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Review article

Involvement of the nitric oxide in melatonin-mediated protection against injury

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ABSTRACT

Melatonin is a hormone mainly synthesized by the pineal gland in vertebrates and known well as an endogenous regulator of circadian and seasonal rhythms. It has been demonstrated that melatonin is involved in many physiological and pathophysiological processes showing antioxidant, anti-apoptotic and anti-inflammatory properties. Nitric oxide (NO) is a free radical gas in the biological system, which is produced by nitric oxide synthase (NOS) family. NO acts as a biological mediator and plays important roles in different systems in humans. The NO/NOS system exerts a broad spectrum of signaling functions. Accumulating evidence has clearly revealed that melatonin regulates NO/NOS system through multiple mechanisms that may influence physiological and pathophysiological processes. This article reviews the latest evidence for the effects of melatonin on NO/NOS regulation in different organs and disease conditions, the potential cellular mechanisms by which melatonin is involved in organ protection are discussed.

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is a neurohormone derived from amino acid tryptophan and principally produced by the pineal gland in vertebrates [1], which was initially studied in terms of its role in endocrine physiology regulating circadian [2]. Considerable evidence indicates that melatonin is involved in a wide variety of physiological and pathological processes and possesses anti-oxidant and anti-inflammatory properties [3,4].

NO is synthesized intracellularly directly by nitric oxide synthase (NOS) using L-arginine as substrate [5]. NOS exists as a family of three distinct isoforms: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS) [5]. nNOS and eNOS are considered constitutively expressed proteins (cNOS), which are dependent on a rise in tissue calcium concentration for activity. iNOS is an inducible, calcium-independent isoform, which was first identified in macrophages and can also be induced in large varieties of cell types [5].

A growing body of evidence indicates that administration of melatonin can regulate NOS gene expression and NO production. The purpose of this review is to present the recent findings on melatonin-induced NO/NOS modulation. Possible cellular mechanisms and intermediate role of NO/NOS in melatonin-mediated organ protection are also discussed.

2. Effects of melatonin on NO/NOS in different organs and disease models

2.1. Vessels

Endothelial cells play an important role in the regulation of vascular activity through the release of NO, which activates soluble guanylate cyclase (sGC), resulting in the production of the cyclic guanosine monophosphate (cGMP) to exert its relaxing effect [6]. eNOS is expressed mainly in endothelial cells. The stimulation of the NO pathway in endothelium contributes to the regional and/or systemic hemodynamic changes [7,8], which can be mediated by melatonin. For example, melatonin increases expression pattern of eNOS and NO production in kidney of alcohol-induced damage [9]. The effect is confirmed in aortas of high-fat diet-fed insulin-resistant mice [10], lung in chronically hypoxic rats [11], cardiac microvascular system [12] and liver [13] following ischemia/reperfusion (I/R) injury, in which melatonin improves endothelial vascular function to exert a protective effect. In addition, melatonin prevents the endothelial dysfunction with ameliorated levels of NO and expressions of eNOS in rats with chronic

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intermittent hypoxia [14], aging mice [15] and in cultured vascular endothelial cells [16]. Moreover, melatonin regulates NOS expression in ischemic brain injury and prevents the injury-induced decrease of eNOS [17]. The studies provide further evidence that melatonin exerts protective effects through up-regulation of eNOS in endothelial cells.

In contrast with the results above, there are studies showing that melatonin inhibits NO/eNOS in endothelial cells. For example, melatonin abolishes hydrogen peroxide-induced eNOS expression in bovine cerebral arteries [18]. In addition, melatonin reduces NO production induced by bradykinin in microvascular endothelial cells [19,20]. It might be interesting to investigate the dual effects on eNOS of melatonin in vessels.

2.2. Brain/nerve

NO is regarded as an atypical neural neurotransmitter in the nervous system, which is synthesized intracellularly directly by nNOS, an isoform of NOS identified and cloned originally from neural tissue. It has been demonstrated that physiological concentrations of melatonin inhibit NO/nNOS activity in the retina [21], hypothalamus [22,23] and cerebellum [24]. The inhibitory effects are confirmed in vitro [23,25]. Interestingly, an in vivo study shows that collecting the hypothalamus from animals at night, when endogenous melatonin levels are elevated, results in a significant decrease of NOS activity [23], since melatonin is a major hormone that regulates body circadian rhythms. In addition, melatonin inhibits directly NOS activity within the enteric nervous system and influences function of gastrointestinal tract [26]. It is well known that activation of *N*-methyl-D-aspartate (NMDA) receptor leads to ultimate NO production through activation of nNOS in neurons. Escames' study shows that melatonin modulates the NMDA-dependent excitatory response in rat striatal neurons, such an effect could be related to its inhibition of nNOS [27]. Another study also demonstrates that melatonin inhibits nNOS activity in both rat striatal homogenate and purified brain [28].

Peripheral nerve injury results in functional changes of lesioned neurons in which expression of nNOS is significantly increased. Following melatonin administration, the nNOS augmentation is successfully suppressed [29]. The same effect is demonstrated in a rat middle cerebral artery occlusion (MCAO) stroke model [30] and hypobaric hypoxic rat hippocampus [31]. In contrast, melatonin ameliorates the constitutive NO production via up-regulation of nNOS expression to protect against hippocampal injury induced by intermittent hypoxia [32]. The overall effect of melatonin treatment is neuroprotective, although the mechanisms of the biphasic effects (enhancing or inhibiting) on regulating nNOS remain to be elucidated.

2.3. Oxidative stress

Oxidative stress is defined as an imbalance between production of reactive oxygen species (ROS) and its elimination by protective mechanisms, referred to as antioxidants. The imbalance in pro-oxidant/antioxidant homeostasis can cause damage of all components of the cell [1]. Oxidative stress plays a key role in the pathogenesis of many diseases. NO can bind superoxide anion radical ($O_2(-*)$ to form peroxynitrite (ONOO–), an important component of ROS. The nitrooxidative stress is involved in many pathogenic processes. Melatonin is considered as a powerful antioxidant and can reduce oxidative stress, which is mainly attributed to its inhibitory effect on the NO pathway.

The major part of NO is synthesized by iNOS, usually after challenge by immunological or inflammatory stimuli. A great deal of evidence indicates that melatonin reduces NO production and/or expression of iNOS, which are confirmed not only in vitro in neurons [33], glial cells [33–38] and macrophages [39], but also in different disease models including I/R or hypoxic injury in kidney [40–43], brain [17,44–48] and heart [49], sepsis [50–57], and injury of liver [58,59] and peripheral nerve [60].

Taken as whole the results indicate that melatonin seems to inhibit inducible NO production and exert anti-oxidative effects, although it has been demonstrated that iNOS expression in brain cortex is increased in the melatonin treatment during ethanol hangover [61].

2.4. Inflammation

iNOS and NO can be induced in various cells, the increase of which has been proposed as a major factor involved in inflammatory process [62]. Accumulating evidence shows that melatonin have anti-inflammatory effects through NO regulation. For example, melatonin is thought to exert anti-inflammatory effects by inhibiting the expression/activity of iNOS and decreasing NO production in local inflammation induced by carrageenan [63] and zymosan-activated plasma [64], pancreatic fluid-induced lung inflammation [65], the age-dependent inflammation [66] and acute inflammatory phase of wounds [67]. LPS can elicit strong immune responses and produce many types of mediators including NO. Melatonin prevents the LPS-induced increase NO/iNOS in microglial cells [68], macrophage [69–73], endothelial cells [74] and vascular smooth muscle cells [75]. Moreover, melatonin exerts anti-inflammatory action in primary rat brain microvascular endothelial cells [76], neuroblastoma cells [77,78] and glial cells [79]. The anti-inflammatory effects are potentially due to the inhibition of iNOS and NO production.

2.5. Pain control by melatonin

A lot has been learned about NO involved in nociception processing [80]. There is considerable evidence that melatonin plays a role in pain modulation [81]. In the post-herpetic neuralgia model, melatonin decreases the NO levels in the brain and spinal cord tissues, which may be the mechanism of its analgesic effects [82]. NOS protein and NO levels are increased in various inflammatory or neuropathic pain models [80]. As mentioned above, melatonin inhibits the iNOS expression and decreases NO production to exert anti-inflammatory and anti-oxidative effects in local inflammation [63,64,83]. It is reasonably assumed that melatonin produces attenuation of nociceptive responses to different noxious stimulus. In addition, NO-cyclic GMP-protein kinase G-K+ channels pathway may be involved in the anti-nociception of melatonin at peripheral receptor level [84]. NO production is tightly controlled by a family of three distinct NOS genes, the expression of which is subjected to differential regulation including melatonin in a cell type- and stimulus-specific manner. Thus the role of regulatory effects of melatonin on NO in nociception is more necessary to make extensive efforts to investigate.

3. Mechanisms of NO regulation by melatonin

3.1. Free radical scavenging

Accumulating evidence shows that melatonin is very effective as a broad spectrum antioxidant. Melatonin is shown to directly scavenge the highly toxic NO and ONOO- anion as well [85]. Based on the analyses of structure-activity relationships, the indole moiety of the melatonin molecule is the reactive center of interaction with oxidants due to its high resonance stability and very low activation energy barrier towards the free radical reactions [85]. NO-scavenging property of melatonin is demonstrated in the endothelium of human umbilical arteries [86–88] in vitro, which is confirmed in a local inflammation model in vivo [83]. The free radical scavenging of melatonin is one of the most important mechanisms of its anti-oxidation effect.

3.2. MT receptor

Some important effects of melatonin are mediated through activation of specific receptors, which include two membrane-associated