Biological Melatonin versus Synthetic Melatonin

Subjects: Nutrition & Dietetics

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Melatonin dietary supplements are widely consumed worldwide, with developed countries as the largest consumers, with an estimated annual growth rate of approximately 10% until 2027, mainly in developing countries. The wide use of melatonin against sleep disorders and particular problems, such as jet lag, has been added to other applications, such as anti-aging, anti-stress, immune system activation, anticancer, and others, which have triggered its use, normally without a prescription. The chemical industry currently covers 100% of the needs of the melatonin market.

dietary supplements GMOs melatonin microorganisms

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is widely used around the world as a dietary supplement. In general, melatonin is used as a sleep aid supplement, a mild tranquilizer, a generalist antioxidant, and an anticancer and anti-aging component, among others [1]. According to the American Psychiatric Association (APA), approximately one-third of adults suffer from insomnia during their lifetime [2]. It manifests itself in incessant problems falling asleep and staying asleep. Therefore, it is very likely that the use of synthetic melatonin will spread. In 2019, the global production of synthetic melatonin, which was around 4000 tons, accounted for around 1.3 billion USD. This vast market is fully assisted by chemical melatonin, whose synthesis process is very cheap, effective, and, therefore, lucrative. The melatonin market is expected to grow at a CAGR (compound annual growth rate) of >10% over the next 5 years. With this considerable increase in demand, the insomnia problems generated by the COVID-19 pandemic have been of great relevance [3]. North America has the highest consumption by far, followed by Europe. The global melatonin market is mainly controlled by a few major companies, such as BASF, Aspen Pharmacare Australia, Nature's Bounty, Pfizer Inc., Natrol LLC, Aurobindo Pharma, and Biotics Research Co. Note that the consumption of melatonin for medical purposes involves about 50% of the synthetic melatonin produced; the rest has chemical and industrial applications [2][4].

Biologically, melatonin is a molecule widely distributed in all kingdoms of living organisms [5]. Discovered in 1958 in the pineal gland of a cow [6] and later in humans [7], it is one of the most studied biomolecules, and its multiple functions are known, mainly in mammals [8][9], but also in fish [10][11][12], poultry [13][14], and invertebrates [15]. In animal and human cells, melatonin acts as an antioxidant—a relevant role attributed to it in 1993 [16][17][18]. Melatonin acts as an interesting cell protector in stressful situations, in various physiological aspects in humans,

and, according to multiple studies, benefits an improvement in different diseases and dysfunctions. **Figure 1** shows some of the protective and regulatory actions of melatonin in humans and presents melatonin as an interesting pleiotropic molecule, standing out due to its relevance, the role of melatonin in the regulation of lipid and glucose metabolism, inducing nocturnal insulin resistance and diurnal insulin sensitivity. This effect seems to be associated with nocturnal fasting and diurnal feeding, preventing excessive weight gain [19]. Researchers also highlight its role as an anti-oncogenic agent, inhibiting the growth, proliferation, and metastasis of several tumors. The treatment of tumors with melatonin improved chemo- and radiotherapy sensitivity, acting as a synergistic molecule in the control of cancer cells. Additionally, melatonin mitigates acute damage to normal cells, protecting them against drug toxicity, possibly by enhancing immune responses [20][21][22]. Among the dysfunctions and diseases where the beneficial effects of melatonin have been studied are neurological ones, such as Alzheimer's, Parkinson's, fibromyalgia, depression, attention-deficit hyperactivity disorder, autism, and migraines; cardiovascular health problems, including hypercholesterolemia, hypertension, metabolic syndrome, and glycemic imbalance; gastrointestinal health problems, such as gastroesophageal reflux, ulcers, and irritable bowel syndrome; immunological health problems, such as multiple sclerosis, autoimmune responses (athletic stress, toxic stress, psoriasis, etc.), sepsis, COVID-19, etc. [3][23][24][25][26]; and also osteopenia [27], sarcopenia [28], pre-eclampsia, fertility, polycystic ovarian syndrome, and menopause, among others [29][30][31][32]. However, even though melatonin is a molecule that has been widely studied since the 1950s, the studies carried out require more clinical and extensive double-blind trials in order to clarify its sometimes confusing pleiotropic action [33][34].

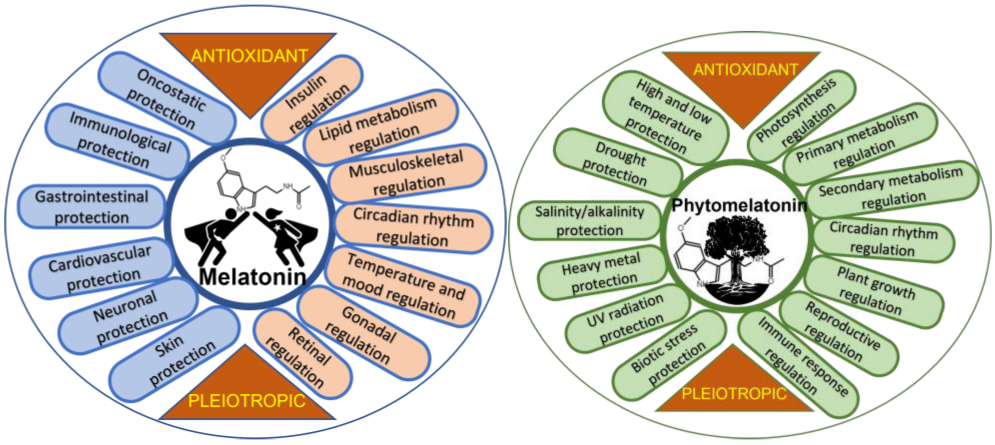

Figure 1. Diagram showing general roles of melatonin in humans and phytomelatonin in plants.

However, melatonin is well known for being the hormone that regulates sleep. Its oscillating levels in the blood flow according to the periods of light and darkness (circadian rhythms) due to the release of melatonin by the pineal gland is one of the most studied and known aspects of this molecule. The increase in blood melatonin levels during the first period of sleep to around 150–220 pmoles/mL acts on sleep initiation, reduces sleep latency and fragmentation, and increases sleep duration and quality [1][35][36]. Melatonin acts as an internal synchronizer of the circadian sleep–wake cycle and seasonal rhythmicity. In this sense, many sleep disorders have been treated with melatonin, including delayed sleep phase syndrome, night shift work sleep disorder, seasonal affective disorder, sleep disorders in the blind and aging, and pathophysiological disorders of children, with notable improvements in

sleep quality [37][38][39][40][41]. The most widespread disorder treated with melatonin is jet lag—a de-phasing in sleep–wake rhythms following trans-oceanic flights [42][43][44][45]. Possibly, the emphasis in studies on its role as a sleep regulator has caused a lack of studies on its possible role in many other physiological and clinical aspects.

Melatonin in plants, so-called phytomelatonin, was discovered simultaneously by three research groups in diverse plant material in 1995 [46][47][48]. The term phytomelatonin, which refers to melatonin of vegetable origin (plants and algae), is used to differentiate it from animal and/or synthetic melatonin. This term is very widespread and is used continuously in studies of phytochemistry, plant physiology, botany, food chemistry, etc., on plant melatonin. In plants, phytomelatonin is also a pleiotropic molecule, presenting multiple roles in diverse physiological responses (**Figure 1**). The regulation by melatonin of aspects such as photosynthesis, including stomatal CO_2 uptake and water economy, carbohydrate, lipid, nitrogen, and sulfur metabolism, and simple phenol, flavonoid, and terpenoid metabolism, has demonstrated crucial interest in the basic and technical processes of vegetative (germination, plant growth, rooting, branching, etc.) and reproductive development, including fertility, parthenocarpy, seed and fruit development, ripening, senescence, and the conservation of fruits and cut flowers [49][50][51][52][53]. Generally, melatonin regulates these processes through the action of the plant hormone network, up/down-regulating several biosynthesis, catabolic, and transcription factors that are plant hormone-related [54][55][56]. One of the aspects of greatest agronomic and biotechnological interest is the role of phytomelatonin as a promoter of tolerance against biotic and abiotic stresses [57][58][59][60][61][62][63][64][65][66][67][68] (**Figure 1**). Currently, phytomelatonin is presented as an interesting eco-friendly tool to control biological diseases and to facilitate the resistance/adaptation of plants to/against climate change.

2. Biosynthesis of Melatonin

Melatonin is an acetylated compound derived from serotonin. Both indolic amines are synthesized from the amino acid tryptophan in a biosynthetic pathway that has been extensively studied in both animals and plants [69][70]. In plants, tryptophan is converted into tryptamine by the enzyme tryptophan decarboxylase (TDC) (**Figure 2**). Tryptamine is then converted into 5-hydroxytryptamine (serotonin) by tryptamine 5-hydroxylase (T5H), an enzyme that has been extensively studied in rice, and which could act with many substrates, although this has not been studied in depth. Serotonin is *N*-acetylated by serotonin *N*-acetyltransferase (SNAT). *N*-acetylserotonin is then methylated by acetylserotonin methyl transferase (ASMT)—a hydroxyindole-*O*-methyltransferase—which generates melatonin. In plants, the methylation of *N*-acetylserotonin can also be performed by caffeic acid *O*-methyltransferase (COMT), a class of enzyme that can act on a variety of substrates, including caffeic acid and quercetin [71]. Serotonin may also be transformed into 5-methoxytryptamine by ASMT/COMT to generate melatonin after the action of SNAT. This route would occur in senescence and/or stress situations [70][72]. Furthermore, melatonin can be generated through the formation of *N*-acetyltryptamine by SNAT, which would be converted into *N*-acetylserotonin by T5H [73], although this route has not been demonstrated, possibly because T5H is the least studied enzyme of the pathway (**Figure 2**). Interestingly, up to four genes encoding histone deacetylases (DAC) have been identified in rice plants that can reverse the steps from serotonin to *N*-acetylserotonin and from 5-

methoxytryptamine to melatonin. DAC, expressed in chloroplast, exhibited enzyme activity toward *N*-acetylserotonin, *N*-acetyltryptamine, and melatonin, with the highest deacetylase activity for *N*-acetyltyramine [74].

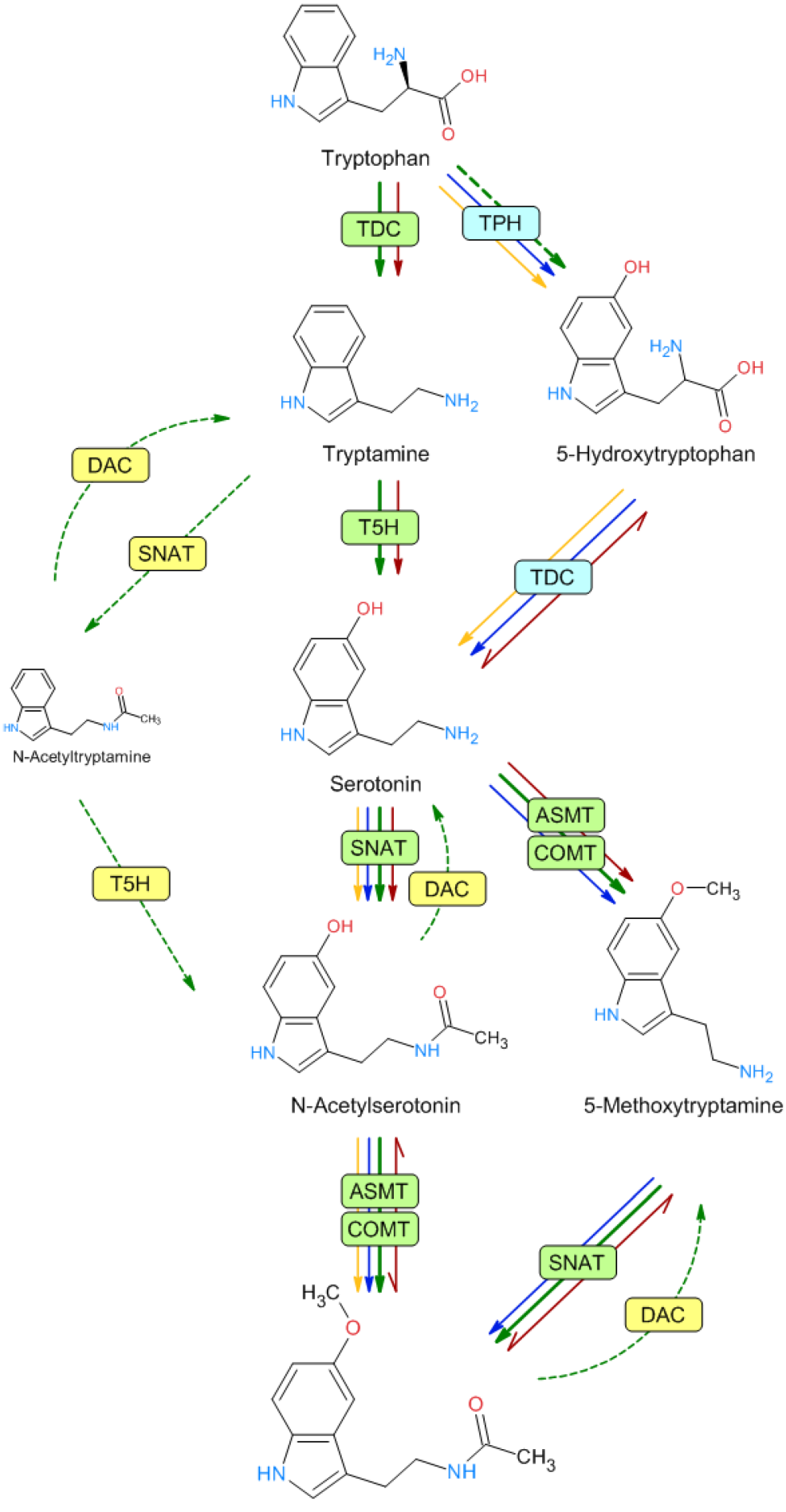
Tryptophan
TDC
TPH
Tryptamine
5-Hydroxytryptophan
DAC
SNAT
T5H
TDC
N-Acetyltryptamine
Serotonin
ASMT
COMT
SNAT
DAC
T5H
N-Acetylserotonin
5-Methoxytryptamine
ASMT
COMT
SNAT
DAC

Figure 2. Biosynthetic melatonin pathways in mammals, plants, and microorganisms. The names of the different enzymes are described in the text. The different arrow colors denote plants (green), animals (blue), bacteria (yellow), and yeasts (red). Dashed lines indicate unproven reactions.

In animal cells, serotonin is formed from 5-hydroxytryptophan after the sequential action of tryptophan hydroxylase (TPH) and TDC. Although TPH has not been detected in plants, the presence of 5-hydroxytryptophan suggested that some enzymatic activity, such as that of TPH, acts to a lesser extent in plant cells. Moreover, melatonin can be generated through the formation of 5-methoxytryptamine, mainly under stress conditions as proposed by several authors, suggesting that the melatonin biosynthesis pathway may follow various alternative routes compared with animal cells, with a greater ability to adapt to metabolic changes in plants [72][75]. All the named enzymes have been detected and characterized in rice and Arabidopsis, except TPH, which is well known in animals but not in plants. Nevertheless, some authors have proposed that T5H can act as a hydroxylase with low substrate specificity and is capable of acting in all the hydroxylation steps described [70][76][77][78][79]. This same broad substrate specificity can also be attributed to SNAT, ASMT, and COMT enzymes. Melatonin intermediates are produced in various subcellular compartments, such as the cytoplasm, endoplasmic reticulum, mitochondria, and chloroplasts, which determine the subsequent enzymatic steps [80][81].

In microorganisms, there are few studies on the melatonin biosynthesis pathway [82]. *Saccharomyces* and bacteria (*Geobacillus*, *Bacillus*, and *Pseudomonas*) produced both serotonin and melatonin at different concentrations [83][84][85][86][87][88][89]. Moreover, the production of melatonin was evidenced by other authors in the cultures of the yeasts *Pichia kluyveri*, *Saccharomyces cerevisiae*, and *S. uvarum* and bacteria (*Agrobacterium*, *Pseudomonas*, *Variovorax*, *Bacillus*, and *Oenococcus*) [85][90][91] and previously in the photosynthetic bacteria *Rhodospirillum rubrum* [92] and *Erythrobacter longus* [93] and *Escherichia coli* [94].

In the yeast *Saccharomyces cerevisiae*, unlike in plants and animals, it seems that the biosynthesis of 5-hydroxytryptophan from tryptophan does not occur. Interestingly, several of the described stages appear to be reversible in *S. cerevisiae*, such as between 5-hydroxytryptophan and serotonin, *N*-acetylserotonin and melatonin, and 5-methoxytryptamine and melatonin [90][95], as detailed in **Figure 2**. In *Bacillus amyloliquefaciens* SB-9 and *Pseudomonas fluorescens* RG11, 5-hydroxytryptophan, serotonin, and *N*-acetylserotonin, but not tryptamine, were detected [85][86]. So, several genes from bacterial origin were used to build a melatonin-producing *Escherichia coli* strain. For example, the DDC gene, encoding an aromatic L-amino acid decarboxylase from *Candidatus Koribacter versatilis* Ellin 345 and *Draconibacterium orientale*, and the AANAT gene, encoding an aralkylamine *N*-acetyltransferase from *Streptomyces griseofuscus*, were assayed [96][97]. Undoubtedly, further studies are needed to elucidate the complete biosynthetic pathways of melatonin in different prokaryotic and eukaryotic microbes [82].

3. Biological Melatonin versus Synthetic Melatonin

Initially, melatonin was obtained for experimental and clinical studies from animal sources (mainly from the pineal gland and urine), with the consequent risk of viral transmission [98][99]. These techniques were withdrawn when melatonin could be obtained by chemical synthesis [100]. Currently, all melatonin used for industrial and medical

purposes is obtained by using chemical synthesis methods. These methods, which presented serious problems in the 1980s, including deaths due to the presence of by-products of synthesis from tryptophan [101], are much safer and more efficient today. However, melatonin preparations have described the presence of a whole set of undesirable by-products due to their toxic nature. **Figure 3** shows three of the most commonly used chemical synthesis routes for melatonin and the by-products that are generated in its synthesis [102]. The synthesis of melatonin from tryptophan derivatives (**Figure 3**, Scheme A) generates toxic by-products that have sometimes caused significant diseases, such as eosinophilia myalgia syndrome [101][103][104], while the most current methods (**Figure 3**, Scheme B) for the synthesis of melatonin from phthalimide [105] raise important doubts about the toxicity of several of the by-products that are generated [106]. In addition, Fischer indole reactions from allylamine (**Figure 3**, Scheme C) present dangerous and toxic reactants [107].

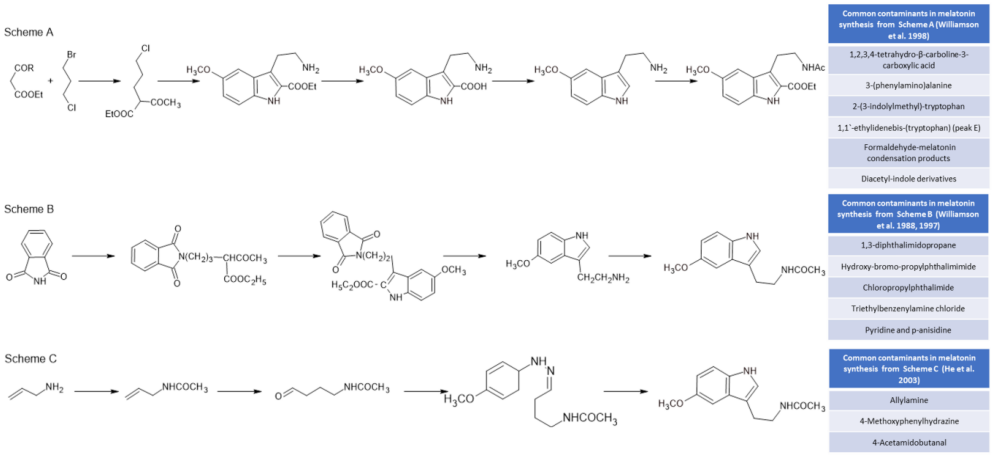

Figure 3. Some chemical melatonin synthesis pathways and their by-products present in synthetic melatonin preparations.

On the other hand, obtaining melatonin from non-animal biological sources is presented as a strong commitment to the future, not to replace synthetic melatonin but to be a more natural complementary and alternative source [108].

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