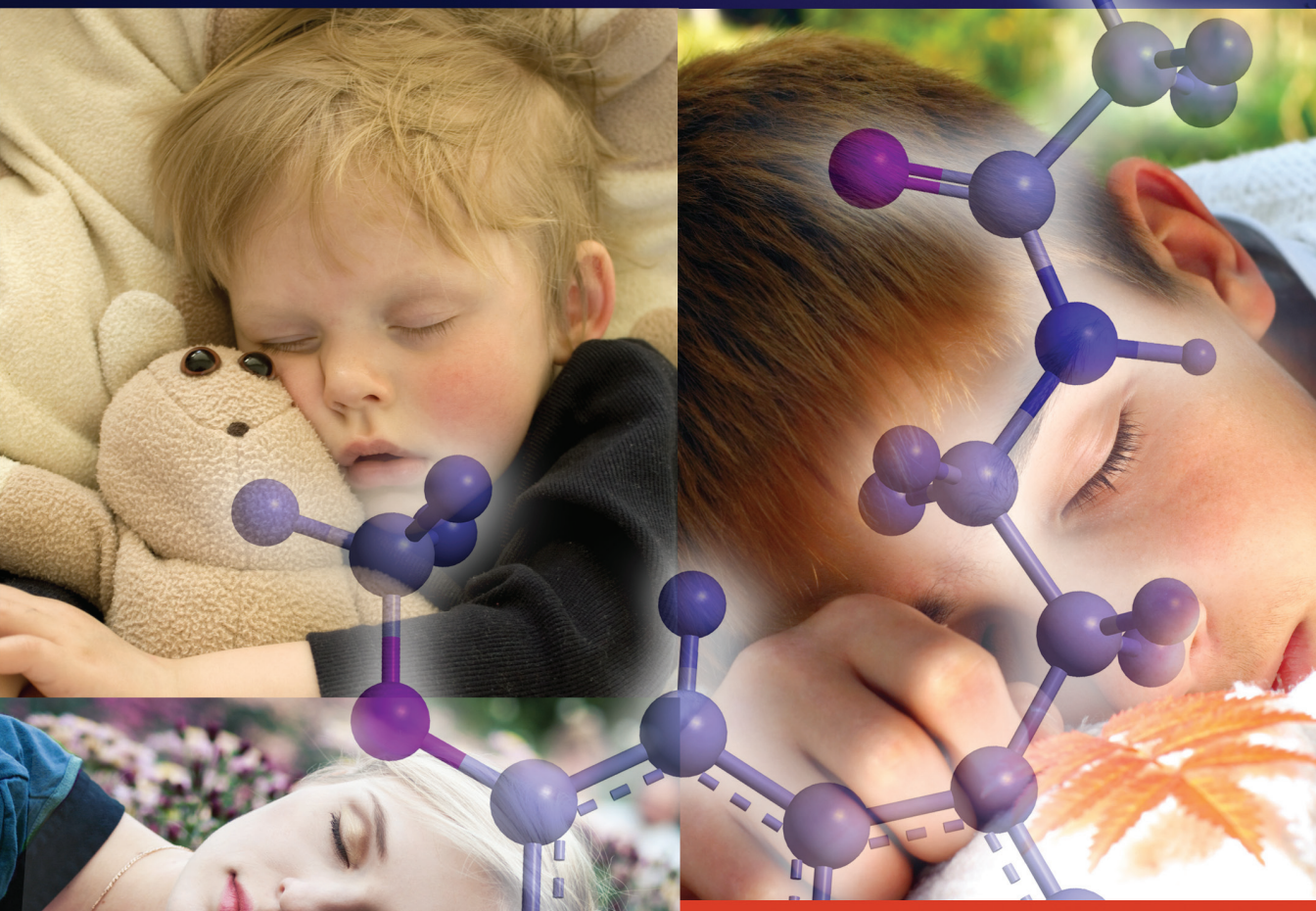
Melatonin in the Promotion of Health

Edited by Ronald Ross Watson



Second Edition

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Preface

Melatonin is a small molecule, a derivative of the amino acid tryptophan, *N*-acetyl-5 methoxytryptamine. It is a potent hormone with well-recognized activities and the potential to influence many bodily functions. It is produced by the pineal gland in the brain and is secreted when the body recognizes darkness. Melatonin has intense effects on the timing of the sleep/awake cycle, regulating the circadian rhythms of several biological functions. This is its most studied biological activity. Melatonin, as a multifunctional hormone, appears to regulate or modulate other functions in humans through the activation of its receptors and works as a strong antioxidant that protects the DNA. Melatonin's antioxidant activity may reduce Parkinson's disease development, prevent cardiac arrhythmia, and, in animals, promote longevity. Studies on human disease prevention and treatment are more limited and will be a major area of review in this book. Aging involves many changes in our physiology, and only a few are understood. It has been recognized that melatonin levels decline with age and may play a role in many age-related changes. Melatonin is a powerful antioxidant with numerous effects on the metabolism and health of older people. Melatonin has been available as a dietary supplement in the United States since 1993 as well as in Canada and some European countries. In moderate doses, melatonin has routinely been found to be safe, with very low toxicity in animals and humans. There is evidence that melatonin supplementation or level in human affect headaches, fertility, attention deficit–hyperactivity disorder, mood disorders, cancer, gall stones, circadian rhythm disorders (sleep). They help prevent ischemic damage, autism, immune regulation, dreaming, oxidant removal, as well as recent but more limited evidence of roles in other human health conditions. The editor selected researchers with experience in studying the role of melatonin in various disease and physiological states primarily in humans. Thus, this book has a wide variety of expert reviews on the biology of melatonin relevant to health in humans. The editor, Dr. Watson, has done research with melatonin as a modulator of immune function and regulator of AIDS in mice.

Section I reviews key areas of melatonin, including a history of melatonin and its use in various therapies, as well as melatonin and circadian rhythms. The focus of Section II relates to melatonin's action in the prevention of diseases such as cardiovascular diseases, reproductive diseases, solar skin damage, diabetes, immune function, uveitis, and gut function, among others. There are three other sections with clinical potential importance and varying levels of study. The key section, Section III, relates to melatonin's action in the treatment of diseases and physiological disorders such as surgery, bone, breast cancer, gastrointestinal function, reproduction, and pancreas diseases. In some cases, the role of melatonin is still controversial in certain human health conditions, and the researchers define what is known for the various conditions being treated. Section IV describes melatonin in the context of sleep and circadian rhythm regulation. Finally, Section V describes how melatonin's actions on physiological functions in humans focus on the effects of loss of pineal function and the subsequent reduction in melatonin production as well as its replacement as a therapy. These include neuroendocrine effects, inflammation, age-related degeneration, collagen synthesis, vascular structure, DNA protection, oxidation, and traumatic stress, among others. Understanding melatonin is critical, as it is a hormone that is sold over the counter as a dietary supplement and is thus readily available to consumers in many countries.

Acknowledgments

Special thanks are extended to Bethany L. Stevens, the editorial assistant to Dr. Ronald Ross Watson. She spent many hours working with the publisher and with the authors of this book. She made it possible for Dr. Watson to function as the editor by lightening his load and taking responsibility for routine questions, format, and style. Support for editorial assistance was provided by Southwest Scientific Editing and Consulting, LLC. The help of Mari Stoddard, an Arizona Health Sciences Librarian, was crucial to finding all the authors and topics that appear in the book.

Editor

Ronald R. Watson attended the University of Idaho but graduated from Brigham Young University in Provo, UT, with a degree in chemistry in 1966. He earned his PhD in biochemistry from Michigan State University in 1971. His postdoctoral schooling in nutrition and microbiology was completed at the Harvard School of Public Health, where he gained two years of postdoctoral research experience in immunology and nutrition.

From 1973 to 1974, Dr. Watson was assistant professor of immunology and performed research at the University of Mississippi Medical Center in Jackson. He was assistant professor of microbiology and immunology at the Indiana University Medical School from 1974 to 1978 and associate professor at Purdue University in the Department of Food and Nutrition from 1978 to 1982. In 1982, Dr. Watson joined the faculty at the University of Arizona Health Sciences Center in the Department of Family and Community Medicine of the School of Medicine. He is currently professor of health promotion sciences in the Mel and Enid Zuckerman Arizona College of Public Health.

Dr. Watson is a member of several national and international nutrition, immunology, cancer, and alcoholism research societies. Among his patents, with more pending, is a dietary supplement using passion fruit peel extract. He has done melatonin research on its effects on mouse AIDS and immune function for 20 years. He edited a previous book on melatonin, *Melatonin in the Promotion of Health* (CRC Press, 1998, 224 pp.). For 30 years, he was funded by the Wallace Research Foundation to study dietary supplements in health promotion. Dr. Watson has edited more than 35 books on nutrition, dietary supplements and over-the-counter agents, and 53 other scientific books. He has published more than 500 research and review articles.

Contributors

Marco Antonioli
Section of Perinatal Psychiatry and Stress
King's College London
London, United Kingdom

Andreas Gunter Bach
Clinic for Diagnostic Radiology
Martin Luther University of Halle-Wittenberg
Halle, Germany

Soyhan Bağci
Department of Neonatology
University of Bonn
Bonn, Germany

Mariano Bizzarri, MD, PhD
Department of Experimental Medicine
University "La Sapienza"
Rome, Italy

Petr Bob
Department of Psychiatry
Charles University
Prague, Czech Republic

Gregory M. Brown
Department of Psychiatry
University of Toronto
Toronto, Ontario, Canada

Iwona Brzozowska
Department of Physiology
Jagiellonian University Medical College
Cracow, Poland

Tomasz Brzozowski
Department of Clinical Physiology
Jagiellonian University Medical College
Cracow, Poland

George A. Bubenik
Department of Integrative Biology
University of Guelph
Guelph, Ontario, Canada

Daniel P. Cardinali
Department of Teaching and Research
Pontifical Catholic University of Argentina
Buenos Aires, Argentina

Livia A. Carvalho
Department of Life Sciences
Roehampton University
London, United Kingdom

Marek Cegielski
Department of Histology and Embryology
Wrocław Medical University
Wrocław, Poland

Krzysztof Celinski
Department of Gastroenterology
Medical University of Lublin
Lublin, Poland

Mónica Chianelli
Department of Human Biochemistry
University of Buenos Aires
Buenos Aires, Argentina

Indrajit Chowdhury
Department of Obstetrics and Gynecology
Morehouse School of Medicine
Atlanta, Georgia

William P. Clafshenkel
Graduate School of Pharmaceutical Sciences
Duquesne University
Pittsburgh, Pennsylvania

Alessandra Cucina
Department of Surgery "P. Valdoni"
University "La Sapienza"
Rome, Italy

Fabrizio D'Anselmi
Department of Surgery "P. Valdoni"
University "La Sapienza"
Rome, Italy
and
Italian Space Agency
Rome, Italy

Vicki L. Davis
Graduate School of Pharmaceutical Sciences
Duquesne University
Pittsburgh, Pennsylvania

Simona Dinicola
Department of Experimental Medicine
University "La Sapienza"
Rome, Italy

Balasunder R. Dodda
Graduate School of Pharmaceutical Sciences
Duquesne University
Pittsburgh, Pennsylvania

Piotr Dzięgiel
Department of Histology and Embryology
Wrocław Medical University
Wrocław, Poland

Tom S. Edrington
USDA–Agricultural Research Service
College Station, Texas

Pedro A. Fernandes
Laboratory of Chronopharmacology
University of São Paulo
São Paulo, Brazil

Diego C. Fernandez
Department of Human Biochemistry
University of Buenos Aires
Buenos Aires, Argentina

Tobias W. Fischer
Department of Dermatology
University of Lübeck
Lübeck, Germany

Eleonora Foglio
Department of Biomedical Sciences and Biotechnologies
University of Brescia
Brescia, Italy

José Luis Guzmán
Department of Agroforestry Sciences
University of Huelva
Huelva, Spain

Khek-Yu Ho
University Medicine Cluster
National University Health System
and
Department of Medicine
National University Hospital
Singapore
and
Department of Medicine
National University of Singapore
Singapore

Dan-Ning Hu
Tissue Culture Center
The New York Eye and Ear Infirmary
New York, New York
and
New York Medical College
New York, New York

Salah A. Ismail
Department of Anesthesia
Suez Canal University
Ismailia, Egypt

Ilona Iżykowska
Department of Histology and Embryology
Wrocław Medical University
Wrocław, Poland

Jolanta Jaworek
Department of Medical Physiology
Jagiellonian University School of Medicine
Cracow, Poland

Birgit C. P. Koch
Erasmus Medical Center
Rotterdam, The Netherlands

Masayuki Kondo
Human Life R&D Institute
Sekisui House, Ltd.
Kyoto, Japan

Peter C. Konturek
Department of Internal Medicine
University Erlangen–Nuremberg
Erlangen, Germany

Stanislaw J. Konturek
Department of Clinical Physiology
Jagiellonian University School of Medicine
Cracow, Poland

Mary P. Kotlarczyk
Graduate School of Pharmaceutical Sciences
Duquesne University
Pittsburgh, Pennsylvania

Mauro Labanca
Department of Biomedical Sciences and Biotechnologies
University of Brescia
Brescia, Italy

Theodore Lialiaris
Laboratory of Genetics
Demokrition University of Thrace University
Alexandroupolis, Greece

Saumen Kumar Maitra
Department of Zoology
Visva-Bharati University
Santiniketan, West Bengal, India

Maria D. Maldonado
Department of Medical Biochemistry and Molecular Biology
University of Seville Medical School
Seville, Spain

Benoît Malpaux
Joint Research Unit:
National Institute of Agronomic Research
National Center for Scientific Research
Universite de Tours, and Haras Nationaux
Nouzilly, France

Regina P. Markus
Laboratory of Chronopharmacology
University of São Paulo
São Paulo, Brazil

Takeshi Morita
Department of Living Environmental Science
Fukuoka Women's University
Fukuoka, Japan

Hany A. Mowafi
Department of Anesthesia
Dammam University
Dammam, Saudi Arabia

María José Munuce
Laboratory of Reproductive Studies
University of Rosario
Rosario, Argentina

J. Elsbeth Nagtegaal
Meander Medical Center
Amersfoort, The Netherlands

David J. Nisbet
USDA–Agricultural Research Service
College Station, Texas

V. Haktan Ozacmak
Department of Physiology
Zonguldak Karaelmas University
Zonguldak, Turkey

Seithikurippu R. Pandi-Perumal
Somnogen Inc.
New York, New York

Alessia Pasqualato
Department of Basic and Applied Medical Sciences
University G. d'Annunzio
Chieti-Pescara, Italy

Ming Pei
Stem Cell and Tissue Engineering Laboratory
West Virginia University
Morgantown, West Virginia

Maria A. Pérez-San-Gregorio
Department of Personality, Evaluation and Psychological Treatment
University of Seville
Seville, Spain

Elmar Peschke
Institute of Anatomy and Cell Biology
Martin Luther University of Halle-Wittenberg
Halle, Germany

Carlos Ponce
Program of Anatomy and Developmental Biology
University of Chile
Santiago, Chile

Sara Proietti
Department of Experimental Medicine
University "La Sapienza"
Rome, Italy

Rita Rezzani
Human Anatomy Section
University of Brescia
Brescia, Italy

Joan E. Roberts
Division of Natural Sciences
Fordham University
New York, New York

Luigi Fabrizio Rodella
Department of Biomedical Sciences and Biotechnologies
University of Brescia
Brescia, Italy

Héctor Rodríguez
Program of Anatomy and Developmental Biology
University of Chile
Santiago, Chile

Richard B. Rosen
New York Eye and Ear Infirmary
New York, New York
and
New York Medical College
New York, New York

Ruth E. Rosenstein
Department of Human Biochemistry
University of Buenos Aires
Buenos Aires, Argentina

Marije Russcher
Resident in Hospital Pharmacy
Meander Medical Center
Amersfoort, The Netherlands

Joanna Rybka
Department of Biochemistry
Nicolaus Copernicus University
Toruń, Poland
and
Ludwik Rydygier Collegium Medicum
Bydgoszcz, Poland
and
Section of Perinatal Psychiatry and Stress
King's College London
London, United Kingdom

Daniel A. Sáenz
Department of Human Biochemistry
University of Buenos Aires
Buenos Aires, Argentina

Alexander Samel
Division of Flight Physiology
Institute of Aerospace Medicine
Cologne, Germany

Pablo H. Sande
Department of Human Biochemistry
University of Buenos Aires
Buenos Aires, Argentina

Luis Sarabia
Program of Anatomy and Developmental Biology
University of Chile
Santiago, Chile

Shalini Sethi
Graduate School of Pharmaceutical Sciences
Duquesne University
Pittsburgh, Pennsylvania

Andrzej T. Slominski
Skin Cancer Division
University of Tennessee
Memphis, Tennessee
and
Department of Pathology and Laboratory Medicine
University of Tennessee Health Science Center
Memphis, Tennessee

Radomir Slominski
Department of Pathology and Laboratory Medicine
University of Tennessee Health Science Center
Memphis, Tennessee

D. Warren Spence
Sleep and Alertness Clinic
Toronto, Ontario, Canada

Ioannis A. Taitzoglou
Faculty of Veterinary Medicine
Aristotle University of Thessaloniki
Thessaloniki, Greece

John E. Tidwell
Department of Orthopaedics
West Virginia University
Morgantown, West Virginia

Ilya Trakht
Department of Medicine
Columbia University
New York, New York

Maria P. Tsantarliotou
Faculty of Veterinary Medicine
Aristotle University of Thessaloniki
Thessaloniki, Greece

G. C. Verster
Department of Psychiatry
University of Stellenbosch
Cape Town, South Africa

Tomoko Wakamura
Department of Human Health Sciences
Kyoto University
Kyoto, Japan

Allan F. Wiechmann
Department of Cell Biology
University of Oklahoma Health Sciences Center
Oklahoma City, Oklahoma

Paula A. Witt-Enderby
Graduate School of Pharmaceutical Sciences
Duquesne University
Pittsburgh, Pennsylvania

Jennie Wong
Department of Medicine
National University of Singapore
Singapore

Mustafa Yildiz
Kosuyolu Heart Center
Istanbul, Turkey

Luis Ángel Zarazaga
Department of Agroforestry Sciences
University of Huelva
Huelva, Spain

1 Melatonin Time Line: From Discovery to Therapy

Indrajit Chowdhury and Saumen Kumar Maitra

CONTENTS

1.1 INTRODUCTION

Discovery of an active substance in any component of a living system has never been so exciting as what happened with the isolation, purification, and characterization of an indole compound from extracts of the pineal gland, which has long been considered as a functional relic of the brain [1]. The credit of such breakthrough research goes to a group led by an American dermatologist, Dr. Aaron Bunsen Lerner, in the Yale University School of Medicine, who extracted only a few milligrams of *N*-acetyl-5-methoxy-serotonin from more than 100,000 cattle pineal glands nearly 53 years ago [2,3]. They named this purified pineal substance melatonin (MEL) in recognition of an initial observation that treatment with crude acetone extract of bovine pineal glands to *Rana pipens* tadpole caused a pronounced lightening (blanching) of their skins (i.e., melanophore-contracting hormone; Greek: μελαζ = black; τονζ = tension, in the sense of contraction), resulting in clear visibility of the larger viscera through the dorsal body wall [4]. Nevertheless, the functional implication of pineal has a history way back to the 19th century, when Huebner [5] reported for the first time that tumor of the human pineal altered pubertal development, indicating a possible role of some factor(s) of pineal origin in influencing reproductive functions. This observation led many scientists in the first half of the 20th century to experimentally examine the association of the pineal with the reproductive status in a variety of animal species with limited compelling evidences [6] (Table 1.1).

TABLE 1.1
Melatonin Time Line

Year	Critical Findings
1898	• Tumor of human pineal is found to be associated with altered pubertal development.
1917	• Injection of bovine pineal extract to *Rana pipens* causes pronounced lightening of their skin.
1958	• MEL is isolated from bovine pineal gland.
1961	• *O*-Methylated by hydroxyindole-*O*-methyltransferase (*S*-adenosyl L-methionine: hydroxyindole *O*-methyltransferase; EC 2.1.1.4; HIOMT) is isolated and characterized.
1964	• L-Aromatic amino acid decarboxylase (aromatic L-amino acid carboxylase, EC 4.1.1.28, AAAD/AADC) is isolated and characterized.
1964	• Role of MEL as an antigonadotropic factor is demonstrated.
1967	• Trp-5-monooxygenase/hydroxylase (L-tryptophan, tetrahydropterin-dine: oxygen oxidoreductase (EC 1.14.16.4, TPH) is isolated and characterized.
1974	• The location of the biological clock, the mind's clock, in mammals is identified in the SCN.
1984	• Arylalkylamine-*N*-acetyltransferase (acetyl CoA: arylalkylamine-*N*-acetyltransferase/serotonin *N*-acetyltransferase; EC 2.3.1.87; AA-NAT) is isolated and characterized.
1982	• Possible role of MEL in breast cancer is demonstrated.
1991	• The antioxidant properties of MEL are demonstrated.
1991	• MEL is suggested as dietary supplement for beneficiary role.
1993	• The MEL rhythm is described as both clock and calendar.
1993	• The increase in MEL secretion in the evening is found correlated with an increase in sleep propensity.
1994	• MEL receptors (MT_1 and MT_2) are cloned.
1996	• The circadian rhythm of MEL production is found regulated by SCN of the anterior hypothalamus.
1999	• MT_1 dual signaling mechanism is identified.
2000	• MEL receptor MT3 is demonstrated as the enzyme quinine reductase 2.
1995–2002	• MEL is found in a wide spectrum of organisms including bacteria, fungi, plants, protozoa, invertebrates, and vertebrates and also in extrapineal sites like the retina, Harderian gland, gut, bone marrow, platelets, and skin in vertebrates.
2005	• MEL agonist agomelatine and ramelteon are launched as an orally active hypnotic for the treatment of transient and chronic insomnia in human.

Note: See text for details and references.

In the past 50 years since the discovery of MEL, we have witnessed an emergence of a plethora of information from studies on a wide group of animals, including vertebrates and invertebrates, and plants subjected to MEL, the most unique and wonder molecule among all the known substances in areas covering endocrinology, pharmacology, physiology, psychology, chronobiology, and environmental biology. The most remarkable feature of this pineal hormone is its synthesis in the synchronization with the environmental light/dark (LD) conditions. In all the animals investigated so far, irrespective of their habit, MEL synthesis in a 24-hour cycle reaches peak during the dark phase. As a hormone, MEL afforded the first opportunity for its use as a chronobiological marker, particularly for those who are engaged in circadian studies. It plays a central role in primary circadian pacemaker (suprachiasmatic nucleus, SCN) to synchronize the body functions to LD cycle of the environment. Accordingly, this hormone is considered as a "chronobiotic molecule," or the "hormone of darkness." Molecular biology study dealing with its biosynthetic enzymes and their genes, molecular regulators, degradation byproducts, and the mechanisms of signaling in a cell has opened up a new chapter in circadian research. Carefully controlled studies in different animals, including the human, have implicated MEL in the control of a wide variety of body functions ranging from aging to aggression, hibernation to hypertension, jet lag to seasonal affective disorders, sleep to stress, and reproduction to tissue regeneration. More recent studies revealed that MEL, because of its lipophilic nature, can cross the plasma membrane of any cell and thereby has free access to all the tissues, organs, and systems in the living body and by acting as a potent scavenger of free radicals may play an important role in combating oxidative stress in a metabolically active cell [7]. Moreover, MEL is known to be involved in complex processes of cellular protection and apoptosis. A rapidly expanding body of literature suggests that MEL as a biomarker of circadian dysfunctions may play pivotal roles in various neurodegenerative or neurological diseases. As a result, MEL has become a potent candidate for investigation in several disciplines of experimental biology and pathophysiology signifying its importance in clinical and therapeutic research. The aim of this review is to track the progress in research from discovery of MEL to its potential use in therapy by focusing the most fascinating, and arguably important, data gathered in recent past. We emphasize mostly on recent review articles, including ours [8], as they included many original findings that contributed to the current state of knowledge.

1.2 UBIQUITOUS DISTRIBUTION

MEL is ubiquitously distributed in the living system and is suggested to represent one of the most primitive biological signals on earth [9]. It is found not only in vertebrates but in all major phylogenetically distant taxa, including bacteria, unicellular eukaryotes, in different parts of plants (including the roots, stems, flowers, and seeds), and in invertebrate as well [10–13]. In vertebrates, MEL is produced predominantly by the pinealocytes of the pineal gland [14]. Additionally, it is produced in several extrapineal tissues and cells including the Harderian gland, extraorbital lachrymal gland, retina, bone marrow cells, platelets, lymphocytes, skin, enterochromaffin cells of gastrointestinal (GI) tract, and in bile in a variety of animal species [15–21]. In the retina, it is produced by photoreceptor cells [14,18]. MEL is released from extrapineal sites, either only poorly or in high amounts (by order of magnitude), as in the case of the GI tract, but without profound chronological consequences [10,15,22]. In bile, MEL concentration is about 1000 times higher than its daytime concentrations in the blood [23].

1.3 BIOSYNTHESIS

In all vertebrates investigated so far, MEL is primarily synthesized in the pinealocytes of pineal gland [20] at night regardless of the diurnal or nocturnal activity of the concerned animals. Biosynthesis of MEL is a four-step phenomenon (Figure 1.1). First, its precursor L-tryptophan (Trp) is taken up by the pinealocyte from the circulation (blood) and is converted to 5-hydroxy-Trp (5-HTP) in the mitochondria by Trp-5-monooxygenase/hydroxylase (L-tryptophan, tetrahydropterin-dine:

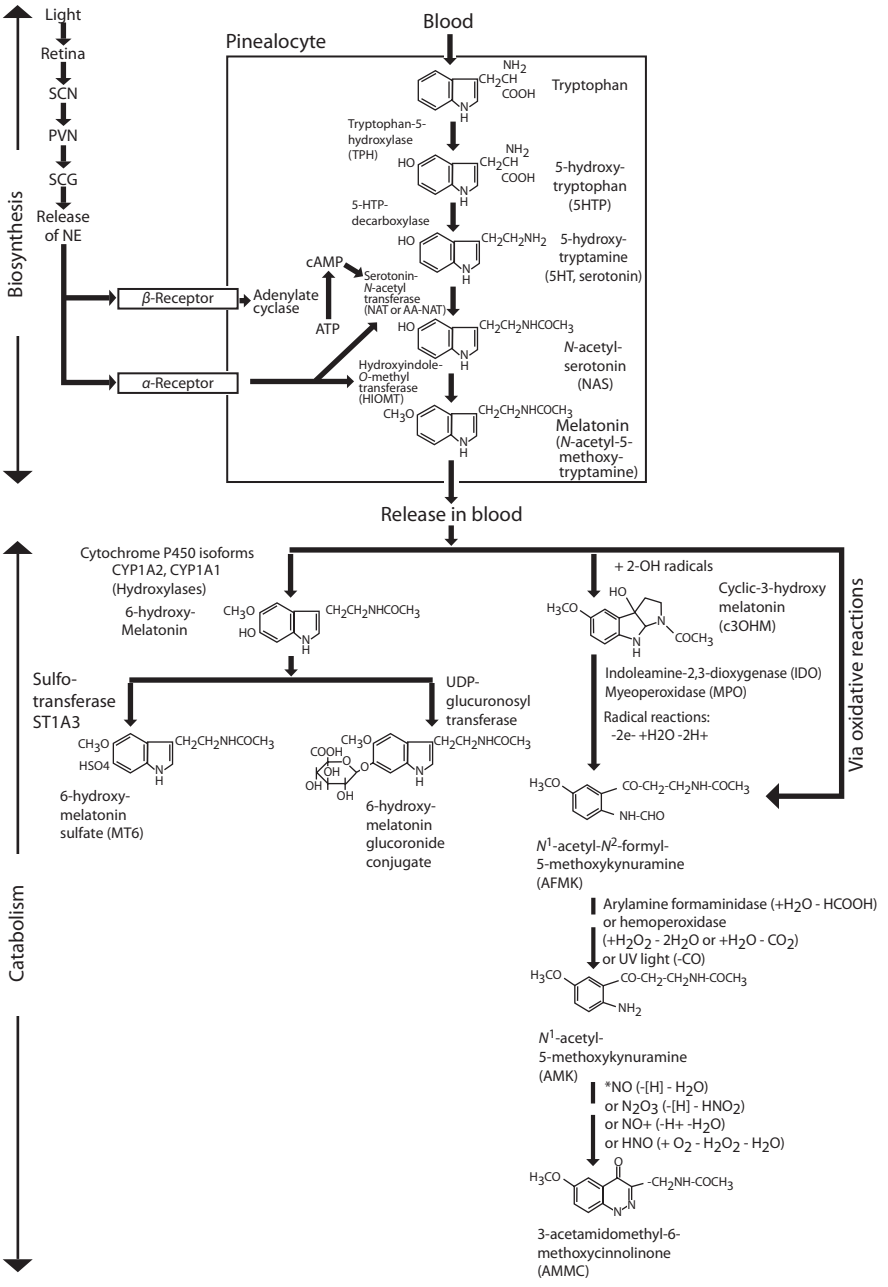

FIGURE 1.1 Schematic representation of the pathway of synthesis, secretion, and catabolism of MEL (from tryptophan to kynurenine). The hormone MEL is synthesized from tryptophan in a four-step pathway, which are under the control of the enzymes tryptophan 5-hydroxylase (TPH), 5-hydroxytryptophan-(5HTP)-decarboxylase, serotonin-*N*-acetyltransferase (NAT), or AA-NAT, and hydroxyindole-*O*-methyl transferase (HIOMT) in the pinealocytes of pineal gland. The rate of MEL formation depends on the multiple molecules that act through multiple mechanisms at different steps of its synthesis. The formation and secretion of AA-NAT and HIOMT are influenced by suprachiasmatic nucleus (SCN) activity via seasonal and circadian timing mechanisms. Under natural environment, MEL is secreted during the night in the healthy human. The catabolism of MEL is through the classical hydroxylation pathway or by kynuric pathway through oxidation of indole core of MEL. During catabolism, various catabolites are formed, which includes N^1-acetyl-5-methoxykynuramine (AMK), N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), glucuronides, etc. (see text for details).

oxygen oxidoreductase, EC 1.14.16.4, TPH) [24] and is then decarboxylated to form serotonin (5-hydroxytryptamine, 5-HT) in the cytosol by L-aromatic amino acid decarboxylase (aromatic L-amino acid carboxylase, EC 4.1.1.28, AAAD/AADC). A fraction of 5-HTP may be methylated into 5-methoxytryptophan [25].

Serotonin is the initial substrate of three different synthetic pathways [26]: (a) 5-HT can be directly *O*-methylated by hydroxyindole-*O*-methyltransferase (*S*-adenosyl L-methionine: hydroxyindole *O*-methyltransferase; EC 2.1.1.4; HIOMT) into 5-methoxytryptamine [27]. (b) 5-HT can be deaminated by monoamine oxidase (amine: oxygen oxidoreductase; EC 1.4.3.4; MAO) into 5-hydroxyindole-acetaldehyde (5-HIAL). This compound is then either successively oxidized into 5-hydroxyindole acetic acid (5-HIAA) by an aldehyde dehydrogenase (aldehyde: NAD^+ oxidoreductase; EC 1.2.1.3) then *O*-methylated by HIOMT to form 5-methoxyindole acetic acid (5-MIAA), or successively reduced into 5-hydroxytryptophol (5-HL) by an alcohol dehydrogenase (alcohol: NAD^+ oxidoreductase; EC 1.1.1.1) then *O*-methylated by HIOMT to form 5-methoxytryptophol (5-ML). (c) The most physiologically important metabolic pathway of 5-HT leads to the synthesis of MEL [28]. 5-HT is first acetylated (*N*-acetylation) by arylalkylamine-*N*-acetyltransferase (acetyl CoA: arylalkylamine-*N*-acetyltransferase/serotonin *N*-acetyltransferase; EC 2.3.1.87; AA-NAT) into *N*-acetyl serotonin (NAS) [29], which plays a key role in MEL biosynthesis. Finally, *N*-acetyl serotonin is *O*-methylated by hydroxyindole-*O*-methyltransferase (*S*-adenosyl-L-methionine: *N*-acetylserotonin-*O*-methyltransferase, EC 2.1.1.4, HIOMT) to form MEL [30].

1.4 REGULATORS OF MEL BIOSYNTHESIS

The rate of MEL formation depends on the multiple molecules that act through multiple mechanisms at different steps of its synthesis. The most important among them are endogenous circadian clock and environmental light, the activity of AA-NAT [18,31], and, to a lesser extent, TPH (which controls the availability of serotonin) [32], HIOMT, noradrenergic regulators, and different peptidergic regulators. AA-NAT switched MEL synthesis on and off with photoperiodic variations in duration, whereas HIOMT tunes the amplitude of the nocturnal MEL synthesis with photoperiodic variation in magnitude. In a few species, nutritional factors such as the availability of tryptophan, folate, and vitamin B6 could also influence MEL synthesis [33–35].

1.4.1 Endogenous Circadian Clock and Environmental Light

The levels of MEL are high during the dark phase of any natural or imposed LD illumination cycle [36]. Studies performed so far in different mammalian models including humans revealed that the magnitude and duration of the nocturnal increase in MEL synthesis is dependent upon the length of the dark phase in a photoperiodic cycle or external geophysical cycle that acts as a "clock" and "calendar" for the entrainment of other biological activities [37], regardless of whether a given species is active during daytime (diurnal), nighttime (nocturnal), or exhibits a crepuscular activity pattern.

The rate and pattern of the nocturnal increase in MEL production depend on species and tissues, among other factors. Light is the dominant environmental factor that controls MEL biosynthesis both in the pineal gland and in the retina. A light signal/pulse of suitable intensity and duration [38] perceived by the retina is transmitted primarily through the retinohypothalamic tract to the SCN, circadian oscillator/master pacemaker of the anterior hypothalamus in brain [39]. Otherwise, constant darkness is sufficient to phase-shift and to synchronize the MEL rhythm in animal species to 24 hours via SCN, which is considered as the major central rhythm-generating system or "clock" in mammals [37,40]. Nerve fibers from the SCN subsequently conveys the signal to the pineal via multisynaptic descending pathways, the subparaventricular zone or paraventricular nucleus, the dorsomedial hypothalamic nucleus, the upper thoracic cell columns of the spinal cord, the superior cervical ganglia, and, finally, the postganglionic adrenergic fibers innervating the pineal gland [41], which itself is a self-sustaining "clock" in some species except in lower vertebrates [42]. The SCN

clock is set to a 24-hour day by the natural LD cycle via retinal light input that then sends circadian signals over a neural pathway including sympathetic nerve terminals that project from the superior cervical ganglia to the pineal gland. Changes in the levels of noradrenaline (NA) released from these fibers ensure proper translation of the light information (via the circadian clock) and thereby driving rhythmic MEL synthesis by the pineal gland [43].

The main photoreceptor pigment for circadian timing appears to be melanopsin in the retinal ganglion cells. Specifically, SCN is the major regulatory site of the activity of AA-NAT, which is the ultimate and key enzyme in the synthesis of MEL from tryptophan (Figure 1.1). Furthermore, the prolonged duration of the night leads to a longer duration of secretion of MEL in most animal species [44]. Ocular light serves to entrain/synchronize the rhythm to 24 hours and suppress secretion at the beginning and/or the end of the dark phase. The amount of light required to suppress MEL secretion during the night varies from species to species, with the time of night, and with the previous light exposure [45]. The amplitude of nocturnal MEL secretion exhibits considerable interindividual differences and is believed to be genetically determined.

1.4.2 Tryptophan Hydroxylase

The mitochondrial enzyme TPH transforms tryptophan to 5-HT and requires a pteridine cofactor, tetrahydrobiopterin (BH4) for its catalytic action. The localization of TPH is restricted to serotonin-synthesizing tissues, including the pineal gland and retina [46,47]. TPH exists in two isoforms, TPH1 found in the pineal and gut, whereas TPH2 is exclusively expressed in the brain [47,48]. The rat pineal *Tph* gene codes for two transcripts of 1.8 and 4 kb [49] with same coding sequences but differ by their 3′ noncoding region length. The promoter region does not have a canonical CRE motif [50], but an inverted CCAAT box and a GC-rich region that binds the transcription factors NF-Y and Sp1, both being essential for *Tph* gene transcription at the basal level and following cyclic adenosine monophosphate (cAMP) treatment [51]. In the pineal gland and retina, *Tph* gene expression (TPH mRNA) and/or TPH enzyme activity display daily variations, low (20%) during the day [52] and high (100%) during the night [53], through post-transcriptional/post-translational mechanisms [53,54]. Thus, TPH represents clock-driven circadian rhythms [47,55,56]. The nocturnal increase in the enzyme activity requires de novo protein synthesis [56]. Exposure to light during the night causes a rapid reduction in nocturnal TPH activity [56–57]. The TPH protein has short half-life (~75 minutes) [58] and is activated through the phosphorylation by the cAMP-dependent protein kinase A (PKA) [59], a Ca^{2+} calmodulin (CaM)-dependent protein kinase ($PKCa^{2+}$/CaM) and PKC [60].

1.4.3 Aromatic Amino Acid Decarboxylase

AAAD is present in large quantities in the cytosolic fraction of the pinealocytes for the synthesis of 5-HT [61]. However, it is not a rate-limiting enzyme, since it is not a specific enzyme to the pineal gland only.

1.4.4 Monoamine Oxidase

MAO activity is detectable in the pinealocytes and in the noradrenergic nerve endings [62]. This differential distribution reflects two types of MAO: type A in the nerve terminals and type B in the pinealocytes. These two types of MAO are characterized by different biochemical properties and sensitivity to inhibitors. It appears that MAO-A is mainly involved in 5-HT oxidation [61]. Consequently, 5-HT exits the pinealocytes for oxidation in the noradrenergic nerve terminals and then returns to the pinealocytes. MAO activity displays day/night variations, with higher values during the day [61].

1.4.5 Alcohol and Aldehyde Dehydrogenases

Neither alcohol nor aldehyde dehydrogenase is saturated by 5-HIAL. Pineal titers of 5-HIAA and 5-HL vary similar to that of 5-HT. The 5-HIAA/5-HL ratio is around 1:6 and is probably related to the lower affinity of alcohol dehydrogenase for its substrate [61].

1.4.6 Arylalkylamine-*N*-Acetyltransferase (Serotonin-*N*-Acetyltransferase)

N-Acetyltransferase, which was first identified as the arylamine-*N*-acetyltransferase (EC 2.3.1.5; NAT), catalyzes *N*-acetylation of 5-HT and is considered as the "rate-limiting enzyme" for the synthesis of MEL, although serotonin availability is one of the major factors that play an important regulatory role in this process [63]. There are two types of *N*-acetyltransferase found in the pineal gland, the arylamine-*N*-acetyltransferase (ANAT) and the arylalkylamine-*N*-acetyltransferase (AA-NAT) named after their best substrates [29]. The affinity of 5-HT is much higher for AA-NAT than A-NAT and only the former enzyme is involved in the rhythmic synthesis of MEL. The cDNA coding for *Aa-nat* has been isolated first in the rat [64,65], then in sheep [66], human [67,68], monkey [69,70], mouse [71], cow [72], Syrian hamster [73], and grass rat [74], with few interspecies differences in the *Aa-nat* gene sequence [69]. A single *Aa-nat* gene has been found in mammalian, avian, and anuran genomes [75]. Teleost fish have two genes: *Aa-nat-1* (homologous to the non-fish *Aa-nats*) and *Aa-nat-2*, primarily expressed in the retina and pineal gland, respectively [76,77]. The *Aa-nat* gene is located on chromosome 11 (position E1.3-2.3) in the mouse, on chromosome 10q32.3 in the rat [78], and on chromosome 17q25 in the human [67].

Aa-nat gene has four exons separated by three introns and encodes for only one transcript (size varies between 1.0 and 1.7 kb according to species). In most species, *Aa-nat* gene expresses in several tissues with a high level in the pineal and the retina and with a much lower level in different nervous tissues (including the pars tuberalis [PT], SCN, hippocampus) and peripheral tissues (including testis and ovaries) [64,67,69,79]. The rat gene has been widely studied. The rat promoter region of the *Aa-nat* gene has one CRE-like sequence (differing by one base from the perfect CRE sequence and named natCRE), an inverted CCAAT box, and an activating protein 1 (AP-1) site [80]. The natCRE site is capable of binding the phosphorylated form of CREB (pCREB), whereas CCAAT box activation by specific binding proteins (CATBP) also appears necessary for large activation of *Aa-nat*. cAMP-induced *Aa-nat* gene transcription requires activation of a CRE–CCAAT complex and appears critical to achieve full stimulation of *Aa-nat* gene expression [81]. Another *cis*-DNA sequence named E-box (able to mediate transcriptional up-regulation via the action of the BMAL1/CLOCK heterodimer) has been identified in the first intron of the rat *Aa-nat* gene [82]. However, transfection of pinealocytes with *Bmal1/Clock* was unable to induce *Aa-nat* transcription, whereas the same kind of transfection in retinal cells led to activation of *Aa-nat* gene expression [82]. The pineal *Aa-nat* gene promoter contains a pineal regulatory element (PIRE) that binds the transcription factor cone–rod homeobox (CRX), which is exclusively expressed in photoreceptors and pinealocytes [83]. Northern blot analysis revealed the presence of high AA-NAT mRNA levels in the pineal glands and retinas in vertebrates [18,84]. In the retina, AA-NAT mRNA has been observed primarily in photoreceptor cells [70,85] and at significantly lower levels in the inner nuclear layer and the ganglion cell layer [85,86]. These findings suggest that, in addition to photoreceptors, other retinal cells may also possess a limited capacity to produce MEL [18].

The vertebrate AA-NATs belong to a superfamily of GCN5-related *N*-acetyltransferases (GNAT), an approximately 23-kDa soluble cytosolic protein with a catalytic core and regulatory regions. The rat AA-NAT protein structure is globular, made of eight β-sheets and five α-helices [87]. Based on the deduced amino acid sequence homology, human AA-NAT is 97% homologous with the monkey, 84% with the sheep, and 90% with the rat. The N-terminal area involved in the binding of the arylalkylamines as an acetyl group donor and facilitates the transfer of the acetyl group, while the C-terminal area with two well-preserved motifs, named A and B, which bind the cofactor acetyl

coenzyme A (AcCoA) and contains phosphorylation sites critical for activation and stabilization of the catalytic core [31,75]. Several well-preserved putative phosphorylation sites for the PKA, the PKC, and the casein kinase of type II are present across species [69]. Phosphorylation on the Thr31 residue of AA-NAT promotes binding with the chaperone protein 14-3-3 proteins with a ratio of 1:1 (AA-NAT/14-3-3 protein). This protein–protein interaction yields a relatively stable complex and leads to conformational changes with the unfolding of the binding site of the two substrates onto the AA-NAT protein [88–91], which reduces the K_m for the arylalkylamine substrates and also protects the enzyme from proteosomal proteolysis [75,76]. Additionally, an intramolecular disulfide bond between the Cys61 and Cys177, formed upon oxidation and cleaved upon reduction, is proposed to act as a catalytic switch for AA-NAT activation [92]. The enzyme has a high affinity for arylalkylamines, such as tryptamine and serotonin, and has a very low activity with regard to arylamines, such as phenylamine [93].

1.4.7 Molecular Mechanism of AA-NAT Regulation

The dynamic changes of AA-NAT synthesis and activity are regulated by complex control systems at different stages of enzyme synthesis and processing, and such systems consist of two basic elements: an autonomous circadian clock and turn-off mechanisms [31,94] (Figure 1.2). The circadian clock is composed of transcriptional/translational feedback loops and is entrained to the environmental lighting conditions. Turn-off mechanisms are responsible for the rapid suppressive effects of light on AA-NAT levels and activity [38,76,96]. At the transcriptional level, the control is through posttranscriptional processes (such as phosphorylation and binding to chaperone proteins) and through regulation of protein degradation velocity by proteosomal proteolysis [18,31,43,89].

The sympathetic neurotransmitter, NA, released from postganglionic fibers that innervate the pineal, is central to rhythmic AA-NAT fluctuations. At night, when the activity of these fibers increases, NA is released and stimulates postsynaptic β_1- and α_1-adrenergic receptors located on pinealocytes. In a process termed *biochemical "AND" gate*, an increase in intracellular Ca^{2+} concentration (resulting from α_1-adrenoceptor stimulation) results in the activation of adenylate cyclase (AC), resulting from β_1-adrenoceptor stimulation by a mechanism involving protein kinase C and calcium/calmodulin protein kinase. This activation causes a rapid and large increase (~100-fold in the rat) of intracellular cAMP level [84,96]. Elevated levels of cAMP, the second messenger that controls MEL biosynthesis in mammals, subsequently activate PKA and exert dual actions on AA-NAT. Thus, during the night or darkness (when cAMP levels are high), AA-NAT is phosphorylated by PKA and forms a complex with 14-3-3 proteins. Within this complex, AA-NAT is catalytically activated and protected from dephosphorylation and degradation [31,90,97]. In humans, the AA-NAT is regulated primarily at a post-transcriptional level, whereas in rodents, the key event appears to be cAMP-dependent phosphorylation of a transcription factor that binds to the AA-NAT promoter [98]. In rhesus monkey and human, the quantity of *Aa-nat* mRNA is high and displays no daily variations, while the enzyme activity increases by up to 10-fold at night [70]. Due to change in MEL content and secretion reflect oscillation in AA-NAT activity, it is also known as "the MEL rhythm-generating enzyme" [31,64,99,100].

The magnitude of the light evoked changes in nocturnal AA-NAT activity and dependent on the duration and intensity of the light pulse, its wavelength (blue and red light being the most and least potent, respectively), tissue, and species examined [101–103]. Studies performed on humans and nonhuman mammals indicate that a novel photoreceptor system that is distinct from the classical visual photoreceptors (cones and rods) and sensitive to the blue portion of visible light (λ_{max} between 446 and 484 nm), primarily involved in MEL-related and other non-image–forming light responses [101–102,104–106]. It is suggested that melanopsin, a newly discovered photo pigment [107–108], plays a primary role in light-induced MEL suppression [108].

Light pulses beginning late in the subjective day and early in the subjective night delay the phase of the MEL/AA-NAT activity circadian rhythm, while pulses beginning during the second half

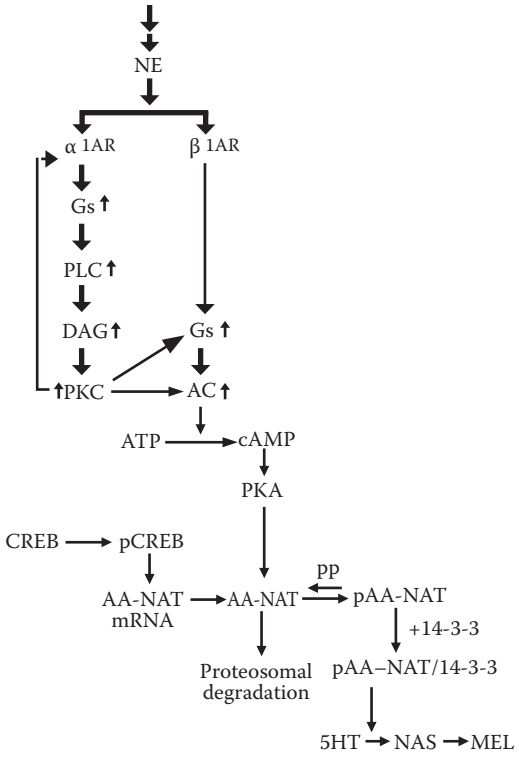

FIGURE 1.2 Schematic representation of the pathway of synthesis and degradation of AA-NAT. The dynamic changes of AA-NAT synthesis and activity are regulated by complex control systems at different stages of enzyme synthesis and processing. At night, NE is released from sympathetic nerves in the perivascular space in the pineal gland. NE interacts with adrenergic receptors on the pinealocyte membrane to increase intracellular levels of cAMP, results in activation of cAMP dependent PKA and phosphorylation of cAMP response element binding protein (CREB), thereby promoting transcription of AA-NAT. Moreover, PKA phosphorylates the AA-NAT protein that increases the affinity of the AA-NAT protein for 14-3-3 proteins. The phosphorylated AA-NAT (pAA-NAT)/14-3-3 regulatory complex protects the pAA-NAT against dephosphorylation by protein phosphatase (PP) and destruction by proteasomal proteolysis. In addition, complex (pAA-NAT)/14-3-3 has higher affinity for serotonin (5-HT) and increases *N*-acetylation of 5-HT resulting in an increase in *N*-acetylserotonin (NAS), which in turn enhances MEL production (see text for details).

of the subjective night produce a phase advance of the rhythm [109–114]. These time-dependent effects can be summarized as a phase response curve (PRC) [115]. The human PRC to light [111] is about 12 hours out of phase with the PRC to MEL [116]. In some reports, pulses of light given during the subjective day did not produce phase shifts [109,117]. However, it remains controversial whether such a "dead zone" exists in humans [116], in which clear evidence is available for the participation of two "oscillators" in the production of phase shifts [118]. The phase-advancing morning light has a greater affect on the MEL/AA-NAT rhythm decline, while evening phase-delaying light has a greater effect on the MEL/AA-NAT rise [119–120].

Exposure to light lowers cAMP, which leads to dephosphorylation of AA-NAT and disruption of the AA-NAT/14-3-3 complex, with a concomitant drop in AA-NAT catalytic activity and rapid proteasomal proteolysis of the enzyme [97,99,121–124]. This is the only cellular mechanism known to control AA-NAT activity in primates [31]. The rapid decline in the activity of AA-NAT with light treatment at night [38] appears to be complex and associated with the control of catabolism through various phosphorylation of AA-NAT by PKA, rho kinase, checkpoint protein 1 (CHK1)

[125], and subsequent association of the phosphorylated AA-NAT with the chaperone-like 14-3-3 protein [89]. This dimerization either drives the complex to a proteasomal mediated proteolysis or protects the complex from breakdown [126]. The retinal AA-NAT enzyme appears to be protected against breakdown during the day [127].

1.4.8 Hydroxyindole-*O*-Methyltransferase

HIOMT catalyzes the final step of MEL synthesis and other 5-methoxyindoles. This enzyme transfers a methyl group from the cofactor *S*-adenosyl-L-methionine to its indolic substrate. HIOMT represents 2%–4% of the pineal protein fraction [128]. The mean daily level of pineal HIOMT activity is about 4.3 ± 0.1 nmol/h/mg protein in humans [129] and ~9 nmol/h/mg protein in rhesus monkey [70], with no significant day/night variation.

The cDNA coding for *Hiomt* was first isolated in the cow [130], then in chicken [131], human [132], rat [133], and monkey [71], indicating large species differences. The rat cDNA displays low homologies with the cDNA of the cow (65%), human (63%), and chicken (59%). The whole cDNA sequence in the rat is 1728 bp long: the coding region contains 1101 bp, the 5′-noncoding region 184 bp and 3′-noncoding region 444 bp [133]. The human *Hiomt* gene is the best studied so far [129,132,134]. It is located in the pseudoautosomal region of the X chromosome and codes for three transcripts containing a transposable long interspersed element 1 (LINE-1) fragment. Two promoters, containing different *cis*-regulatory elements, have been characterized: one promoter A, whose expression appears restricted to the retina (contains the CCAATTAG sequence able to recognize transcription factors specific for the retina), and one promoter B, containing a CRE and an AP-1 site, whose strong expression is induced by a pineal specific regulatory element [134] like CRX. Indeed, PIRE and the CRX binding site have been reported in the promoter of human *Hiomt* [83]. The putative amino acid sequence of the rat HIOMT displays 60% homology with human. In the rat, the translated protein is made of 367 amino acids with putative sites of phosphorylation for PKC, type II casein kinase, and tyrosine kinase [133]. In different species studied so far, the enzyme displays a high molecular mass (76–78 kDa) and is made up of two similar subunits of about 39 kDa each. Immunochemical studies have revealed a large heterogeneity in the protein structure and enzymatic properties [135]. The HIOMT is very stable ($t_{1/2}$ > 24 hours) protein [129,136].

HIOMT activity has also been measured in the retina and the Harderian gland, although at much lower levels [129–134,137,138]. A very weak HIOMT activity has been demonstrated in the duodenum and colon (enterochromaffin cells) [139], in the human retinoblastoma Y79 cell line [129,136], and in ovaries [140]. RT-PCR experiments have also shown the presence of HIOMT mRNA in human platelets and the testis [141]. In contrast to AA-NAT, the nocturnal increase in pineal HIOMT activity is very low. In rats, pineal HIOMT activity displays a weak but significant nocturnal increase (by 40%–50%) [142]. This increase activity persists in constant darkness (D/D) and is inhibited in constant light (L/L) [142].

Hiomt gene expression is already high during the day but still displays a twofold increase at night that persists in D/D [133,142]. Light exposure at night rapidly decrease ($t_{1/2}$ = 20 minutes) the level of *Hiomt* mRNA [142]. A β-adrenergic receptor (β-AR) agonist stimulates daytime levels of *Hiomt* mRNA, whereas a β-AR antagonist inhibits it [133,142]. The short-term regulation of the enzyme appears to involve Ca^{2+} and PKC-dependent mechanisms [143], suggesting the activity and expression of HIOMT is regulated by different neurotransmitters using different mechanisms [144]. The decrease in enzyme activity corresponds to a reduction of the quantity of protein and decrease can be abolished by daily injections of a noradrenergic agonist [143,145]. The long-term regulation of HIOMT activity is due to the high stability of the protein ($t_{1/2}$ > 24 hour) [128,136,146] and depends upon noradrenergic control of *Hiomt* gene expression [142].

Demonstration of specific regulation of HIOMT activity in different mammalian species strongly suggests that HIOMT is involved in the rhythmic synthesis of MEL, especially the long term/seasonal rhythm in the nocturnal MEL peak pattern, which is an important indicator of the

transmission of photoperiodic information. During the day, AA-NAT activity is lower than HIOMT activity and would be the limiting factor for the synthesis of MEL. The increase in AA-NAT activity at the beginning of the night thus induces the increase in MEL synthesis. During the night, however, HIOMT activity is lower than AA-NAT activity and would thus become the limiting enzyme for MEL production. Consequently, any variation in nighttime HIOMT activity should modulate the rate of MEL synthesis (the amplitude of the nocturnal MEL peak) [26]. Thus, HIOMT displays an important role in the photoperiodic control of pineal metabolic activity.

1.4.9 Noradrenergic and Other Regulators

Studies have shown that the pattern of MEL synthesis and secretions (the duration and amplitude of the nocturnal peak) coincide with target sensitivity, suggesting a complex control supported by the presence of multiple transmitters/neurotransmitters and their receptors in the pinealocyte/pineal gland. Therefore, the pinealocyte is orchestrating the functions of numerous neurotransmitters in the regulation of MEL synthesis. A dense noradrenergic innervation of postganglionic sympathetic nerve fibers that ends at the pineal gland release norepinephrine (NE) and plays a crucial role in the control of MEL synthesis [147]. Noradrenergic regulation of MEL synthesis in mammalian species (including Syrian hamsters, Siberian hamsters, European hamsters, mice, sheeps, cows, monkeys, and humans) has been well studied [26]. A marked species difference exists in the nocturnal NE stimulation of MEL synthesis. Limited studies in humans and monkeys suggest a "sheep-like" regulation. In humans, there is a large interindividual variability in the daily pattern of MEL synthesis, which also varies depending on age [148]. The nocturnal elevations in noradrenergic (NA) stimulation, via β- and α_1-adrenergic receptor of pinealocytes, increase the intracellular concentration of cAMP, which in turn activates AA-NAT [98], with the resulting MEL rhythm characterized by high levels at night. Stimulation of α-adrenergic receptors potentiates β-stimulation and requires the participation of other molecules such as calcium ions, phosphatidyl-inositol, diacyl-glycerol, and protein kinase C [149]. Thus, the synthesis and release of MEL are stimulated by darkness (~80% synthesis of MEL) and are inhibited by light. There is an immediate increase in circulating MEL at the onset of darkness [109]. Daytime β1-AR stimulation does not stimulate MEL synthesis [150], but its nocturnal synthesis can be inhibited by a β1-AR antagonist [151]. These findings suggest that NE is probably an important neurotransmitter regulating daily MEL synthesis. Since different mammalian species are not fully responsive to NE, it appears possible that other transmitters like 5-hydroxytryptamine, monoamine oxidase, cortisol, corticosterone, aldosterone, testosterone, and estradiol might play role to obtain a full MEL response, but their exact molecular mechanisms are not yet well characterized. Notably, the majority of the studies are restricted in the rat, mice, hamsters, and cell lines.

MEL synthesis, secretions, and catabolism are also regulated by a wide range of peptides including vasoactive intestinal peptide (VIP), pituitary adenylate cylcase (PACAP), histidine isoleucine peptide (HIP), neuropeptide Y (NPY), vasopressin, oxytocin, substance P, calcitonin gene related peptide (CGRP), secretoneurin (SN), hypocretin, natriuretic peptides (atrial, ANP; brain, BNP, and C-type, CNP), angiotensin, opiate peptide, and gonadotropin-releasing hormone (GnRH) [26]. However, most of this information emerged from the studies on nonprimates or cell lines.

1.5 SECRETION

Under natural environment, MEL is secreted during the night in the healthy human, as in all other species. MEL, being a lipophilic molecule, it is not stored but directly released by diffusion out of the pineal gland and released into the cerebrospinal fluid and the circulation. Although the eye contributes significantly to circulating MEL levels in a few species (sea bass, frog, quail, pigeon), retinal MEL acts primarily within the eye [18,152]. In humans, serum concentrations of MEL is low during the day (10–20 pg/ml) and is significantly higher at night (80–120 pg/ml), with peak

between 02:00 and 04:00 h, when measured with high-specificity assay. The onset of secretion usually takes place around 21:00–02:00 h and the offset around 07:00–09:00 h in adults in the temperate zone [109]. The concentrations of MEL in saliva are approximately one third of those in plasma. Minimum concentrations in both fluids are usually below 5 pg/ml. The most striking characteristic of the human MEL is its reproducibility from day to day and from week to week in normal individuals, rather like a hormonal fingerprint [109]. A large variability in the amplitude of the rhythm between subjects and the nighttime production of hormone can differ by three orders of magnitude among individuals.

1.5.1 Factors Affecting the MEL Rhythm

The concentration of MEL varies in relation to the stage of development, puberty, menstrual cycle, and age of the individuals. Its peak values in blood may also vary from one individual to the other and depend on their age, sex, and disease [20]. Just after birth, very little MEL or 6-sulfatoxymelatonin (MT6s) is detectable in body fluids. A robust MEL rhythm appears around 6 to 8 weeks of age [153]. The development of MEL production is markedly delayed in premature infants [153,154]. The amplitude of serum level of MEL increases rapidly thereafter and reaches a highest peak in between 1 and 3 years of age [95,155]. The increment is much greater at night (54–75 pg/ml). Subsequently, a steady decrease (80%) occurs, reaching a mean adult concentration in mid to late teens, with a major decline before puberty, becomes relatively stable until 35 to 40 years, and a final decline in amplitude doesn't take place until low levels (16–40 pg/ml) are seen in old (~70 years) age [45,156,157]. During childhood, the decline in MEL production may be due to the increasing size of the human body [158,159].

Although MEL concentration is low during precocious puberty, higher MEL is noted in delayed puberty and hypothalamic amenorrhea compared with age-matched control [155] and abnormal MEL secretion in patients with premenstrual tension [160]. Low MEL is associated with cardiovascular diseases and diabetic autonomic neuropathology [161]. A potent reduction in nocturnal MEL together with an increase in daytime levels has been found in patients with Alzheimer's disease (AD), and these pathomorphological processes include dysfunction of SCN innervation to the pineal gland, degenerative changes in the SCN [162], and insufficient environmental illumination [163], a life condition frequently found in older residents of nursing homes. The plasma MEL profiles in blind people also fluctuate and may be categorized into three groups: (a) entrained with a normal phase, (b) entrained with an abnormal phase, and (c) free-running, with a circadian period (tau) different from 24 hours [164]. The majority of totally blind subjects have free running circadian rhythms and cyclic (non–24-hour) sleep/wake disorders. These are characterized by a period of good sleep followed by a period of poor sleep (short night-sleep duration) when the MEL rhythm is in an abnormal phase position (e.g., peaks during the day) [164,165]. This has been associated with increased napping and reduced alertness and performance during the day [164,166]. Appropriately timed daily doses of MEL have been shown to improve night sleep and reduce daytime napping as well as to entrain the free-running circadian rhythms [167–169].

1.6 CATABOLISM

Pineal MEL is released in the cerebrospinal fluid in the third ventricle via the pineal recess and attains levels up to 20–30 times higher than in blood but rapidly diminishes with increasing distance from the pineal gland [170]. This observation suggests that MEL is taken up by brain tissues. In the blood, 50%–75% of total MEL is bound reversibly to albumin and glycoproteins. The half-life of MEL is biexponential, with a first distribution half-life of 2 minutes and a second of 20 minutes [9,171] in the bloodstream [172]. The half-life of exogenous MEL is about 12–48 minutes [173–175].

The mechanism of catabolism of MEL is less understood, with the exception of the conjugation steps that account for ~70% of the ingested dose. MEL in all cells is metabolized nonenzymatically

and also by free radicals as an oxidant. It is converted into cyclic 3-hydroxymelatonin (c3OHM) when it directly scavenges two hydroxyl radicals [176]. More than 90% of circulating MEL is primarily metabolized through the classical hydroxylation pathway in human and rodent liver by microsomal enzymes, cytochrome P_{450} monooxygenases (isoenzymes CYP1A2, CYP1A1, or CYP1B1) to 6-hydroxy-MEL [177,178], which undergoes further conjugation with either sulfate, catalyzed by sulfotransferase ST1A3, to form 6-sulfatoxy-MEL (aMT6s), and eliminated in the urine [109,179], or glucuronic acid, catalyzed by UDP-glucuronosyltransferase, to form 6-hydroxymelatonin glucuronide (in mouse) [9,171,180]. Urinary aMT6s excretion closely reflects the plasma MEL profile and is frequently used for evaluation of MEL rhythm in humans [109,181]. The magnitude of the light evoked changes in nocturnal aMT6s concentration is dependent on the duration and intensity of the light pulse, its wavelength (blue and red light being the most and least potent, respectively), tissue, and the species examined [94]. The appearance and peak plasma levels of aMT6s are delayed by 1 to 2 hours, and the morning decline by 3 to 4 hours [182]. In urine, 50%–80% of aMT6s appears in overnight sample (2400 to 0800 h), and levels are low but rarely undetectable in the afternoon and early evening [109]. In healthy full-term infants, rhythmic aMT6s excretion in urine is detected at 5–12 weeks of life [154,183]. At 24 weeks of age, total aMT6s excretion is 25% of adult levels [183]. Several other metabolites (approximately 30% of overall MEL) are also formed by the oxidative pyrrole ring cleavage [125]. Within the brain, MEL is degraded via oxidative catabolism by indoleamine-2,3-dioxygenase (IDO; EC 1.13.11.1.7) and/or myeloperoxidase (MPO; EC 1.11.1.7) leads to the formation of unstable intermediary kynurenine derivative, N^1-acetyle-N^2-formyl-5-methoxy-kynurenine (AFMK). IDO and MPO have micromolar range affinity with MEL. AFMK is further deformylated to the more stable metabolized either spontaneously or enzymatically by kynunerine formamidase (arylamine formamidase or hemoperoxidase) to N^1-acetyl-5-methoxy-kynunerine (AMK) [181,184,185]. AFMK is the primitive and primary active metabolite of MEL [185] (Figure 1.1).

Metabolic breakdown of retinal MEL is different from that of the MEL synthesized by the pineal gland. Initially, aryl acylamidase (aryl-acylamide amidohydrolase) catalyzes the deacetylation of MEL to 5-methoxytryptamine. Subsequently, 5-methoxytryptamine is metabolized via the same pathway as indoleamines and catecholamines, with deamination by monoamine oxidase to form 5-methoxyindole acetaldehyde, and its further oxidation to 5-methoxyindoleacetic acid or reduction to 5-methoxytryptophol [186]. Thus, 90% clearance rate of MEL is associated with excretion in the urine in metabolized form and in small quantities (~10%) in unmetabolized form.

1.7 MEL RECEPTORS

Being a lipophilic molecule, MEL has free access to all cells, all tissues, and all organs of the body. MEL has in addition acquired autocoid (act like local *hormones* near the site of synthesis, have a short half-life, and one that is not waste), paracoid, and hormonal properties [187]. The response of a cell to MEL may be due to receptor mediated, protein mediated (activate endogenous antioxidant enzymes) or non-protein–mediated (interact with different cations and metals) actions (see later section of this review). The development of high affinity radioligand binding assays of 2-[^{125}I] iodo-MEL, autoradiography, and studies with putative MEL receptor agonist and antagonists have allowed to identify, characterize, and demonstrate distribution patterns of MEL receptors in various central and pheripheral tissues [188–190]. The first MEL receptor was cloned from *Xenopus laevis* immortalized melanophore (dermal melanophore) mRNAs by using a cDNA library construct [191], but expressed only in nonmammalian species (birds and fish). Subsequently, human MEL receptors have been cloned [192].

Two forms of high-affinity MEL receptors have been identified on the basis of pharmacological and kinetic differences in 2-[^{125}I] iodo MEL binding assay [192,193]. According to the classification of nomenclature committee of IUPHAR (http://www.iuphar-db.org/iuphar-rd) [194], the high-affinity MT receptors are designated as MT_1 and MT_2, corresponding to the subtypes previously

known as Mel_{1a} and Mel_{1b}, respectively [191,192,195]. In humans, MT_1 receptor is mapped to chromosome 4q35.1 and consists of 350 amino acids [196]. The gene for MT_2 receptor is located into chromosome 11q21-22 and cDNA encodes a protein containing 362 amino acids. Human MT_1 and MT_2 receptors show high homology at the amino acid level (60% overall and 70% within transmembrane [TM] domain) and have similar affinity for MEL [192,195,197]. These membrane-bound receptors (MT_1 and MT_2) belong to the superfamily of guanidine triphosphate binding proteins or G protein-coupled receptors (GPCR) containing the typical seven TM α-helices (TMI– TMVII) domains linked by three alternating intracellular (IC1–IC3) and three extra cellular (EC1–EC3) loops [198,199], and share high (~55%) homology in their amino acid sequences [193,200–202] (Figure 1.3). Glycosylation sites have been detected in the N-terminal region and, more importantly, the fourth IC loops of either receptor contain palmitoylable cystein residues [203], and the C-terminal domains putative phosphorylation sites for PKA and PKC as well as casein kinases I and II (CK I and II) [193]. Site-directed and chimeric receptor mutagenesis studies have identified residues critical for MEL binding to the MT_1 and MT_2 receptors [96,204,205]. Moreover, site-directed mutagenesis study [203] showed that neither the replacement of the palmitoylation site by alanine nor the truncation of the C-terminal domain containing the phosphorylation sites altered receptor affinity. However, the presence of both the lipid anchor and the C-terminal tail are required for G protein interaction, as judged from the lack of the cAMP response. The phosphorylation, but not the palmitoylation, sites are required for internalization indicating the unusual internalization process via phosphorylation and β-arrestin binding. In the brain, SCN is the main target of MEL, but the receptor density is ~4 fmol/mg protein, which is ~100 times lower than 5-HT receptors in other brain areas [206].

The human MT_2 has a lower affinity (K_d = 160 pmol/l) for ^{125}I-MEL as compared with the human MT_1 (K_d = 20–40 pmol/l), but the binding characteristics of the two are generally similar, such as, both are of high affinity and the agonist binding is guanosine triphosphate-sensitive [193]. MT_1 and MT_2 differ in their affinities to the natural ligand. For the human MT_1 and MT_2 receptors, K_i values are 80.7 and 383 pM, respectively [207]. However, higher inhibition or dissociation constants for MT receptors are also known [208]. Based on $MT_{1/2}$ receptors analogy to other GPCRs, it is suggested that MEL receptors form both homo- and heterodimers [209].

An additional cloned MEL-related receptor (GPR50) has around 40% sequence identity with $MT_{1/2}$ receptors, but is incapable of binding MEL [210]. At present, this receptor is classified as an orphan GPCR cytosolic enzyme, quinone reductase 2 (QR2, NRH-quinone-oxidoreductase 2, NQO_2; NRH, dihydronicotinamide riboside) and represented by MT_3 (previously known as Mel1c) [211,212]. Unlike $MT_{1/2}$ receptors, MT_3 is not a GPCR. MT_3 belongs to a group of reductase that participates in the protection against oxidative stress by preventing electron transfer reactions of quinines.

MEL is also ligand for transcription factors belonging to the retinoid-related orphan nuclear hormone receptor superfamily. These receptors are cloned and named retinoid Z receptor (RZR) and retinoid acid receptor (ROR)–related orphan receptor [213,214]. The RZR/ROR family consists of three subtypes: α, β, and γ. The ROR-α1, ROR-α2, and RZR/ROR-β have low affinity with MEL (K_d values in the lower nano molar range), and are widely expressed in the central and peripheral nervous systems and cancer tissues [213,215–217]. In addition, at elevated concentration of MEL interacts weakly with several intracellular proteins such as calmodulin (CaM) [218,219], calreticulin [220,221], or tubulin [222] and antagonizes the binding of Ca^{2+} to calmodulin [223].

1.7.1 Receptor Distribution

MT_1 and MT_2 are expressed both singly and together in various cells and tissues of the body [193,195] (Table 1.2). The density of MEL receptors not only varies with species and location, but also with the lighting regime, time of the day, tissues, developmental, or endocrine status of the species, MEL concentration in the plasma and aging or disease [224]. In birds and lower vertebrates, MEL

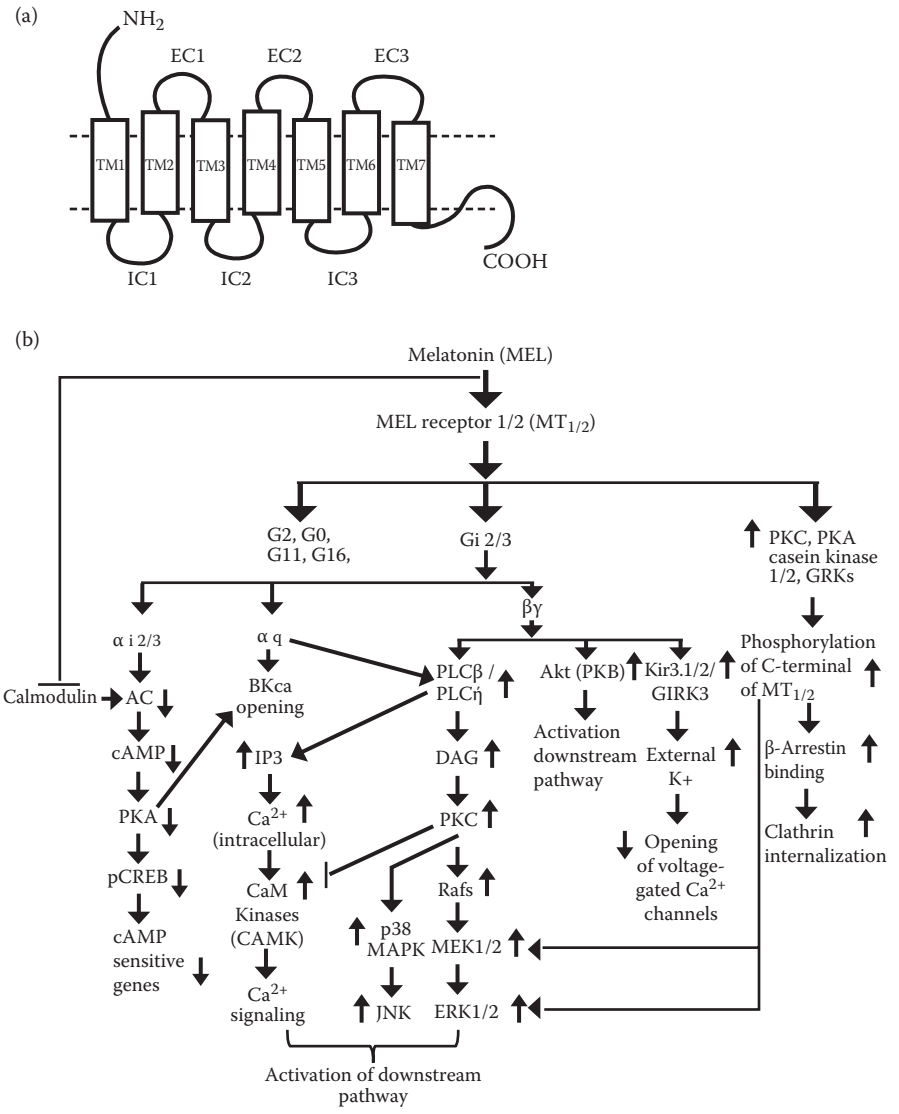

FIGURE 1.3 (a) Schematic representation of the structure of MEL receptors (MT_1 and MT_2). The membrane-bound MT_1 and MT_2 receptors belong to the superfamily of guanidine triphosphate–binding proteins or GPCR containing the typical seven transmembrane (TM) α-helices (TMI–TMVII) domains linked by three alternating intracellular (IC1–IC3), three extra cellular (EC1–EC3) loops, an extracellular N-terminal tail and a cytosolic C-terminal tail. (b) An overview of parallel and cross-signaling pathways of MT_1 and MT_2 receptors. In relationship with the function of MT_1 and MT_2 receptors, MEL influences second messenger cascades that vary in cell, tissue, and species-specific ways. These cascades include AC/cAMP/PKA/cAMP response element-binding protein (CREB) pathway, phospholipase C (PLC) β and PLC-η pathway, Ras/Rafs/MEK1/2/ERK1/2 pathway, PI3K/Akt (PKB) pathway, Kir3 (GIRK) inward rectifier potassium channels, GC/cGMP/PKG pathway, and receptor-independent signaling through Ca^{2+} binding proteins (see text for details). Abbreviations: Gα subunits and Gβγ dimers are depicted for Gi and Gq proteins; AC, adenylyl cyclase; Akt, homolog of kinase from retrovirus AKT8; Ca^{2+}, intracellular calcium; CaM, calmodulin; cAMP, cyclic adenosine 3′,5′-monophosphate; cGMP, cyclic guanosine 3′,5′-monophosphate; DAG, diacyl glycerol; ERK, extracellular signal-regulated kinase; IP3, inositol 1,4,5-tris-phosphate; MAP kinase: mitogen-activated protein kinase; MEK, MAP ERK kinase; pCREB, phosphorylated cAMP/Ca^{2+} response element-binding protein; PI3K, phosphoinositide 3-kinase; PLC, phospholipase C; PK, protein kinase; Raf, homolog of retroviral kinase, the product of oncogene v-raf; ↑, up-regulation or rise; ↓, down-regulation or decrease.

TABLE 1.2
Functional Attribute to Activation of Different Types of MEL Receptors in Humans

Site of Activity	Activation of MT_1 Receptor	Activation of MT_2 Receptor
SCN	• Inhibition of neuronal firing rate that modulates (entrains) the circadian rhythm (phase shift) and induce sleep • Inhibition of the cAMP response element-binding protein phosphorylation	• Induction of phase shifts in circadian rhythms
PT	• Inhibition of prolactin secretion	–
Central dopaminergic system	• Modulation of dopamine synthesis, release, and activation of dopamine receptors	–
Hippocampus	• Memory, excitation, and inhibition of neuronal activity; enhancement of seizure threshold via depression of GABAa receptor	• Same as MT_1 receptors
Cerebellum	• Interactions with glutamatergic synapses	• Same as MT_1 receptors
Retinal cells	• Inhibition of stimulation evoked release of dopamine, modulation of rod phototransduction pathways and photoreceptor functions, adaptation to low light intensities	• Same as MT_1 receptors
Cardiac ventricular wall	• Modulation of β-adrenergic receptor mediated cAMP signaling and stimulate voltage-activated calcium current	• Same as MT_1 receptors
Coronary, cerebral, and systemic artery	• Induction of vasoconstriction	• Induction of vasodilation
Prostate epithelial cells	• Suppression of DNA synthesis	–
Prostate cancer cells (LNCaP)	• Antiproliferative effect	–
Breast cancer cells	• Inhibition of proliferation in ER-α-positive cells (MCF-7)	–
Myometrium	• Modulation of uterine contraction, lower expression of MT_1 receptors in pregnant woman	• Same as MT_1 receptors, lower expression of MT_2 receptors
Granulosa cells	• Increase in LH receptor mRNA levels (luteal phase) and hCG stimulated progesterone levels; decrease in GnRH receptor levels	• Same as MT_1 receptors
Gallbladder epithelia	• Gallbladder contraction	• Same as MT_1 receptors
Pancreatic cancer cell lines (MIA, PANC)	• Regulation of acid/base homeostasis (stimulation of HCO_3 secretion)	
Skin and skin cells	• Antiproliferative effects on melanoma cells and normal cells	• Same as MT_1 receptors
Duodenal enterocytes	–	• Stimulation of HCO_3 secretion via neural stimulation
Adipocytes	• Lowering of GLUT4 mRNA and glucose uptake	• Same as MT_1 receptors
Choriocarcinoma	• Antiproliferative effects	
Immune system	–	• Increase splenocyte proliferation • Inhibition of leukotrine B4-induced leukocyte adhesion

Note: See text for details and references.
Activation of MT_3 (QR2) receptors: immunostimulatory effects.
Protection against a potential toxification mechanism.

receptors are widely distributed in the central nervous system (CNS) [195,225–227]. On the other hand, the distribution of MEL receptors is more restricted in mammals, and the level of expression is markedly weaker than in nonmammalian species. In mammals, most of the [^{125}I] MEL binding observed by in vitro autoradiography and physiological responses to MEL reflect MT_1 receptors, and MT_1 is more prevalent than the MT_2. The highest expression of MEL receptors in mammals (including human) has been found in the PT of the anterior pituitary (adenohypophysis).

Functional MT_1 receptors have been widely localized in the SCN [193,228], cerebellum [229], hippocampus [230], central dopaminergic pathways (including substantia nigra, ventral tegmental area, nucleus accumbens, caudate–putamen) [231], PT, ovary [232,233], testis [234], mammary gland [235], retina [236], coronary blood vessels and aorta [237,238], liver and kidney [239], gallbladder [240], skin [241], adrenal gland, and the immune system (T and B lymphocytes) [242]. The presence of MT_1 mRNA has also been demonstrated in the cerebral cortex, thalamus, hippocampus, cerebellum, cornea, and retina [198,243,244].

MT_2 receptors are more restrictedly expressed, being found mainly in the brain (hippocampus, SCN, and cerebellum of human) and retina, although their presence has also been detected in the lung, cardiac, aortic and coronary tissue, myometrium and granulosa cells, immune cells, duodenum, and adipocytes [21,193,198,239,243,246–248]. Moreover, both MT_1 and MT_2 receptors have been reported in the retina, cornea, ciliary body, lens, choroids, and sclera [243,249]. Both MT_1 and MT_2 receptors are widely distributed in the cancer tissues and cells, including breast, ovarian, endometrial, colon, hepatoma, melanoma, and prostate cancers [224].

The MT_3 receptor is expressed in the liver, kidney, brain, heart, lung, intestine, muscle, brown adipose tissue, and eye [250,251]. *In situ* hybridization studies have demonstrated mRNA expression of the RZR/ROR-β receptors in the rat brain, pineal, and neurons of several sensory regions including the dorsal horn of the spinal cord, but not in regions involved in motor control [252].

1.7.2 Mechanism of Action of MEL at Cellular Level

The SCN is the putative site of circadian action of MEL, and the hypophyseal PT is the putative site for its reproductive effects (Figure 1.3). In the SCN, MEL affects under both *in vivo* and *in vitro* conditions, circadian phase, and amplitude. MT_1 acts by suppressing neuronal firing activity by mediating a pertussis toxin (an inhibitor of Gαi)-sensitive vasoconstriction through BKca, whereas MT_2 inducing phase shifts by mediating vasodilatation [193,253,254]. However, the receptor subtypes are complementary in their actions and can, to a limited extent, mutually substitute for each other. In the extreme, one of these receptors can be exceptionally missing, as shown in two Siberian hamsters, *Phodopus sungorus* and *Ph. campbelli*, which lack active MT_2 receptors [255].

Depending on the tissue and species, MEL can activate different second messenger cascades acting on the same receptor subtype. By using recombinant MEL receptors, it has been shown that the predominant cellular effect of the MEL is the inhibition of forskolin-stimulated cAMP accumulation in the SCN and PT [256]. This effect of MEL is pertussis toxin sensitive, indicating coupling of the receptor to a G_i protein [257]. However, a cholera toxin (inhibitor of Gsα subunit)–sensitive component also mediates the inhibition of forskolin-stimulated cAMP accumulation [258], implying coupling through a G_0 protein. Thus, the classical effect of MT_1 and MT_2 receptors are primarily coupled, in an inhibitory manner, to the AC → cAMP → PKA signaling pathway, via a pertussis toxin sensitive G_i protein [193,195,198,254,255,260]. The decrease in cAMP production reduces the uptake of linoelic acid, an essential and major fatty acid, by specific fatty acid transporters. Co-precipitation experiments showed that the MT_1 receptor is coupled to different G proteins that mediate AC inhibition and phopholipase Cβ activation. Thus, MT_1 receptor activation leads to activation of a large variety of G proteins including $G_{i\alpha 2}$, $G_{i\alpha 3}$, and $G_{\alpha q/11}$ proteins [261], and $G_{i\alpha s}$, $G_{\alpha z}$, and $G_{\alpha 16}$ [262,263]. Moreover, activation of MT_1 receptors leads to activation of phospholipase Cβ (PLC-β), with a concomitant increase of inositol-(1,4,5)-triphosphate (IP3), cytosolic Ca^{2+} and 1,2-diacylglycerol [198,243,253,264]. In addition, activated MT_1 receptors inhibit cAMP

responsive element binding protein (CREB) phosphorylation, a nuclear transcriptional activator of cAMP-sensitive gene factor [265–268], and also inhibit the formation of immediate early gene products, c-Fos and Jun B [269]. The functional significance of this differential G protein coupling has further deciphered that G_{i2} and G_{i3} proteins mediate AC inhibition through a pertussis toxin–insensitive $G_{q/11}$ protein and are coupled to phospholipase Cβ activity in cell lines (HEK293, Cos-7, CHO cells) through stably expressing MT_1 receptors [261,264]. Parallel signaling processes are observed through other G proteins, including G_0, G_z, or G_{16}. This stimulatory effect is independent of an interaction with G_i or G_s proteins and associated with a calcium–calmodulin (CaM) signal transduction pathway and c-Jun N-terminal kinase activation [263,272].

Furthermore, stimulation of recombinant human MT_1 receptors causes not only the inhibition of forskolin-induced cAMP accumulation [259,261,264], but also potentiates the prostaglandin $F_{2\alpha}$–induced release of arachidonate and hydrolysis of phosphoinositide [259]. Activation of the MT_1 receptor induces a transient elevation in cytosolic calcium ion concentration and in inositol phosphate accumulation [261,264]. Stimulation of the MT_1 receptor is associated further with increased phosphorylation of mitogen-activated protein kinase (MEK1/2) and extracellular signal–regulated kinase (ERK1/2) [263,271]. In addition, MT_1 receptors regulate other ion fluxes and specific ion channels, such as increase in potassium conductance by activating inward rectifier potassium channels (Kir3/GIRK or Ca^{2+} activated K^+ channel, BKca) [272], and potentiate prostaglandin $F_{2\alpha}$- and ATP-mediated stimulation of PLC activity [259,264]. Both processes may involve activation of membrane-bound βγ-subunits released by G_i proteins. Thus, MEL restores the metabolic functions of cells with improvement in several aspects of Ca^{2+}-signaling such as the amplitude and frequency, the size of intracellular Ca^{2+} pool, capacitative Ca^{2+} entry, and the mitochondrial potential [273,274].

Activation of the recombinant MT_2 receptor couples to a number of signal transduction pathways including phosphoinositide production, inhibition of the formation of cAMP via the AC, and cGMP levels following the soluble guanylate cyclase pathway. Additionally, MEL increases the PKC activity through MT_2 receptor in rat SCN [253,275]. Expression of human MT_1 and MT_2 receptors in COS-7 cells demonstrates that activation of these receptors stimulates c-Jun N-terminal kinase (JNK) activity via pertussis toxin–sensitive and insensitive G proteins [263]. Moreover, despite the low expression of both MT_1 and MT_2 receptors in peripheral tissues/cells, they inhibit AC through αi, and activate other parallel signaling cascades through different G-protein subforms and also βγ heterodimers [193,198,224,259,261,268,276]. A critical step in MEL action through $MT_{1/2}$ is increased intracellular Ca^{2+} that leads to activation of PKCα [259,277,278]. PKC activation by MEL attenuates specific cellular functions such as androgen-dependent gene expression in prostate cell [279]. Furthermore, MEL blocks calmodulin (CaM) interactions with its target enzymes through induction of CaM phosphorylation by PKCα [280]. CaM Kinase II plays a regulatory role in the maintenance of CREB phosphorylation in the spinal cord [281].

A third mechanism of the biological effects of MEL is through MT_3 receptor, which is identified with lower MEL affinity, very rapid ligand association/dissociation kinetics, and widely distributed in various tissues of the body [282]. Mass spectrometry and enzymatic data confirmed that MT_3 is quinine reductase 2 (QR2), a known detoxifying enzyme [283]. The MT_3 receptor modulates calcium and CaM activity. Calcium-activated CaM is involved in the initiation of the S and M phases of the cell cycle, cell cycle–related gene expression, and the reentry of quiescent cells from G_0 back into the cell cycle [282]. Becker-Andre et al. [213] demonstrated that beside MT receptors, MEL has genomic action through a novel class of orphan nuclear receptors of the retinoic acid receptor family. ROR-α1 and ROR-α2 receptors are involved in immune modulation and inflammatory reactions [284]. MEL also acts as an intracrine, paracrine, or an autocrine manner in the pineal gland, eye, lymphocytes, gut, bone marrow, skin, and gonads for the local coordination [15,17,200,249,251,285–287]. The role of RZR/ROR-β as a transcription factor in sensory system is also suggested. The mechanism of actions of MEL at the cellular level has been schematically presented in Figure 1.3.

1.8 PHYSIOLOGICAL DIVERSITY AND THE THERAPEUTIC POTENTIALS

The amphiphilic (lipophilic and hydrophilic) character and pleiotropic nature of MEL with wide range of distributions of its receptors ascribe for multipotent physiological or pathophysiological functions of this hormone in humans. Although most of the studies in order to provide experimental evidence for the roles of MEL in human have used pharmacological doses of MEL (1 μM and above), a few studies confirmed these functions clinically or perfect experimentally as opposed to physiological doses (below the nanomolar range) of MEL [201,288]. Since several recent publications including ours [8] have reviewed the current status on multiple physiological functions including the autocrine–paracrine role of MEL, the current treatise emphasizes only a few of them, which proved important in the context of human health.

1.8.1 Synchronization of Rhythmic Body Functions with the Environment

Biological rhythmicity is fundamental to various physiological processes, and these rhythms are described according to frequency, period length, amplitude, and phase [289]. Circadian frequency implies that one repetition occurs every 24 hours. In humans, a few physiological variables (ACTH, cortisol, FSH, LH, MEL, prolactin, TSH, lymphocytes, and eosinophil counts) attain peak levels during sleep. Catecholamines and blood pressure surges occur during the transition from sleep to wakefulness. Other variables (blood viscosity, platelet adhesiveness, erythrocyte count, body temperature, blood levels of insulin and cholesterol, fifth Ewing variant [FEV], an E-twenty-six [ETS] transcription factor) that are assumed to be involved in the transcription of gene(s) in the serotonergic pathway and to play a role in early brain development attain peak activity during wakefulness, while gastric secretion and WBC count peak during the transition from wakefulness to sleep [289]. Circadian rhythms are generated by the SCN in the hypothalamus and are influenced by a variety of factors including sleep, cyclical hormone secretion, and daily rhythms of core body temperature [290]. Chronobiological disorders are the result of these rhythms failing to remain synchronized with environmental rhythms or discrepancy occurs when external time cues are removed. In human circadian periodicity has a frequency of 23.8 to 27.1 hours (~24.2 hours/cycle) with the body functions and is an inherited characteristic. This circadian frequency is closely related to diurnal preference and the early or late timing of the circadian system (MEL, cBT) in a normal entrained situation [291] (Figure 1.4).

The most important entrainment factor is environmental light and others include food, hormones (MEL, thyroid, and adrenal cortex hormones), social factors, and physical activity, although these are not as potent as photic stimulation [289,292]. MEL acts as an endogenous synchronizer either in stabilizing rhythms (circadian) of body functions or in reinforcing them. Hence, MEL is called a "chronobiotic" molecule [293]. It is also considered as a "neuroendocrine transducer" or "hormone of darkness" or "biological night," which is exclusively involved in signaling the "length of night" or "time of day" and "time of year" to all tissues. In humans, the exogenous administration of MEL changes the timing of rhythms by increasing sleepiness, wake electroencephalogram (EEG) theta activity, rapid eye movement (REM) sleep propensity, sleep propensity, endogenous MEL, and decreasing core body temperature, which ultimately leads to sleep [44,294,295]. This phase-shifting effect of MEL depends upon its time of administration. MEL phase-advances the circadian clock when given during the evening and the first half of the night, whereas the circadian rhythms are phase-delayed during the second half of the night or at early daytime. Thus, the exogenous administration of MEL at night (23:30 h) has a sedative effect, but earlier administration (18:00 h) causes the effect of temazepam [296]. The magnitude of phase advance or phase delay depends on the dose of MEL [297]. As MEL crosses the placenta, it plays an active role in synchronizing the fetal biological clock [9]. Thus, a practical definition of MEL would be "a substance that adjusts the timing of internal biological rhythms" or more specifically "a substance that adjusts the timing of the central biological clock" as chronological pacemaker or *zeitgeber* (time cue) as calendar function

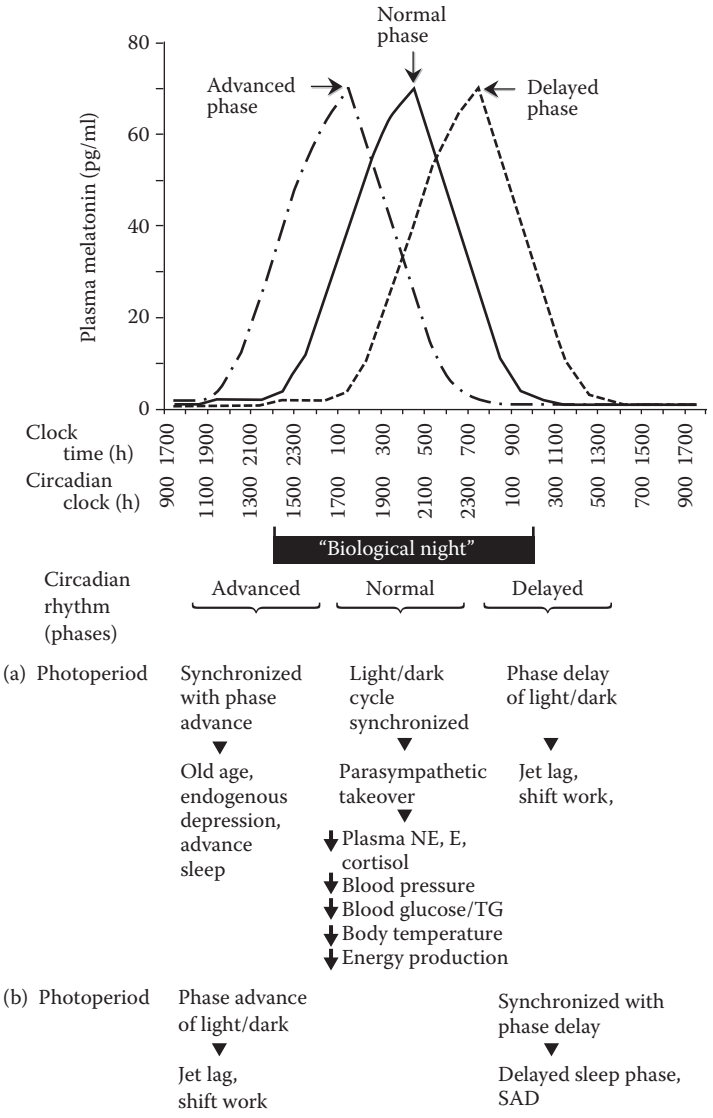

FIGURE 1.4 Schematic representation of the normal endogenous profile of plasma MEL secretions defining biological night, phase advance and delays, based on published data on phase response curves. (a) and (b) represent normal circadian rhythm disorders in relations to photoperiod (see text for details).

[298], although the appropriate zeitgeber circadian oscillators are found in every organ and indeed in every cell in the body [299].

The chronobiotic effect of MEL is caused by its direct influence on the electrical and metabolic activity of the SCN as shown by *in vitro* as well as *in vivo* experiments [293,300]. MEL, through various receptors, induces a differential influence on clock genes. All of the currently known mammalian clock genes have been cloned during the last two decades (1987–2005). These include the three mammalian homologues of *period* (*Per1*, *Per2*, and *Per3*), circadian locomotors output cycles kaput (*clock*), brain and muscle ARNT-like 1 (*Bmal*1/Mop3), the two homologues of *Drosophila cryptochrome* (*Cry1* and *Cry2*) and casein kinase 1ε (CK1ε). Human clock genes show high similarity to those of other mammalian clock genes. *Per1*, *Per2, Per3, clock*, *Bmal1* (*Mop3*), *Cry1*, and

Cry2 genes are expressed in all peripheral tissues [301,302]. The clock genes are observed from flies to humans, but the functional roles played by some of their corresponding products differ between insects and mammals. In humans, the PT and SCN clock genes expression pattern show 24-hour rhythmicity. *Per1* is activated at the beginning of the light phase and Cry1 at the onset of the dark phase. Long or short photoperiod information is encoded within the SCN. Thus, synthesis of MEL is driven by the SCN and conveys the photoperiodic information to the PT by virtue of its secretion pattern. This phenomenon, in turn, influences the pattern of expression of the clock genes *Per1* and *Cry1* within the PT, providing a means of translating the MEL signal for the control of body rhythm or rhythmic synthesis and secretions of hormones [303]. However, amplitude modulation is unrelated to clock gene expression in the SCN [190,303]. The circadian regulation is determined by and interacts with neurotransmitter functions too. The highest concentrations of serotonin in the CNS is in the SCN [304,305], and its turnover exhibits marked circadian and seasonal rhythmicity, which is rapidly stimulated by light exposure [305].

At the cellular level, circadian rhythms of other clock genes are driven by the interlocking self-regulatory interaction. The positive arm of the circuit is the heterodimer of the proteins CLOCK:BMAL1. This complex binds E-box elements (CACGTG/T) at the promoter region of *Per1–2* and *Cry1–2*, including their transcription. The negative regulators are the translated CRY and PER proteins that complex with CK1ε and translocate into the nucleus, interacting with the CLOCK:BMAL1 complex, thus blocking their own transcription. Phosphorylation of PER and CRY proteins by CK1ε controls their proteosomal degradation, delaying the formation of CRY:PER complex and determining the length of the cycle. Additional regulators are negative loop generated by the transcription of the differentiated embryo chondrocyte (*dec1/sharp2/stra13*) and *dec2* (*sharp1*) genes, which are driven by CLOCK:BAML1 via E-boxes in their promoters. The basic helix–loop–helix proteins differentiated embryo chondrocyte 1 (DEC1) and DEC2 may block circadian gene expression, in part by the formation of a nonfunctional heterodimer with BMAL1, which inhibits the expression of all genes dependent on an E-box as well as plays a role in light induction of genes in the SCN. The circadian oscillation of clock gene expression controls the expression of genes involved in multiple cellular functions in a 24-hour cycle (clock control genes, CCGs) by at least two mechanisms, the direct interaction with E-boxes in the promoters of these genes and the regulation of other CCGs that in turn regulate transcription factors, like albumin D site-binding protein [306]. These studies reveal that MEL is an effective chronobiotic molecule that synchronizes rhythmic body functions with the environmental variables and clock genes.

1.8.2 Regulation of Sleep/Wakefulness Cycle

Two-process model of sleep regulation considers the timing and architecture of sleep to be a consequence of a homeostatic process of rising sleep pressure and the duration of prior wakefulness that is dissipated during the sleep period and is a function of circadian pacemaker [307]. Over the past decade, two important protocols have been developed to understand circadian and sleep homeostatic processes in human [308]. Based on these protocols, a strong relationship has been found between sleep and MEL levels [294]. Several epidemiological studies have confirmed the concept of the need for 7–8 hours of sleep per night, as this amount of sleep is associated with the lowest mortality and morbidity [309]. Both nocturnal MEL levels and the quality of sleep decline at puberty and in older people [310] (Table 1.3). The nocturnal peak is almost lost with age, which contributes to the homeostatic sleep drive decrease and need for daytime naps with poor nighttime sleep (mainly due to circadian loss). The period of sleep tends to become shorter and the quality of sleep poorer with decrease amplitude of the circadian rhythm and waking in a 12-hour light/12-hour dark cycle and, some time with phase advancement of circadian rhythm, caused delayed sleep phase syndrome, advance sleep phase syndrome, irregular sleep wake pattern, and non–24-hour sleep–wake syndrome [308,311,312]. Even a short period of sleep deprivation causes abnormal endocrine responses, leading to further complications such as impaired glucose tolerance, increased blood

TABLE 1.3
Relationship among Human Age, Plasma Concentrations of MEL, and Duration of Sleep per Day

Age	Plasma MEL (pg/ml)	Average sleep / day (hour)
Newborn–1 month	~5–10	Up to 18
1–12 month	~110	14–18
1–3 years	~110	12–15
3–5 years	~130	11–13
5–10 years	~160–170	9–11
10–18 years	~80–105	9–11
18–40 years	~85	7–8
41–50 years	~80	7–8
51–65 years	~60–75	7–8
65–70 years	~60	7–8
>70 years	~28–40	Irregular

Note: See text for details and references.

pressure, sympathetic activation, reduced levels of leptin, and increased inflammatory markers [309]. Similarly, cross-meridian flights involve disorganization of biological rhythm (jet lag) caused by the rapid change of environment and associated LD cues. Eastward travel affects sleep latency, while westward travel affects sleep maintenance. Delays in the LD cycle produce fewer symptoms, and therefore, westward flights tend to be less disruptive [312–314]. Jet lag exacerbates existing mood disorders (depressed mood) with reduced alertness, loss of appetite, poor psychomotor coordination, and reduced cognitive skills.

The clock genes in the SCN gradually adapt to phase-shift of the LD cycle (as found in shift work, trans-meridian flight). Peripheral clocks in the muscle, liver, pancreas, kidney, heart, lung, and mononuclear leukocytes are entrained directly by the SCN through some neurohormonal signals, glucocorticoids, retinoic acid, growth factors, and other zeitgeber, such as body temperature and feeding time, which resynchronize their clock genes at their own rates [315,316]. Circadian clocks in peripheral tissues/cells are resynchronized on their own. This results in a "double desynchronization"—"internal desynchronization" between different clocks in the body and brain and "external desynchronization" between the timing of body rhythms with respect to the LD cycle. This temporal orchestra of "jet lags" (sleep disturbance, mental inefficiency, or daytime fatigue) can be corrected by MEL taken at local bedtime only after arrival (between 10 pm and midnight), and effectiveness could be attenuated by the appropriate use of bright light [317]. Moreover, patient or animal model with primary insomnia (wakefulness and inability to fall asleep before 02:00 to 03:00 am), narcolepsy (a disorder of disturbed circadian sleep/wake rhythm and REM (sleep deficit), and sleep disorders in children (hyperactivity disorder) can be successfully corrected by treating or using wide pharmacological doses (0.5–50 mg) of MEL, but 5 mg seems to be the most effective dose and should be taken close to the target bedtime at the destination (22:00–24:00 h) [318]. Doses higher than 5 mg appear to have no further benefits, although higher secretion of MEL causes maximum sleepiness and fatigue at night [312,314,319]. Thus, MEL can be used at local bedtime to help in resynchronization of the circadian oscillator with the new environment for coordination of circadian rhythms and sleep function [318,319]. These studies indicate that application of MEL in optimum dose and schedule can successfully solve sleep-related problems in different age group of human.

1.8.3 Regulation of Mental State, Behavior, and Brain Functions

The pineal gland promotes homeostatic equilibrium through MEL and acts as a "tranquilizing organ" in stabilizing electrical activity of the CNS and causes rapid synchronization of the EEG. The classic endogenous, or nonseasonal, depression is characterized by insomnia (early morning awakening), appetite suppression, weight loss, and advanced onset of nocturnal MEL release, which begins in the spring and persists through the summer or through the winter during the period of light-phase shortening [320] (Figure 1.4). Similarly seasonal affective disorder (SAD) is characterized by late sleep, morning hypersomnia, increased appetite, and retarded onset of nocturnal MEL release, which peaks in the fall and spring [295]. Such phenomena are associated with individuals with low nocturnal MEL levels and major depressive/panic disorders [321]. Some patients experience major depressive disorder with seasonal pattern (MDD-SP), which is characterized by depressive episodes that occur at the same time every year [322,323]. The mood usually worsens as the duration of light hours is reduced in the winter. The circadian rhythms in these patients are usually in a phase-delay status. A supposition of strong link among MEL levels, pineal function, and mood disorders in these patients is strengthened by epidemiologic and chronobiological evidences. Moreover, in patients with depression, disrupted sleep is of major significance [324,325] with an increase in cortisol and temperature, and decrease in MEL amplitude [326,327]. In practice, between 40%–95% of subjects with depression have poor sleep quality [324,326,327]. These finding have been corroborated by physiological criteria from polysomnographic studies. About 40%–60% of outpatients and 90% of inpatients with depression exhibit polysomnographic abnormalities [326]. There is a casual relationship between insomnia and depression such as insomnia increases the risk of onset of depression and persistent insomnia is associated with a 40-fold increase in risk of depression. Moreover, insomnia increases the risk of recurrence of depression, whereas a stable sleep/wake rhythm and good sleep habits are essential in the prevention of further relapses in depressed patients [324]. Insomnia could also contribute to worse in the symptoms already caused by depression, such as irritability, decreased cognitive functioning, and poor executive functioning [290]. The treatment with different doses (>1 mg/day) of MEL in mentally depressed individuals at night prolongs the nocturnal MEL rise and helps in recovering SAD by changing the expression of clock gene and by changing the expression of *Per2* gene in bipolar or classic depression [321,328] and finally changing the quality and duration of sleep. However, the use of large doses of MEL in morning or early afternoon represents no clear effects [329]. In children, MEL is well tolerated and does appear to reduce the time to sleep onset as well as in minimizing nighttime awakening [290]. MEL is effective in hyperactive and neurologically compromised children and developmental gains have been reported after treatment with MEL (2.5–5 mg approximately 30–60 minutes before bedtime) [330].

Phototherapy as an adjuvant may accelerate responses to antidepressants among patients with depression [331]. MEL secretion has been shown to be wavelength-dependent as exposure to monochromatic light at 460 nm produced a twofold greater circadian phase delay [105]. These results are further confirmed by measuring brain 5-HT and Trp levels which rise after MEL administration and are directly linked with an array of neuropsychiatric phenomenon [332]. The diminished central 5-HT, as indicated by low levels of serotonin marker 5-hydroxy indole acetic acid (5-HIAA) in cerebrospinal fluid, is associated with impulsiveness, aggression and autoaggregation, alcoholism, compulsive gambling, overeating, and other obsessive–compulsive behaviors [333]. Moreover, the requirement of intact β-receptor function for MEL synthesis and stimulatory effect of norepinephrine on MEL synthesis and release demonstrate a direct relation of MEL to depression [334].

Administration of tricyclic antidepressants (TCA) at night also exerts sedative effects with increased MEL synthesis through binding with β-adrenergic receptors of pinealocyte and increased cAMP production, which in turn activates AA-NAT and enhanced MEL rhythm amplitude. TCA also inhibits cytochrome p450 enzyme CYP1A2, which metabolizes MEL in the hepatocytes and increases endogenous MEL levels [335,336]. Finally, it supports the serotonergic system to change or elevate mood, reduce aggression, increase the pain threshold, reduce anxiety, relieve insomnia,

improve impulse control, and ameliorate obsessive–compulsive syndromes. However, higher doses or long term use of TCA has side effects by blocking muscarinic, histaminergic, and adrenergic receptors because of its joined benzene ring structure. In consideration to the quality of sleep and inducing onset of sleep with negligible daytime consequences, a schedule of treatment of insomnia patients with 2 mg MEL at night has been found to be equal to the treatment with zopiclone 5 mg at night [337]. Unfortunately, exogenous MEL has a very short half-life and is quickly cleared from the circulation.

Collectively, available information suggests that appropriate exogenous MEL administration can restore human neurological disorders with direct impact on general health of elderly people [338]. However, in some isolated cases, it has been demonstrated that a long-term use of MEL causes psychomotor disturbance, increased seizure risk, blood clotting abnormalities, headache, insomnia, GI tract effects, delayed puberty, and hypogonadism [339]. High doses of MEL in healthy subjects also cause drowsiness, decreased attention, and prolonged reaction time [322].

1.8.4 Scavenger of Free Radicals

The amphiphilic character of MEL helps it to cross both in the lipid and aqueous subcellular compartments in all morphological and physical barriers or hemato-encephalic barrier (blood–brain barrier [BBB], placenta) and reaches all tissues of the body within a very short period [9,340,341]. Therefore, the antioxidant properties of MEL appear to effect and perform a very important receptor-independent metabolic function as a multifaceted scavenger of free radicals. The antioxidant effects of MEL have been well described and included both direct and indirect effects with equal efficiency in multiple sites (nucleus, cytosol, and membranes) of the cell [305]. MEL is a more potent antioxidant than vitamins C (exclusively water soluble) and E (exclusively lipid soluble) [342]. Free radicals are defined as molecules or molecular fragments containing one or more unpaired electrons in their atomic or molecular orbital. Radicals and their nonradical related species are referred to as reactive oxygen and nitrogen species (ROS and RNS, respectively) and are products of normal cellular metabolism. The first indication that MEL may be a direct free radical scavenger was reported by Ianas et al. [343]. Two years later, Tan et al. [344,345] provided strong evidence that MEL is highly effective in detoxifying the highly reactive hydroxyl radical (•OH). MEL detoxifies a variety of free radicals and reactivates oxygen intermediates including the hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), peroxy radicals (LOO•), peroxynitrite anion ($ONOO^-$), singlet oxygen ($^1O_2$), nitric oxide (NO•), superoxide anions (O_2•–), and lipid peroxidation. MEL neutralizes H_2O_2 by three different ways, through directly interacting with H_2O_2, by enhancing H_2O_2-catabolizing enzymes, or through its catabolic products (Figure 1.5).

MEL is itself not a direct free radical scavenger, but catabolites of MEL that formed during these interactions (namely AFMK and with considerably higher efficacy, AMK) [346,347] are excellent scavengers of toxic reactants. AFMK is produced by both enzymatic and non-enzymatic mechanisms [22] and mainly by myeloperoxidase (MPO) [125]. AFMK is capable of donating two electrons and, therefore, acts as a direct free radical scavenger in its own capacity [340]. The potent scavenger, AMK, consumes additional radicals in primary and secondary reactions [348]. Interestingly, AMK interacts not only with ROS but also with RNS [349]. MEL scavenges •OH by contributing an electron and, thereby rendering the radical nonreactive, becomes itself a radical, the indolyl cation radical [350]. This product is not very reactive and is, therefore, nontoxic to the cell [351]. The indolyl cation radical then scavenges the O_2–• forming AFMK, which is excreted in the urine. Each MEL molecule also scavenges two •OHs and generates the product, cyclic-3-hydroxyMEL and is directly excreted in the urine [352] as the classic antioxidant. The ratio of scavenger to radicals neutralized is 1:1.

The evidence that MEL neutralizes $^1O_2$ was first provided by Cagnoli et al. [353]. Subsequent study revealed that this function is performed through the formation of AFMK [354]. MEL scavenges the LOO•, which is produced during lipid peroxidation and able to propagate the chain reaction.

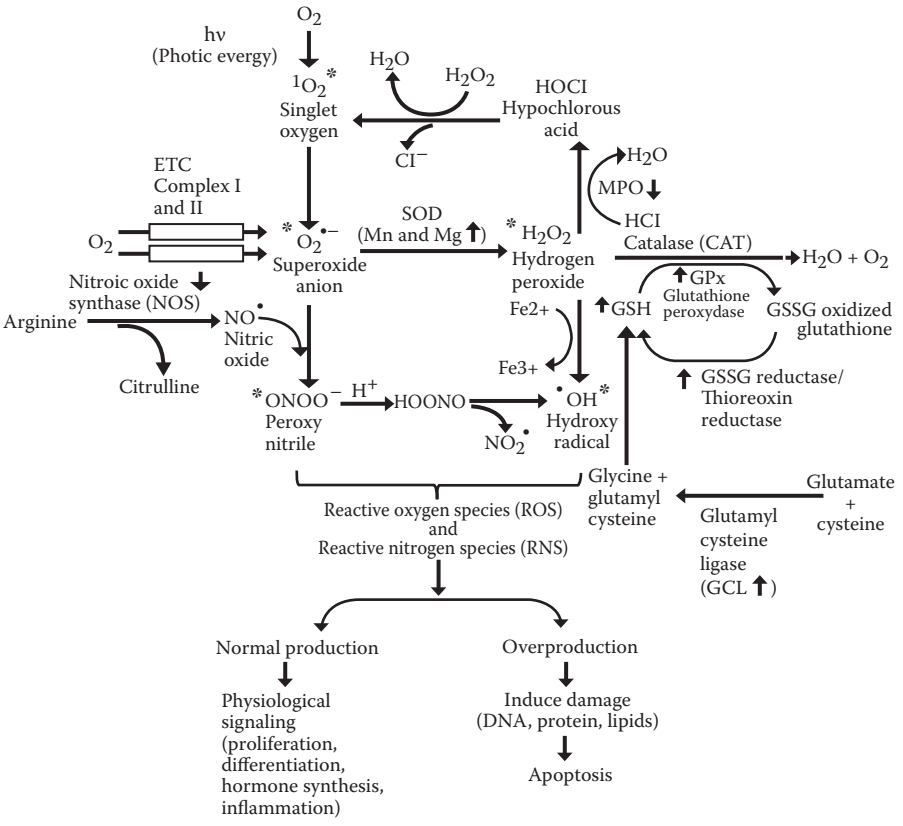

FIGURE 1.5 Schematic representation of the pathways of generation and neutralization of ROS and RNS by MEL. The main cellular source of ROS and RNS and the interrelationship between the antioxidant defenses by MEL. MEL directly scavenges the reactants marked with an asterisk. Abbreviations: •OH, hydroxyl radical; H_2O_2, hydrogen peroxide; LOO•, peroxy radicals; $ONOO^-$, peroxynitrite anion; $^1O_2$, singlet oxygen; NO•, nitric oxide; $O_2^{\bullet-}$, superoxide anions; GPx, glutathione peroxidise; GRd, glutathione reductase; G6PD, glucose-6-phosphate dehydrogenase; SOD, superoxide dismutase, both MnSOD and CuZnSOD, CAT, catalase; NOS, nitric oxide synthase; GSH, glutathione (see text for details).

Moreover, MEL is more effective than vitamin E in neutralizing LOO• and inhibiting lipid peroxydation [355]. Furthermore, MEL protective effects are a consequence of its ability to reduce NO• formation and scavenge $ONOO^-$ and associated oxidants. Although, inherently nonreactive, NO• quickly couples with O_2–• to form $ONOO^-$ [356]. Thus, by scavenging NO•, MEL reduces its generation under some circumstances by inhibiting the activity of its rate-limiting enzyme, nitric oxide synthases (c) [357,358]. The role of MEL in this process supports its anti-inflammatory ability.

The broad spectrum antioxidant activity of MEL also includes an indirect effect by up-regulating several antioxidative enzymes, and down-regulating pro-oxidant enzymes and 5- and 12-lipooxygenases [359], glutathione peroxidase (GPx) [360,361], glutathione reductase (GRd) [361], glucose-6-phospahte dehydrogenase (G6PD) [340,362], superoxide dismutase (SOD, both manganese–superoxide dismutase, MnSOD, and CuZnSOD) [363–365], catalase (CAT) [340,362] NOS [176] in particular. SOD plays a role in the dismutation of O_2–• from cells, thereby lowering the formation of highly reactive and damaging $ONOO^-$ [356]. GPx and catalase enzymes are involved in converting H_2O_2 to nontoxic products (water) in the body [366]. However, reduced glutathione (GSH) is oxidized to its disulfide, GSSG. GSSG is rapidly reduced back to GSH by GRd, thereby helping to maintain high levels of reduced glutathione [367].

MEL acts as a metal chelator. Limson et al. [368] demonstrated that MEL forms complexes with aluminum (III), cadmium (II), copper (II), iron (III), lead, and zinc. It forms complexes with lithium, potassium, sodium, and calcium [369]. The metal chelating ability of MEL increases with increasing concentrations of MEL. Thus, as a metal chelator, MEL acts as a neuroprotector agent.

MEL via AFMK pathway is highly efficient; a single MEL molecule scavenges up to 10 ROS or RNS. During oxidative stress, MEL level declines as it is metabolized by interaction with different reactive species [370]. More AFMK is produced during high oxidative stress along with cyclic-3-hydroxyMEL [370]. Likewise, AMK is 25% more potent than MEL in the inhibition of neuronal NOS (nNOS) [370]. Moreover, 6-OH-MEL (6-OHM) is also an effective free radical scavenger [371] as it binds with iron (III) and converts to iron (II), which is a more biologically usable form of iron [372].

Due to its strong antioxidant property, MEL protects membrane lipid, cytosolic proteins, nuclear DNA, and mitochondria from free radical damages. In mitochondria, MEL accumulates in high concentrations and stabilizes inner mitochondrial membrane thereby improving electron transport chain (ETC) activity [373–376]. It increases the activity of the brain and liver mitochondria respiratory complex I and IV, whereas the activities of complex II and III are not affected [377]. The high redox potential of MEL (0.94 V) suggests that it may interact with the respiratory complexes of the ETC and may donate and accept electrons, thereby increasing electron flow, an effect not possessed by other antioxidants [355,378]. MEL interacts, at high affinity (K_i = 150 pM) with a binding site at the amphipathic ramp of Complex I, thereby presumably modulating electron flux [276]. Thus, MEL protects the mitochondria from oxidative damage, reducing oxygen consumption concomitantly with its concentration, inhibits any increase in oxygen flux in the presence of an excess of ADP, reduces the membrane potential and consequently inhibits the production of superoxide anion and H_2O_2 [355]. At the same time, MEL maintains the efficiency of oxidative phosphorylation and ATP synthesis while increasing the activity of the respiratory complexes.

The protective effects of MEL and catabolic products on lipid peroxidation are induced by oxidative stress in mitochondrial membrane. MEL at micromolar concentration prevents the oxidation depletion of cariolipin (CL), which is particularly rich in unsaturated fatty acids (~80% represented by linoleic acid in heart tissue), and in addition CL is located near to the sites of ROS production. CL molecules are particularly susceptible to peroxidation by LOO• [355]. MEL prevents Ca^{2+}/peroxidized CL-dependent mitochondrial permeability transition pore (MPTP) opening and mitochondrial cytochrome c release. It also exerts antiapoptotic actions by up-regulating anti–apoptotic B-cell lymphoma (Bcl-) proteins, preventing Bax translocation, and directly inhibiting the MPTP at low affinity (K_i = 0.8 μM) [276]. This effect of MEL may have important implications in those pathophysiological situations that are characterized by alterations of Ca^{2+} homeostasis and accumulation of peroxidized CL in mitochondria [355]. Further, the functional alterations of the mitochondrial ETC complexes, as noted in heart ischemia/ reperfusion, and in the heart and brain of old animals, may increase the electron leak from the ETC, generating more O_2−• and perpetuating a cycle of oxygen radical–induced damage that ultimately leads to mitochondrial dysfunction because of oxidation/depletion of CL molecules [355]. AMK exerts its effects on electron flux through the respiratory chain and improves ATP synthesis in conjugation with the rise in complex I and IV activities [379]. Thus, MEL can be used in the treatment of several neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, Huntington's chorea, amyotrophic ateral sclerosis, and aging, which are caused by three major and frequently interrelated processes, namely glutamate excitotoxicity, free radical–mediated nerve injury, and mitochondrial dysfunction [347,380–382]. These studies showed disruption of nocturnal surge of MEL in ischaemic stroke patients and patients with acute cerebral hemorrhage and reduction in the degree of tissue damage against ischemic injury through direct free radical scavenging or by indirect antioxidant activities in these patients following exogenous administration of MEL, which is suggested to have the neuroprotective role in MEL in strokes [383].

The oxidative stress is an important hallmark in disorganizing the cortical actin cytoskeletal assembly and disruption of accompanied subcellular intricate fibrous network composed of

microtubules, microfilaments, and intermediate filaments (IF) as well as by associated proteins [384]. MEL prevents cytoskeletal structure disruption followed by cell shape changes and increased lipid peroxidation or apoptosis induced by okadaic acid of physiological plasma and cerebrospinal fluid [385]. In this process, PKC participates as an important signaling molecule as demonstrated by a PKC inhibitor (bisindolylmaleimide) abolished cytoskeltal re-establishment elicited by MEL, while the PKC agonist (PMA) reorganized microtubules and microfilaments [386]. Thus, MEL may be a suitable compound in the treatment of neurodegenerative diseases as a cytoskeletal modulator and also as a free radical scavenger [332,387].

The antioxidant properties of MEL help in reducing blood cholesterol (~38%) mainly by inhibiting copper-induced oxidation of low-density lipoprotein (LDL), with reduction in blood pressure and catecholamine levels via relaxation of smooth muscles in the aortic walls, thereby potentially contributing to an antiatherosclerotic effect on the cardiovascular system [388]. Likewise, exogenous MEL administration causes vasodilatation, decreasing internal artery pulsatile index, increases the cardiac vagal tone, decreases circulating norepinephrine levels, and finally reduces blood pressure in hypertensive patients [389]. MEL's antioxidant properties can also be additive to the treatment of gastric ulcer, bowel syndrome, ulcerative colitis, diarrhea, and glycerol-induced renal failure [390,391]. Taken together, MEL may act as a potent free radical scavenger, by neutralizing hydroxyl and peroxyl radicals among others, preventing lipid membrane peroxidation, apoptosis, and protecting the DNA from the damage induced by free radicals [392].

1.8.5 Antinociceptive, Anti-Inflammatory, and Immunomodulatory Function

The role of MEL as an immunomodulator in the regulation of proliferation, differentiation, and functions of lymphoid tissues has been known for nearly the past three decades [285,393–395]. The nocturnal rise in blood MEL levels in human is associated with increased production of interleukins (IL, including IL-1, IL-2, IL-6, IL-12), thymosin 1a, thymulin, and tumor necrosis factor-alpha (TNF-α). MEL production has been identified in lymphocytes and associated with IL-2 secretions [17,395]. The IL-2 is required for T-cell proliferation and clonal expansion, secretion of interferon γ (IFN γ), B-cell maturation and differentiation, and natural killer (NK) cell activation. All these factors are involved in allorecognition and responses [396]. In humans, MEL represents an inflammatory stimulus to monocytes, and it is suggested that monocyte function is directed toward an inflammatory phenotype during the night, when production of IL-12 is increased and IL-10 is decreased [397]. Furthermore, MEL implants enhance a defined T helper 2 (Th2)-based immune response under *in vivo* conditions and MEL supplementation enhances antigen presentation from antigen-presenting cells (APC) to T-cells via the specific up-regulation of major histocompatibility complex (MHC) class II molecules, and further increases TNF-α and IL-1 secretions [398]. MEL augments peripheral blood mononuclear cell (PBMC) proliferation *in vitro*, via the inhibition of production of T-helper type 2 (Th2) cytokines (IL-10) [399]. MEL may increase chemotaxis via the up-regulation of the chemokine production at the sites of inflammation, a process that may be synergistic with TNF-α [400]. In rodents, postnatal pinealectomy suppresses immunity and thymic atrophy, whereas exogenous MEL treatment increases cell-mediated immune function by increasing NK cell activity [401]. MEL limits the expression and activity of matrix metalloproteinases (MMP-2 and MMP-9) through the regulation of MAPK, JNK, ERK1/2, and the high-mobility group box 1 protein expression (HMGB1), thereby, reducing proinflammatory effects of TNF-α [402,403]. Collectively, these studies suggest potential role of MEL as a novel adjuvant immunomodulatory agent, in the adaptive processes of allo-recognition and allo-response [404]. Thus, MEL can be used as a potent therapeutic molecule to prevent graft rejection after organ transplantation. Since all organ transplant recipients are treated with continuous and permanent immunosuppression, this in turn increases the risk of infection and neoplasia formation [396].

MEL acts on immunocompetent cells (monocytes, B-lymphocytes, NK lymphocytes, T-helper lymphocytes, cytotoxic T lymphocytes) through MT_1 and RZR/ROR-α orphan nuclear receptor

family and enhances cytokine production/secretions, cell proliferation, or oncostasis [405,406]. In B-lymphocytes, MEL binds to the RZR/ROR-α receptors to down-regulate the gene expression of 5-lipoxygenase (5-LOX), which is an important enzyme in allergic and inflammatory diseases like asthma and arthritis [284,407]. Immunomodulatory effects of MEL are observed in humans with bronchial asthma. On the other hand, exogenous MEL has an adverse effect in patients with asthma [347]. The nocturnal asthma is associated with elevation and phase delay of peak serum MEL levels [408]. In adjuvant-induced arthritis, both prophylactic and therapeutic MEL administrations inhibit the inflammatory response [409].

MEL also plays a role in pain modulation through $MT_{1/2}$ receptors as activation induces an antinociceptive effect at spinal and supraspinal levels under conditions of acute and inflammatory pain [410]. This effect agrees with localization of $MT_{1/2}$ receptors in thalamus, hypothalamus, dorsal horn of the spinal cord, spinal trigeminal tract, and trigeminal nucleus. The activation of $MT_{1/2}$ receptors leads to reduced cAMP formation and reduced non-reception. In addition, MEL is able to activate opioid receptors indirectly, to open several K^+ channels and to inhibit expression of 5 lipoxygenase, and cyclooxygenase 2 [410]. Thus, MEL and the immune system are linked by complex bidirectional communication. The immune system, in turn, appears to reciprocally regulate the pineal gland functions, mainly via cytokines produced by activated immune cells and depends on age, sex, and species [285,393–395].

1.8.6 Influences on Endocrine Network

MEL affects the synthesis, secretion, and action of steroid as well as nonsteroid hormones. It influences the adenohypophysial activity, which is dependent on the age and sex of the species, as well as the concentration of the hormone. There are substantial in favor of the conjecture that MEL modifies synthesis and secretion of different adenohypophysial hormones such as growth hormone/somatotrophin (GH), thyrotropin (TSH), and adrenocorticotropin (ACTH) either by directly influencing the secretory activity of the cells in the anterior pituitary or indirectly by influencing the hypothalamic neurons producing the respective neurohormones that stimulate or inhibit the release of adequate adenohypophysial hormones [411,412]. MEL has a positive phase relation with the synthesis of prolactin and negative with GH [411–413] and is involved in the regulation of calcium and phosphorus metabolism by stimulating the parathyroid gland or inhibiting calcitonin release and prostaglandin synthesis [414]. A similar change in prolactin levels is observed with the nocturnal increase and morning decrease as MEL levels. Administration of MEL also stimulates prolactin secretion [415]. However, the relationship between MEL and GH are poorly understood.

MEL affects the activity of pituitary–adrenal axis by modulating the peripheral action of corticoids [411,412]. Pinealectomy leads to adrenal hypertrophy, which is reversed by administration of exogenous MEL [339]. Therefore, it is proposed that MEL acts as a corticotrophin-releasing factor inhibitor with disinhibition of the pituitary–adrenal axis in major depression, since pineal MEL levels are low and unable to modulate its influence on adrenal gland [416]. Similar phenomenon (low MEL level) is found in patients with Cushing diseases (hyperadrenocorticism) [417] and hypercortisolemia, which is further linked to several aspects of aging and age-associated phenomena, including glucose intolerance, atherogenesis, impaired immune functions, and cancer [418]. Nocturnal MEL levels decline or are almost completely lost with age in humans [157,419]. This close reciprocal relation of MEL and corticoids or loss of MEL rhythmicity may be responsible for the pituitary/adrenal axis disinhibition that has been described as a characteristic of aging. The adrenals of elderly humans are apparently hypersensitive to adrenocorticotropic hormone, and midnight corticoid levels (low in young) are markedly elevated at old age [420]. Thus, MEL has phasic inhibitory effects on both the release of corticoids and their peripheral actions by immune depression, hypercatabolism, thymic involution, and adrenal suppression [421], which finally delays aging. However, there are no sufficient direct experimental evidences to demonstrate a relationship between MEL and hypothalamic–pituitary–adrenal axis.

There is a strong relationship between the functions of pineal gland and hypothalamic–hypophysial–thyroid axis [422]. But there are no sufficient data on the existence of such relationship in humans. Most of the studies in relation to thyroid and MEL are in other mammalian or nonmammalian vertebrate species.

The sex steroid production at different stages of ovarian follicular maturation is influenced by MEL. Adriaens et al. [423] have demonstrated that MEL (100 μM) increased progesterone (P) and androstenedione (A) production in mouse preantral follicles after incubation for 12 days, and MEL at a dose (100 ng/ml) stimulates P and A production in 30-hour cultures of porcine antral follicles, whereas estradiol (E_2) levels are not changed [424]. MEL also inhibits CYP 11A and CYP 17 expression but not that of CYP 19. Despite an increase in P production, the expression of CYP 11A is significantly inhibited. Further studies suggested that, at the time point studied, CYP 11A is transcriptionally inhibited as a result of feedback inhibition by high levels of P secreted into the culture medium. In contrast, MEL (0.1–10 ng/mL) decreases P, E_2, and cAMP production by hamster preovulatory follicles after 24 hours of incubation with hCG [425]. When theca cells and granulosa cells (GCs) are separated, MEL reduces P production by theca, whereas it does not influence the GCs. MEL may directly suppress follicular (thecal) steroidogenesis at an early stage in the steroid synthesis pathway through cAMP modulation. Moreover, MEL blocks the expression of steroidogenic acute regulatory protein (StAR) [426]. It is believed that StAR protein determines the translocation of cholesterol across the intermembrane space into the inner membrane where P450scc cleaves cholesterol into pregnenolone. MEL (10 nM) treatment for 3 hours reduces the StAR protein expression stimulated by hCG in mouse Leydig tumor cells. An *in vitro* study clearly demonstrated that MEL accelerates the action of maturation inducing hormone (MIH) in denuded fish oocytes [427]. However, the direct action of MEL on follicular steroid production is complicated; it seems to depend on the cell type (theca cell or GC), duration of treatment (acute- or long-term response), experimental model (cell culture or follicle culture), species, and the dose of hormone [428].

Locally produced growth factors, such as the insulin-like growth factors (IGFs, and members of the transforming growth factor β (TGF-β)) superfamily (inhibins, activins, and bone morphogenetic proteins, BMPs), work in concert with gonadotropins (FSH, LH) throughout the follicular growth continuum [428]. Insulin-like growth factors are produced by GCs and theca cells during follicle development [429]. Insulin-like growth factors are mitogenic and anti-apoptotic peptides which promote differentiation and also cause insulin-like metabolic effects mediated by binding to specific high-affinity membrane receptors. The IGF-I and IGF-II stimulate DNA synthesis and E_2 and P secretion by human GCs and granulosa–luteal cells [434]. Insulin-like growth factor I is an anti-apoptotic in ovarian follicles, whereas ovarian apoptosis is enhanced by IGF binding protein [430]. MEL (0.01-10 μg/mL) stimulates IGF-I production by cultured human GCs [431]. Recently, Picinato et al. [432] demonstrated that MEL (0.1 μM) induces IGF-I receptor and activates two intracellular signaling pathways: the PI3 K/AKT, which is mainly involved in cell metabolism, and MEK/ERKs that participate in cell proliferation, growth, and differentiation.

The TGF-β superfamily is expressed by ovarian cells and oocytes in a developmental, stage-related manner and functions as intra-ovarian regulators of follicle development. In humans, TGF-β is produced by both thecal cells and GCs [433]. The TGF-β stimulates FSH receptor expression [434], amplifies FSH-induced aromatase activity, and P production and LH receptor induction in GCs [435-437]. Interestingly, MEL enhances synthesis of TGF-β1 in human benign prostate epithelial cells [438]. Similarly, TGF-β1 immunostaining of multinuclear chondrocytes are dramatically increased in degenerated intervertebral disk tissue after exogenous MEL treatment (30 mg/kg of body weight daily at 5 pm to 6 pm for 4 weeks) in rats [439]. MEL treatment (5 mg/kg intraperitoneal injection) upregulates the level of gene expression of TGF-β in mouse peritoneal cells [406]. There is increasing evidence to support a critical role of TGF-β superfamily members BMP and growth and differentiation factor-9 (GDF-9) in growing antral follicles. BM-15 and GDF-9, exclusively produced by oocytes, may exert their effects by regulating the actions of gonadotropins. The BMP-15 has been shown to attenuate FSH actions on rat GCs by suppressing FSH receptor

expression [440]. The GDF-9 reduced FSH-stimulated P and E_2 production and attenuated FSH-induced LH receptor formation [441]. These studies suggest an important interaction between MEL and BMP-15 and GDF-9 in the growing follicle.

1.8.7 Influences on Reproduction

The role of MEL in the regulation of reproduction is one of the major areas that received wide attention for different mammalian–nonmammalian animal species as well as in humans [7,428,442–444]. Obviously, MEL is per se neither antigonadotrophic nor progonadotrophic. Rather, the changing duration of the nocturnal MEL message is a passive signal that provides the hypothalamic–pituitary–gonadal axis information as to the time of year (calendar information) [445]. The reproductive axis uses the seasonally dependent MEL rhythm to adjust testicular and ovarian physiology accordingly. MEL has an inverse relationship with the hypothalamic GnRH, which is a decapeptide, released in pulsatile fashion from neurosecretory cells in the hypothalamus and acts on GnRH-receptors (GnRH Rs) in the pituitary to regulate the production and release of gonadotropins (follicle stimulating hormone, FSH; luteinizing hormone, LH) from the anterior pituitary. Traditionally, LH has been used as a surrogate marker of GnRH pulse generator activity in the human, based on its validation in animal models as a faithful mirror of GnRH secretions [446]. GnRH has been detected in human embryonic brain extracts as early as 4–5 weeks [451]. By 9 weeks gestation, GnRH neurons have been demonstrated in the fetal hypothalamus, although functional connections between these neurons and the portal system are not established until 16 weeks [451]. LH and FSH, being first detectable in the pituitary at 10 weeks, are measurable in peripheral blood by 12 weeks, reach a peak in midgestation, and then decreased toward term with the development of functioning gonadal negative feedback mechanisms [452].

During the neonatal period, there is a clear evidence of GnRH secretion in the study showing the persistence of pulsatile secretions of gonadotropins (FSH and LH) [453] followed by a decrease by approximately 6 months postnatal life in boys and 1–2 year in girls to the low levels that are present until the onset of puberty [454,455] (Table 1.4), whereas in neonatal period (~1–12 months), both boys and girls exhibit very low levels of plasma MEL (~5–10 pg/ml). The ultrasensitive LH assays have revealed that pulsatile GnRH secretion continues throughout the childhood period, albeit at a markedly reduced amplitude [456], when MEL level (~110–130 pg/ml) reaches its peak at night. The precise mechanism responsible for reversibly restraining the hypothalamic GnRH pulse generator at this time has not yet been elucidated. However, it is likely to involve a process that inhibits GnRH release rather than its synthesis as demonstrated in primates that abundant GnRH mRNA and protein are present within the appropriate hypothalamic neurons at an equivalent developmental stage [457]. In contrast, it has demonstrated that MEL down-regulates GnRH gene expression in a cyclical pattern over a 24-hour period through MEL receptors MT_1, MT_2, ROR-α, and RZR-β [458]. In neonatal pituitary cells, MEL inhibits GnRH-induced calcium signaling and gonadotropin (FSH, LH) secretion through MT_1 and MT_2 receptors. Further, MEL (1 nM) treatment in GT1/7 cells results in significant down-regulation of rat GnRH (rGnRH)-I mRNA rhythmic expression in a 24 h-cycle [459]. The potential regulator elements of MEL are localized to five regions, including -1827 to -1819 bp, -1780 to -1772 bp, -1746 to -1738 bp, -1736 to -1728 bp, and -1697 to -1689 bp, within the rGnRH-I enhancer [460]. These regions have been found to bind a number of transcription factors, such as Oct-1, GATA-4, and Otx2. In addition, two direct repeats of consensus binding sites for orphan nuclear receptors, including retinoic acid receptor-related orphan receptor/retinoid Z receptor, and COUP-TFI, and also other consensus binding sites for AP-1 and CCAAT-enhancer-binding protein (C/EBPb), are found within the -1736 to -1728 bp region [461]. Super-shift assays have demonstrated that only COUP-TFI and C/EBPb bind to this enhancer region of the rGnRH-I gene. Thus, the hypothalamic–pituitary–gonadal axis, which is already active during fetal life, remains quiescent until the age of ~10 years due to high levels of MEL (~160–170 pg/ml at night). This inhibitory

TABLE 1.4
Relationship between Human Age and Plasma Concentrations (UI/l) of Gonadotropins (FSH and LH)

	Male		Female	
Age	**LH**	**FSH**	**LH**	**FSH**
Day 1 to 1 month	~0.21–3.0	~0.25–1.50	~0.10–0.50	~0.20–6.70
1–3 months	~0.10–0.60	~0.10–0.90	~0.15–0.65	~1.10–2.50
4–6 months	~0.10–0.60	~0.10–0.90	<0.07–0.31	~0.50–1.60
7–24 months	~0.10–0.60	~0.10–0.90	<0.07–0.11	~0.40–0.75
2–10 year	~0.10–0.60	~0.10–0.90	<0.07	<0.10–0.50
Puberty				
Tanner stage I (preadolescent: elevation of papilla only, B1)	~0.30–1.00	~0.70–1.50	~0.30–1.00	<0.07–0.15
Tanner stage II (breast bud stage: elevation of breast and papilla, enlargement of areola diameter, B2)	~2.00–3.00	~1.10–1.70	~0.80–2.50	~1.30–4.20
Tanner stage III (further enlargement and elevation of breast and areola, without separation of contours, B3)	~4.50–5.50	~2.00–3.5	~3.50–5.80	~4.50–6.80
Tanner stage IV (projection of areola and papilla to form a secondary mound above levels of the breast, B4)	~4.00–8.00	~3.00–7.00	~5.00–11.00	~3.30–6.00
Tanner stage V (mature stage, projection of papilla only, recession of the areola to the general contour of the breast, B5)	~4.00–5.00	~1.50–4.50	~2.00–6.00	~3.00–5.00
18–40 years	~2.00–6.00	~3.5–5.00	a. Follicular phase ~2.40–12.60 b. Midcycle ~14.00–95.60 c. Luteal phase ~1.00–11.5	a. Follicular phase ~3.50–12.50 b. Midcycle ~4.70–21.50 c. Luteal phase ~1.70–7.50
>40 years	~0.80–8.00	~0.70–11.00	Menopause ~7.00–40.00 Postmenopause ~8.00–58.00	Menopause ~11.00–>20.00 Postmenopause ~25.00–134.00

Note: For details, see the works of Seminara et al. [446], Bergada et al. [447], Chada et al. [448], Phillips et al. [449], and Resende et al. [450].

role of MEL on the hypothalamus is responsible for sexual inhibition. However, other peptidergic regulators are also involved in this process.

Commensurate with the onset of puberty, there is a sleep-entrained reactivation of the reproductive axis characterized by a marked increase in amplitude of GnRH-induced LH pulse with much more modest changes in frequency with decrease in MEL levels (~80–105 pg/ml) [446]. This nocturnal

augmentation of LH secretion stimulates secretion of sex steroids and inhibin from gonads at night with subsequent decrease to pubertal levels during the day [462]. As puberty progresses, secretion of gonadotropins occurs during both day and night. Moreover, the decline of plasma MEL below a threshold value (~115 pg/ml) may constitute the activation of signals for the hypothalamic pulsatile (increase in the amplitude and frequency of GnRH pulses) secretion of GnRH and reactivation of the pulsatile secretion of LH and FSH that are crucial subsequent pubertal changes (~10 years age onward) [411,412,463]. Furthermore, the inhibitory effects of MEL on GnRH action gradually decline due to decreased expression of functional MEL receptors [464]. The low concentration of MEL would result in premature activation of the hypothalamic GnRH secretion and the occurrence of precocious puberty [465]. These effects are further clinically demonstrated in humans showing that acute oral doses of MEL amplify LH pulses in early follicular phase, stimulate prolactin secretion and vasopressin secretions [283].

In adulthood, both sexes secrete gonadotropins in a pulsatile fashion, but in different patterns. In the adult male, wide variations in LH interpulse interval (2-hour frequency) have been reported with parallel changes in sex steroids [446]. In the female, the reproductive axis is under more dynamic regulation, with a complex series of changes in GnRH pulse frequency occurring throughout the menstrual cycle [446]. In the early follicular phase, the GnRH pulse frequency starts at approximately 90 minutes, shifts to 60 minutes in the mid- and late-follicular phases and, with the appearance of P secretions in the luteal phase, shows to cyclic changes approximately every 4–6 hours. By the late luteal phase, the GnRH pulse generator is active only at interval of 6–8 h, but accelerates once again during the luteal–follicular transition to approximately hourly intervals during the day, acquiring the sleep-induced slowing typical of the early follicular phase [446,466]. Furthermore, administration of exogenous MEL in combination with P to women reportedly induces a reduction in LH secretion, blocks ovulation, and in the luteal phase increases P without affecting FSH or inhibiting E_2 [467]. Acute suppression of LH levels is also observed in men after MEL treatment [468]. These effects may be mediated by MEL's ability to influence hypothalamic gonadotropin release [469]. Thus, decrease in MEL levels at the pubertal phase promotes GnRH dependent pubertal maturation and sexual development.

In contrast to hypothalamic action of MEL, a direct effect as an autocrine/paracrine manner on reproductive axis is also known [470]. Studies in human GCs showed that both types of MEL receptors (MT_1 and MT_2) are present, and MEL up-regulates LH mRNA-receptor too [470,471]. LH is essential for the initiation of leuteinization. Furthermore, MEL treatment enhances the human chorionic gonadotropin (hCG) stimulated P secretion with an inhibition of GnRH and GnRH receptor (GnRH-R) expressions. In the female reproductive tract P plays key roles in ovulation, implantation, and the maintenance of pregnancy by regulating GC function and follicle rupture during ovulation [472]. Ovulation is a complex process by which a preovulatory follicle ruptures and releases a fertilizable oocyte into the oviductal lumen. This process occurs as a result of a dynamic interaction between the LH surge and local factors including steroids, NO, prostaglandins, and peptides in a time-dependent manner. The LH surge triggers structural and biochemical changes that lead to rupture of the Graafian follicles, resulting in expulsion of the oocyte and subsequent development of a CL. After hCG injection, follicular steroidogenesis quickly shifts from E_2 dominance to P dominance by the inhibition of 17α-hydroxylase-$C_{17–20}$ lyase activity [473]. This acute increase of P production is essential for luteinization and ovulation. Progesterone and E_2 concentrations are significantly higher in the large follicles (size > 18 mm, P ~10 μg/ml; E_2 ~512 ng/ml; T ~5ng/ml) than in the small follicles (size < 10 mm, P ~3.3 μg/ml; E_2 ~299 ng/ml; T ~7.5 ng/ml) of humans, and likewise, MEL concentrations are also higher in the large follicles (MEL ~123 pg/ml) compared with smaller follicles (~54 pg/ml) [428]. Interestingly, there is a positive correlation between follicular P and MEL concentrations [474]. Elevated concentrations of MEL in preovulatory follicle may be involved in P production, resulting in luteinization and ovulation.

Activation of the LH receptor in follicular cells by the preovulatory LH surge causes ovulation and rapidly initiates a program of terminal differentiation of the ovulated follicle into a CL

through a process termed luteinization. Formation of the CL is initiated by a series of morphological and biochemical changes in cells of the theca interna and granulosa of the preovulatory follicle. Remarkably, transformation of GCs into luteal cells occurs within a few hours [475]. Not only structural changes, but genomic alterations also lead to the terminal differentiation of follicular cells into P-producing luteal cells. Progesterone receptor (PR) and cyclooxygenase 2 (COX-2) gene expression are induced after LH/hCG surge in GCs of ovulating follicles [476,477]. Ovulation is similar to a local inflammatory response [478], with both RNS and ROS being generated in this process. Both endothelial NO synthase (eNOS) and inducible NO synthase (iNOS) are present in the oocytes and thecal cells of the mouse [479]. The major source of ROS appears to be inflammatory cells including macrophages and neutrophils, as they are present in the ovary at ovulation [480,481] and they generate tremendous numbers of free radicals. These radicals act not only in the regulation of ovulation but also induce apoptosis of ovarian cells [482,483]. Thus, MEL, as well as its metabolites, acts as broad-spectrum antioxidants and exhibit free radical scavenger properties. Further, MEL concentrations in human ovarian follicular fluid (FF) obtained from the antra of Graafian follicles are significantly higher (>112 pg/ml) than those in simultaneously collected plasma samples [474]. Therefore, MEL and its metabolites quench ROS as well as RNS (see section MEL role as scavenger). Elevated MEL in preovulatory follicles is likely to protect GCs and the oocyte from free radicals that are induced during ovulation. MEL also prevents ovarian GnRH induced regression of corpus luteum [411,412,484]. Additionally, MEL's epigenetic efficacy through nuclear MEL receptor inhibits DNA methyl transferase by masking target sequences or by blocking the active site of the enzyme protects ovarian follicles [485].

In males, sperm are also protected from oxidative damage by MEL or its metabolites. Diazinon is a widely used organophosphorus pesticide that is toxic to sperm acting via free radical mechanisms. Exposure of any species to this toxin results in abnormal spermatogenesis. MEL has been found to be protective against many other free radical–generating toxic molecules [486]. In mouse, MEL treatment preserved spermatogenesis that was interrupted due to diazinon exposure [487]. Under other conditions in which testicular free radical generation is accelerated, MEL markedly reduces the level of gonadal oxidative stress [487,488]. For mammalian spermatozoa to be capable of fertilizing an oocyte, they must be capacitated and thereafter undergo an acrosome reaction and hyperactivation, which is a specialized movement of the flagellum of the sperm that permits it to penetrate the zona pellucidum. Human seminal fluid contains MEL [489] and spermatozoa reportedly possess membrane MEL receptors [490]. In hamsters, the addition of nighttime serum MEL (1 nM) resulted in significant hyperactivation of sperm within 1–2 hours [491]. This activation response was inhibited by the addition of the MTNR1A/MTNR1B MT receptor antagonist luzindole to the incubation medium, while the addition of specific MTNR1B antagonists was incapable of altering the hyperactivation response to MEL. The ability of MEL to stimulate flagellar motility is important given that this response is a requirement for the successful penetration of the zona pellucidum and fertilization of the egg. The physiological MEL concentrations in the seminal fluid of humans do not correlate with sperm motility. Further studies are needed to clarify the role of MEL in sperm activation and function in humans and other species.

MEL is also involved in myometrial cell functions. Human births occur more frequently at night when MEL levels are at their maximum than during the day. Schlabritz-Loutsevitch et al. [492] demonstrated that the two events are related and that myometrial cell possesses MEL receptors. Use of RT-PCR and *in situ* hybridization methods revealed that human myometrial cell membranes of uterine tissues have both MTNR1A and MTNR1B membrane receptors, and they are G_i protein linked to AC as in other cells. These receptors are also present in rat myometrial cells [493]. In myometrial cells collected from women during labor, the expression levels of the MTNR1B MEL receptor are markedly up-regulated. Moreover, in these cells, the actions of MEL synergize with those of oxytocin to promote muscle contractions and gap junction activity that is important in the coordination of myometrial contractions [494]. These observations are confirmed further by the study on cultured uterine cells in which the interactive effects of oxytocin and MEL are coordinated

and lead to forceful myometrial contractions that enhance successful parturition [494]. These studies demonstrate a strong role of MEL in the maintenance of the hypothalamic–pituitary–gonadal axis.

1.8.8 Oncostatic Effects

The study on the influences of MEL on cancer has a long history. Cohen et al. [495] first put forward the theory on the possible role of the pineal gland on the etiology of breast cancer and suggested that a decrease in pineal function (reduction in MEL secretion) could induce a state of relative hyperestrogenism and the early and prolonged exposure of the breast tissue to the estrogens could be involved in the etiology of breast carcinogenesis. A few years later, Tamarkin et al. [496] described a relationship between plasma MEL concentration and breast cancer. There are also low incidences of breast cancer in blind women [497,498]. Subsequent studies have shown reduced levels of MEL in patients with certain types of cancers compared with normal healthy people of the same age [499–501]. The nighttime plasma MEL levels are lower in women with estrogen receptor–positive breast cancer than in estrogen receptor negative breast cancer and even lower than in healthy control women. Notably, women with the lowest peak MEL concentrations have the highest concentrations of estrogen receptors [496]. These oncostatic effects of MEL are evident further from the study showing consequent reduction of hormones such as prolactin and to a large extent estradiol, which are responsible for normal and pathological growth of the mammary epithelium [502]. Later, it has been demonstrated that MEL has antiproliferative effect through receptors MT_1 and MT_2 on various types of cancers including breast, lung, metastatic renal cell carcinoma, hepatocellular carcinoma, brain metastases from solid tumors, ovarian carcinoma, human neuroblastoma cells, bladder carcinoma, and erythroleukemia with tumor growth [500,503,504]. In these studies, the incidence of metastases has shown physiological to pharmacological effects of MEL. The antiproliferative effects of MEL are related to its modulatory effects on the cell cycle. In MCF-7 human breast cancer cells, MEL, in the presence of normal serum or estradiol, has been shown to retard or block the progression of cells from G_0–G_1 into S phase [505,506]. It has been demonstrated that exposure of rats with hematomas or human breast cancer xenografts to light during each 12 hours of dark phase resulted in a dose-dependent suppression of nocturnal MEL levels in blood and a stimulation of tumor growth [503]. Similarly, the treatment of prostate cancer cells with pharmacological doses of MEL significantly reduces the number of prostate cancer cells and stopped cell cycle progression in both androgen-dependent (LNCaP) and androgen-independent (PC3) epithelial prostate cancer cells and induced cellular differentiation [507]. Thus, it is evident that MEL not only reduces cell proliferation but also their metastatic capacity. MEL, at nanomolar concentrations, increases the expression of p53 and p21WAF1 [508,509], cell surface adhesion proteins (E-cadherin and β-integrin [510], and increases gap junctional intercellular communication between adjacent epithelial cells [511].

It has been demonstrated further that MEL through the action on the neuroendocrine reproductive axis may down-regulate the expression of estrogen receptor α (ER-α) and finally inhibits the binding of estradiol–ER complex to the estrogen response element (ERE) on DNA [506,512], and these effects depend on its binding to a high-affinity membrane-bound receptor coupled to G_i proteins [235,513,514]. MEL also shifts forskolin- and estrogen-induced elevation of cAMP levels by 57% and 45%, respectively, thereby affecting signal transduction mechanisms in human breast cancer cells [515]. cAMP and other protein kinase activators have been documented to synergize with steroid hormone–occupied receptors, leading to enhanced ER-mediated transcription through phosphorylation of the ER or associated transcription factors [516]. Estrogen-activated AC markedly increases the concentration of cAMP in ER-responsive breast cancer cells in culture in a manner that does not require new mRNA or protein synthesis, and is mediated by a high-affinity hormone binder (possibly ER). MEL, after its binding to $MT_{1/2}$ receptors, inhibits the AC and decreases cAMP, thus counteracting the effects of estrogens [508].

Another possible link between MEL and the estrogen signaling pathway is calmodulin (CaM). ER-α (not ER-β) has a CaM binding site and interacts with CaM [517]. The binding of CaM to ER-α stimulates the phosphorylation of the receptor, thus facilitating the binding of the estrogen as well as the binding of the estradiol–ER complex to the ERE [517,518]. MEL modulates the Ca^{2+}/CaM signaling pathway either by changing the intracellular Ca^{2+} concentration via activation of its G-protein coupled $MT_{1/2}$ receptors or through a direct interaction with CaM [218,519]. This is an antiestrogenic effect of MEL [506].

MEL treatment also showed MT_1/MT_2-dependent inhibition of uptake of fatty acids in general and of linoleic acid in particular, thereby preventing the formation of its mitogenic metabolite, 13-hydroxyoctadecadienonic acid [250]. At the same time, MEL also inhibits the fatty acid growth-factor uptake by cancer cells, inhibits telomerase activity by reducing telomere length, which causes apoptosis in cancer cells, inhibits endothelin-1 synthesis, an angiogenic factor that promotes blood vessel growth in tumors and finally modulates the expression of tumor suppressor gene, *TP53*, or inhibit transcriptional expression of cyclin D1. The action of MEL at different levels of signaling pathways in a tumor cell collectively promotes the idea that MEL may be considered as a supportive anticancer drug in the prevention and treatment of cancer, but the exact mechanisms of MEL action on cancer remain unknown.

1.9 MEL AS DIETARY SUPPLEMENT

Available literature providing evidence that various age-related or different physiological changes may be modulated by the decrease in MEL led few researchers and physician to suggest a beneficiary role of MEL as a dietary supplement against general age-related deterioration of health [520]. Degenerative conditions such as Alzheimer's disease and deterioration of cognitive function and behavior are strongly associated with low levels of MEL [311]. An obvious outcome of such observations is the suggestion that the required amount of MEL can be obtained from various plant sources (root, leaves, fruits, and seeds) in different species [19] (Table 1.5). It is expected that an efficient uptake of MEL from food should influence plasma MEL concentration, which is basically very high at night and is below the level of 10pg/ml during the day [22].

1.10 PHARMACOLOGICAL STRATEGIES IN USING MEL AS A THERAPEUTIC AGENT

Even though the MEL is a pleiotropic molecule that mediates the induction of a wide variety of physiological processes, a major constrain in the therapeutic use of MEL is its short half-life. Thus, pharmacological strategies need to be developed to overcome this shortcoming. The most effective tool of such development may be the strategy of slow-release of MEL preparations or the use of a MEL analog with a longer half-life ($t_{1/2}$ = 8–10 hours) than endogenous MEL, which might have greater effect on melatoninergic receptors in the SCN and other regions of the brain and can mimic the physiological profile with improvement in sleep quality, daytime alertness, sleep-onset latency, and general quality of life [311,532]. In the current scenario, there are only two applications for an improved therapeutic use of MEL: agomelatine (Valdoxan®, Melitor®), for the treatment of depression [533], and ramelteon (Rozerem®), approved by the Food and Drug Administration (FDA) for the treatment of primary chronic insomnia characterized by difficulty with sleep onset [534]. In addition, at present, phase II and III studies on tasimelteon (VEC-162, a high-affinity agonist of human MT_1 and MT_2 receptors) are in progress to demonstrate that the drug may have therapeutic potential for transient insomnia in circadian rhythm sleep disorders in general and for improved sleep latency, sleep efficiency, and sleep maintenance in particular [535]. These MEL agonists appear to be the most successful in clinical applications in chronobiology to date.

A more targeted therapeutic use of MEL is attempted in the form of ramelteon, which is synthesized by Takeda Chemical Industrial Ltd. (Osaka, Japan). Ramelteon has a very high affinity for

TABLE 1.5
Sources of MEL in Plants, Which May Be Used for Humans as Dietary Supplement

Source	MEL Content (pg/g)	References
White mustard seed	189,000	Manchester et al., 2000 [521]
Black mustard seed	129,000	Manchester et al., 2000 [521]
Turmeric	120,000	Chen et al., 2003 [522]
Wolf berry seed	103,000	Manchester et al., 2000 [521]
Fenugreek seed	43,000	Manchester et al., 2000 [521]
Almond seed	39,000	Manchester et al., 2000 [521]
Sunflower seed	29,000	Manchester et al., 2000 [521]
Fennel seed	28,000	Manchester et al., 2000 [521]
Alfalfa seed	16,000	Manchester et al., 2000 [521]
Green cadamone seed	15,000	Manchester et al., 2000 [521]
Tart cherries	2060–13,460	Burkhardt et al., 2001 [523]
Fax seed	12,000	Manchester et al., 2000 [521]
Anise seed	7,000	Manchester et al., 2000 [521]
Coriander seed	7,000	Manchester et al., 2000 [521]
Celery seed	7,000	Manchester et al., 2000 [521]
Poppy seed	6,000	Manchester et al., 2000 [521]
Tall fescue	5,288	Hattori et al., 1995 [524]
Walnuts	3,500	Reiter et al., 2005a,b [525,526]
Milk thistle seed	2,000	Manchester et al., 2000 [521]
Oat	1,796	Hattori et al., 1995 [524]
Sweet corn	1,366	Hattori et al., 1995 [524]
Tomato	1,067–1399	Pape and Luning, 2006 [527]
Rice	1,006	Hattori et al., 1995 [524]
Grape skin	5–965	Iriti et al., 2006 [528]
Japanese radish	657	Hattori et al., 1995 [524]
Japanese ashitaba	624	Hattori et al., 1995 [524]
Ginger	584	Hattori et al., 1995 [524]
Banana	466	Dubbels et al., 1995 [529]
Chungiku	417	Hattori et al., 1995 [524]
Barley	378	Hattori et al., 1995 [524]
Wheat	125	Hernandez-Ruiz et al., 2005 [530]
Olive oil	71–119 (pg/ml)	De La Puetra et al., 2007 [531]
Chinese cabbage	113	Hattori et al., 1995 [524]
Cabbage	107	Hattori et al., 1995 [524]
Welsh onion	86	Hattori et al., 1995 [524]
Cucumber	86	Dubbels et al., 1995 [529]
Carrot	55	Hattori et al., 1995 [524]
Tarro	55	Hattori et al., 1995 [524]
Japanese butterbur	50	Hattori et al., 1995 [524]
Apple	48	Hattori et al., 1995 [524]
Indian spinach	39	Hattori et al., 1995 [524]
Pineapple	36	Hattori et al., 1995 [524]
Canary grass	27	Hernandez-Ruiz et al., 2005 [530]
Strawberry	12	Hattori et al., 1995 [524]
Asparagus	10	Hattori et al., 1995 [524]

human MT_1 and MT_2 receptors, and a negligible affinity for MT_3 binding sites and for a large number of other receptors, including NA, GABA, glutamate, serotonin, histamine, acetylcholine, dopamine, and opioid receptors [207]. The half-life of circulating ramelteon is in the range of 1–2 hours, which is much longer than that of endogenous human MEL [536]. Ramelteon is metabolized in the liver by hepatic cytochrome p450 monooxygenases (CYP1A2, CYP2C, and CYP3A isoforms). Four distinct metabolites (M-I, M-II, M-III, and M-IV) of ramelteon are formed [536]. Among these, the metabolite M-II exerts a selective action on MT_1 and MT_2 as does the parent compound, but with an affinity of only 10% of ramelteon itself. Since, the metabolite M-II circulates at much higher concentrations than ramelteon resulting in a 20- to 100-fold greater mean systemic exposure, it is likely to most effectively contribute to biological action of the drug [536]. Ramelteon does not appear to significantly alter sleep architecture [537]. It reduces the evening circadian arousal signal and thus enhances the ability to fall asleep and stay asleep during the early part of the night [538,539]. The improvement in sleep-onset latency with ramelteon treatment is similar to that of MEL; however, ramelteon does not improve the patient's perceived sleep quality and next-day performance compared with placebo [534]. Ramelteon shows no evidence of accumulation after multiple dosing [536] and does not produce next-day residual effects [537]. It may be used at a dose of 8 mg at night as an approval of FDA for long-term use in adults. In contrast to commonly uses hypnotic drugs, ramelteon lacks abuse liability and does not impair motor and cognitive function. Ramelteon also has benign side effect profile, but some hormonal changes such as reduced testosterone have been reported [539].

Agomelatine is another pharmaceutical agent that is a potent and novel antidepressant agonist of MEL MT_1 and MT_2 receptors [540] and an antagonist of the serotonin 5-HT2c receptor subtype [541] and is endowed with antidepressant properties [311,327,542–545]. Like MEL, agomelatine causes inhibition of SCN neuronal activity, but its action is more prolonged than that of MEL due to its higher binding affinity to MT_1 receptor in the SCN [546]. Clinical studies of patients with MDD have demonstrated that the symptoms of depression are significantly improved with agomelatine compared with placebo, and agomelatine appears to be quite efficacious in treating MDD as other antidepressants but with fewer adverse effects [533,543,545]. Rapid onset of improved REM sleep quality without daytime sedation is achieved with oral administration (agomelatine ~5 or 100 mg/day) together with effective antidepressant and anxiolytic activity. In addition, polysomnographic studies have shown that agomelatine reduces sleep latency, decreases waking after sleep onset, and improves sleep stability, as measured by changes in the cyclic alternating pattern [543,547,548]. No significant discontinuation symptoms are known [327]. Agomelatonin has proven antidepressant effects with rapid efficacy in improving subjective sleep compared with several commonly used antidepressants [327,543]. Chronic treatment with agomelatonin leads to increased cell proliferation and neurogenesis in the ventral dentate gyrus in animals, suggesting similar effects in humans [327]. Agomelatonin has a strong restoring effect on the disrupted circadian rhythm in SAD [312].

1.11 CONCLUSIONS

MEL, a tiny tryptophan derivative in the bovine pineal extract discovered by a dermatologist just 53 years back, drew first scientific attention for its ability of blanching the skin in tadpole. Since then, the data already gathered from carefully controlled studies in a large number of animals and even plants have clearly implicated this wonder molecule in the control mechanisms of a wide variety of physiological and psychological activities of human beings. As an obvious outcome, MEL is now considered as a potent candidate for therapeutic use in the treatment of a diverse range of diseases mostly because of its recognition as a chronobiotic pleiotrophic molecule with multifaceted effects. Convincing reports do suggest that MEL causes modulation of body functions at various levels of hierarchy. The daily rhythm in MEL may have synchronized all physiological functions. But information on MEL synthesis in extrapineal sites at low or almost no circadian dynamics also indicate its additional noncircadian functions by the formation of bioactive metabolites and/or by

specific subcellular actions. Although the roles and signaling mechanisms of tissue MEL are poorly understood to date, it seems that the coexistence of endocrine, paracrine, autocrine, and intracrine actions of MEL goes beyond local feedback effects common to other hormones, which are mostly mediated by the same receptors, or variants of them, as found in the respective target organs [276]. Additional binding sites different from membrane receptors may be of importance particularly for extrapineal MEL. As a whole, MEL displays a remarkable contextual diversity of functions, reaching from the control of circadian pacemakers and hypothalamic/pituitary axes to vasomotor effects and exhibits various facets of immunomodulation, antioxidant actions (expression of genes relevant to redox metabolism), direct and indirect antiapoptotic effects, interference with NO signaling, other antiexcitatory actions via ion channels and neurotransmitter systems, and modulation of mitochondrial electron flux. Its efficacy and safety may eventually drive its use in universally effective clinical applications and an adjuvant therapy for future treatment of different diseases as a supportive molecule to act together with other medicine. Moreover, melatoninergic agonists have a longer half-life than MEL and are widely useful in the modulation of sleep and depression.

In some countries (United States, China, Argentina, Poland), MEL is sold as a dietary supplement in health food and grocery stores/drug stores, but not as a drug, since all potential risks and/or advantages of MEL are yet to be clearly known. The effects of MEL at clinically relevant concentrations or under pathological situations are also required to be demonstrated in appropriate studies. Further detailed clinical investigation of the crosstalk and transactivation of different pathways would certainly help in understanding the mechanisms of action of MEL as a drug, allowing the design of powerful therapeutic agents for pathophysiological healing. Collectively, the current state of knowledge extends strong support to the contention of Ebadi et al. in 1989 [549] that, "the research for and discovery of how MEL, with its apparent omnipotent effects, brings forth a wide range of functions may raise the exciting prospect of providing new avenues of treating numerous diseases, thus replacing old treatments which sustain life but diminish its quality."

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