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Aeromedical Aspects of Melatonin–An Overview

Donald C. Sanders
Arvind K. Chaturvedi
Jerry R. Hordinsky

Civil Aeromedical Institute
Federal Aviation Administration
Oklahoma City, OK 73125

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16. Abstract

Melatonin, a pineal hormone present in the blood of humans and other species, has a distinct diurnal variation in its biosynthesis and, therefore, in its concentration. This variation has suggested the possibility of a regulatory function in day/night-dependent physiological processes, such as sleep, and has led scientists to explore the effects of administered melatonin on the modulation of circadian rhythms. For the self-treatment of sleep disorders and other benefits, melatonin usage has been extolled to the extent that 20 million new consumers were added to the U.S. retail market in 1995. Its principal aeromedical application has been in the experimental treatment of jet lag effects. For aircraft passengers, melatonin administration at destination-bedtime appears to improve sleep quality and to decrease the time required to reestablish normal circadian rhythms. For international aircrews, who travel through multiple time zones without time to adapt to new environments, taking melatonin prior to arriving home may further impair already disturbed circadian rhythms. Its use to adjust to shiftwork changes by air traffic controllers, aircraft maintenance workers, and support personnel is even more controversial. Limited studies suggest that giving this hormone to shift workers should be done only under controlled conditions and that taking it at the wrong time may actually impair job performance. Because of its possible interaction with certain medications and the changes in its concentrations observed in some clinical conditions, the practitioner must exercise caution during the medical certification of airmen. The variations in the concentration of melatonin can be effectively determined by radioimmunoassay, high-performance liquid chromatography, and gas chromatography-mass spectroscopy analytical techniques. These techniques are capable of measuring the human daytime (10 pg/ml) and nighttime (30-120 pg/ml) melatonin in plasma/serum. Melatonin measurements in victims of accidental deaths may allow forensic scientists and accident investigators to utilize the relationship between its concentration and the time of day when death occurred. The most accurate estimations of the time of death result from analysis of melatonin content of the whole pineal body, whereas less accurate estimates are obtained from serum and urine analyses—pineal levels of melatonin are unlikely to be altered by exogenous melatonin, but its blood and urine levels would change. Its high blood levels in a daytime crash victim would suggest exogenous supplementation. The possible interfering effects of postmortem biochemical processes on melatonin concentrations in whole blood and in other tissues are not well understood, and there is a need for the continuing research into melatonin's chronobiological properties to define its proper applications and limitations. The indiscriminate use of melatonin by aviation professionals may pose unacceptable safety risks for air travel.

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AEROMEDICAL ASPECTS OF MELATONIN—AN OVERVIEW

INTRODUCTION

Confronted with the widespread use of melatonin for the self-treatment of sleep disorders and other alleged health benefits, scientists have voiced their concerns by urging the National Institutes of Health to support clinical trials to determine its efficacy and long-term safety. The extent of melatonin use was illustrated by the 20 million new U.S. consumers in 1995 and by an estimated U.S. retail market of $200 million to $350 million annually (Lamberg, 1996; Rawls, 1996). The increasing use of melatonin to promote sleep and to minimize jet lag effects stems from enthusiastic and sometimes extravagant claims in the media and in popular books, while the inability to patent this endogenously-produced neurohormone has tended to limit the amount of support and incentive for pharmaceutical research. Since this substance occurs naturally in some foods, it can be sold in the U.S. as a dietary supplement without premarket approval from the Food and Drug Administration, in contrast to its formal banning of sale by some European governments (Moody, 1996). Indeed, this is one of the few biologically-active chemicals on which basic animal research has been more extensive than clinical research (Redman et al., 1983; Tamarkin et al., 1985; Pang et al., 1990; Lerchl and Schlatt, 1992; Lamberg, 1996).

Saper (1996), of the Harvard Medical School, felt that individuals taking melatonin risk potentially dangerous long term effects, which have not been adequately studied. This author relates melatonin self-treatment to a similar fad involving "food supplement" tryptophan several years ago, where a batch of the unregulated amino acid contained a contaminant that caused neuromuscular damage. Since melatonin is not approved as a therapeutic agent, there is no standardization of dosage and no certification of the quality of commercial preparations.

Although the Federal Aviation Administration (FAA) offers no formal guidance to pilots or to their medical practitioners, the use of melatonin is cautioned (Aerospace Medical Association, 1996). Collectively, the lay press implies a fairly active interest in the use of melatonin by aviation workers and air travelers, but surveys of such use by pilots and passengers have not been documented. Two anonymous surveys conducted during the early 1997 Aircraft Cabin Safety Workshops for Flight Attendants at the FAA's Civil Aeromedical Institute in Oklahoma City, Oklahoma, revealed a fairly low actual use of melatonin during the preceding 3 months (3 of 40; 2 of 22). However, a seasoned business traveler, K. E. Grubbs, Jr., Editor-in-Chief of *World Trade*, is quoted in its May 1997 edition: "...As we pass the melatonin, hope for more and cheaper supersonic passenger planes, and pray for closer-to-home international airports, we should ponder for a moment...." Perhaps the editor speaks for a large segment of the traveling population who have compelling reasons to feel that melatonin use is an option in grueling travel settings.

Since melatonin usage by aviation professionals can reasonably be expected to follow general population trends, it is prudent to consider the related safety and side-effects, including any residual attention deficits in persons charged with aviation safety. Therefore, issues of jet lag, shift work, and medical certification are emphasized under clinical perspectives of this article. Melatonin's biochemical pharmacology, analytical methods, and postmortem toxicology are also provided as background information potentially needed by clinicians and aircraft accident investigators. The more general aspects of its use in humans are discussed in several extensive review articles available in the literature (Miles and Philbrick, 1987; Arendt, 1988; Claustrat et al., 1990; Webb and Puig-Domingo, 1995; Lamberg, 1996; Brzezinski, 1997).

CHEMISTRY, BIOSYNTHESIS, AND METABOLISM

Lerner et al. (1958) first isolated a few micrograms of *N*-acetyl-5-methoxytryptamine from kilogram quantities of bovine pineal glands and named it melatonin because of its biological activity on the melanocytes in frog skin. It is a pale yellow solid, melting at 116-118°C (Budavari, 1989) and has been synthesized from 5-methoxyindole (Szmuszkovicz et al., 1960). Melatonin was originally thought to be biosynthesized only in the pineal gland, but subsequent

studies have shown that it is also produced in the retina, Harderian glands, and gastrointestinal tract (Huether, 1994). The rhythmic nature of the synthesis and secretion of pineal melatonin are controlled by the light-dark environment, acting through the hypothalamic suprachiasmatic nuclei (SCN). These light-dark signals are transmitted by a neural pathway from the retina, through the SCN, preganglionic neurones of the upper thoracic spinal cord, and postganglionic sympathetic fibers from the superior cervical ganglia, to the pineal gland (Webb and Puig-Domingo, 1995). Thus, the pineal melatonin synthesis is inhibited by light and stimulated by darkness, and it is independent of sleep (Deacon et al., 1993; Miyauchi, 1993; Webb and Puig-Domingo, 1995). The gastrointestinal tract contributions to circulating melatonin are more likely to be affected by food intake (tryptophan availability) and nutritional state (Huether, 1994).

As shown in Fig. 1, starting from the amino acid tryptophan, melatonin biosynthesis occurs in four basic steps (Miles and Philbrick, 1987; Claustrat et al., 1990). The major regulating step in the synthesis is the *N*-acetyltransferase-mediated conversion of serotonin to *N*-acetylserotonin, the step inhibited by light and stimulated by darkness, thereby creating a marked rhythm in the pineal output. It is generally conceded that the direct secretion into blood is the major route for the transport of endogenous melatonin to its targets. There does not appear to be any storage site, so the circulating melatonin levels essentially parallel pineal activity. Studies of pinealectomized animals indicate that the extrapineal sources of melatonin synthesis may be able to maintain the relatively low daytime levels in circulation, but the expected nighttime elevations are much lower (Huether, 1994).

Melatonin is rapidly metabolized in the liver to 6-hydroxymelatonin, which is then conjugated as 60-70% sulfate and 20-30% glucuronide (Webb and Puig-Domingo, 1995). Circulating plasma melatonin has a relatively short half-life, with about 90% being cleared during a single hepatic passage (Huether, 1994). A small fraction is biotransformed into N^{γ}-acetyl-5-methoxykynurenamine in the brain (Hirata et al., 1974). These metabolic routes are outlined in Fig. 2. The 24-hour urinary excretion of conjugated 6-hydroxymelatonin (6-sulfatoxymelatonin) has been correlated with the corresponding 24-hour plasma melatonin profile (Arendt et al, 1985; Markey et al., 1985; Bojkowski et al., 1987), thus providing a non-invasive method for measuring pineal function in normal subjects. However, the metabolic clearance rate of plasma melatonin in persons with liver dysfunction is much reduced (Iguchi et al., 1982a).

PHARMACOLOGY

Normal melatonin secretion patterns display a distinct circadian rhythm resulting in very low daytime serum concentrations (10 pg/ml) and nocturnal levels that can range from 30 to 120 pg/ml (Iguchi et al., 1982b; Bojkowski et al., 1987; Miles and Philbrick, 1987; Claustrat et al., 1990). Salivary melatonin levels range from 27 to 32% of the corresponding plasma levels (Miles et al., 1987). Secretion patterns also display seasonal phase delays with the evening melatonin rise in winter being delayed approximately 1.5 hours relative to that in the summer (Illnerová et al., 1985; Arendt, 1988). Pineal melatonin secretion is highest in young children, decreases in adults, and continues to decrease with age, where little melatonin secretion can be observed. Iguchi et al. (1982b) found an approximately linear correlation for daytime serum levels from 10 pg/ml down to 2 pg/ml in 81 human subjects from 1 to 92 years old. During the same study, they compared nighttime peak serum melatonin levels for two age groups (26 and 84 years) and found 83 and 12 pg/ml, respectively.

The relationship between circulating melatonin levels and light-dark cycles led to research directed toward the therapeutic administration of melatonin to treat certain sleep disorders (James et al., 1990; Waldhauser et al., 1990; Dollins et al., 1994; Jan and Espezel, 1995). Early studies varied considerably in the dosage of the hormone and its time of administration, factors now known to be critical in the interpretation of the observed response (Lewy and Sack, 1993). High oral dosages (100 mg) of crystalline melatonin have been well tolerated in humans (Cagnacci et al., 1991). Doses as large as 300 mg/day for 30 days were given to subjects in one study of pituitary-ovarian function (Voordouw et al., 1992). Pharmacokinetic studies reveal that serum melatonin levels return to the basal level within 4 hours after a 2-mg oral dose and have a mean elimination half-life of 0.54 to 0.67 hour (Dollins et al., 1994; Aldous et al., 1985). After an 80-mg dose, the level increased from 17 pg/ml (basal level) to 25800 pg/ml within 1 hour and decreased to 203 pg/ml in 10 hours (Waldhauser et al., 1990). While large differences in its interindividual absorption exist, representative examples

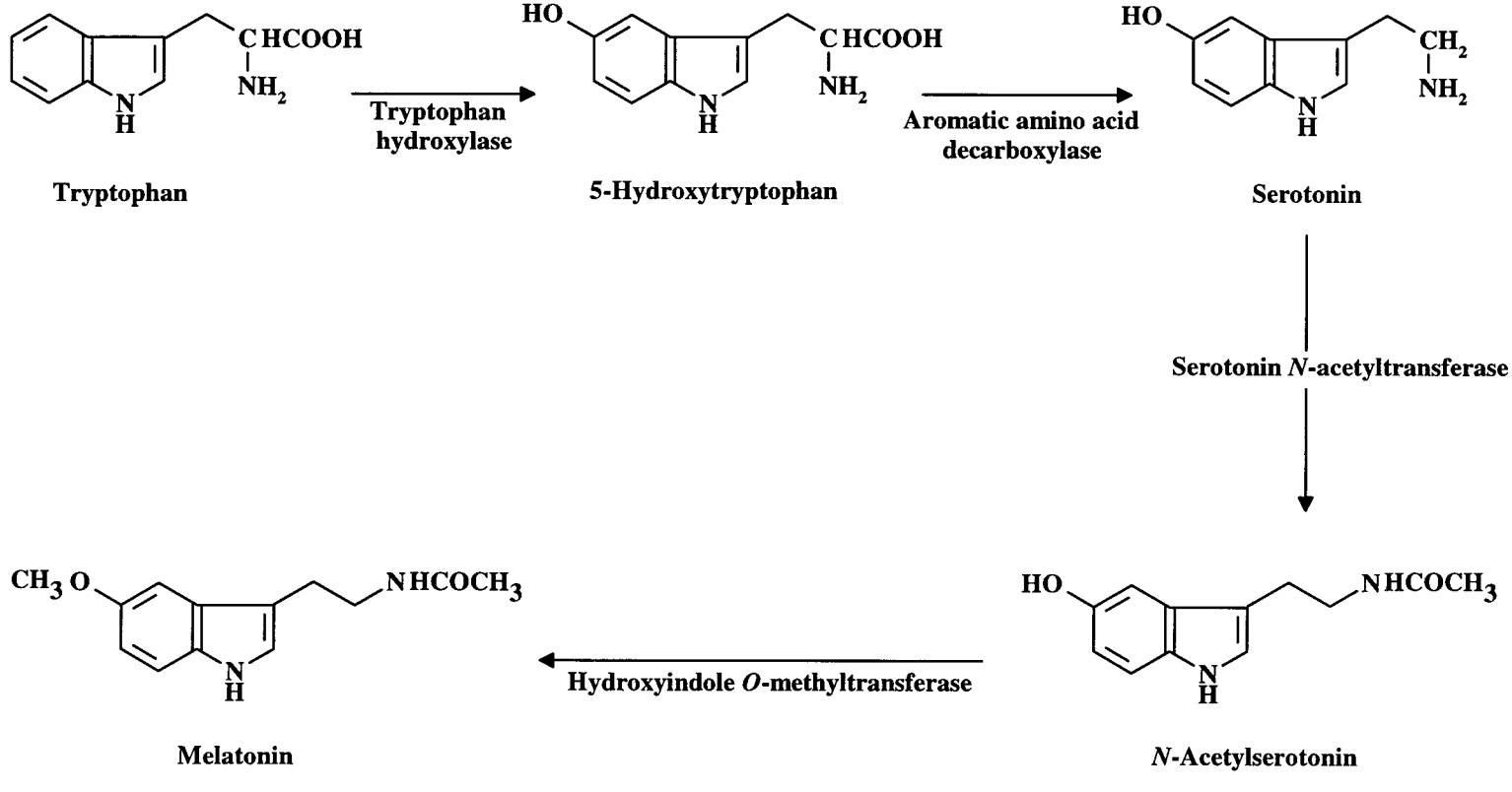

Fig. 1. Bioformation of melatonin.

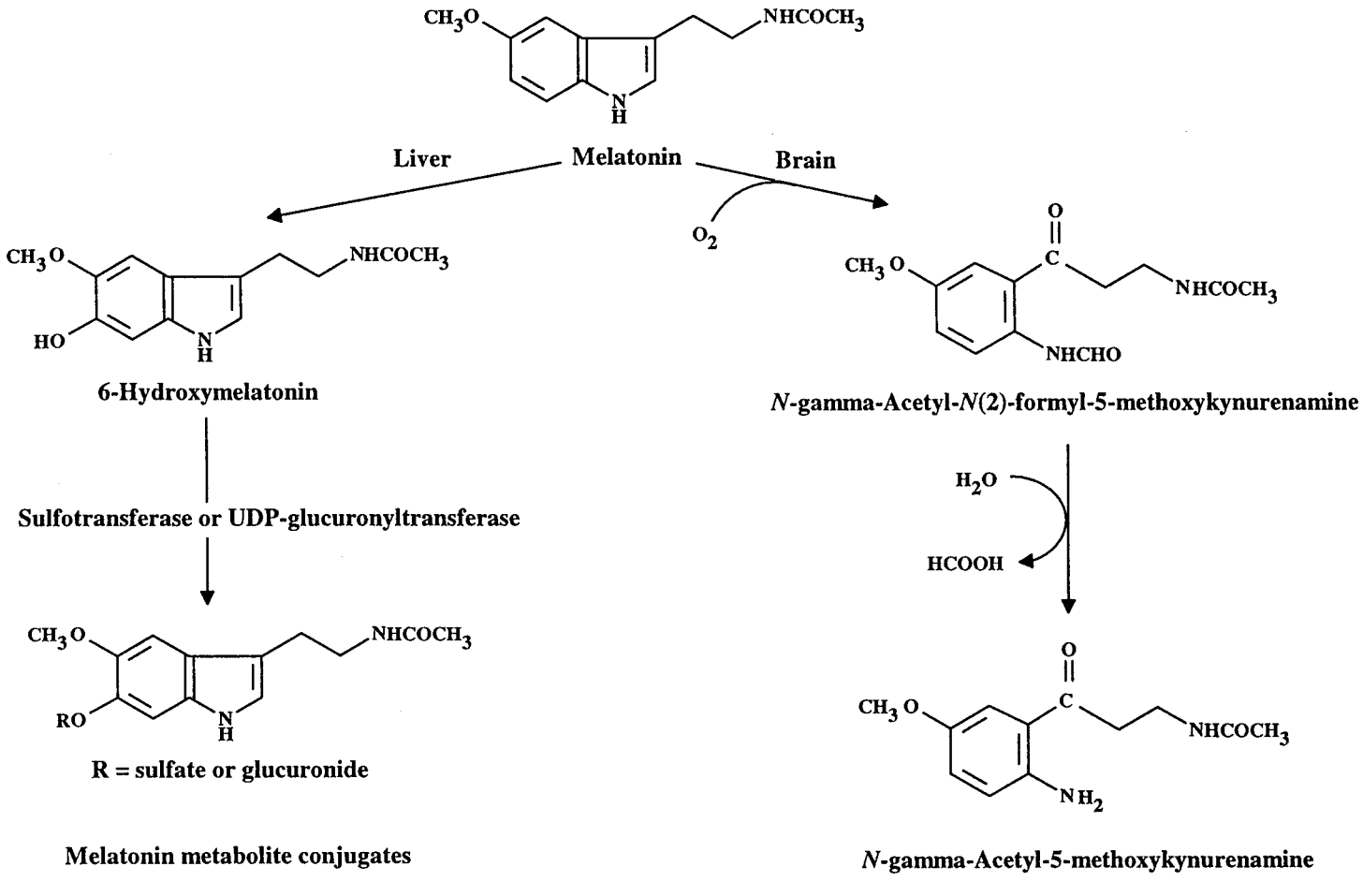

Fig. 2. Biotransformation of melatonin.

of dose/1-hour serum levels are 0.1mg/50 pg/ml, 0.3 mg/120 pg/ml, 1 mg/400 pg/ml, 2 mg/1900 pg/ml, 10 mg/6300 pg/ml, and 80 mg/25800 pg/ml (Dollins et al., 1994; Aldous et al., 1985; Waldhauser et al., 1990). Unlike the sustained blood levels observed from endogenous release, oral doses produce a rapid increase in blood concentration followed by a rapid decrease.

Experimental sleep laboratory investigations indicate that melatonin exerts some sedative and hypnotic effects by accelerating sleep initiation and improving sleep maintenance, without hangover problems on the following morning (Waldhauser et al., 1990). When 2 to10 mg doses were given at bedtime to children suffering from chronic sleep disorders, researchers reported that melatonin administration fully or partially corrected the sleep-wake cycle (Jan and Espezel, 1995). However, similar doses given to adult patients with insomnia proved ineffective (James et al., 1990). Daytime administration of 0.1 to 0.3 mg generated peak serum melatonin concentrations within the normal nocturnal ranges of untreated people. These and higher doses produce measurable hypnotic effects, independent of the circadian time signal synchronizing action. The development of a parenteral sustained release pharmaceutical preparation producing an extended plasma melatonin plateau at physiological levels may define whether it acts primarily as a hypnotic or actually speeds up resynchronization of circadian rhythms (Claustrat et al., 1992). A submucosal patch as described by Bénès et al. (1993) can simulate the rise, plateau, and decrease of endogenous plasma melatonin, providing a near-physiological delivery system for future research.

Animal studies continue to reveal melatonin effects worthy of further study in their applications to humans. Melatonin treatment appears to decrease brain serotonin release leading to hypotension and bradycardia in rats (Chuang et al., 1993). It has also been shown to exaggerate the development of collagen-induced arthritis in mice (Hansson et al., 1992) and to reduce *in vitro* insulin secretion from rat pancreas (Atkins et al., 1973). Indications of melatonin's function as a neuroimmunoregulatory and anti-stress hormone in mice were noted in a study by Pierpaoli and Maestroni (1987). Recent studies by Reppert at the Harvard Medical School (cited by Barinaga, 1997) indicate that melatonin's principal action may be the suppression of SCN neuron activity. Different receptors in mouse brain have been identified for melatonin's SCN-inhibition and phase-shifting activities. Since the resultant phase shifts are short (< 1 hr), it appears that melatonin's sleep-inducing effects are caused by its SCN-suppression activity.

CLINICAL PERSPECTIVES

The medical practitioner must interpret melatonin use in the context of varied user expectations. Some users have been inspired by the claims that melatonin is a "magic bullet" that reverses the aging process, helps in the treatment of cancer, improves sex, activates the body's immune response, and cures sleep disorders (Pierpaoli and Maestroni 1987; Hearn, 1995; Pierpaoli et al., 1995; Dille 1996; Lamberg 1996). Although this hormone is ostensibly the most potent known hydroxyl radical scavenger (Poeggeler et al., 1994; Reiter, 1995a, b), and a prominent theory attributes the rate of aging to accumulated free radical damage especially in DNA, such dramatic protection has only been demonstrated in rodents (Tan et al., 1993, 1994). Its anti-cancer activity is based on laboratory reports of the inhibition of mitosis in tumor growth and, conversely, on the metastasis-enhancing effects of pinealectomy (Miles and Philbrick, 1987). Clinical studies have been mostly on patients with advanced cancer, where melatonin was given in large (20 to 40 mg/day) doses in combination with radiotherapy or chemotherapy. Some of the preliminary results have been encouraging, but confirmation is needed with larger groups observed over a longer period of time (Brzezinski, 1997). Similarly, claims for improved sex, enhanced immune response, or a cure-all for sleep disorders have not been scientifically validated through clinical trials in humans. Whether or not these claims prove valid, the indiscriminate use of melatonin poses a fundamental safety issue in air travel, based on its known sedative/hypnotic effects. Therefore, specific clinical studies and issues related to jet lag, shift work, and airman medical certification are discussed in the following subsections.

Jet Lag

It is not surprising that melatonin, with its promising ability to shift circadian rhythms, might be considered for the treatment of diurnal rhythm disruptions caused by travel through time zones. Strughold (1952) described this phenomenon in terms of the physiological readiness of rapidly-transported military troops and in the treatment adjustment for

air-evacuated casualties. Certainly, the advent of commercial jet aircraft has made it possible to transport passengers across multiple time zones to areas where the day-night cycle is different from the cycle that existed at takeoff. This rapid travel has lead to "jet lag," which is generally described as transient insomnia, degraded performance, and a general lack of well being. Jet lag is attributed to lack of sleep and to disturbed 24-hour rhythmic functions. It is usually more severe in eastward flights, where the night period occurs before the subject's normal bedtime, than on westward flights, where the day period is extended and sleep is delayed. Arendt and Marks (1983) first suggested that melatonin might hasten the resynchronization of human 24-hour rhythms after time zone changes. Arendt and co-workers (1986, 1987) performed a double-blind study on 17 human subjects who flew from London to San Francisco, remained 14 days in San Francisco, and returned to London. Daily doses of 3 mg of melatonin were taken at 1800 hours local time for 3 days before the return (eastward) flight and for 4 days after returning. Jet lag was considered to be significantly less severe in the melatonin-treated group, with improvements in sleep quality, decreased sleep latency, and more rapid resynchronization of endogenous melatonin and cortisol rhythms.

Subsequently, Petrie et al. (1989) reported a study of 20 subjects flown from Auckland, New Zealand, to London (eastward) and returning after a 3-week interval. All subjects took 5 mg of melatonin daily (or a placebo) between 1000 and 1200 hours for 3 days before the flight, once during the flight, and daily between 2200 and 2400 hours (destination time) for 3 days after arrival. Effects were determined by visual analog ratings of jet lag and tiredness, by a profile from a moods states questionnaire for the measurement of vigor-activity and fatigue-inertia, and by a retrospective rating of jet lag on Day 10 after arrival. Subjects taking melatonin reported less jet lag and took less time to recover than the controls from the shifts across 12 time zones in both directions.

A double-blind study with 61 subjects, flying from the UK to Australia/New Zealand and return, indicated that melatonin treatment significantly improved self-rated jet lag (Skene et al., 1989). On the eastward flight, subjects took 5 mg of melatonin daily at a local time corresponding to 0200 hours at the destination time zone for 2 days before arrival, then daily for 4 days after arrival at local bedtime. On the return flight (westward), 5 mg of melatonin or a placebo was taken daily at the local bedtime for 4 days after arrival. Evening tiredness was only significantly increased after the 4 days of administration, suggesting an effect on timing mechanisms of fatigue instead of the pharmacological hypnotic effect. Infrequent side effects included drowsiness, headache, and nausea.

A simplified protocol was used by Claustrat et al. (1992) for a study wherein 40 volunteers were selected for their sensitivity to eastward jet lag. With no predeparture treatment, each subject took 8 mg of melatonin (or placebo) at 2200 hours on the day of the nocturnal flight (USA to France) and for 3 consecutive days between 2200 and 2300 hours in France. Thirty self-rating questionnaires, completed on Day 7 after return, rated melatonin treatment ($n = 15$) at the median of efficiency scores (visual analogue scale) of 73, whereas the median of 48 was observed with the placebo-treated subjects ($n = 15$). Two cases of side effects were related to the melatonin's hypnotic activity. A single case of tachycardia and 2 cases of "heavy head" were considered to be of minor consequence.

Unlike passengers, international aircrews are commonly required to travel through a number of time zones without time to adapt to the new environment before continuing to the next sector. The resulting circadian desynchronization can result in performance decrements and could possibly compromise the safety of airline operations. The crews work during flight, making them less able than the passengers to alter their sleep and rest patterns. Melatonin administration to aircrews prior to their arrival at home might simply add a confusing cue to an already disturbed circadian rhythm (Petrie et al., 1993). Härmä et al. (1993) measured salivary melatonin in airline flight attendants on a 4-day trip over 10 time zones and found that subjects had disturbed melatonin secretion for 7-8 days in connection with each transmeridian flight. Petrie et al. (1993) evaluated the timing of melatonin dosage in 52 members of an Air New Zealand cabin crew on a 9-day duty schedule flying from Auckland to Los Angeles to London and returning by the same route. In the study, Group 1 received 5 mg of melatonin on each of 3 days prior to returning to New Zealand (0700-0800 Los Angeles time) and on each of 5 days at home (2200-2400 New Zealand time). Group 2 received placebo during the preflight and flight periods and were given melatonin only after arrival in New Zealand. Group 3 received only placebo. The group receiving melatonin only following

the flight (Group 2) showed significantly lower visual analogue ratings of jet lag, compared with the "early" melatonin or placebo group (Group 1 or 3). In these aircrews, melatonin taken prior to returning home resulted in worse adjustment. These authors suggest that melatonin administration before arrival does not re-entrain the disrupted circadian rhythm. In a less formal study, a research group in Italy confirmed that prior treatment was unnecessary and that a single 5-mg melatonin dose on the evening of arrival was adequate to prevent the classic symptoms of jet lag (Lino et al., 1993).

The efficacy of melatonin in preventing sleep loss and cognitive degradation after travel across 8 time zones was recently tested in a military study of U.S. Army personnel (29 male aircrew) deployed on a training mission to the Middle East (Comperatore et al., 1996). Melatonin was given orally in dosages of 10 mg/day for 3 days pre-travel, once during travel, and for 5 days after arrival. The melatonin-treated subjects exhibited fewer post-deployment errors in both dual-vigilance and reaction time tasks than their placebo-treated counterparts and were able to extend their sleep periods under noisy crowded conditions. In this and other studies, it is difficult to unequivocally determine whether the indicated improvements in overcoming jet lag effects are due to melatonin's hypnotic activity or chronobiotic property. Redfern (1992) suggests that we are probably asking too much of any "jet lag pill" to expect resynchronization, at the same rate, of all physiological functions to new time cues following phase shift and that a single pharmacological entity capable of this re-entrainment is still in the future.

Shift Work

Many of the problems manifested in jet lag are also present in shift work. The diurnal nature of humans makes it harder to remain alert at night and to sleep in the daytime, when the reversed light-dark periods provide cues that conflict with normal behavior patterns. The slow and uneven adjustment of the human internal circadian rhythm to a change in work schedule is limited by the amount to which it can adjust on a daily basis. This adjustment is also directional—that is, it is easier to postpone the sleep-wake cycle than to advance the cycle (Moline et al., 1992).

The demands of the aviation environment require round-the-clock activity of both pilots and support personnel. Pilots with evening departures need to sleep during the day at a time usually out of phase with their biological clocks. Maintenance workers and air traffic control specialists frequently work rapidly-rotating shifts (Schroeder and Goulden, 1983; Melton and Bartanowicz, 1986; Della Rocco and Cruz, 1995; 1996). Individuals employ a variety of sleep strategies to cope with the disturbances of their circadian rhythms (Sasaki et al., 1986), and concern has been expressed about melatonin's use by shift workers, citing its residual effects on mood, interest, and efficiency (Lieberman et al., 1984; Dille, 1996).

In a study of 17 volunteer police officers working 7 successive night shifts, 5 mg of melatonin, taken at the desired bedtime, increased rated sleep quality and duration relative to baseline and placebo conditions (Folkhard et al., 1993). The data also suggested increased alertness, especially in the early morning, but there was no evidence that melatonin treatment had resulted in a phase delay of the alertness rhythm. Midshift performance showed a reduced memory scanning speed and an increased perception of mental workload relative to baseline and placebo conditions, suggesting that melatonin's effects on performance may need careful evaluation. Josephine Arendt, a co-author in that 1993 study and pioneer researcher on melatonin, is cited by Lamberg (1996) as saying, "But we would not dream of giving melatonin to shift workers except in experimental conditions. Timing is critical. Taking melatonin at the wrong time may disrupt biological rhythms. Melatonin-induced sleepiness may impair driving or job performance." Thus, it would appear that the use of melatonin by shift workers to speed adjustments to new schedules will require further evaluation.

Medical Certification

The aeromedical practitioner charged with assessing the implications of melatonin use must keep in mind the related current societal applications and clinical observations, including any effects of exogenous melatonin on the endocrine system. Melatonin secretion does not change during a woman's normal menstrual cycle (Brzezinski, 1997), nor did a dose of 10 mg of melatonin to female subjects change the menstrual characteristics (Kirby et al., 1997). Acute oral doses of melatonin have been associated with stimulation of prolactin levels and some increase in growth hormone levels, but no significant effects on follicle stimulating hormone, luteinizing hormone, thyroid stimulating hormone, and testosterone

(Valcavi et al., 1987; Terzolo et al., 1991; Webb and Puig-Domingo, 1995). In a study of healthy young women over a period of four months, a 300-mg daily dose of melatonin reportedly did suppress luteinizing hormone at mid-cycle and demonstrated some ovulatory inhibition (Brzezinski, 1997). This is a very high dose and lesser doses are more likely to be selected for self-administration. Several disease states – Klinefelters syndrome, Turners syndrome, psoriasis vulgaris, spina bifida occulta, and sarcoidosis – have been associated with disrupted melatonin profiles, both in terms of rhythm and magnitude (Miles and Philbrick, 1987). Although individuals with these disorders may not make up a significant portion of the airman applicant pool, the effects of exogenous melatonin in these disease states could be presumed to differ from healthy subjects. Therefore, from a practitioner's perspective, caution and special endocrinological evaluation may be warranted in the high-dose users or in the low-dose, long-term users.

Melatonin findings in neuropsychiatric conditions are also of interest. In a review, Webb and Puig-Domingo (1995) reported 3 studies that (i) indicated increases in the cortisol/melatonin ratio and disturbed melatonin rhythms in depressed individuals (Claustrat et al., 1984), (ii) observed very low melatonin levels in chronic schizophrenics (Ferrier et al., 1982), and (iii) proposed low nighttime levels of melatonin as a trait marker for major depressive disorders (Beck-Friis et al., 1985). However, high doses of melatonin worsened moderate to severe clinical depression (Carman et al., 1976), leading to the discontinuation of the treatment. Ethanol ingestion has also been shown to inhibit nocturnal melatonin secretion (Röjdmark et al., 1993), and it has been suggested that urinary melatonin can be used as a marker of alcohol abuse (Murialdo et al., 1991).

Finally, one must reemphasize that melatonin's interactions with many clinical conditions and with prevalent medications (inclusive of those certifiable within the airman population) are scarcely documented. Also, the effects of chronic melatonin use have not been adequately studied, and additional research is needed in these areas if competent clinical evaluations are to be performed. These observations are strongly supported by the National Institutes of Health (Lamberg, 1996) and the National Institute on Aging (Rawls, 1996).

ANALYTICAL METHODS

Melatonin analytical methods vary from techniques that measure nanogram quantities in the entire pineal body to picogram concentrations in plasma and urine. Current studies on human pineal function depend heavily on plasma and urine measurements. After accounting for variations in early analytical values due to developing methodology, normal daytime human plasma melatonin levels are now considered to be less than 20 pg/ml with nighttime peak levels of 40-80 pg/ml (Arendt, 1986). Analytical techniques in current use are radioimmunoassay (RIA), high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS).

RIA methods have undergone many refinements in recent years, making this technique reliable, sensitive, and specific. A modified extraction procedure coupled with a commercial RIA kit was used to analyze melatonin in 0.1 to 5.0 ml of serum with 96-104% recovery (Sieghart et al., 1987). Sturner et al. (1990) used a single chloroform extraction of body fluids with an RIA procedure to study melatonin concentrations in Sudden Infant Death Syndrome. A significant degree of interference from calcium heparin has been reported in a study of anticoagulants and extraction techniques by Plebani and co-workers (1990). A direct saliva RIA method using ^{125}I-2-iodomelatonin was described by English et al. (1993). An enzyme-linked immunosorbent assay (ELISA) was evaluated in comparison with a routinely-used RIA method (Chegini et al., 1995). This ELISA method utilized a smaller serum volume (0.1 ml) and was technically simpler, but required a 10-fold concentration step to detect the low daytime melatonin levels. Both RIA and ELISA methods were easily able to detect melatonin in concentrations less than 10 pg/ml of suitable samples.

Compared to RIA methods, HPLC techniques offer a non-bioassay for melatonin and have been used to validate the specificity of RIA analyses. More recently, HPLC was used for the quantitative analyses of melatonin in tissue extracts (Peniston-Bird et al., 1993). Early HPLC methods utilized an electrochemical detector with its concomitant limitations on the mobile phase composition, but they possessed the advantage of shorter analysis time without requiring radioactive reagents (Mefford and Barchas, 1980; Sagara et al., 1988; Raynaud and Pévet, 1991; Vieira

et al., 1992). Later studies, using fluorescence detection, allowed a more robust methodology with a simple methanol-water (Peniston-Bird et al., 1993) or ethyl acetate (Vitale et al., 1996) mobile phase, giving relatively rapid separation and column regeneration. HPLC's principal disadvantage is that larger volumes of sample are required when measuring low daytime concentrations. Depending on the specific method, detection limits are about 2-20 pg/ml in plasma with 0.2-2.0 ml sample sizes and 7 pg/ml in urine with an 8.0 ml sample size.

Originally, melatonin values obtained by GC-MS served primarily as standards for the validation of other methods. Until recently, the extensive sample workup, low throughput, and expense of the assay have limited GC-MS use for routine analytical applications. Extraction methods varied, but the conversion of melatonin to the fluorinated derivative using pentafluoropropionic anhydride is widely employed for gas chromatographic separation (Kennaway et al., 1977; Greiner and Chan, 1978; Lewy and Markey, 1978; Tetsuo et al., 1981; Skene et al., 1983; Fourtillan et al., 1994). A 150-fold increase in the sensitivity of GC-MS for electron-capturing derivatives was provided by the technique of negative chemical ionization (Lewy and Markey, 1978). This technique utilizes a deuterated internal standard and detects melatonin as low as 1 pg/ml in plasma. Skene et al. (1983) simplified the extraction and derivatization procedures suitable for analysis using the double focusing magnetic mass spectrometer. Melatonin was measured at 0.5 pg/ml levels in plasma using the gas chromatography/negative ion chemical ionization mass spectrometry procedure with methane as the reagent gas (Fourtillan et al., 1994). This highly sensitive assay used a simple liquid-liquid extraction procedure and allowed a laboratory throughput of 50 samples/day. GC-MS techniques have also been described for the quantitation of the 6-hydroxymelatonin conjugates in urine (Fellenberg et al., 1980; Tetsuo et al., 1981).

In general, GC-MS appears to be the analytical technique of choice based on its high sensitivity and small sample size requirement. The specificity of GC-MS methods has also resolved the somewhat ambiguous data that surrounded the early measurements of melatonin in biomatrices and should further improve the understanding of pineal function. However, the applicability of these methods has not been fully established in the analysis of postmortem whole blood and other tissue specimens.

POSTMORTEM TOXICOLOGY

In forensic toxicology, postmortem levels of drugs and their metabolites are used to determine the antemortem or perimortem physical state of victims. The significance of postmortem analysis for melatonin has not been definitively established. While melatonin is endogenously produced, it has a strong potential to be deliberately ingested, and this will present problems in the interpretation of the postmortem analytical results. One possible application of postmortem analysis has been suggested by Mikami et al. (1994), who investigated the quantitation of melatonin in cadavers as a means of estimating the time of death. These authors relate the melatonin content of the pineal body to the hourly segment of the day in which death occurred. In selected cadavers, where time of death was known, they measured melatonin concentrations in the pineal body (75 samples), serum (27 samples), and urine (14 samples). Postmortem time intervals for the collection of these specimens varied from 3.5 to 48 hours. After determining that melatonin in human cerebral cortex decreases to approximately one-third of its initial concentration in 24 hours and remains at that level for 7 days, the investigators extrapolated the original values to represent a 0.5-hour postmortem interval. They proposed the following criteria for the estimation of time of death in relation to melatonin concentration in (ng/pineal body)/time of death: (0-0.5)/1100-1500; (0.5-1)/0500-1900; (1-2)/inconclusive; (2-5)/1000-0100; (5-15)/1800-0900; (>15)/1900-0500. Attempts to correlate serum and urinary melatonin levels with time of death were less precise. Serum melatonin levels in excess of 100 pg/ml were related to death between 2200 and 0100 hours and urinary concentrations over 35 pg/ml were related to death in the 1800-0600 hour period. These authors feel that the serum and urinary criteria can be used without any adjustment to estimate time of death, since they and earlier workers (Sturner et al., 1990) found no significant change in the concentration of melatonin in serum or urine as a function of the postmortem interval.

In the literature, no technique could be found that can differentiate endogenous melatonin from ingested melatonin. The amount of melatonin in the pineal gland is unlikely to be affected by ingested melatonin, but the level in circulation would be increased. It is known that serum melatonin levels vary with age, time of the day, and season (Iguchi et al., 1982b;

Illnerová et al., 1985), and it has been speculated that postmortem serum levels of melatonin approximate its antemortem levels (Sturner et al., 1990). Melatonin is biosynthesized through the serotonin pathway (Fig. 1), and increases in the levels of neurotransmitters are known to occur during excitement and stress, but it is not established whether these physiological states have any influence in altering the melatonin levels in the circulation. Therefore, some uncertainty exists about changes in melatonin levels during panic situations in relation to normal physiological conditions.

Stanley and Brown (1988) studied the postmortem levels of melatonin in the pineal glands of suicide victims and compared them to the melatonin levels in a control group matched for suddenness and violence of deaths. After segregating the values by the time of day in which death occurred, they found that the melatonin content was significantly lower in the pineal glands of suicide victims than in their corresponding "controls," which may be related to the low melatonin levels observed in acutely depressed patients (Beck-Friis et al., 1985).

Most published concentrations of circulating melatonin are presented as serum or plasma values, but no detailed information is available on the whole blood level of this neurohormone. Development of a suitable analytical method for whole blood and other tissues appears to be warranted, and melatonin distribution in whole blood components is also needed. Such information could be applied to the interpretation of results obtained from postmortem blood samples, particularly when cleaner samples (e.g., plasma and serum) are not available.

CONCLUSIONS

Melatonin appears to be beneficial in alleviating perceived jet lag effects when taken in the usual 2-3 mg dosage after arrival at a stable light-dark cycle environment. Timing of ingestion (before normal bedtime at destination) is important. Melatonin taken during a transmeridian flight may further destabilize the individual's circadian rhythm; side effects, such as sleepiness, make its use inadvisable for on-duty flight crews. While melatonin use by flight crews after transmeridian flight is not proscribed, care should be taken to avoid entering duty status with any residual effects. A 24-hour interval between ingestion and flying should allow circulating melatonin concentrations to return to normal levels. Melatonin use by air traffic controllers and other aviation personnel whose duties involve shift work should be self-regulated by similar considerations. Off duty use to improve sleep at the end of a shift change may prove to be practical, but its use should be discontinued in ample time to prevent residual effects during duty hours. Medical certification of airmen who are high-dose or long-term melatonin users may require careful evaluation. The watchword here is "caution," at least until both the efficacy and the clinical relationships of exogenous melatonin with the human endocrine system are better defined.

Current analytical methods are adequate to determine even the low daytime levels of melatonin in serum/plasma and urine. Where cost and usage permit, GC-MS appears to be the preferred technique because of its extreme sensitivity and specificity. Other well-validated methods (HPLC and RIA) may be better suited for infrequent use. Care should be taken in interpreting some of the early literature values for melatonin obtained before the development of adequately validated analytical methods.

Utilization of melatonin quantitation in the pineal body as a means of determining time-of-day in which aircraft accident victims expired may have a specific but limited utility. Most air crashes are well documented chronologically, but for the occasional unobserved accident in an isolated location, the knowledge of approximate time-of-day might assist the investigator in defining the possible contributions of weather and visibility existing at that time. Determination of melatonin levels in serum or urine provides even less specific information. A high concentration in either one raises the question of whether that level is caused by ingested or endogenously produced melatonin. Certainly, finding high levels of melatonin in the blood of a daytime crash victim would suggest possible ingestion, and such levels would require very careful medicolegal interpretations.

Despite extensive research, there are still unanswered questions about the influence of ingested melatonin on certain physiological and psychological conditions, particularly when used in combination with other medications. There are also unresolved differences of opinion among investigators related to the efficacy and safety of melatonin self-treatment for some popularized user expectations. Clearly, much remains to be determined before precise clinical guidelines can be formulated for melatonin use in the aviation environment.

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