neurons?

Melatonin influences dopamine metabolism;

Four major dopaminergic systems are represented in this current investigation - the tuberoinfundibular (median eminence), the tuberohypophyseal (posterior pituitary), the nigrostriatal (caudate n.), and the mesolimbic (amygdala). Unlike the nigrostriatal, mesolimbic or tuberohypophyseal systems, the tuberoinfundibular dopaminergic system is characterized by an absence of terminal autoreceptors, no post-synaptic membrane and an absence of a high-affinity

amine reuptake mechanism (Demarest and Moore, 1979; Annunziato and Weiner, 1980). Dopamine is released from the tuberoinfundibular terminals directly into the hypophyseal vessels, via exocytosis.

The current investigation has provided strong evidence that melatonin administration reduced dopamine content in the median eminence (Table 2; Fig.7). The accumulation of DA was greatly inhibited in melatonin-treated hamsters (Fig. 8) suggesting that this hormone markedly inhibits DA synthesis in tuberoinfundibular neurons. In the median eminence of intact hamsters, the accumulation of dopamine after pargyline administration, was reduced to 22% of controls, while in ovariectomized hamsters the accumulation was reduced to 9% of controls by melatonin.

The data showing significant inhibitory effects of melatonin on DA synthesis in median eminence/arcuate tissue (Fig. 8), in ovariectomized hamsters provide evidence that melatonin-induced inhibition of DA synthesis occurred independently of changes in blood concentrations of ovarian steroids. Since ovariectomy increased the differences in DA synthesis between control and melatonin-injected hamsters, it appears that the presence of the ovaries partially masked the effects of melatonin in intact hamsters.

The marked increase in DA synthesis observed in the median eminence/arcuate region in ovariectomized hamsters (Fig. 8) could be the result of an increase in the activity

of the enzyme, tyrosine hydroxylase. Beattie et.al, (1972), demonstrated a substantial increase in tyrosine hydroxylase activity in the hypothalamus of the female rat following ovariectomy. Increases in the activity of this enzyme have also been reported in the median eminence of the gonadectomized male rat (Kizer et al., 1974). One interpretation of the melatonin-induced inhibition of DA synthesis in ovariectomized hamsters (Fig. 8) is that melatonin administration decreased the activity of this enzyme. A mechanism by which melatonin could achieve this is via an inhibition of cAMP synthesis (Vanecek and Vollrath, 1990; Weaver et al., 1991).

Concentrations of DA metabolites, DOPAC and HVA in median eminence/ arcuate tissue were not significantly influenced by either ovariectomy or melatonin administration. It should be noted that because there is no reuptake mechanism for the DA that is released from terminals into portal vessels, the concentrations of DOPAC and HVA are relatively low in the median eminence region. The levels of these metabolites therefore do not represent a good method of measuring turnover. Released tuberoinfundibular DA is not subjected to degradation by the enzyme catechol-O-methyltransferase (COMT), since there are no synapses or post-synaptic membranes in this system.

Data derived from the posterior pituitary showed an overall inhibitory effect of melatonin treatment on DA content as well as on the content of the dopamine

metabolite DOPAC (Table 5). Since the tuberohypophyseal neurons, like the tuberoinfundibular DA neurons, originate in the arcuate nucleus, the data raise the question of whether melatonin acts at this site, or on neurons impinging on this nucleus. The DA data of the posterior pituitary should be interpreted with caution however, since analysis of variance showed no significant effect of either gonadectomy or of melatonin administration on DA synthesis.

The fact that there were no significant effects of melatonin administration on DA content or turnover in caudate nucleus of intact or of ovariectomized hamsters (Table 7; Fig. 12) suggest that DA metabolism within the striatum was not influenced by melatonin in the same manner as in the median eminence. However, it is possible that a compensatory mechanism may be involved in DA activity in the nigrostriatal dopaminergic system.

In the amygdala tissue punches, DA accumulation was reduced to approximately 7% of controls in ovariectomized hamsters with a significant ($p < .05$) increase in the concentrations of the dopamine metabolites DOPAC and HVA (Table 10). Evidence that melatonin had any effect on DA metabolism within the amygdala, was limited to the data showing a significant difference between HVA concentrations of saline and melatonin injected hamsters that were gonadectomized. Melatonin administration appeared to reverse the effects of ovariectomy. Since the products of COMT catabolism (3MT and normetanephrine) were not measured

in the current study, a complete account of DA metabolism is not presented.

Melatonin has been reported to inhibit DA synthesis and release in vitro. It has been shown to inhibit DA synthesis and release from retinal dopaminergic cells (Dubocovich, 1983; Dubocovich et al., 1985; Besharse et al., 1988) in rats and rabbits. Melatonin was demonstrated to inhibit DA release from hypothalamic tissue slices, but not striatal slices (Zisapel et al., 1982). Zisapel and Laudon, (1983) suggested that melatonin decreases DA release by reducing calcium entry into presynaptic nerve endings.

Several researchers describe an antagonistic relationship between light and melatonin in the retina of both mammalian and non-mammalian species. Light has been shown to increase the concentrations, synthesis, and the release of DA from retinal dopaminergic cells (Iuvone, 1978, 1984; Nowak et al., 1989; Iuvone, 1990). Nowak et al., (1989), suggested that DA activity in the retina is synchronized to the light-dark cycle.

Melatonin influences norepinephrine metabolism

Although no significant effects of melatonin administration upon NE concentrations were detected in any brain region studied (Table 4) (median eminence, posterior pituitary, amygdala and striatum), NE accumulation after pargyline was significantly reduced by melatonin, in both

median eminence/arcuate tissue and in amygdala of ovariectomized hamsters (Fig. 9, 15). This inhibition was not observed in intact hamsters. These data suggest that melatonin administration reduces NE synthesis in female hamsters and that detection of this effect is masked in intact hamsters by cyclic variations in ovarian steroids. Since the median eminence region of the hypothalamus has been identified as a site at which ovarian hormones influence NE turnover (Crowley, 1982; Babu and Vijayan, 1984), the gonadectomized hamster has provided a model essential for studying effects of melatonin administration on NE metabolism independent of circulating levels of estradiol and progesterone. Since sex steroid-binding cells are also found in extrahypothalamic brain regions such as the amygdala, the rationale for using ovariectomized hamsters for studying the effects of melatonin can again be emphasized.

Steger and colleagues (1985), suggested that daily late afternoon melatonin injections would have neuroendocrine and neurochemical actions similar to those induced by short photoperiod. Decreased NE turnover was demonstrated in median eminence and mediobasal hypothalamus of male Syrian hamsters exposed to a short photoperiod, (Steger et al., 1984, 1985, 1986; Benson, 1987). The present data provide direct evidence that melatonin administration inhibits catecholamine synthesis in the median eminence of female hamsters.

In experiments performed on the mouse, Fang and Dubocovich (1990), demonstrated that activation of melatonin receptor sites in the hypothalamus (not hippocampus or frontal cortex), decreased NE turnover. The melatonin agonist, 6-chloromelatonin, retarded the depletion rate of NE elicited by the tyrosine hydroxylase inhibitor, alpha-methyl-para-tyrosine, suggesting that melatonin reduced NE turnover in the hypothalamus of the mouse. The melatonin antagonist, luzindole, significantly accelerated the depletion rate of NE in the hypothalamus. In further experimentation, Dubocovich et al., (1990), demonstrated that the "anti-depressant-like" luzindole, reduced the immobility of the behavioral despair test by blocking in vivo effects of endogenous melatonin. (This is a test used to screen drugs with anti-depressant-like activity; Porsolt et al., 1977, 1978). Various antidepressant drugs with different neurochemical actions were shown to antagonize the melatonin-induced behavioral effects when injected into the nucleus accumbens of the ventral striatum in rats, (Gaffori and Van Ree, 1985).

Again the question is raised of melatonin's mechanism of action. The data in the present study showing melatonin-induced inhibition of catecholamine (NE and DA) accumulation (Fig. 8, 9, 15) in ovariectomized hamsters suggests that melatonin inhibited the activity of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis. Stimulatory effects of light and inhibitory

effects of darkness on tyrosine hydroxylase activity have previously been observed in rat and rabbit retina (Iuvone, 1978; Dubocovich et al., 1985).

Ovariectomy in female hamsters

The utilization of ovariectomized hamsters was necessary in the present study to determine whether melatonin modified monoamine metabolism independently of the presence of ovarian hormones. Shifting concentrations of estradiol and progesterone over the course of the estrous cycle cause alterations in transmitter metabolism. Melatonin treatment of the intact hamster produces an anestrous animal with an altered steroid hormone environment characterized by low circulating levels of serum estradiol and abnormal progesterone surges (Jorgenson and Schwartz, 1987; Vriend et al., 1987). Although melatonin administration produces anestrous in female hamsters, this treatment does not produce an animal with the same steroid mileu as that found in ovariectomized hamsters. Thus the gonadectomized hamster provided a model essential for studying effects of melatonin administration on monoaminergic metabolism independent of changes in circulating levels of estradiol and progesterone.

The process of ovariectomy in hamsters maintained in long photoperiod produces marked increases in the pituitary content of LH (Table 1) and FSH. Pituitary prolactin concentrations, (Table 1), however, decrease (Reiter et

al., 1974; Stetson et al., 1978). Serum levels of prolactin are also decreased following ovariectomy (Fig. 1). Ovariectomy, however, does not prevent the daily gonadotropin surges that are found in acyclic hamsters maintained in short photoperiod (Jorgenson and Schwartz, 1987). This suggests that the periodicity of the gonadotropin surges may be regulated by a neural clock-timed release mechanism (Stetson et al., 1978), although some workers disagree with this theoretical perspective (Jorgenson and Schwartz, 1987).

Melatonin's modulation of hypothalamic neurotransmitter metabolism and the regulation of anterior pituitary hormone release:

Does melatonin inhibit 5HT stimulation of prolactin?

Melatonin's dramatic antigonadotropic effects in Syrian hamsters are hypothesized to be due to its ability to modify the metabolism of neurotransmitters which regulate the activity of hypothalamic releasing factors and anterior pituitary hormones.

One interpretation of the data in the current investigation is that reduced serum and pituitary prolactin (PRL) in melatonin-treated hamsters (Table 1; Fig. 1), resulted from a melatonin-induced decrease in the release of 5HT in the mediobasal hypothalamus. Serotonergic stimulation of prolactin release is well documented in many species (Clemens et al., 1978; Van de Kar, 1982, 1989), and

it has been demonstrated that stimulation of brain stem raphe nuclei elicits a prolactin surge (Advis et al., 1979; Van de Kar and Bethea, 1982). Prolactin release can be stimulated via administration of 5HT agonists. Conversely, the disruption of 5HT neurotransmission induced by treatment with 5HT antagonists, significantly decreases serum PRL (Korden et al., 1973; Lawson and Gala, 1978). Although no significant effects of melatonin in 5HT synthesis was detected in the present study (Fig. 5), a highly significant increase in 5HIAA in the hypothalamus of melatonin treated hamsters was observed (Table 3; Fig. 6).

Diurnal variations in serotonergic metabolism must be recognized when interpreting the present data. Enhanced daytime 5HT concentrations have been demonstrated (Hery et al., 1972; Ferraro and Steger, 1990), in rats and hamsters. Increased brain 5HIAA concentrations were shown to occur at night, during darkness, in the rat (Hery et al., 1972). Ferraro and Steger (1990) have concluded that 5HT activity is synchronized to the environmental light-dark cycle but is not driven by an endogenous circadian pacemaker (like the SCN). Rao and colleagues (1989), demonstrated in a clinical study, that bright lights enhanced blood 5HT levels throughout the daytime. The serotonergic data obtained from this present investigation must therefore be interpreted with caution since the time of animal sacrifice occurred during the light period when endogenous 5HT levels are normally increased. Effects on 5HT content, turnover

and oxidative metabolism may be different when animals are sacrificed in the dark, during or near the timing of the acrophase of the endogenous melatonin circadian rhythm.

Melatonin and DA inhibition of PRL release

The current data showing concomitant melatonin-induced inhibition of tuberoinfundibular dopamine (DA) (Table 2; Fig. 7, 8) and melatonin-induced inhibition of serum and pituitary levels of PRL (Table 1; Fig. 1) seems paradoxical since the prolactin release inhibitory activity of dopamine is well documented (Gudelsky, 1981; Ben-Jonathon, 1985). Explanations for this particular phenomenon are not immediately obvious. One interpretation of this concurrent melatonin-induced decrease in dopamine turnover (Fig. 8) and in pituitary prolactin secretion (Fig. 1) is that the decreased DA synthesis observed in melatonin-treated hamsters resulted from reduced positive feedback effects of PRL on tuberoinfundibular DA neurons (Gudelsky et al., 1976; Gudelsky, 1981). Tuberoinfundibular DA is partially regulated by basal PRL levels. However, ovariectomy by itself reduced PRL levels (Fig.1) yet markedly increased DA content and synthesis - therefore some other explanation should be considered (Fig. 7, 8).

Reduced levels of DA arriving at the anterior pituitary may contribute to the development of an increase in DA receptor density or up-regulation of D2 receptors. This could then lead to an accentuated lactotrope responsiveness

to DA (Ben-Jonathon, 1985; Steger et al; 1985) in melatonin-treated hamsters (Steger and Gay-Primal, 1990). Cheung and Weiner (1976, 1978) and Cheung et al., (1981) demonstrated a supersensitivity of anterior pituitary DA receptors following destruction of the mediobasal hypothalamus. Steger and colleagues (1985), demonstrated an enhanced pituitary sensitivity to DA inhibition in male hamsters exposed to short photoperiod. Less DA was required to suppress PRL in short photoperiod and this effect could be reversed by the presence of an ectopic pituitary graft from hamsters maintained in a long photoperiod (14L/10D). Although the up-regulation hypothesis could partially explain a maintenance of concomitant low tuberinfundibular DA and low PRL levels, it does not satisfactorily explain how the prolactin levels were initially decreased by melatonin administration. Alterations in DA turnover and the development of up-regulated or supersensitive D2 receptors, could provide a compensatory homeostatic mechanism by which hamsters eventually become refractory to melatonin or short photoperiod.

Another interpretation of the data in the present investigation is that the inhibition of NE turnover in the median eminence may also have contributed to the decrease in secretion of PRL, since NE is considered to facilitate the hormone's release (Weiner and Ganong, 1978; Andersson et al., 1981, 1983).

An alternative explanation for the data from the present study is that DA may be the important transmitter in short-term regulation of PRL, but that long-term inhibition of the hormone by light deprivation may be the result of a non-dopaminergic mechanism (Blask and Orstead, 1986). This suggests that a combination of transmitters may be involved in the regulation of PRL secretion. Such an interpretation may involve serotonergic neurons which originate from the brain stem raphe nuclei (Vriend, 1989), and then impinge upon catecholaminergic neurons and inhibit or enhance the release of DA into the hypophyseal portal vessels (Pilott and Porter, 1981). Pilott and Porter (1981) have reported their evidence for 5HT inhibition of DA release into portal plasma. Kiss and Halasz, (1986), documented their discovery of synaptic connections between serotonergic nerve terminals and tyrosine hydroxylase containing neurons.

If melatonin is inhibiting PRL secretion primarily through the serotonergic system, the decreased serum PRL could reduce DA turnover via a reduced positive feedback effect.

Neurotransmitter regulation of LH secretion

It is well documented that NE is the principal neurotransmitter involved in gonadotropin (LH, FSH) release (Weiner and Ganong, 1978; Meites and Sonntag, 1981).

The decreased NE turnover in median eminence (Fig. 9)

found in the GX/Melatonin group of hamsters in the present study, is consistent with the hypothesis that melatonin interferes with normal cycles of LH secretion (Fig. 2) and elevates pituitary LH concentrations (Table 1).

There is some controversy regarding the modulatory role of DA in LH secretion. Some reports suggest an inhibitory effect (Simpkins et al., 1980; Meites and Sonntag, 1981) on LHRH and hence LH secretion by DA while others report a stimulatory one especially in conjunction with NE (Negro-Vilar, 1982).

Several reports suggest that 5HT plays an inhibitory role in LH release (Weiner and Ganong, 1978; Meites and Sonntag, 1981; Kalra and Kalra, 1983), however other studies report evidence for a stimulatory role for 5HT in LH release. This view is supported by findings that stimulation of serotonergic raphe neurons enhances LH secretion (Waloch et al., 1981). The present data showing increased 5HIAA in mediobasal hypothalamus of melatonin-treated hamsters suggests that melatonin induces an increase in daytime oxidative metabolism of serotonin. The present data are consistent with a role for 5HT in melatonin-induced regulation of LH but further studies are required to determine its precise role in LH release.

Clemons et al., (1980) demonstrated that the melatonin receptor agonists, 6-chloromelatonin and 6-fluoromelatonin, inhibit the release of LH and the ovulatory process in the rat. Thus in the present study, melatonin-induced changes

in NE, DA, and/or 5HT metabolism could contribute to the cessation of estrous cyclicity observed in intact melatonin-treated hamsters.

Neurotransmitter regulation of T4 release

Inhibition of noradrenergic neurotransmission has been demonstrated to decrease basal TSH levels (Krulich et al., 1977). Increases in NE turnover elevates TSH levels especially after acute exposure to cold (Jobin et al., 1975). The overall decrease in T4 (Fig. 3) shown in the present experiment, is consistent with the data demonstrating an inhibition of NE accumulation after pargyline in the ovariectomized hamsters treated with melatonin in median eminence (Fig. 9).

Reports of both stimulatory and inhibitory effects of brain 5HT on TSH secretion have been documented (Krulich et al., 1979; Smythe et al., 1979). The data showing increased 5HIAA content (Table 3, Fig. 6) in median eminence/arcuate region of the hypothalamus (probably the result of increased oxidative metabolism) suggest that melatonin-induced changes in 5HT release could interfere with normal cycles of TSH release and thus reduce daytime levels of T4. Further studies on the effects of melatonin on 5HT release and its effects on TSH and T4 secretion are required.

Dopaminergic inhibition of TSH secretion is documented in the literature (Foord et al., 1980; Ben-Jonathon, 1985).

It has been suggested that dopamine inhibits both PRL and TSH through activation of similar receptors in the anterior pituitary (Foord et al., 1983; Tuomisto and Mannisto, 1985). An additional hypothesis is that melatonin decreases the release of TRH from the PVN of the hypothalamus. This would then account for the low serum levels of both PRL and T4 in this study since it is well-documented that TRH is involved in the release of PRL and TSH (Fig. 1, 3).

Melatonin binding - theories of mechanism of action

Specific melatonin binding has been reported in several regions in various species of mammals. The regions of high-affinity binding that have been most reported in the literature include the SCN and the median eminence of the hypothalamus in addition to the pars tuberalis region of the pituitary. Other areas with melatonin binding include midbrain (ventral raphe complex), PVN of the thalamus as well as the hippocampus (Bittman and Weaver, 1990).

Various researchers have reported locating high-affinity melatonin binding sites in the pars tuberalis in sheep (deReviers et al., 1989; Morgan and Williams, 1989; Morgan et al., 1989), the ferret (Weaver and Reppert, 1990) and the ewe (Bittman and Weaver, 1990). It is within this region that by far the greatest binding sites were demonstrated in these studies. Weaver and Reppert (1990) suggest that the pars tuberalis plays an important role in

the mediation of melatonin's neuroendocrine effects. They hypothesize that melatonin does not act in the brain but rather acts through the hypothalamus without necessarily binding to it. They suggest that melatonin activates the secretion of an unknown substance from the pars tuberalis, which travels to the external layer of the median eminence and acts on tuberoinfundibular terminals located there. Weaver and Reppert (1990) however, have not excluded the action of low-affinity melatonin receptors and the effects of their activation.

The pars tuberalis is in very close proximity to the median eminence region of the hypothalamus. In rodents, such as the hamster, there is some difficulty in differentiating binding in these two regions. The question of whether melatonin is acting in one of these adjacent regions more predominately than the other, in the Syrian hamster, presently remains a controversial one.

Hypotheses relating the results from the present experiment with the melatonin binding data include the interpretations that this indoleamine acts on either: one site of action, several sites or on many structures throughout the body via a more generalized mechanism of action. If the first interpretation is correct, the most plausible single site of action might be the brainstem raphe nuclei - a region rich in serotonergic cell bodies. These neurons project throughout the brain to areas that include the median eminence, striatum and amygdala - all

which were analyzed in the present investigation. Our data demonstrate significant effects of melatonin on the serotonergic system in all three of these regions. One interpretation of the catecholaminergic data that would be consistent with this single site hypothesis is that serotonergic terminals impinging on noradrenergic and dopaminergic neurons, are modulating their activity. There is some support from the literature for binding in the midbrain region, however, in one study (Bittman and Weaver, 1990), this was restricted to the ventral raphe complex and the inferior colliculus only.

The interpretation that melatonin acts on several hypothalamic as well as extrahypothalamic sites is consistent with the data demonstrating binding in the SCN, the median eminence (Vanacek and Jansky, 1989; Williams et al., 1989), hippocampus (Anis et al., 1989), the PVN of the thalamus (Morgan and Williams, 1989), and the retina (Dubocovich and Takahashi, 1987; Dubocovich, 1987). If melatonin acted on the PVN of the hypothalamus (Pickard and Turek, 1983), and inhibited the release of TRH, the hormone which stimulates TSH and PRL release, this would explain some of the hormone data of the present investigation. Unfortunately, the binding data does not well support this hypothesis. However, this does not exclude the possibility that neurons projecting from areas that bind melatonin, such as the SCN, are terminating in the PVN.

There is some evidence showing melatonin binding in the

hippocampal region. Anis et al., (1989), demonstrated that morning administration of melatonin (without concomitant late afternoon injections) produced a significant decrease or down-regulation of [125 I] iodomelatonin binding sites. This occurred only in the hippocampus, suggesting that it may be involved in the counterinhibitory effects produced with administration of melatonin in the morning. The hippocampus is adjacent to the amygdala, an area which has been demonstrated to be significantly effected by melatonin administration in this study. This could represent a promising region for future investigation.

A current hypothesis concerning the mechanism of action of melatonin is that high affinity melatonin binding sites activate guanine nucleotide binding proteins (G proteins) which regulate adenylate cyclase (Niles, 1990). According to this model, melatonin receptors are coupled to G proteins which inhibit adenylate cyclase.

Conclusions

The current investigation is the first study to demonstrate significant effects of daily late afternoon melatonin injections upon monoamine metabolism in the mediobasal hypothalamus of the female Syrian hamster. Melatonin markedly inhibited DA and NE synthesis as well as increased daytime oxidative metabolism of 5HT in this region.

Melatonin's inhibition of NE and 5HT synthesis in the amygdala and its stimulation of oxidative metabolism in the caudate nucleus demonstrate that the effects of melatonin on monoamine metabolism are not limited to the mediobasal hypothalamus.

The data showing that melatonin administration modifies transmitter metabolism in the ovariectomized hamster provides evidence that some major effects of melatonin on monoamine metabolism occur independently of changes in circulating levels of ovarian steroid hormones.

Melatonin-induced interference with PRL and LH secretion can be accounted for by melatonin-induced changes in synthesis and release of monoamines which influence the secretion of hypothalamic hormones. Although other investigators have suggested that the neurochemical effects of short photoperiod can be duplicated by late afternoon administration of melatonin, the present study is the first to test this hypothesis directly. If melatonin affects the neurotransmitter systems and hormones in humans as it has been demonstrated to do in Syrian hamsters in the current investigation, a much closer examination of melatonin's neurochemical and neuroendocrine effects in man is warranted.

LITERATURE CITED

Advis, J. P., Simpkins, J. W., Bennett, J., and Meites, J. (1979). Serotonergic control of prolactin release in male rats. Life Sci. 24:359-366.

Aldegunde, M., Duran, R., Alfonso, M., Miguez, I., and Veira, J. (1987). Effect of long-term pinealectomy on serum LH levels and on cerebral serotonin metabolism in adult male rats. Rev. Espanol. Fisiol. 43:351-354.

Andersson, K., Eneroth, P., and Agnati, L. F. (1981). Nicotine-induced increases of noradrenaline turnover in discrete noradrenaline nerve terminal systems of the hypothalamus and the median eminence of the rat and their relationship to changes in the secretion of adenohypophyseal hormones. Acta Physiol. Scand. 113:227-231.

Andersson, K., Nilsen, O. G., Toftgard, R., Eneroth, P., Gustafson, J. A., Battistini, N., and Agnati, L. (1983). Increased amine turnover in several hypothalamic noradrenaline nerve terminal systems and changes in prolactin secretion in the male rat by exposure to various concentrations of toluene. Neurotoxicology 4:43-56.

Anis, Y., Nir, I., and Zisapel, N. (1989). Diurnal variations in melatonin binding sites in the hamster brain: impact of melatonin. Mol. Cell. Endocrinol. 67:121-129.

Annunziato, L., and Weiner, R. I. (1980). Characteristics of dopamine uptake and 3,4-dihydroxyphenylacetic acid (DOPAC) formation in the dopaminergic terminals of the neurointermediate lobe of the pituitary gland. Neuroendocrinology 31:8-12.

Anton-Tay, F., Chou, C., Anton, S., and Wurtman, R. J. (1968). Brain serotonin concentration: Elevation following intraperitoneal administration of melatonin. Science 162:277-278.

Arendt, J. (1988). Melatonin (Review). Clin. Endocrinol. 29:205-229.

Arendt, J. (1989). Melatonin: A new probe in psychiatric investigation. Brit. J. Psychiat. 155:585-590.

Axelrod, J., and Weissbach, H. (1960). Enzymatic-o-methylation of n-acetylserotonin to melatonin. Science 131:1312.

Axelrod, J., Shein, H. M., and Wurtman, R. J. (1969). Stimulation of 14C melatonin synthesis from 14C tryptophan by noradrenaline in rat pineal in organ culture. Proc. Nat. Acad. Sci. USA 69:2547-2550.

Axelrod, J. (1974). The pineal gland: A neurochemical transducer. Science 184:1341-1348.

Babu, N. G., and Vijayan, E. (1984). Hypothalamic tyrosine hydroxylase activity and plasma gonadotropin and prolactin levels in ovariectomized-steroid treated rats. Brain Res. Bull. 12:555-558.

Bast, J. D., and Greenwald, G. S. (1974). Serum profiles of follicle-stimulating hormone, luteinizing hormone and prolactin during the estrous cycle of the hamster. Endocrinology 94:1295-1299.

Beattie, C. W., Rodgers, C. H., and Soyka, L. F. (1972). Influence of ovariectomy and ovarian steroids on

hypothalamic tyrosine hydroxylase activity in the rat. Endocrinology 91:276-280.

Ben-Jonathon, N. (1985). Dopamine: A prolactin-inhibiting hormone. Endocrinol. Rev. 6:564-589.

Benson, B. (1987). Temporal changes in medial basal hypothalamic catecholamines in male Syrian hamsters exposed to short photoperiod. Exp. Brain Res. 65:371-376.

Berdtson, W. E., and Desjardins, C. (1974). Circulating LH and FSH levels and testicular function in hamsters during light deprivation and subsequent photoperiodic stimulation. Endocrinology 95:195-205.

Besharse, J. C., Iuvone, P. M., and Pierce, M. E. (1988). Regulation of rhythmic photoreceptor metabolism: a role for post-receptoral neurons. Prog. Retinal Res. 7:21-61.

Bhatnagar, K. P. (1990). Comparative morphology of the pineal gland. In: Biological Rhythms, Mood Disorders, Light Therapy and the Pineal Gland. Shafii, M.; Shafii, S. L.; editors. Amer. Psychiat. Press. pp. 5-37.

Bittman, E. L. (1978). Photoperiodic influences on testicular regression in the golden hamster: termination of scotorefractoriness. Biol. Reprod. 18:871-877.

Bittman, E. L., and Zucker, I. (1981). Photoperiodic termination of hamster refractoriness: participation of the pineal gland. Biol. Reprod. 24:568-572.

Bittman, E. L., and Weaver, D. R. (1990). The distribution of melatonin binding sites in neuroendocrine tissues of the ewe. Biol. Reprod. 43:986-993.

Blask, D. E., and Orstead, K. M. (1986). Dopamine inhibition of prolactin release but not synthesis in the male Syrian hamster: In vitro studies. (1986). Life Sci. 38:1915-1921.

Brainard, G. C., Richardson, B. A., King, T. S., and Reiter, R. J (1984). The influence of different light spectra on the suppression of pineal melatonin content in the Syrian hamster. Brain Res. 294:333-339.

Brainard, G. C., Lewy, A. J., Menaker, M., Fredrickson, R. H., Miller, L. S., Weleber, R. G., Cassone, V., and Hudson, D. (1985). Effect of light wavelength on the suppression of nocturnal plasma melatonin in normal volunteers. Ann. N.Y. Acad. Sci. 453:376-378.

Bridges, R. S. and Goldman, B. D. (1975). Diurnal rhythms in gonadotropins and progesterone in lactating and photoperiod-induced acyclic hamsters. Biol. Reprod. 13:617-622.

Cardinali, D. P., and Wurtman, R. J. (1972). Hydroxyindole-O -methyl transferases in rat pineal, retina and Harderian gland. Endocrinology 91:247-252.

Cardinali, D. P., Vacas, M. I., and Boyer, E. E. (1978). High affinity binding of melatonin in bovine medial basal hypothalamus. I.R.C.S. Med. Sci. Biochem. 6:357.

Cardinali, D. P., Vacas, M. I., and Boyer, E. E. (1979). Specific binding of melatonin in bovine brain. Endocrinology 103: 437-441.

Carlson, L. L., Weaver, D. R., and Reppert, S. M. (1989). Melatonin signal transduction in hamster brain: inhibition of adenylyl cyclase through a pertussis toxin-sensitive G-protein. Endocrinology 125:2670-2676.

Cassone, V. M., Roberts, M. H., and Moore, R. Y. (1987). Melatonin inhibits metabolic activity in the rats suprachiasmatic nuclei. Neurosc. Lett. 81:29-34.

Cassone, V. (1990). Effects of melatonin on vertebrate circadian systems. Trends Neurosci. 13:457-464.

Cena, V., Halperin, J. I., Yeandle, S., and Klein, D. C. (1991). Norepinephrine stimulates potassium efflux from pinealocytes: Evidence for involvement of biochemical "AND" gate operated calcium and adenosine 3′, 5′-monophosphate. Endocrinology 128:559-569.

Chamberlain, R. S., and Herman, B. H. (1990). A novel biochemical model linking dysfunctions in brain melatonin, propiomelanocortin, peptides, and serotonin in autism. Biol. Psychiat. 28:773-793.

Checkley, S. A. and Palazidou, E. (1988). Melatonin and antidepressant drugs: clinical pharmacology. In: Melatonin: Clinical Perspectives. Miles, A., Philbrick, D. R., and Thompson, C. editors. Oxford University Press. N.Y. pp. 190-204.

Cheung, C. Y., and Weiner, R. I. (1976). Supersensitivity of anterior pituitary to dopamine receptors involved in the inhibition of prolactin secretion following destruction of the medial basal hypothalamus. Endocrinology 99:914-917.

Cheung, C. Y., and Weiner, R. I. (1978). In vitro supersensitivity of the anterior pituitary to dopamine inhibition of prolactin secretion. Endocrinology 102:1614-1620.

Cheung, C. Y., Kuhn, R. W., and Weiner, R. I. (1981). Increased responsiveness of the dopamine-mediated inhibition of prolactin synthesis after destruction of the medial basal hypothalamus. Endocrinology 108:747-751.

Chu, E. W., Wurtman, R. J., and Axelrod, J. (1964). An inhibitory effect of melatonin on the estrous phase of the estrous cycle of the rodent. Endocrinology 75:238-242.

Clemens, J. A., Roush, M. E., and Fuller, R. W. (1978). Evidence that serotonin neurons stimulate secretion of prolactin releasing factor. Life Sci. 22:2209-2214.

Clemens J. A., Flaugh, M. E., Parli, J., and Sawyer, B. D. (1980). Inhibition of luteinizing hormone release and ovulation by 6-chloro and 6-fluoromelatonin. Neuroendocrinology 3:83-87.

Cohen, M., Roselle, D., Chabner, T. J., Shmidt, S. and Lippman, M. (1978). Evidence for a cytoplasmic

melatonin receptor. Nature 274:894-895.

Collin, J. P., and Oksche A. (1981). Structural and functional relationships in the nonmammalian pineal gland. In: The Pineal Gland: (Vol.I: Anatomy and Biochemistry). Reiter, R. J. editor, C.R.C. Press, Inc., Boca Raton, Florida. pp. 27-67.

Cooper, J. R., Bloom, F. E., and Roth, R. H. (1982). The Biochemical Basis of Neuropharmacology. Oxford University Press, N.Y.

Cramer, O. M., Parker, C. R., and Porter, J. C. (1979). Estrogen inhibition of dopamine release into hypophysial portal blood. Endocrinology 104:419-422.

Cramer, O. M., Parker, C. R., and Porter, J. C. (1979). Stimulation of dopamine release into hypophysial portal blood by administration of progesterone. Endocrinology 105:929-933.

Crowley, W. R. (1982). Effects of ovarian hormones on norepinephrine and dopamine turnover in individual hypothalamic and extrahypothalamic nuclei. Neuorendocrinology 34:381-386.

Daniolos, A., Lerner, A. B., and Lerner, M. R. (1990). Action of light on frog pigment cells in culture. Pigment Cell Research 3:38-43.

Deguchi, T., and Axelrod, J. (1973). Superinduction of serotonin N-acetyltransferase and supersensitivity of adenyl cyclase to catecholamines in denervated pineal gland. Molec. Pharmacol. 9:612-618.

Demarest, K. T. and Moore, K. E. (1979). Lack of high affinity transport system for dopamine in the median eminence and the posterior pituitary. Brain Res. 171:545-551.

Demarest, K. T., and Moore K. E. (1979). Comparison of dopamine synthesis regulation in terminals of nigrostriatal, mesolimbic, tuberoinfundibular and tuberohypophyseal neurons. J. Neural Transm. 46:263-277.

Demarest, K. T., and Moore, K. E. (1981). Sexual differences in the sensitivity of tuberoinfundibular dopamine neurons to the actions of prolactin. Neuroendocrinology 33:230-234.

Demarest, K. T., Riegle, G. D., and Moore, K. E. (1984). Long-term treatment with estradiol induces reversible alterations in tuberoinfundibular dopaminergic neurons: a decreased responsiveness to prolactin. Neuroendocrinology 39:193-200.

deReviers, M. M., Ravault, J. P., Tillet, Y., and Pelletier, J. 1989. Melatonin binding sites in the sheep pars tuberalis. Neurosci. Lett. 100:89-93.

Descartes, R. (1662). Des Homine. Figuris et Latinitate Donatus a Schuyl. F. Lugduni Batavorum.

Donham, R. S., Dipinto, M. N., and Stetson, M. H. (1984). Twenty-four hour rhythm of gonadotropin release induces cyclic progesterone secretion by the ovary of prepubertal and adult golden hamsters. Endocrinology

114:821-826.

Donofrio, R. J., Reiter, R. J., Sorrentio, S., Blask, D. E., and Talbot, J. A. (1973). A method for measurement of prolactin in the hamster by means of radioimmunoassay. Neuroendocrinology. 13:79-92.

Dubocovich, M. L. (1983). Melatonin is a potent modulator of dopamine release in the retina. Nature 306:782-784.

Dubocovitch, M. L., Lucas, R. C., and Takahashi, J. S. (1985). Light-dependent regulation or dopamine receptors in mammalian retina. Brain Res. 335:321-325.

Dubocovich, M. L. (1987). Luzindole (N-0774) antagonized the melatonin-induced inhibition of 3H-dopamine from rabbit retina. Soc. Neurosci. Abstr. 2904:1038.

Dubocovich,, M. L., and Takahashi J. S. (1987). Use of 2-[125I]-iodomelatonin to characterize melatonin binding sites in chicken retina. Proc. Natl. Acad. Sci. USA 84:3916-3920.

Dubocovitch M. L., Mogilnicka, E., and Areso, P. M. (1990). Antidepressant-like activity of the melatonin receptor antagonist, luzindole (N-0774), in the mouse behavioral despair test. Eur. J. Pharmacol. 182:313-325.

Ebadi, M. (1984). Regulation of the synthesis of melatonin and its significance to neuroendocrinology. In: The Pineal Gland. Reiter, R. J. editor. Raven Press. N.Y.

Eikenburg, D. C., Ravitz, A. J., Gudelsky, G. A., and Moore, K. E. (1977). Effects of estrogen on prolactin and tuberoinfundibular dopaminergic neurons. J. Neural Transm. 40:235-244.

Fang, J-M., and Dubocovitch, M. L. (1990). Activation of melatonin receptor sites retarded the depletion of norepinephrine following inhibition of synthesis in the C3H/HeN mouse hypothalamus. J. Neurochem. 55:76-82.

Ferraro, J. S. and Steger, R. W. (1990). Diurnal variations in brain serotonin are driven by the photic cycle and are not circadian in nature. Brain Res. 512:121-124.

Foord, S. M., Peters, J., Scanlon, M. F., Rees Smith, B., and Hall, R. (1980). Dopaminergic control of TSH secretion in isolated rat pituitary cells. FEBS. Lett. 121:257-259.

Foord, S. M., Peters, J. R., Dieguez, C., Scanlon, M. F., and Hall, R. (1983). Dopamine receptors on intact anterior pituitary cells in culture: functional association with the inhibition of prolactin and thyrotropin. Endocrinology 112:1567-1577.

Gaffori, O., and Van Ree, J. M. (1985). Serotonin and antidepressant drugs antagonize melatonin-induced behavioral changes after injection into the nucleus accumbens of rats. Neuropharmacology 24:237-244.

Giarman, N. J., and Day, M. (1959). Presence of biogenic amines in the bovine pineal body. Biochem. Pharmacol.

1:235-239.

Gibbs, F. P., and Vriend, J. (1983). Counterantigonadotropic effect of melatonin administration via the drinking water. Endocrinology 113:1447-1451.

Goldman, H., and Wurtman, R. J. (1964). Flow of blood to the pineal body of the rat. Nature 203:87-88.

Guchhait, R. B., and Grau, J. E. (1978). Biosynthesis of S-adenosyl-L-methionine in the rat pineal gland. J. Neurochem. 31:921-925.

Gudelsky, G. A., Simpkins, J., Meites, G. P., Meites, J., and Moore, K. E. (1976). Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinology 22:206-215.

Gudelsky, G. A., Annunziato, L., and Moore, K. E. (1977). Increase in dopamine content of the rat median eminence after long-term ovariectomy and its reversal by estrogen replacement. Endocrinology 101:1894-1897.

Gudelsky, G. A., Nansel, D. D., and Porter, J. C. (1981). Role of estrogen in the dopaminergic control of prolactin secretion. Endocrinology 108:440-444.

Gudelsky, G. A. (1981). Tuberoinfundibular dopamine neurons and the regulation of prolactin secretion. Psychoneuroendocrinology 6:3-16.

Hastings, M. H., Vance, G., and Maywood, E. 1989. Phylogeny and function of the pineal. Experientia

45:903-909.

Hendrickson, S. E., Wagoner, N., and Cowan, W. M. (1972). Autoradiographic and electron microscopic study of retino-hypothalamic connections. Z. Zellerforcsh. 125:1-26.

Hery, F., Rouer, E., and Glowinski, J. (1972). Daily variations of serotonin metabolism in the rat brain. Brain Res. 43:445-465.

Hirata, F., Hayaishi, O., Tokuyama, T., and Senoh, S. (1974). In vitro and in vivo formation of two new metabolites of melatonin. J. Biol. Chem. 249:1311-1313.

Hoffman, R. A., and Reiter, R. J. (1965). Pineal gland: influence on gonads of male hamsters. Science 148: 1609-1611.

Hubbard, C. J., and Greenwald, G. S. (1983). In vitro effects of luteinizing hormone on the induced atretic Graafian follicles in the hamster. Biol. Reprod. 28:849-859.

Iuvone, P. M., Galli, C. L., Garrison-Gund C K., and Neff, N. H. (1978). Light stimulates tyrosine hydroxylase activity and DA synthesis in retinal amacrine neurons. Science 202:901-902.

Iuvone, P. M. (1984). Regulation of retinal dopamine biosynthesis and tyrosine hydroxylase activity by light. Fed. Proc. 43:2709-2713.

Iuvone, P. M. (1990). Development of melatonin synthesis

in chiken retina: Regulation of serotonin N-acetyltransferase activity by light, circadian oscillators and cyclic AMP. J. Neurochem. 54:1562-1568.

Jackson, F. L., Heindel, J. J., Preslock, J. P., and Berkowitz, A. S. (1984). Alterations in hypothalamic content of luteinizing hormone-releasing hormone associated with pineal-mediated testicular regression in the golden hamster. Biol. Reprod. 31:436-445.

James, M. D., Hole, D. R., and Wilson, C. A. (1989). Differential involvement of 5-hydroxytryptamine (5HT) in specific hypothalamic areas in the mediation of steroid-induced changes in gonadotropin release and sexual behavior in female rats. Neuroendocrinology 49: 561-569.

Jobin, M., Ferland, L., Cote, J., and Labrie, F. (1975). Effect of exposure to cold on hypothalamic TRH activity and plasma levels of TSH and prolactin in the rat. Neuroendocrinolgy 18:204-212.

Jorgenson, K. L., and Schwartz N. B. (1987). Dynamic pituitary and ovarian changes occuring during the anestrus to estrus transition in the golden hamster. Endocrinology 120:34-42.

Kalra, S. P., and Kalra, P. S. (1983). Neural regulation of luteinizing hormone secretion in the rat. Endocrinol. Rev. 4:311-351.

Kappers, J. A. (1960). The development, topographical

relations and innervation of the epiphysis cerebri in the albino rat. Zeitschrift fur Zellforsch 52:163-215.

Kappers, J. A. (1960). Innervation of the epiphysis cerebri in the albino rat. Anat. Rec. 136:220-221.

King, T. S., Steger, R. W., Steinlechner, S., and Reiter, R. J. (1984). Day-night differences in estimated rates of 5-hydroxytryptamine turnover in the rat pineal gland. Exp. Brain Res. 54:432-436.

Kiss, J., and Halasz, B. (1986). Synaptic connections between serotonergic axon terminals and tyrosine hydroxylaseimmunoreactive neurons in the arcuate nucleus of the rat hypothalamus. A combination of electron microscopic autoradiography and immunocytochemistry. Brain Res. 364:284-294.

Kizer, J. S., Palkovits, M., Ziven, J., Browstein, M., Saavedra, J. M., and Kopin, I. J. (1974). The effect of endocrinological manipulations on tyrosine hydroxylase and dopamine-Beta-hydroxylase activities in individual hypothalamic nuclei of the adult male rat. Endocrinology 95:799-812.

Klein, D. C., Berg, G. R. and Weller, J. (1970). Melatonin synthesis: adenosine 3′, 5′ monophosphate and norepinephrine stimulate N-acetyltransferase. Science 168:979-980.

Klein, D. C., and Weller, J. (1972). Rapid light-induced decrease in pineal serotonin N-acetyltransferase activity. Science 177:532-533.

Klein, D. C., Namboodirir, M. A., and Auerbach, D. A. (1981). The melatonin generating system: developmental aspects. FEBS. Lett. 28:1975-1986.

Klein, D. C., (1985). Photoneural regulation of the mammalian pineal gland. In: Photoperiodism, Melatonin, and the Pineal. Everd, D., Clark, S. editors. Ciba Foundation Symposium. Pittman; London. pp. 38-55.

Kopin, I. J., Pare, C. M., Axelrod, J., and Weissbach, H. (1961). The fate of melatonin in animals. J. Biol. Chem. 236: 3072-3075.

Kordon, C., Blake, C. A., Terkel, J., and Sawyer, C. H. (1973). Participation of serotonin-containing neurons in the suckling-induced rise in plasma prolactin levels in lactating rats. Neuroendocrinology 13:213-223.

Krause, D. N., and Dubocovitch, M. L. (1990). Regulatory sites in the melatonin system of mammals. Trends Neurosci. 13:464-470.

Krstic, R. (1976). A combined scanning and transmission electron microscopic study and electron probe microanalysis of human pineal acervuli. Cell Tiss. Res. 174:129-137.

Krstic, R. (1986). Pineal calcification: its mechanism and significance. J. Neural Transm. 21:415-432.

Krulich, L., Giachetti, A., Marchlewska-Koj, A., Hefco, E., and Jameson, H. E. (1977). On the role of the central noradrenergic and dopaminergic systems in the regulation of TSH secretion in the rat. Endocrinology

100:496-505.

Krulich, L., Vijayan, E., Coppings, R. J., Giachetti, A., McCann S. M., and Mayfield, M. A. (1979). On the role of the central serotonergic system in the regulation of the secretion of thyrotropin and prolactin: thyrotropin-inhibiting and prolactin-releasing effects of 5-hydroxytryptamine and quipazine in the male rat. Endocrinology 105:276-283.

Kuhn, D. M., Wolf, W. A., and Youdim, M. B. (1986). Serotonin neurochemistry revisited: a new look at some old axioms. Neurochem. Int. 8:141-154.

Launay, J. M., Lemaitre, B. J., and Husson, H. P. (1982). Melatonin synthesis by rabbit platelets. Life Sci. 31: 1487-1494.

Lawson, D. M., and Gala, R. R. (1978). The influence of pharmacological manipulation of serotonergic and dopaminergic mechanisms on plasma prolactin in ovariectomized, estrogen-treated rats. Endocrinology 102:973-981.

Le Gros Clark, W. E. (1939/40). The nervous and vascular relations of the pineal gland. J. Anat. 74:471-492.

Lerner, A. B., Case, J. D., Takahashi, Y., Lee, Y., and Mori, W. (1958). Isolation of melatonin, the pineal gland factor that lightens melanocytes. J. Amer. Chem. Soc. 80:2587-2591.

Lerner, A. B., Case, J. D., and Heinzelmann, R. V. (1959) Structure of melatonin. J. Amer. Chem. Soc.

81:6084-6085.

Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A., and Markey, S. D. (1980). Light suppresses melatonin secretion in humans. Science 210:1267-1269.

Lewy, A. J., Kern, H. A., Rosenthal, N. E., and Wehr, T. A. (1982). Bright artificial light treatment of a manic-depressive patient with a seasonal mood cycle. Amer. J. Psychiat. 139:1496-1498.

Li, K., Reiter, R. J., Vaughan, M. K., Oaknin, S., Troiani, M. E., and Esquifino, A. I., (1987). Elevated ambient temperature retards the atrophic response of the neuroendocrine-reproductive axis of male Syrian hamsters to either daily afternoon melatonin injections to short photoperiod exposure. Neuroendocrinology 45:356-362.

McCord, C. P., and Allen, F. P. (1917). Evidences associating pineal gland function with alterations in pigmentation. J. Exp. Zool. 23:207-224.

Meites, J., and Sonntag, W. E. (1981). Hypothalamic hypophysiotropic hormones and neurotransmitters regulation: current views. Annu. Rev. Pharmacol. Toxicol. 21:295-322.

Miles, A., and Grey, J. E. (1988). Melatonin and schizophrenia - a biocemical link. In: Melatonin: Clinical Perspectives. Miles, A., Philbrick, R. S., Thompson, C. editors. Oxford University Press. pp.243-252.

Miles, A. (1989). Melatonin: perspectives in the life sciences. (Minireview). Life Sci. 44:375-385.

Moore, R. Y., and Lenn, N. J. (1972). A retinohypothalamic projection in the rat. J. Comp. Neurol. 146:1-14.

Moore, R. Y. (1978). The innervation of the mammalian pineal gland. Progr. Reprod. Biol. 4:1-29.

Moore, R. Y. (1983). Organization and function of a central nervous system circadian oscillator: The suprachiasmatic hypothalamic nucleus. Fed. Proc. 42:2783-2789.

Morgan, R. J., Williams, L. M., Lawson, W., and Riddoch, G. (1988). Stimulation of melatonin synthesis in ovine pineals in vitro. J. Neurochem. 50:75-81.

Morgan, P. J., Williams, L. M., Davidson, G., Lawson, W., and Howell, E. (1989). Melatonin receptors on ovine pars tuberalis: Characterization and autoradiographic localization. J. Neuroendocrinol. 1:1-4.

Morgan, P. J., Lawson, W., Davidson, G., and Howell, H. E. (1989). Melatonin inhibits cyclic AMP production in cultured ovine pars tuberalis cells. J. Mol. Endocrinol. 3:R5-R8.

Morgan, P. J., and Williams, L. M. (1989). Central melatonin receptors: Implications for a mode of action. Experientia 45:955-965.

Negro-Vilar, A., Advis, J. P., Ojeda, S. R., and McCann, S. M. (1982). Pulsatile luteinizing hormone (LH)

patterns in ovariectomized rats: Involvement of norepinephrine and dopamine in the release of LH-releasing hormone and LH. Endocrinology 111:932-938.

Niles, L. P. (1990). GTP modulates [125I]iodomelatonin binding to a picomolar-affinity site in the Syrian hamster hypothalamus. Eur. J. Pharmacol. 189:95-98.

Norman, R. L., and Greenwald, G. S. (1971). Effect of phenobarbital, hypophysectomy and x-irradiation on preovulatory progesterone levels in the cyclic hamster. Endocrinology 89:598-605.

Nowak, J. Z., Zurawska, E., and Zawilska, J. (1989). Melatonin and its generating system in vertebrate retina: Circadian rhythm, effect of environmental lighting and interaction with dopamine. Neurochem. Int. 14 (4):397-406.

Oksche, A. (1965). Survey of the developmental and comparative morphology of the pineal gland. In: Progress in Brain Research (10) Kappers, A. J., Schade, J. P. editors. Elsevier, Amsterdam.

Oren, D., Brainard, G., Joseph-Vanderpool, J., Johnston, S., and Rosenthal, N. (1989). Effect of different light wavelengths in SAD. Society for Light Treatment and Biological Rhythms. Bethesda, MD (June, 1989).

Pevet, P., Vivien-Roels, B., and Masson-Pevet, M. (1989). Low temperature in golden hamster accelerates the gonadal atrophy induced by short photoperiod but does not affect the daily pattern of melatonin secretion.

J. Neural Transm. 76:119-128.

Pichard, G. E., and Turek, F. W. (1983). The hypothalamic paraventricular nucleus mediates the photoperiodic control of reproduction but not the effects of light on the circadian rhythm of activity. Neurosci. Lett. 43:67-72.

Pickard, G. E., Ralph, M. R., and Menaker, M. (1987). The intergeniculate leaflet partially mediates effects of light on circadian rhythms. J. Bio. Rhythms 2:35-56.

Pickering, D. S., Niles, L. P. (1990). Pharmacological characterization of melatonin binding sites in Syrian hamster hypothalamus. Eur. J. Pharmacol. 175:71-77.

Pilotte, N. S., and Porter, J. C. (1981). Dopamine in hypophysial portal plasma and prolactin in systemic plasma of rats treated with 5-hydroxytryptamine. Endocrinology 108:2137-2141.

Porsolt, R. D., Bertin, A., and Jalre, M. (1977). Behavioral despair in mice: A primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229:327-336.

Porsolt, R. D., Anton, G., Blavet, N., and Jalre, M. (1978). Behavioral despair in rats: A new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47:379-391.

Quay, W. B. (1963). Circadian rhythms in rat pineal serotonin and its modification by estrous cycle and

photoperiod. Gen. Comp. Endocrinol. 3:473-479.

Quay, W. B. (1964). Circadian and estrous rhythms in pineal melatonin and 5-hydroxyindole-3-acetic acid. Proc. Soc. Exp. Biol. Med. 115:710-713.

Quay, W. B. (1972). Pineal vasoconstriction at daily onset of light: its physiological correlates and control. Physiologist 15:241.

Quay, W. B. (1974). Pineal Chemistry. Thomas, Springfield.

Ralph, C. (1975). The pineal gland and geographical distribution of animals. Int. J. Biom. 19:289-303.

Ralph, M. R., Davis, F. C., and Menaker, M. (1988). Suprachiasmatic transplantation restores donor specific circadian rhythms to arrhythmic hosts. Soc. Neurosc. Abstr. 14:462.

Rao, M. L., Muller-Oerlinghausen, B., Mackert, A., Stieglitz, R. D., Strebel, B., and Volz, H. P. (1990). The influence of phototherapy on serotonin and melatonin in non-seasonal depression. Pharmacopsychiatry 23:155-158.

Raymond, V., Beaulieu, M., Labrie, F., and Boissier, J. (1978). Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. Science 200:1173-1175.

Reiter, R. J., and Hester, R. J. (1966). Interrelationships of the pineal gland, the superior cervical ganglia and the photoperiod in the regulation

of the endocrine systems of hamsters. Endocrinology 79:1168-1170.

Reiter, R. J. (1968). Morphological studies on the reproductive organs of blinded male hamsters and the effects of pinealectomy or superior cervical ganglionectomy. Anat. Rec. 160:13-24.

Reiter, R. J. (1969). Changes in reproductive organs of cold-exposed and light-deprived female hamsters (Mesocricetus auratus). J. Reprod. Fertil. 16:217-222.

Reiter, R. J. (1969). Pineal function in long-term blinded male and female golden hamsters. Gen. Comp. Endocrinol. 12:460-468.

Reiter, R. J. (1972). Evidence for refractoriness of the pituitary-gonadal axis to the pineal gland in golden hamsters and its possible implications in animal reproductive rhythms. Anat. Rec. 173:365-372.

Reiter, R. J., and Johnson, L. Y. (1974). Depressed action of the pineal gland on pituitary luteinizing hormone and prolactin in male hamsters. Horm. Res. 5:311-320.

Reiter, R. J., Blask, D. E., and Johnson, L. Y. (1974). Influence of ovariectomy and estrogen and/or progesterone treatment on pituitary and plasma LH and prolactin levels in female hamsters. Endocrine Res. Commun. 1:181-191.

Reiter, R. J., Vaughan, M. K., Blask, D. E., and Johnson, L. Y. (1975). Pineal methoxyindoles: new evidence

concerning their function in the control of pineal mediated changes in the reproductive physiology of male golden hamsters. Endocrinology 96:206-213.

Reiter, R. J., Blask, D. E., Johnson, L. Y., Rudeen, P. K., Vaughan, M. K., and Waring, P. J. (1976). Melatonin inhibition of reproduction in the male hamster: Its dependency on time of day of administration and on an intact and sympathetically innervated pineal gland. Neuroendocrinology 22:107-116.

Reiter, R. J. (1980). The pineal and its hormones in the control of reproduction in mammals. Endocrine Rev. 1:109-131.

Reiter, R. J. (1981). Reproductive effects of the pineal indoles in the Syrian hamster and albino rat. In: The Pineal Gland (Vol.II: Reproductive Effects). Reiter, R. J. editor, C.R.C. Press Inc., Boca Raton, Florida. pp. 45-81.

Reiter, R. J., Hurlburt, E. C., Brainard, G. C., Steinlechner, S., and Richardson B. A. (1983). Influence of light irradiance on hydroxyindole-O-methyltransferase activity, serotonin-N-acetyltransferase activity and radioimmunoassayable melatonin levels in the pineal gland of the diurnally active Richardson's ground squirrel. Brain Res. 288:151-157.

Reiter, R. J., Brit, J. H., and Armstrong, J. D. (1987).

Absence of a nocturnal rise in either NE, NAT, HIOMT or melatonin in the pineal gland of the domestic pig kept under natural environmental photoperiods. Neurosci. Lett. 81:171-176.

Reppert, S. M., Weaver, D. R., Rivkees, S. A., and Stopa, E. G. (1988). Putative melatonin receptors in a human biological clock. Science 242:78-81.

Rollag, M. D., Panke, E. S., Trakulrungsi, W., Trakulrungsi, C., and Reiter, R. J. (1980). Quantification of daily melatonin synthesis in the hamster pineal gland. Endocrinology 106:231-236.

Romero, J. A., Zatz, M., and Axelrod, J. (1975). Beta-adrenergic stimulation of pineal N-acetyltransferase: Adenosine 3', 5'-cyclic monophosphate stimulates both RNA and protein synthesis. Pro. Natl. Acad. Sci. USA. 72:2107-2111.

Romero, J. A. and Axelrod, J. (1975). Regulation of sensitivity to beta-adrenergic stimulation in induction of pineal N-acetyltransferase. Proc. Natl. Acad. Sci. USA. 72:1661-1665.

Rosenthal, N. E., Sack, D. A., Gillin, J. C., Lewy, A. J. Goodwin, F. K., Davenport, Y., Newsome, D. A., and Wehr, T. A. (1984). Seasonal affective disorder: A description of the syndrome and preliminary findings with light therapy. Arch. Gen. Psychiat. 4:72-80.

Rusak, B. (1980). Suprachiasmatic lesions prevent an antigonadal effect of melatonin. Biol. Reprod.

22:148-158.

Rusak, B. (1989). The mammalian circadian system: models and physiology. J. Biol. Rhythms 4:121-134.

Schneider, H. P., McCann, S. M. (1970) Monoamines and indoleamines and control of LH secretion. Endocrinology 86: 1127-1133.

Seegal, R. F., and Goldman, B. D. (1975). Effects of photoperiod on cyclicity and serum gonadotropins in the Syrian hamster. Biol. Reprod. 12:223-231.

Silavin, S. L., and Greenwald, G. S. (1982). In-vitro progesterone production by ovarian interstitial cells from hypophysectomized hamsters. J. Reprod. Fertil. 66:291-298.

Simpkins, J. W., Kalra, P. S., and Kalra, S. P. (1980). Temporal alterations in luteinizing hormone-releasing hormone concentrations in several discrete brain regions: effects of estrogen, progesterone and norepinephrine synthesis inhibition. Endocrinology 107:573-577.

Sitaram, B. R., and Lees, G. J. (1978). Diurnal rhythm and turnover of tryptophan hydroxylase in the pineal gland of the rat. J. Neurochem. 31: 1021-1026.

Siuciak, J. A., Fang, J., and Dubocovich, M. L. (1990). Autoradiographic localization of 2-[125I] iodomelatonin binding sites in the brains of C3H/HeN and C57BL/6J strains of mice. Eur. J. Pharmacol. 180:387-390.

Smythe, G. A., Bradshaw, J. E., Cai, W. Y., and Symons, R.

G. (1982). Hypothalamic serotonergic stimulation of thyrotropin secretion and related brain hormone and drug interactions in the rat. Endocrinology 111: 1181-1191.

Soares, M. J., Colosi, P., and Talamantes, F. (1983). Development of a homologous radioimmunoassay for secreted hamster prolactin. Proc. Soc. Exp. Biol. Med. 172:379-381.

Stankov, B., Lucini, V., Mariani, M., Scaglione, F., Demartini, G., and Fraschini, F. (1990). Alpha-1 adrenoceptor involvement in the control of melatonin secretion in the golden hamster. J. Pineal Res. 9:21-28.

Stankov, B., and Reiter, R. J. (1990). Melatonin receptors: Current status, facts, and hypotheses. Life Sci. 46:971-982.

Steger, R. W., Bartke, A., Goldman, B. D., Soares, M. J., and Talamantes, F. (1983). Effects of short photoperiod on the ability of golden hamster pituitaries to secrete prolactin and gonadotropins in vitro. Biol. Reprod. 29:872-878.

Steger, R. W., Bartke, A., Matt, K. S., Soares, M. J., and Talamantes, F. (1984). Neuroendocrine changes in male hamsters following photostimulation. J. Exp. Zool. 229:467-474.

Steger, R. W., Matt, K., Klemcke, H. R., and Bartke, A. (1985). Interactions of photoperiod and ectopic

pituitary grafts on hypothalamic and pituitary function in male hamsters. Neuroendocrinology 41:89-96.

Steger, R. W., Matt, K. S., and Bartke, A. (1985). Neuroendocrine regulation of seasonal reproductive activity in the male golden hamster. Neurosci. Biobehav. Rev. 9:191-201.

Steger, R. W., Matt, K., and Bartke, A. (1986). Interactions of testosterone and short photoperiod exposure on the neuroendocrine axis of the male Syrian hamster. Neuroendocrinology 43:69-74.

Steger, R. W., Dennis, C., VanAbbema, A., and Gay-Primel, E. (1990). Alterations in hypothalamic serotonin metabolism in male hamsters with photoperiod-induced testicular regression. Brain Res. 514:11-14.

Steger, R. W., and Gay-Primel, E. (1990). Effects of melatonin injections on the ability of golden hamster pituitaries to secrete prolactin and luteinizing hormone. Biol. Reprod. 42:217-221.

Steinlechner, S., Steger, R. W., King, T. S., and Reiter, R. J. (1983). Diurnal variation in the serotonin content and turnover in the pineal gland of the Syrian hamster. Neurosci. Lett. 35:167-172.

Steinlechner, S., Stieglitz, A., Heldmaier, G. (1988). Acute cold exposure reduces sensitivity of the Djungarian hamster pineal gland to light at night. In: Abstracts of the 14th Congress of the European Society of Comparative Endocrinology. p. 51.

Stetson, M. H., Watson-Whitmyre, M. and Matt, K. S. (1978). Cyclic gonadotropin release in the presence and absence of estrogenic feedback in ovariectomized golden hamsters. Biol. Reprod. 19:40-50.

Stetson, M. H., Watson-Whitmyre, M., and Tate-Ostroff, B. (1983). The role of the pineal and its hormone melatonin in the termination of photorefractoriness in golden hamsters. Biol. Reprod. 29:689-696.

Sugden, D., and Morris, R. D. (1979). Changes in regional brain levels of tryptophan, 5-hydroxytryptamine, 5HIAA, DA, and NA after pinealectomy in the rat. J. Neurochem. 32:1593-1595.

Tamarkin, L., Westron, W. K., Hamill, A. I., and Goldman, B. D. (1976). Effect of melatonin on the reproductive systems of male and female Syrian hamsters: A diurnal rhythm in sensitivity to melatonin. Endocrinology 99:1534-1541.

Tamarkin. L., Hutchison, J. S., and Goldman, B. D. (1976). Regulation of serum gonadotropins by photoperiod and testicular hormone in the Syrian hamster. Endocrinology 99:1528-1533.

Terranova, P. F., Martin, N. C., and Chien, S. (1982). Theca is the source of progesterone in experimentally induced atretic follicles of the hamster. Biol. Reprod. 26:721-727.

Trakulrungsi, C., Reiter, R. J., Trakulrungsi, W. K., Vaughan, M. K., and Waring-Ellis, P. J. (1979).

Interaction of daily injections and subcutaneous resevoirs of melatonin on the reproductive physiology of female Syrian hamsters. Acta Endocrinol. 91:59-69.

Tuomisto, J., and Mannisto, P. (1985). Neurotransmitter regulation of anterior pituitary hormones. Pharmacol. Rev. 37:249- 332.

Van de Kar, L. D., and Bethea, C. L. (1982). Pharmacological evidence that serotonergic stimulation of prolactin secretion is mediated via dorsal raphe nucleus. Neuroendocrinology 35:225-230.

Van de Kar, L. D., Lorens, S. A., Urban, J. H., and Bethea, C. L. (1989). Effect of selective serotonin (5HT) agonists on prolactin secretion. Neuropharmacology 28:299-305.

Van de Kar, L. D. (1989). Neuroendocrine aspects of the serotonergic hypothesis of depression. Neurosci. Biobehav. Rev. 13:237- 246.

Vanecek, J., Pavlik, A., and Illnerova, H. (1987). Hypothalamic melatonin receptor sites revealed by autoradiography. Brain Res. 435: 359-363.

Vanecek, J. (1988). Melatonin binding sites. J. Neurochem. 51:1436-1440.

Vanecek, J., and Jansky, L. (1989). Short days induce changes in specific melatonin binding in hamster median eminence and anterior pituitary. Brain Res. 477:387-390.

Vanecek, J., and Vollrath, L. (1990). Developmental

changes and daily rhythm in melatonin-induced inhibition of 3', 5'-cyclic AMP accumulation in the rat pituitary. Endocrinology 126:1509-1513.

Vaughan, G. M., Pelham, R. W., Pang, S. F., Loughlin, L., Wilson, K. M., Sandock, K. L., Vaughan, M. K., Koslow, S. H., and Reiter, R. J. (1976). Nocturnal elevation of plasma melatonin and urinary 5-hydroxyindoleacetic acid in young men: attempts at modification by brief changes in environmental lighting and sleep and by autonomic drugs. J. Clin. Endocrinol. Metab. 42:752-764.

Vaughan, M. K., Powanda, M. C., Brainard, G. C., Johnson, L. Y., and Reiter, R. J. (1982). Effects of blinding or afternoon melatonin injections on plasma cholesterol, triglycerides, glucose, TSH, and thyroid hormone levels in male and female Syrian hamsters. In: The Pineal and its Hormones. Reiter, R. J. editor. Alan R. Liss Inc. N.Y. pp. 177-186.

Vollrath, L. (1973). Synaptic ribbons of a mammalian pineal gland. Circadian changes. Z. Zellforsche 145:171-183.

Vollrath, L. (1981). The Pineal Organ. in handbuch der mikroskopischen Anatomie des Menschen. Oksche, A., Vollrath, L. editors. Vol VI/7 (Berlin, Springer).

Vriend, J., and Reiter, R. J. (1977). Free thyroxine index in normal, melatonin-treated and blind hamsters. Hor. Metab. Res. 9:231-234.

Vriend, J., Richardson, B. A., Vaughan, M. K., Johnson, L. Y., and Reiter, R. J. (1982). Effects of melatonin on thyroid physiology of female hamsters. Neuroendocrinology 35:79-85.

Vriend, J. (1983). Evidence for pineal gland modulation of the neuroendocrine-thyroid axis. Neuroendocrinology 36:68-78.

Vriend, J. Bertalanffy, F. D., and Ralcewicz, T. A. (1987). The effect of melatonin and hypothyroidism on estradiol and gonadotropin levels in female Syrian hamsters. Biol. Reprod. 36:719-728.

Vriend, J., and Steiner, M. (1988). Melatonin and thyroid function. In: Melatonin: Clinical Perspectives. Miles, A., Philbrick, D. R., Thompson, C. editors. N.Y. Oxford University Press. pp. 92-117.

Vriend, J. (1989). Endocrine effects of blinding in male Syrian hamsters are associated with increased hypothalamic 5HIAA/5HT ratios. J. Pineal Res. 7:401-409.

Vriend, J., Sheppard, M. S., and Borer, K. T. (1991). Melatonin increases serum growth hormone and insulin-like growth factor I (IGF-I) levels in male Syrian hamsters via hypothalamic neurotransmitters. Growth (in press).

Waloch, M., Gilman, D., Whitmoyer, D., and Sawyer, C. H. (1981). The effects of stimulation and lesion of raphe nuclei on luteinizing hormone release in estrogen-

progesterone-treated ovariectomized rats. Brain Res. 217:305-313.

Wang, P. S., and Porter, J. C. (1986). Hormonal modulation of the quantity and in situ activity of tyrosine hydroxylase in neurites of the median eminence. Proc. Natl. Acad. Sci. USA. 83:9804-9806.

Weaver, D. R., and Reppert, S. M. (1990). Melatonin receptors are present in the ferret pars tuberalis and pars distalis, but not in brain. Endocrinology 127:2607-2609.

Weaver, D. R., Provencio, I., Carlson, L. L., and Reppert, S. M. (1991). Melatonin receptors and signal transduction in photorefractory Siberian hamsters. Endocrinology 128:1086-1092.

Webb, S. M., Champney, T. H., Lewinski, A. K., and Reiter, R. J. (1985). Photoreceptor damage and eye pigmentation: influence on the sensitivity of rat pineal N-acetyltransferase activity and melatonin to light at night. Neuroendocrinology 40:205-209.

Wehr, T., Skwerer, R., Jacobsen, F., Sack, D., and Rosenthal, N. (1987). Eye-versus-skin phototherapy of seasonal affective disorder. Am. J. Psychiat. 144:753-757.

Weiner, R. I., and Ganong, W. F. (1978). Role of brain monoamines and histamine in regulation of anterior pituitary secretion. Physiol. Rev. 58:905-976.

Weissbach, H., Redfield, B. G., and Axelrod, J. (1960).

Biosynthesis of melatonin: enzymatic conversion of serotonin to N-acetylserotonin. Biochim. Biophys. Acta 43:352-353.

Wetterburg, L. (1978). Melatonin in humans. Physiological and clinical studies. J. Neural Trans. (Suppl.) S13:289-310.

Wiechmann, A. F. (1986). Melatonin: Parallels in pineal gland and retina. (Review) Exp. Eye Res. 42:507-527.

Wilkinson, M., Arendt, J., Bradtke, J., De Ziegler, D. (1977). Determination of a dark-induced increase of pineal N-acetyltransferase activity and simultaneous radioimmunoassay of melatonin in pineal serum and pituitary tissue of the male rat. J. Endocrinol. 72:243-244.

Williams, L. M., Morgan, P. J., Hastings, M. H., Lawson, W. (1989). Melatonin receptor sites in the Syrian hamster brain and pituitary: Localization and characterization using [I-125] Iodomelatonin. J. Neuroendocrinol. 1(5):315-320.

Wolfe, D. E. (1965). The epiphyseal cell: an electron microscopic study of its intercellular relationships and intracellular morphology in the pineal body and other areas of brain tissue. Progr. Brain Res. 10:332-386.

Wurtman, R. J., Axelrod, J., and Philips, L. (1963). Melatonin synthesis in the pineal gland: control by light. Science 142:1071-1073.

Wurtman, R. J., and Axelrod, J. (1965). The pineal gland. Scient. Am. 213:50-60.

Zatz, M. (1981). Pharmacology of the rat pineal gland. In: The Pineal Gland: (Vol. I: Anatomy and Biochemistry). Reiter, R. J. editor, C.R.C. Press, Inc., Bota Raton, Florida. pp. 229-242.

Zisapel, N., Egozi, Y., and Laudon, M. (1982). Inhibition of dopamine release by melatonin: regional distribution in the rat brain. Brain Res. 246:161-163.

Zisapel, N., and Laudon, M. (1983). Inhibition by melatonin of dopamine release from rat hypothalamus: regulation of calcium entry. Brain Res. 272:378-381.

A P P E N D I X

TABLE 1: Pituitary content of PRL and LH after 10 weeks of daily afternoon melatonin (M) injections in both intact and ovariectomized (GX) hamsters; pargyline administration 2 hours prior to sacrifice.

Anova of PRL data:

Pargyline	$F = 0.09$
Melatonin	$F = 114.50$ ($p < .001$)
Ovariectomy	$F = 65.66$ ($p < .001$)
Melatonin/Ovariectomy Interaction	$F = 71.31$ ($p < .001$)

Anova of LH data:

Pargyline	$F = 7.54$ ($p < .01$)
Melatonin	$F = 4.87$ ($p < .05$)
Ovariectomy	$F = 51.27$ ($p < .001$)
Pargyline/Ovariectomy Interaction	$F = 4.42$ ($p < .05$)

TABLE 1: Pituitary content of PRL and LH after 10 weeks of daily afternoon melatonin (M) injections in intact and ovariectomized (GX) hamsters; pargyline (P) administration 2 hrs. prior to sacrifice.

Treatment	PRL μg/mg s.e.	LH ng/mg s.e.
Saline (parg)	9.87 ± 0.57 9.69 ± 0.12	120 ± 11 155 ± 19
Melatonin (parg)	1.38 ± 0.26** 1.29 ± 0.18**	173 ± 18 207 ± 33
GX - saline (parg)	1.79 ± 0.44** 1.89 ± 0.39**	401 ± 93* 507 ± 103**
GX-melatonin (parg)	1.31 ± 0.36** 0.79 ± 0.15**	431 ± 105** 843 ± 120**

* $p < .05$ compared to saline controls
** $p < .01$ compared to saline controls

TABLE 2: Concentrations of monoamines and metabolites in median eminence.

Treatment	Serotonin pg/mg ± s.e.	Dopamine pg/mg ± s.e.	HVA pg/mg ± s.e.	Dopac pg/mg ± s.e.
Saline	1016 ± 50	981 ± 131	105 ± 19	85 ± 4
(parg)	1727 ± 97	1302 ± 179	ND	ND
Melatonin	1094 ± 73	736 ± 103	87 ± 7	102 ± 12
(parg)	1992 ± 164	807 ± 62*	ND	ND
GX-Saline	805 ± 48*	993 ± 83	87 ± 5	98 ± 10
(parg)	1557 ± 151	2238 ± 324	ND	ND
GX-Melatonin	1019 ± 116	992 ± 166	94 ± 9	101 ± 13
(parg)	1799 ± 66	1102 ± 179**	ND	ND

Significant pargyline-induced increases in serotonin ($F = 166.86$, $p < .001$) and in dopamine ($F = 13.67$, $p < .001$) concentrations were observed.

* $p < .05$, compared to saline-injected controls
** $p < .01$, compared to ovariectomized (GX) controls.

TABLE 3. EFFECTS OF MELATONIN ON CONCENTRATIONS OF 5HIAA IN MEDIAN EMINENCE AND STRIATUM

TREATMENT	5HIAA CONCENTRATIONS IN MEDIAN EMINENCE pg/mg ± S.E.	5HIAA CONCENTRATIONS IN STRIATUM pg/mg ± S.E.
Saline	398 ± 25	489 ± 18
(Pargyline)	121 ± 18	240 ± 24
Melatonin	455 ± 13	577 ± 41
(Pargyline)	110 ± 13	205 ± 29
GX - Saline	418 ± 25	451 ± 21
(Pargyline)	109 ± 16	207 ± 17
GX - Melatonin	468 ± 28	521 ± 17*
(Pargyline)	96 ± 9	221 ± 16
Pargyline	P F = 545.00***	P F = 285.91***
Melatonin	M F = 2.21	M F = 3.92
Ovariectomy	O F = .01	O F = 2.52
	P/M F = 5.60*	P/M F = 6.78*

* P < .05 compared to ovariectomized (GX) controls

TABLE 4: EFFECTS OF MELATONIN ON CONCENTRATIONS OF NOREPINEPHRINE (NE)

Treatment	NE in Median Eminence pg/mg + S.E.	NE in Posterior Pituitary pg/mg + S.E.	NE in Striatum pg/mg + S.E.	NE in Amygdala pg/mg + S.E.
Saline (Pargyline)	1546 ± 279 1720 ± 293	188 ± 17 217 ± 32	277 ± 27 385 ± 107	853 ± 24 957 ± 65
Melatonin (Pargyline)	1537 ± 294 1785 ± 288	205 ± 12 149 ± 15	292 ± 32 307 ± 21	854 ± 54 963 ± 66
GX-Saline (Pargyline)	1402 ± 382 1884 ± 300	206 ± 27 238 ± 37	273 ± 50 416 ± 74	852 ± 47 1092 ± 63
GX - Melatonin (Pargyline)	1681 ± 347 1595 ± 318	152 ± 16 213 ± 28	289 ± 15 303 ± 41	905 ± 38 873 ± 49
Pargyline Melatonin Ovariectomy	P F = 5.52* M F = 0.01 O F = 0.03	P F = 0.83 M F = 3.29 O F = 0.49	P F = 3.32 M F = 1.11 O F = 0.02	P F = 6.83* M F = 0.97 O F = 0.35

TABLE 5: Concentrations of DA and Dopac in posterior pituitary

Treatment	Dopamine pg/mg ± s.e.	Dopac pg/mg ± s.e.
Saline (parg)	1096 ± 240 1341 ± 95	758 ± 139 ND
Melatonin (parg)	828 ± 171 867 ± 151*	404 ± 46* ND
GX-Saline (parg)	1167 ± 233 1388 ± 166	553 ± 156 ND
GX-Melatonin (parg)	740 ± 147 1263 ± 199	359 ± 82 ND

* $p < .05$ compared to respective controls

TABLE 6: EFFECTS OF MELATONIN ON DA AND NE ACCUMULATION AFTER PARGYLINE IN THE POSTERIOR PITUITARY

Treatment	Accumulation of DA after Pargyline pg/mg/hr + S.E.	Accumulation of NE after Pargyline pg/mg/hr + S.E.
Controls	123 ± 48	17 ± 16
Melatonin	20 ± 76	- 19 ± 8
Ovariectomized	110 ± 83	16 ± 19
Ovariectomized and Melatonin	262 ± 100	31 ± 14*
Melatonin (M) Ovariectomy (O) Interaction (I)	M F = 0.09 O F = 1.89 I F = 2.31	M F = 0.47 O F = 2.75 I F = 2.91

P < .05 compared to intact melatonin-treated hamsters

TABLE 7: Concentrations of monoamines and metabolites in striatum.

Treatment	[1]Serotonin pg/mg ± s.e.	[1]Dopamine ng/mg ± s.e.	[2]HVA pg/mg ± s.e.	[2]Dopac pg/mg ± s.e.
Saline	526 ± 43	14.68 ± 0.94	1215 ± 109	1543 ± 104
(parg)	1133 ± 96	24.95 ± 1.62	74 ± 16	265 ± 34
Melatonin	664 ± 67	17.60 ± 1.25	1416 ± 122	1666 ± 140
(parg)	1000 ± 79	23.82 ± 2.12	68 ± 14	286 ± 19
GX-Saline	536 ± 42	18.31 ± 1.09	1247 ± 105	1809 ± 135
(parg)	1167 ± 121	24.84 ± 0.94	53 ± 11	299 ± 27
GX-Melatonin	643 ± 30	18.45 ± 1.06	1255 ± 65	1747 ± 100
(parg)	1228 ± 96	26.22 ± 1.05	56 ± 12	254 ± 61

[1]Significant pargyline-induced increases in serotonin ($F = 92.66$, $p < .001$) and in dopamine ($F = 68.15$, $p < .001$) concentrations were observed.

[2]Significant pargyline-induced decreases in the dopamine metabolites, HVA ($F = 554.40$, $p < .001$) and Dopac ($F = 495.98$, $p < .001$) were observed.

TABLE 8: EFFECTS OF MELATONIN ON DA AND NE ACCUMULATION AFTER PARGYLINE IN STRIATUM

Treatment	Accumulation of DA after Pargyline pg/mg/hr + S.E.	Accumulation of NE after Pargyline pg/mg/hr + S.E.
Controls	5,118 ± 812	1 ± 7
Melatonin	3,109 ± 1,630	8 ± 10
Ovariectomized	3,263 ± 1,680	72 ± 37
Ovariectomized and Melatonin	3,885 ± 1,900	7 ± 21
Melatonin Ovariectomy Interaction	M F = 0.84 O F = 0.51 I F = 3.03	M F = 1.63 O F = 2.34 I F = 2.42

TABLE 9: Effects of melatonin on serotonin concentrations in amygdala of female hamsters.

Treatment	pg 5HT/mg ± s.e.	pg 5HT/mg ± s.e. (after pargyline)
Controls	1452 ± 161	2403 ± 224
Melatonin	1536 ± 70	2584 ± 238
Ovariectomized	1369 ± 82	2710 ± 74
Ovariectomized + melatonin	1548 ± 97	2315 ± 129

Pargyline F = 95.92***
Melatonin F = 0.01
Ovariectomy F = 0.01

TABLE 10: EFFECTS OF MELATONIN ON DOPAMINE METABOLISM IN AMYGDALA

Treatment	DA Concentrations in Amygdala (pg/mg)	DA Accumulation after Pargyline (pg/mg/hr)	DOPAC Concentrations in Amygdala (pg/mg)	HVA Concentrations in Amygdala (pg/mg)
Control	978 ± 183	602 ± 213	248 ± 21	169 ± 36
(Pargyline)	2182 ± 427		202 ± 19	84 ± 24
Melatonin	1293 ± 277	296 ± 161	259 ± 31	164 ± 24
(Pargyline)	1885 ± 323		182 ± 16	53 ± 10
GX - Saline	1959 ± 312	42 ± 104*	318 ± 15*	278 ± 21*
(Pargyline)	2044 ± 208		160 ± 18	49 ± 13
GX - Melatonin	1481 ± 204	170 ± 185	283 ± 13	165 ± 24
(Pargyline)	2086 ± 415		205 ± 13	78 ± 21
Pargyline Melatonin Ovariectomy	P F = 7.86** M F = 0.22 O F = 1.93	Mel F = 0.27 Ovx F = 4.05	P F = 43.47*** M F = 0.00 O F = 1.87 P/O F = 4.30* P/M/O F = 4.26*	P F = 66.00*** M F = 3.66 O F = 2.50 P/M/O F = 7.03*

* P<.05 compared to intact controls