Review

Neuroprotective Effect of Melatonin: A Novel Therapy against Perinatal Hypoxia-Ischemia

Daniel Alonso-Alconada *, Antonia Álvarez, Olatz Arteaga, Agustín Martínez-Ibargüen and Enrique Hilario

Department of Cell Biology and Histology, School of Medicine and Dentistry,
University of the Basque Country, Barrio Sarriena s/n, Leioa 48940, Bizkaia, Spain;
E-Mails: antoniaangeles.alvarez@ehu.es (A.A.); olatz_b@hotmail.com (O.A.);
agustin.martinez@ehu.es (A.M.-I.); enrique.hilario@ehu.es (E.H.)

* Author to whom correspondence should be addressed; E-Mail: daniel.alonsoa@ehu.es;
Tel.: +34-946-013-294 (ext. 123); Fax: +34-946-013-266.

Abstract: One of the most common causes of mortality and morbidity in children is perinatal hypoxia-ischemia (HI). In spite of the advances in neonatology, its incidence is not diminishing, generating a pediatric population that will require an extended amount of chronic care throughout their lifetime. For this reason, new and more effective neuroprotective strategies are urgently required, in order to minimize as much as possible the neurological consequences of this encephalopathy. In this sense, interest has grown in the neuroprotective possibilities of melatonin, as this hormone may help to maintain cell survival through the modulation of a wide range of physiological functions. Although some of the mechanisms by which melatonin is neuroprotective after neonatal asphyxia remain a subject of investigation, this review tries to summarize some of the most recent advances related with its use as a therapeutic drug against perinatal hypoxic-ischemic brain injury, supporting the high interest in this indoleamine as a future feasible strategy for cerebral asphyctic events.

1. Introduction

The main function of the pineal gland in all species is to transduce information concerning light-dark cycles to body physiology, particularly for organization of body rhythms via its main hormone melatonin [1]. Based on the ability of melatonin (*N*-acetyl-5-methoxytryptamine) and its metabolites to scavenge a wide variety of free radicals (FR), it is not surprising to consider it as one of its most important functions in living organisms leading to protect them from oxidative stress [2,3]. Acting as a direct scavenger, this neurohormone is able to remove FR, such as singlet oxygen, superoxide anion radical, hydroperoxide, hydroxyl radical and the lipid peroxide radical [2,4]. Moreover, a single melatonin molecule may generate products in a scavenger cascade, which may collectively eliminate up to ten FR [4]. Melatonin can develop indirect antioxidant actions through the improvement of the mitochondrial efficiency [5], the stimulation of the gene expression and the activation of some of the most important antioxidant enzymes, including superoxide dismutase (SOD), catalase, glucose-6-phosphate dehydrogenase, glutathione reductase and glutathione peroxidase [6] and also with the strengthening of the antioxidant effect of substances, like glutathione, vitamin E and vitamin C [7].

The brain is particularly sensitive to FR damage due to its high utilization of oxygen, its relatively poorly developed antioxidant defense and its high amount of easily oxidizable fatty acids. Thus, the use of melatonin as pharmacological agent against neurodegenerative disorders, such as Huntington's disease, Alzheimer's disease and Parkinsonism and also against ischemic brain injury/stroke, has been extensively evaluated. With an incidence of 2–6/1,000 term births [8], perinatal hypoxia-ischemia (HI) remains the single most important cause of brain injury in the newborn, leading to death or lifelong disability [9,10]. Despite the improvements in perinatal care, significant neurological sequelae can occur in as many as 50%–75% of these asphyctic children, which may suffer from long-term neurological consequences, such as cerebral palsy, mental retardation and epilepsy [11–13]. Asphyxia is also associated with attention deficits and hyperactivity in children and adolescents [14,15]. During the last decade, melatonin has started to be considered as an attractive option in order to minimize as much as possible the neurological sequelae from hypoxic-ischemic brain injury. It easily crosses both the placental and the blood-brain barrier, reaching subcellular compartments with a low toxicity and high efficacy [16–18], and even at high supra-physiological concentrations, there appear to be no adverse side-effects [19], making it a relatively-safe therapy that could be administered to babies.

2. Brain Protection

Following cerebral asphyxia, HI starts out a multi-faceted cascade of events that ultimately causes cell death and often damages the whole brain [20]. Everything begins when the reduction in oxygen and blood supply induces a decrease in oxidative phosphorylation and the neonate's brain converts to anaerobic metabolism in an effort to sustain functional ability. Anaerobic metabolism leads to a rapid depletion of ATP, accumulation of lactic acid and failure of ion pumps, resulting in a massive entry of sodium, calcium and water into the cells. Afterwards, multiple and diverse downstream biochemical reactions aggravate the pathogenesis of hypoxic-ischemic brain damage, being the most important, among others, the production of reactive oxygen species leading to oxidative stress, the massive increase

in free cytosolic calcium concentrations and the drop in mitochondrial function triggering the activation of apoptotic pathways, DNA fragmentation and cell death. After ischemic brain injury/stroke, melatonin has showed a remarkable capacity to reduce infarct volume and/or inhibit neuronal cell death after in different mammalian species and using different experimental models [21–33].

Figure 1. Nissl-stained (**A**–**C**), myelin basic protein (**D**–**F**) and glial fibrillary acidic protein (**G**–**I**) immunolabeled brain sections corresponding to the surrounding areas of the CA1 region of the hippocampus and the external capsule showing cell loss (**B**), myelination deficit (**E**) and reactive gliosis (**H**) after hypoxia-ischemia and recovery after melatonin administration. Seven-day old rats were subjected to hypoxia-ischemia (left common carotid artery ligated and then 8% oxygen for 2 h) and sacrificed seven days after the injury. Pups without ischemia or hypoxia served as controls (Sham group). Bar: 100 μm.
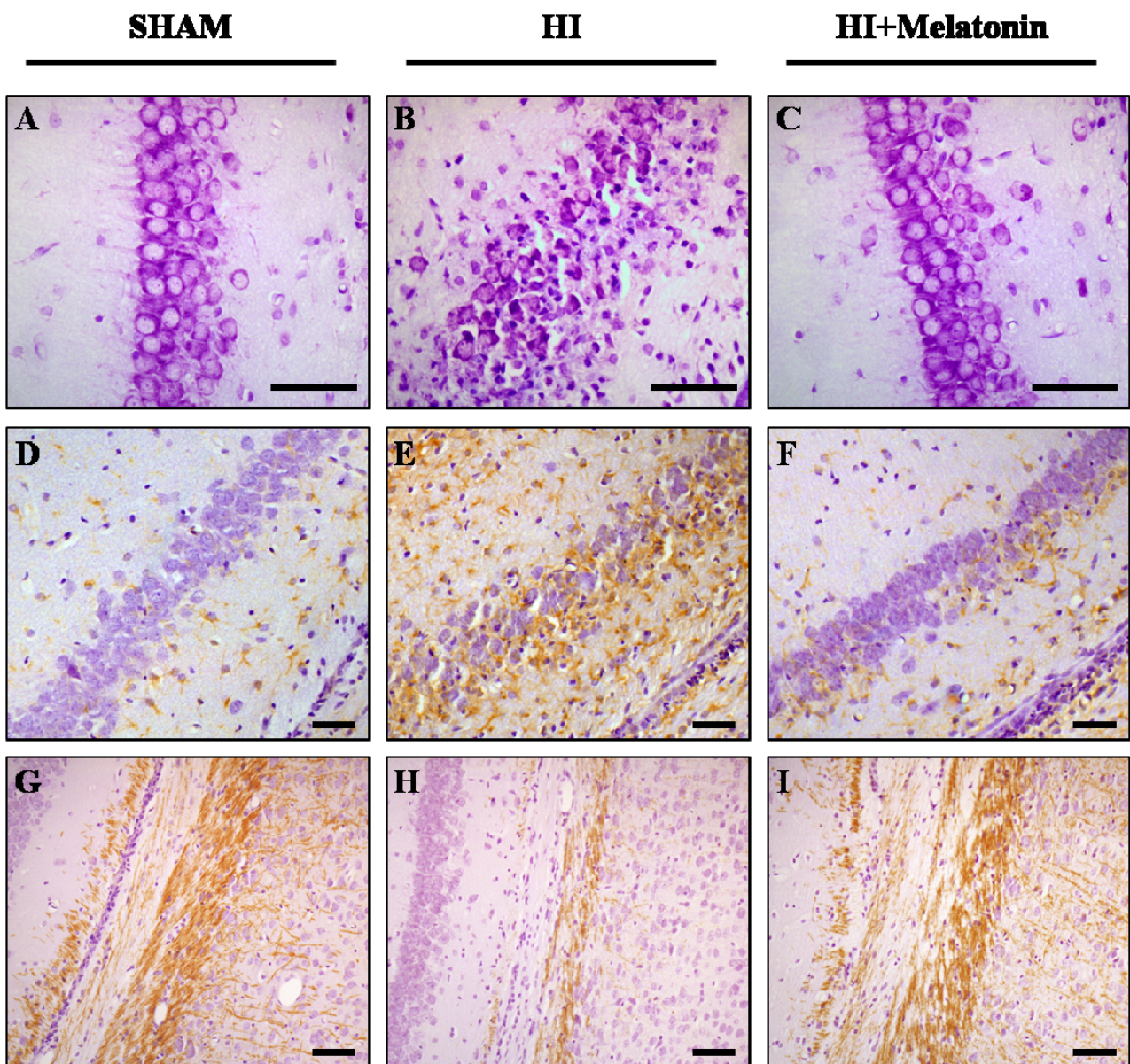
Melatonin administration after neonatal HI has been shown to reduce infarct volume both administered before or after the injury [34–36]. Indeed, melatonin was able to decrease sensorimotor asymmetry and learning deficits, thus protecting the pups from the long-term consequences of neonatal asphyxia [34]. While virtually every cell is affected by asphyxia, they do not respond in the same way during HI, being neurons the most sensitive cells to the lack of oxygen and showing a selective vulnerability [37–39]. Histological analysis demonstrated an increase in the number of morphologically well preserved neurons in melatonin-treated animals in the CA1, CA2–CA3 areas and dentate gyrus of the hippocampus and parietal cortex when compared with the hypoxic-ischemic group [33,40,41] (Figure 1). Even though astrocytes are more resistant than neurons to oxygen deprivation, their death may give rise to a new wage of neuronal death due to their role in the maintenance of the homeostatic state of neurons. Therefore, astrocytes can modulate in a significant manner the extension and degree of severity of the damage [42,43], either conferring neuroprotection by scavenging reactive oxygen species and also assisting with reconstruction from brain injury [44] or leading to deficiencies in the myelination processes, neuronal signaling impairment and an increase the inflammatory response [45,46]. Melatonin has demonstrated to reduce the expression of the glial fibrillary acidic protein [33] (Figure 1), whose accumulation is related with the creation of new astrocytic processes and reactive gliosis [43]. In addition to neurons, oligodendrocytes are particularly vulnerable to asphyxia, affecting myelination that gives rise to white matter lesions and damaging gray matter oligodendrocyte progenitors [47]. An abnormal decrease in the expression of myelin basic protein leading to myelination deficit is considered hallmark of inflammation-associated diffuse white matter damage [48,49]. In this sense, several groups have suggested that melatonin may be of therapeutic value in ameliorating hypoxic-ischemic damage to the developing white matter through normalization of the myelination process [33,50–52] (Figure 1).

3. Antioxidant

The high incidence of hypoxic-ischemic brain lesions in newborns can be partly attributed to the fact that the developing brain is especially vulnerable to oxidative stress. After neonatal asphyxia, reperfusion brings about the overproduction of FR leading to oxidative stress, as the antioxidant capacity of immature neurons is easily overwhelmed by hypoxia-induced reactive oxygen species. The newborn brain is especially vulnerable to oxidative imbalance, due to its increased fatty acid content, higher concentrations of free iron, high rates of oxygen consumption, low concentrations of antioxidant, an imbalance of antioxidant enzymes, as for example catalase CuZn-SOD-1, mitochondrial SOD-2 and glutathione peroxidase and oxygen-induced vasoconstriction, leading to reduced brain perfusion, among others [53–55].

When membrane lipoproteins and polyunsaturated fatty acids suffer attacks from FR, many oxygenated compounds, particularly aldehydes, such as malondialdehyde (MDA), are produced. Thus, the evaluation of lipid peroxidation is a useful tool to evaluate oxidative stress leading to brain damage. Melatonin has been able to abolish lipid peroxidation in late-gestation fetal sheep in respond to umbilical cord occlusion [56] and to avoid the rise in MDA induced by hypoxia in rat pups [57] and asphyxiated human newborns [58]. Deferoxamine-chelatable free iron, isoprostanes, neuroprostanes and neurofurans are also quantitative biomarkers of oxidative damage [59–61], and after melatonin

administration, their levels were significantly lower than those in hypoxic-ischemic rats [62,63]. These results were similar to those observed in fetal sheep after umbilical cord occlusion, where the production of 8-isoprostanes was attenuated [64]. On the other hand, melatonin may prevent protein oxidation in the brain tissue of hypoxic neonatal rats [65], as terminal products of protein exposure to FR are considered reliable markers of the degree of protein damage in oxidative stress [66]. Additionally, the activity of the antioxidative enzyme catalase is maintained [57], hydroxyl formation reduced [56] and nitrite/nitrate levels reduced [58] in different animals models subjected to hypoxia.

4. Anti-Apoptotic

Under pathophysiological conditions, one of the most important key regulators of apoptotic cell death is mitochondrial impairment, as the disruption of its membrane integrity and loss of membrane potential can determinate cell survival by overproduction of reactive oxygen species, abnormal calcium homeostasis and release of apoptotic proteins. In several studies demonstrating anti-apoptotic actions, melatonin prevented cytochrome *c* release [32,67,68], reduced or blocked caspase-1 and caspase-3 activation [32,67,69–73], increased the expression of anti-apoptotic proteins Bcl-2 [71,74,75] and Bcl-xL[70], diminished Bad [31,72] and Bax [71] pro-apoptotic proteins, inhibited poly-ADP-ribose-polymerase cleavage [72], avoided mitochondrial permeability transition pore opening, thus counteracting the collapse of the mitochondrial membrane potential [67,69], and decreased the number of TUNEL-positive cells/DNA breaks [31,32,72,75–78]. In the central nervous system, melatonin can also generate anti-excitatory effects on neurons through the modulation of gamma-aminobutyric acid and glutamate receptors [79,80], inducing a decrease in cytosolic calcium concentrations [81,82].

Shortly after a hypoxic-ischemic event, reactive oxygen species overproduction can start out a harmful multi-faceted cascade that includes lipid peroxidation, protein oxidation and DNA fragmentation, ultimately damaging vital cellular components as nucleic acids, cell membranes and mitochondria, resulting in subsequent cell death in the immature brain [83–85]. In this regard, we have recently shown that HI can develop a widespread increase in reactive oxygen species after perinatal asphyxia, an overproduction correlated with the number of, as well as with the distribution of apoptotic cells [86].

Melatonin may be an effective prophylactic agent for use in late pregnancy to protect against mitochondrial-induced cell death after a hypoxic-ischemic event at birth. Given to pregnant rats, it prevented oxidative mitochondria damage after ischemia-reperfusion in premature fetal rat brain [87] by means of the maintenance of the number of intact mitochondria and the respiratory control index (an indicator of mitochondrial respiratory activity), as well as the reduction in thiobarbituric acid-reactive substances concentration (a marker of oxidative stress) [40,41]. Hutton *et al.* studied caspase-3 activation and fractin in order to evaluate the anti-apoptotic effect of melatonin in a model of birth asphyxia in the spiny mouse, showing lower levels after its administration [88]. Accordingly, Fu *et al.* demonstrated the inhibition of caspase-3 activation, the induction of Bcl-2 expression and the increase Bcl-2/Bax ratio in a model of hypoxia *in vitro* [89]. Melatonin administration not only generates a neuroprotective effect when administered before the hypoxic-ischemic event, but also when given after the onset of the injury. Using the terminal deoxynucleotidyl transferase dUTP nick

end labeling method (TUNEL) to detect DNA fragmentation and apoptotic figures, melatonin-treated neonatal animals have shown a reduction in TUNEL-positive cells per unit area in neonatal sheep [64] and in rats [33,35,36].

As shown above, melatonin can exert a wide range of antiapoptotic effects, mainly targeting mitochondria, but it can also enhance cell survival pathways leading to cell rescue. For instance, protection from cerebral ischemic injury was attributed to the maintenance of signaling via the MAP kinase pathway, leading to the prevention Bad dephosphorylation [31]. Furthermore, melatonin can target PI3K/Akt pathway [70,72,90–92], mTOR [93] or the forkhead transcription factor, pAFX [91], and also restore JNK1/2 and ERK 1/2 phosphorylated levels [72,90], thereby preventing the proapoptotic actions of the dephosphorylated proteins.

5. Anti-Inflammatory

In recent years, the use of melatonin has started to be considered as another meaningful tool against inflammatory response in an effort to improve the clinical course of illnesses, which have an inflammatory etiology. The strategy mediated by melatonin and its main metabolites 6-hydroxy, *N*1-acetyl-*N*2-formyl-5-metho, *N*1-acetyl-5-methoxykynuramine and cyclic 3-hydroxy melatonin, encompasses the downregulation of some inflammation-related molecules, such as cytokines interleukin-6, interleukin-8 and tumor necrosis factor-α [94–100], 5-lipoxygenase [101], cyclooxygenase [102,103] and prostaglandin [104–106], an important reduction of nitric oxide (NO) and MDA levels [107] and also the inhibition of neuronal (nNOS) [108–111] and inducible (iNOS) nitric oxide synthases [111,112]. Even though NO participates in diverse processes acting as a physiological messenger, an excess in its concentrations can induce energy depletion, liberation of excitotoxic amino acids and a high ability to react with other FR. Downregulation of nNOS may contribute to the maintenance of electron transport chain function [109,110,113–115], thus protecting from NO-mediated mitochondrial impairment and cell damage [116–119]. From its part, counteraction to iNOS can avoid lipid peroxidation, shifts the glutathione redox state and boosts energy efficiency and ATP production in mitochondria [118,120,121]. Regarding the field of ischemic brain injury, melatonin and its metabolites have been able to reverse the inflammatory response and edema after stroke suppressing the production of inflammatory cytokines [102,122,123], reducing NOS [124], preventing the translocation of NF-κB to the nucleus [125,126] and decreasing cyclooxygenase-2 gene expression [127], molecular changes correlated with a reduction in the size of brain infarcts.

After perinatal asphyxia, FR also stimulate ischemic cells to secrete inflammatory cytokines and chemokines, which in turn can generate a wide variety of cytotoxic agents, including more cytokines, matrix metalloproteases, NO and more reactive oxygen species. These molecules can dismantle both blood brain barrier and extracellular matrix, allowing the blood, soluble elements and peripheral inflammatory cells to penetrate the brain, resulting in the exacerbation of the damage. Melatonin may be beneficial, as it reduces NO production, vascular endothelial growth factor concentration and, hence, vascular permeability that results increased after hypoxic exposure [128]. Prophylactic maternal treatment with melatonin has also demonstrated a reduction in central nervous system inflammation, by limiting macrophage infiltration and glial cell activation in a model of birth asphyxia in the spiny mouse [88]. Indeed, a reduced number of ED1 positive cells, a marker of activated

microglia-macrophages, was found in neonatal rats treated with melatonin when comparing with pups without treatment [63].

Table. Summary of the experimental evidence regarding the beneficial effects of melatonin.

Target	Effect	References
Brain Protection		
Infarct volume	↓	[34–36]
Sensorimotor asymmetry	↓	[34]
Learning deficits	↓	[34]
Morphologically well preserved neurons	↑	[33,40,41]
GFAP expression	↓	[33]
MBP expression	↑	[33,50–52]
Antioxidant		
Lipid peroxidation and MDA production	↓	[56–58]
Iso- and neuroprostanes and neurofurans	↓	[62–64]
Protein oxidation	↓	[65]
Catalase's activity	→	[57]
Hydroxyl formation	↓	[56]
Nitrite/nitrate levels	↓	[58]
Anti-apoptotic		
Cytochrome c release	↓	[32,67,68]
Caspase-1 and Caspase-3 activation	↓	[32,67,69–73,88,89]
Bcl-xL and Bcl-2 expression	↑	[70,71,74,75,89]
Bax expression	↓	[71]
Poly-ADP-ribose-polymerase cleavage	↓	[72]
Mitochondrial transition pore opening	↓	[67,69]
TUNEL-positive cells/DNA breaks	↓	[31–33,35,36,64,72,75–78]
Cytosolic calcium concentrations	↓	[81,82]
Oxidative mitochondria damage	↓	[87]
Mitochondrial respiratory activity	→	[40,41]
Oxidative stress	↓	[40,41]
Fractin levels	↓	[88]
Bcl-2/Bax ratio	↑	[89]
MAP kinase, JNK1/2 and ERK 1/2	→	[31,72,90]
Bad dephosphorylation	↓	[31]
Anti-inflammatory		
Interleukin-6, Interleukin -8 and Tumor Necrosis Factor- α	↓	[94–100,102,122,123,125,126]
5-lipoxygenase and Cyclooxyenase-2	↓	[101–103,127]
Prostaglandin	↓	[104,106]
NO, nNOS, iNOS and VEGF	↓	[107–112,124,128]
Macrophage infiltration	↓	[88]
ED1 positive cells	↓	[63]

6. Conclusions

Nowadays, there are convincing evidences demonstrating that melatonin treatment is highly effective against hypoxic-ischemic brain injury in different animal models by reducing infarct volume and neuronal loss, minimizing lipid and protein peroxidation, blocking some apoptotic pathways, inhibiting FR production and decreasing inflammation. Melatonin supplementation, which has a benign safety profile, may help to reduce complications in the neonatal period that are associated with short gestation [129] and has demonstrated not only neuroprotective actions against HI in animal models, but also in preliminary clinical trials [57,130–132].

Nevertheless, the complexity of neonatal hypoxic-ischemic pathophysiology determines that successful neuroprotection could be achieved only by multi-therapeutic approaches and optimizing therapy for neonatal brain injury will require capitalizing on multiple pathways, which prevent cell death. The use of synergic strategies, such as the association between hypothermia and other therapeutic drugs, may lead to a larger neuroprotective effect on the brain thus improving the neonatal outcome. In this regard, Robertson *et al.* have recently shown that melatonin administration to newborn piglets augments hypothermic neuroprotection by improving cerebral energy metabolism and by reducing brain damage [133].

Melatonin's protective actions include not only its direct free radical scavenging, but also the interaction of its receptors and several yet-undefined functions, so the mechanisms underlying its neuroprotective benefits are not yet fully elucidated. Moreover, its variable oral absorption and rapid metabolization [134–136], the search for an appropriate dosage to obtain an antioxidant effect without desensitize melatonin receptors and its different pharmacokinetic profile when comparing preterm infants with adults (the half-life of melatonin in neonates is approximately 15 h, while in adults, it is around 45–60 min) [137], highlight the work that needs to be done before melatonin comes into clinical practice in a neonatal or pediatric critical care unit.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Arendt, J. *Melatonin and the Mammalian Pineal Gland*; Chapman & Hall: London, UK, 1995.
2. Tan, D.X.; Manchester, L.C.; Terron, M.P.; Flores, L.J.; Reiter, R.J. One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.* **2007**, *42*, 28–42.
3. Tan, D.X.; Hardeland, R.; Manchester, L.C.; Paredes, S.D.; Korkmaz, A.; Sainz, R.M.; Mayo, J.C.; Fuentes-Broto, L.; Reiter, R.J. The changing biological roles of melatonin during evolution: From an antioxidant to signals of darkness, sexual selection and fitness. *Biol. Rev. Camb. Philos. Soc.* **2010**, *85*, 607–623.

4. Rosen, J.; Than, N.N.; Koch, D.; Poeggeler, B.; Laatsch, H.; Hardeland, R. Interactions of melatonin and its metabolites with the ABTS cation radical: Extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. *J. Pineal Res*. **2006**, *41*, 374–381.

5. Acuna-Castroviejo, D.; Martin, M.; Macias, M.; Escames, G.; Leon, J.; Khaldy, H.; Reiter, R.J. Melatonin, mitochondria, and cellular bioenergetics. *J. Pineal Res*. **2001**, *30*, 65–74.

6. Tomas-Zapico, C.; Coto-Montes, A. A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. *J. Pineal Res*. **2005**, *39*, 99–104.

7. Reiter, R.J.; Tan, D.X.; Osuna, C.; Gitto, E. Actions of melatonin in the reduction of oxidative stress. A Review. *J. Biomed. Sci*. **2000**, *7*, 444–458.

8. De Haan, M.; Wyatt, J.S.; Roth, S.; Vargha-Khadem, F.; Gadian, D.; Mishkin, M. Brain and cognitive-behavioural development after asphyxia at term birth. *Dev. Sci*. **2006**, *9*, 350–358.

9. Du Plessis, A.J.; Volpe, J.J. Perinatal brain injury in the preterm and term newborn. *Curr. Opin. Neurol*. **2002**, *15*, 151–157.

10. Hamrick, S.E.; Ferriero, D.M. The injury response in the term newborn brain: Can we neuroprotect? *Curr. Opin. Neurol*. **2003**, *16*, 147–154.

11. Volpe, J.J. Perinatal brain injury: From pathogenesis to neuroprotection. *Ment. Retard. Dev. Disabil. Res. Rev*. **2001**, *7*, 56–64.

12. Low, J.A. Determining the contribution of asphyxia to brain damage in the neonate. *J. Obstet. Gynaecol. Res*. **2004**, *30*, 276–286.

13. Vannucci, S.J.; Hagberg, H. Hypoxia-ischemia in the immature brain. *J. Exp. Biol*. **2004**, *207*, 3149–3154.

14. Maneru, C.; Junque, C.; Botet, F.; Tallada, M.; Guardia, J. Neuropsychological long-term sequelae of perinatal asphyxia. *Brain Inj*. **2001**, *15*, 1029–1039.

15. Yager, J.Y.; Armstrong, E.A.; Black, A.M. Treatment of the term newborn with brain injury: Simplicity as the mother of invention. *Pediatr. Neurol*. **2009**, *40*, 237–243.

16. Vitte, P.A.; Harthe, C.; Lestage, P.; Claustrat, B.; Bobillier, P. Plasma, cerebrospinal fluid, and brain distribution of 14C-melatonin in Rat: A biochemical and autoradiographic study. *J. Pineal Res*. **1988**, *5*, 437–453.

17. Menendez-Pelaez, A.; Reiter, R.J. Distribution of melatonin in mammalian tissues: The relative importance of nuclear versus cytosolic localization. *J. Pineal Res*. **1993**, *15*, 59–69.

18. Gupta, Y.K.; Gupta, M.; Kohli, K. Neuroprotective role of melatonin in oxidative stress vulnerable brain. *Indian J. Physiol. Pharmacol*. **2003**, *47*, 373–386.

19. Rees, S.; Harding, R.; Walker, D. The biological basis of injury and neuroprotection in the fetal and neonatal brain. *Int. J. Dev. Neurosci*. **2011**, *29*, 551–563.

20. Hilario, E.; Alvarez, A.; Alvarez, F.J.; Gastiasoro, E.; Valls-i-Soler, A.; Cellular mechanisms in perinatal hypoxic-ischemic brain injury. *Curr. Pediatr. Rev*. **2006**, *2*, 131–141.

21. Manev, H.; Uz, T.; Kharlamov, A.; Joo, J.Y. Increased brain damage after stroke or excitotoxic seizures in melatonin-deficient rats. *FASEB J*. **1996**, *10*, 1546–1551.

22. Li, X.J.; Zhang, L.M.; Gu, J.; Zhang, A.Z.; Sun, F.Y. Melatonin decreases production of hydroxyl radical during cerebral ischemia-reperfusion. *Zhongguo Yao Li Xue Bao* **1997**, *18*, 394–396.

23. Cho, S.; Joh, T.H.; Baik, H.H.; Dibinis, C.; Volpe, B.T. Melatonin administration protects CA1 hippocampal neurons after transient forebrain ischemia in rats. *Brain Res.* **1997**, *755*, 335–338.

24. Kilic, E.; Ozdemir, Y.G.; Bolay, H.; Kelestimur, H.; Dalkara, T. Pinealectomy aggravates and melatonin administration attenuates brain damage in focal ischemia. *J. Cereb. Blood Flow MeTable* **1999**, *19*, 511–516.

25. Wakatsuki, A.; Okatani, Y.; Izumiya, C.; Ikenoue, N. Melatonin protects against ischemia and reperfusion-induced oxidative lipid and DNA damage in fetal rat brain. *J. Pineal Res.* **1999**, *26*, 147–152.

26. Joo, J.Y.; Uz, T.; Manev, H. Opposite effects of pinealectomy and melatonin administration on brain damage following cerebral focal ischemia in rat. *Restor. Neurol. Neurosci.* **1998**, *13*, 185–191.

27. Cuzzocrea, S.; Costantino, G.; Gitto, E.; Mazzon, E.; Fulia, F.; Serraino, I.; Cordaro, S.; Barberi, I.; De Sarro, A.; Caputi, A.P. Protective effects of melatonin in ischemic brain injury. *J. Pineal Res.* **2000**, *29*, 217–227.

28. Letechipia-Vallejo, G.; Gonzalez-Burgos, I.; Cervantes, M. Neuroprotective effect of melatonin on brain damage induced by acute global cerebral ischemia in cats. *Arch. Med. Res.* **2001**, *32*, 186–192.

29. Zhang, J.; Guo, J.D.; Xing, S.H.; Gu, S.L.; Dai, T.J. The protective effects of melatonin on global cerebral ischemia-reperfusion injury in gerbils. *Yao Xue Xue Bao* **2002**, *37*, 329–333.

30. Pei, Z.; Pang, S.F.; Cheung, R.T. Administration of melatonin after onset of ischemia reduces the volume of cerebral infarction in a rat middle cerebral artery occlusion stroke model. *Stroke* **2003**, *34*, 770–775.

31. Koh, P.O. Melatonin attenuates the focal cerebral ischemic injury by inhibiting the dissociation of pBad from 14-3-3. *J. Pineal Res.* **2008**, *44*, 101–106.

32. Wang, X.; Figueroa, B.E.; Stavrovskaya, I.G.; Zhang, Y.; Sirianni, A.C.; Zhu, S.; Day, A.L.; Kristal, B.S.; Friedlander, R.M. Methazolamide and melatonin inhibit mitochondrial cytochrome C release and are neuroprotective in experimental models of ischemic injury. *Stroke* **2009**, *40*, 1877–1885.

33. Alonso-Alconada, D.; Alvarez, A.; Lacalle, J.; Hilario, E. Histological study of the protective effect of melatonin on neural cells after neonatal hypoxia-ischemia. *Histol. Histopathol.* **2012**, *27*, 771–783.

34. Carloni, S.; Perrone, S.; Buonocore, G.; Longini, M.; Proietti, F.; Balduini, W. Melatonin protects from the long-term consequences of a neonatal hypoxic-ischemic brain injury in rats. *J. Pineal Res.* **2008**, *44*, 157–164.

35. Cetinkaya, M.; Alkan, T.; Ozyener, F.; Kafa, I.M.; Kurt, M.A.; Koksal, N. Possible neuroprotective effects of magnesium sulfate and melatonin as both pre- and post-treatment in a neonatal hypoxic-ischemic rat model. *Neonatology* **2011**, *99*, 302–310.

36. Ozyener, F.; Cetinkaya, M.; Alkan, T.; Goren, B.; Kafa, I.M.; Kurt, M.A.; Koksal, N. Neuroprotective effects of melatonin administered alone or in combination with topiramate in neonatal hypoxic-ischemic rat model. *Restor. Neurol. Neurosci.* **2012**, *30*, 435–444.

37. Mattson, M.P.; Guthrie, P.B.; Kater, S.B. Intrinsic factors in the selective vulnerability of hippocampal pyramidal neurons. *Prog. Clin. Biol. Res.* **1989**, *317*, 333–351.

38. Johnston, M.V. Selective vulnerability in the neonatal brain. *Ann. Neurol.* **1998**, *44*, 155–156.

39. Northington, F.J.; Ferriero, D.M.; Graham, E.M.; Traystman, R.J.; Martin, L.J. Early neurodegeneration after hypoxia-ischemia in neonatal rat is necrosis while delayed neuronal death is apoptosis. *Neurobiol. Dis.* **2001**, *8*, 207–219.

40. Hamada, F.; Watanabe, K.; Wakatsuki, A.; Nagai, R.; Shinohara, K.; Hayashi, Y.; Imamura, R.; Fukaya, T. Therapeutic effects of maternal melatonin administration on ischemia/reperfusion-induced oxidative cerebral damage in neonatal rats. *Neonatology* **2010**, *98*, 33–40.

41. Watanabe, K.; Hamada, F.; Wakatsuki, A.; Nagai, R.; Shinohara, K.; Hayashi, Y.; Imamura, R.; Fukaya, T. Prophylactic administration of melatonin to the mother throughout pregnancy can protect against oxidative cerebral damage in neonatal rats. *J. Matern. Fetal. Neonatal Med.* **2012**, *25*, 1254–1259.

42. Takuma, K.; Baba, A.; Matsuda, T. Astrocyte apoptosis: Implications for neuroprotection. *Prog. Neurobiol.* **2004**, *72*, 111–127.

43. Panickar, K.S.; Norenberg, M.D. Astrocytes in cerebral ischemic injury: Morphological and general considerations. *Glia* **2005**, *50*, 287–298.

44. Sizonenko, S.V.; Camm, E.J.; Dayer, A.; Kiss, J.Z. Glial responses to neonatal hypoxic-ischemic injury in the rat cerebral cortex. *Int. J. Dev. Neurosci.* **2008**, *26*, 37–45.

45. Huang, Z.; Liu, J.; Cheung, P.Y.; Chen, C. Long-term cognitive impairment and myelination deficiency in a rat model of perinatal hypoxic-ischemic brain injury. *Brain Res.* **2009**, *1301*, 100–109.

46. Xiong, M.; Yang, Y.; Chen, G.Q.; Zhou, W.H. Post-ischemic hypothermia for 24h in P7 rats rescues hippocampal neuron: Association with decreased astrocyte activation and inflammatory cytokine expression. *Brain Res. Bull.* **2009**, *79*, 351–357.

47. Rothstein, R.P.; Levison, S.W. Gray matter oligodendrocyte progenitors and neurons die caspase-3 mediated deaths subsequent to mild perinatal hypoxic/ischemic insults. *Dev. Neurosci.* **2005**, *27*, 149–159.

48. Inder, T.E.; Wells, S.J.; Mogridge, N.B.; Spencer, C.; Volpe, J.J. Defining the nature of the cerebral abnormalities in the premature infant: A qualitative magnetic resonance imaging study. *J. Pediatr.* **2003**, *143*, 171–179.

49. Wang, X.; Hagberg, H.; Zhu, C.; Jacobsson, B.; Mallard, C. Effects of intrauterine inflammation on the developing mouse brain. *Brain Res.* **2007**, *1144*, 180–185.

50. Olivier, P.; Fontaine, R.H.; Loron, G.; van Steenwinckel, J.; Biran, V.; Massonneau, V.; Kaindl, A.; Dalous, J.; Charriaut-Marlangue, C.; Aigrot, M.S. *et al.* Melatonin promotes oligodendroglial maturation of injured white matter in neonatal rats. *PLoS One* **2009**, *4*, e7128.

51. Kaur, C.; Sivakumar, V.; Ling, E.A. Melatonin protects periventricular white matter from damage due to hypoxia. *J. Pineal Res.* **2010**, *48*, 185–193.

52. Villapol, S.; Fau, S.; Renolleau, S.; Biran, V.; Charriaut-Marlangue, C.; Baud, O. Melatonin promotes myelination by decreasing white matter inflammation after neonatal stroke. *Pediatr. Res.* **2011**, *69*, 51–55.

53. McLean, C.; Ferriero, D. Mechanisms of hypoxic-ischemic injury in the term infant. *Semin. Perinatol.* **2004**, *28*, 425–432.

54. Sheldon, R.A.; Jiang, X.; Francisco, C.; Christen, S.; Vexler, Z.S.; Tauber, M.G.; Ferriero, D.M. Manipulation of antioxidant pathways in neonatal murine brain. *Pediatr. Res*. **2004**, *56*, 656–662.

55. McQuillen, P.S.; Ferriero, D.M. Selective vulnerability in the developing central nervous system. *Pediatr. Neurol*. **2004**, *30*, 227–235.

56. Miller, S.L.; Yan, E.B.; Castillo-Melendez, M.; Jenkin, G.; Walker, D.W. Melatonin provides neuroprotection in the late-gestation fetal sheep brain in response to umbilical cord occlusion. *Dev. Neurosci*. **2005**, *27*, 200–210.

57. Tutunculer, F.; Eskiocak, S.; Basaran, U.N.; Ekuklu, G.; Ayvaz, S.; Vatansever, U. The protective role of melatonin in experimental hypoxic brain damage. *Pediatr. Int*. **2005**, *47*, 434–439.

58. Fulia, F.; Gitto, E.; Cuzzocrea, S.; Reiter, R.J.; Dugo, L.; Gitto, P.; Barberi, S.; Cordaro, S.; Barberi, I. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: Reduction by melatonin. *J. Pineal Res*. **2001**, *31*, 343–349.

59. Kohen, R.; Nyska, A. Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol*. **2002**, *30*, 620–650.

60. Arneson, K.O.; Roberts, L.J., 2nd. Measurement of products of docosahexaenoic acid peroxidation, neuroprostanes, and neurofurans. *Methods Enzymol*. **2007**, *433*, 127–143.

61. Song, W.L.; Lawson, J.A.; Reilly, D.; Rokach, J.; Chang, C.T.; Giasson, B.; FitzGerald, G.A. Neurofurans, novel indices of oxidant stress derived from docosahexaenoic acid. *J. Biol. Chem*. **2008**, *283*, 6–16.

62. Signorini, C.; Ciccoli, L.; Leoncini, S.; Carloni, S.; Perrone, S.; Comporti, M.; Balduini, W.; Buonocore, G. Free iron, total F-isoprostanes and total F-neuroprostanes in a model of neonatal hypoxic-ischemic encephalopathy: Neuroprotective effect of melatonin. *J. Pineal Res*. **2009**, *46*, 148–154.

63. Balduini, W.; Carloni, S.; Perrone, S.; Bertrando, S.; Tataranno, M.L.; Negro, S.; Proietti, F.; Longini, M.; Buonocore, G. The use of melatonin in hypoxic-ischemic brain damage: An experimental study. *J. Matern. Fetal. Neonatal Med*. **2012**, *25*, 119–124.

64. Welin, A.K.; Svedin, P.; Lapatto, R.; Sultan, B.; Hagberg, H.; Gressens, P.; Kjellmer, I.; Mallard, C. Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. *Pediatr. Res*. **2007**, *61*, 153–158.

65. Eskiocak, S.; Tutunculer, F.; Basaran, U.N.; Taskiran, A.; Cakir, E. The Effect of melatonin on protein oxidation and nitric oxide in the brain tissue of hypoxic neonatal rats. *Brain Dev.* **2007**, *29*, 19–24.

66. Witko-Sarsat, V.; Friedlander, M.; Capeillere-Blandin, C.; Nguyen-Khoa, T.; Nguyen, A.T.; Zingraff, J.; Jungers, P.; Descamps-Latscha, B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int*. **1996**, *49*, 1304–1313.

67. Andrabi, S.A.; Sayeed, I.; Siemen, D.; Wolf, G.; Horn, T.F. Direct Inhibition of the mitochondrial permeability transition pore: A possible mechanism responsible for anti-apoptotic effects of melatonin. *FASEB J.* **2004**, *18*, 869–871.

68. Wang, X.; Zhu, S.; Pei, Z.; Drozda, M.; Stavrovskaya, I.G.; Del Signore, S.J.; Cormier, K.; Shimony, E.M.; Wang, H.; Ferrante, R.J. *et al*. Inhibitors of cytochrome c release with therapeutic potential for huntington's disease. *J. Neurosci*. **2008**, *28*, 9473–9485.

69. Jou, M.J.; Peng, T.I.; Reiter, R.J.; Jou, S.B.; Wu, H.Y.; Wen, S.T. Visualization of the antioxidative effects of melatonin at the mitochondrial level during oxidative stress-induced apoptosis of rat brain astrocytes. *J. Pineal Res*. **2004**, *37*, 55–70.

70. Kilic, E.; Kilic, U.; Reiter, R.J.; Bassetti, C.L.; Hermann, D.M. Tissue-plasminogen activator-induced ischemic brain injury is reversed by melatonin: Role of inos and akt. *J. Pineal Res*. **2005**, *39*, 151–155.

71. Jang, M.H.; Jung, S.B.; Lee, M.H.; Kim, C.J.; Oh, Y.T.; Kang, I.; Kim, J.; Kim, E.H. Melatonin attenuates amyloid beta25–35-induced apoptosis in mouse microglial bv2 cells. *Neurosci. Lett*. **2005**, *380*, 26–31.

72. Ebadi, M.; Sharma, S.K.; Ghafourifar, P.; Brown-Borg, H.; El Refaey, H. Peroxynitrite in the pathogenesis of parkinson's disease and the neuroprotective role of metallothioneins. *Methods Enzymol*. **2005**, *396*, 276–298.

73. Alvira, D.; Tajes, M.; Verdaguer, E.; Acuna-Castroviejo, D.; Folch, J.; Camins, A.; Pallas, M. Inhibition of the cdk5/p25 fragment formation may explain the antiapoptotic effects of melatonin in an experimental model of parkinson's disease. *J. Pineal Res*. **2006**, *40*, 251–258.

74. Ling, X.; Zhang, L.M.; Lu, S.D.; Li, X.J.; Sun, F.Y. Protective effect of melatonin on injuried cerebral neurons is associated with bcl-2 protein over-expression. *Zhongguo Yao Li Xue Bao* **1999**, *20*, 409–414.

75. Sun, F.Y.; Lin, X.; Mao, L.Z.; Ge, W.H.; Zhang, L.M.; Huang, Y.L.; Gu, J. Neuroprotection by melatonin against ischemic neuronal injury associated with modulation of DNA damage and repair in the rat following a transient cerebral ischemia. *J. Pineal Res*. **2002**, *33*, 48–56.

76. Chung, S.Y.; Han, S.H. Melatonin attenuates kainic acid-induced hippocampal neurodegeneration and oxidative stress through microglial inhibition. *J. Pineal Res*. **2003**, *34*, 95–102.

77. Feng, Z.; Cheng, Y.; Zhang, J.T. Long-term effects of melatonin or 17 beta-estradiol on improving spatial memory performance in cognitively impaired, ovariectomized adult rats. *J. Pineal Res*. **2004**, *37*, 198–206.

78. Deng, Y.Q.; Xu, G.G.; Duan, P.; Zhang, Q.; Wang, J.Z. Effects of melatonin on wortmannin-induced TAU hyperphosphorylation. *Acta Pharmacol. Sin*. **2005**, *26*, 519–526.

79. Rosenstein, R.E.; Cardinali, D.P. Central gabaergic mechanisms as targets for melatonin activity in brain. *Neurochem. Int*. **1990**, *17*, 373–379.

80. Molina-Carballo, A.; Munoz-Hoyos, A.; Sanchez-Forte, M.; Uberos-Fernandez, J.; Moreno-Madrid, F.; Acuna-Castroviejo, D. Melatonin increases following convulsive seizures may be related to its anticonvulsant properties at physiological concentrations. *Neuropediatrics* **2007**, *38*, 122–125.

81. Prada, C.; Udin, S.B.; Wiechmann, A.F.; Zhdanova, I.V. Stimulation of melatonin receptors decreases calcium levels in xenopus tectal cells by activating gaba(c) receptors. *J. Neurophysiol*. **2005**, *94*, 968–978.

82. Prada, C.; Udin, S.B. Melatonin decreases calcium levels in retinotectal axons of xenopus laevis by indirect activation of group iii metabotropic glutamate receptors. *Brain Res*. **2005**, *1053*, 67–76.

83. Buonocore, G.; Perrone, S.; Bracci, R. Free radicals and brain damage in the newborn. *Biol. Neonate* **2001**, *79*, 180–186.

84. Blomgren, K.; Hagberg, H. Free radicals, mitochondria, and hypoxia-ischemia in the developing brain. *Free Radic. Biol. Med*. **2006**, *40*, 388–397.

85. Kumar, A.; Mittal, R.; Khanna, H.D.; Basu, S. Free radical injury and blood-brain barrier permeability in hypoxic-ischemic encephalopathy. *Pediatrics* **2008**, *122*, 722–727.

86. Alonso-Alconada, D.; Hilario, E.; Alvarez, F.J.; Alvarez, A. Apoptotic cell death correlates with ros overproduction and early cytokine expression after hypoxia-ischemia in fetal lambs. *Reprod. Sci*. **2012**, *19*, 754–763.

87. Watanabe, K.; Wakatsuki, A.; Shinohara, K.; Ikenoue, N.; Yokota, K.; Fukaya, T. Maternally administered melatonin protects against ischemia and reperfusion-induced oxidative mitochondrial damage in premature fetal rat brain. *J. Pineal Res*. **2004**, *37*, 276–280.

88. Hutton, L.C.; Abbass, M.; Dickinson, H.; Ireland, Z.; Walker, D.W. Neuroprotective properties of melatonin in a model of birth asphyxia in the spiny mouse (*Acomys cahirinus*). *Dev. Neurosci*. **2009**, *31*, 437–451.

89. Fu, J.; Zhao, S.D.; Liu, H.J.; Yuan, Q.H.; Liu, S.M.; Zhang, Y.M.; Ling, E.A.; Hao, A.J. Melatonin promotes proliferation and differentiation of neural stem cells subjected to hypoxia *in vitro*. *J. Pineal Res.* **2011**, *51*, 104–112.

90. Kilic, U.; Kilic, E.; Reiter, R.J.; Bassetti, C.L.; Hermann, D.M. Signal transduction pathways involved in melatonin-induced neuroprotection after focal cerebral ischemia in mice. *J. Pineal Res.* **2005**, *38*, 67–71.

91. Koh, P.O. Melatonin prevents the injury-induced decline of akt/forkhead transcription factors phosphorylation. *J. Pineal Res*. **2008**, *45*, 199–203.

92. Zhou, J.; Zhang, S.; Zhao, X.; Wei, T. Melatonin impairs nadph oxidase assembly and decreases superoxide anion production in microglia exposed to amyloid-beta1–42. *J. Pineal Res*. **2008**, *45*, 157–165.

93. Koh, P.O. Melatonin prevents ischemic brain injury through activation of the mtor/p70s6 kinase signaling pathway. *Neurosci. Lett*. **2008**, *444*, 74–78.

94. Fjaerli, O.; Lund, T.; Osterud, B. The effect of melatonin on cellular activation processes in human blood. *J. Pineal Res.* **1999**, *26*, 50–55.

95. Baykal, A.; Iskit, A.B.; Hamaloglu, E.; Guc, M.O.; Hascelik, G.; Sayek, I. Melatonin modulates mesenteric blood flow and TNFα concentrations after lipopolysaccharide challenge. *Eur. J. Surg*. **2000**, *166*, 722–727.

96. Silva, S.O.; Rodrigues, M.R.; Ximenes, V.F.; Bueno-da-Silva, A.E.; Amarante-Mendes, G.P.; Campa, A. Neutrophils as a specific target for melatonin and kynuramines: Effects on cytokine release. *J. Neuroimmunol*. **2004**, *156*, 146–152.

97. Wang, H.; Wei, W.; Shen, Y.X.; Dong, C.; Zhang, L.L.; Wang, N.P.; Yue, L.; Xu, S.Y. Protective effect of melatonin against liver injury in mice induced by bacillus calmette-guerin plus lipopolysaccharide. *World J. Gastroenterol*. **2004**, *10*, 2690–2696.

98. Perianayagam, M.C.; Oxenkrug, G.F.; Jaber, B.L. Immune-modulating effects of melatonin, *N*-acetylserotonin, and *N*-acetyldopamine. *Ann. N.Y. Acad. Sci*. **2005**, *1053*, 386–393.

99. Carrillo-Vico, A.; Lardone, P.J.; Fernandez-Santos, J.M.; Martin-Lacave, I.; Calvo, J.R.; Karasek, M.; Guerrero, J.M. Human lymphocyte-synthesized melatonin is involved in the regulation of the interleukin-2/interleukin-2 receptor system. *J. Clin. Endocrinol. MeTable* **2005**, *90*, 992–1000.

100. Gitto, E.; Reiter, R.J.; Sabatino, G.; Buonocore, G.; Romeo, C.; Gitto, P.; Bugge, C.; Trimarchi, G.; Barberi, I. Correlation among cytokines, bronchopulmonary dysplasia and modality of ventilation in preterm newborns: Improvement with melatonin treatment. *J. Pineal Res*. **2005**, *39*, 287–293.

101. Steinhilber, D.; Brungs, M.; Werz, O.; Wiesenberg, I.; Danielsson, C.; Kahlen, J.P.; Nayeri, S.; Schrader, M.; Carlberg, C. The nuclear receptor for melatonin represses 5-lipoxygenase gene expression in human B lymphocytes. *J. Biol. Chem.* **1995**, *270*, 7037–7040.

102. Mayo, J.C.; Sainz, R.M.; Tan, D.X.; Hardeland, R.; Leon, J.; Rodriguez, C.; Reiter, R.J. Anti-inflammatory actions of melatonin and its metabolites, *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK) and *N*1-acetyl-5-methoxykynuramine (AMK), in macrophages. *J. Neuroimmunol*. **2005**, *165*, 139–149.

103. Deng, W.G.; Tang, S.T.; Tseng, H.P.; Wu, K.K. Melatonin suppresses macrophage cyclooxygenase-2 and inducible nitric oxide synthase expression by inhibiting p52 acetylation and binding. *Blood* **2006**, *108*, 518–524.

104. Cardinali, D.P.; Ritta, M.N.; Fuentes, A.M.; Gimeno, M.F.; Gimeno, A.L. Prostaglandin E release by rat medial basal hypothalamus *in vitro*. Inhibition by melatonin at submicromolar concentrations. *Eur. J. Pharmacol*. **1980**, *67*, 151–153.

105. Cardinali, D.P.; Ritta, M.N. The role of prostaglandins in neuroendocrine junctions: Studies in the pineal gland and the hypothalamus. *Neuroendocrinology* **1983**, *36*, 152–160.

106. Carrillo-Vico, A.; Garcia-Maurino, S.; Calvo, J.R.; Guerrero, J.M. Melatonin counteracts the inhibitory effect of PGE2 on IL-2 production in human lymphocytes via its mt1 membrane receptor. *FASEB J.* **2003**, *17*, 755–757.

107. Bilici, D.; Akpinar, E.; Kiziltunc, A. Protective effect of melatonin in carrageenan-induced acute local inflammation. *Pharmacol. Res*. **2002**, *46*, 133–139.

108. Acuna-Castroviejo, D.; Escames, G.; Lopez, L.C.; Hitos, A.B.; Leon, J. Melatonin and nitric oxide: Two required antagonists for mitochondrial homeostasis. *Endocrine* **2005**, *27*, 159–168.

109. Leon, J.; Macias, M.; Escames, G.; Camacho, E.; Khaldy, H.; Martin, M.; Espinosa, A.; Gallo, M.A.; Acuna-Castroviejo, D. Structure-related inhibition of calmodulin-dependent neuronal nitric-oxide synthase activity by melatonin and synthetic kynurenines. *Mol. Pharmacol*. **2000**, *58*, 967–975.

110. Leon, J.; Escames, G.; Rodriguez, M.I.; Lopez, L.C.; Tapias, V.; Entrena, A.; Camacho, E.; Carrion, M.D.; Gallo, M.A.; Espinosa, A. *et al*. Inhibition of neuronal nitric oxide synthase activity by *N*1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin. *J. Neurochem*. **2006**, *98*, 2023–2033.

111. Jimenez-Ortega, V.; Cano, P.; Cardinali, D.P.; Esquifino, A.I. 24-Hour variation in gene expression of redox pathway enzymes in rat hypothalamus: Effect of melatonin treatment. *Redox Rep*. **2009**, *14*, 132–138.

112. Tapias, V.; Escames, G.; Lopez, L.C.; Lopez, A.; Camacho, E.; Carrion, M.D.; Entrena, A.; Gallo, M.A.; Espinosa, A.; Acuna-Castroviejo, D. Melatonin and its brain metabolite N(1)-acetyl-5-methoxykynuramine prevent mitochondrial nitric oxide synthase induction in parkinsonian mice. *J. Neurosci. Res*. **2009**, *87*, 3002–3010.

113. Leon, J.; Vives, F.; Crespo, E.; Camacho, E.; Espinosa, A.; Gallo, M.A.; Escames, G.; Acuna-Castroviejo, D. Modification of nitric oxide synthase activity and neuronal response in rat striatum by melatonin and kynurenine derivatives. *J. Neuroendocrinol.* **1998**, *10*, 297–302.

114. Chandrasekaran, A.; Ponnambalam, G.; Kaur, C. Domoic acid-induced neurotoxicity in the hippocampus of adult rats. *Neurotox Res.* **2004**, *6*, 105–117.

115. Escames, G.; Khaldy, H.; Leon, J.; Gonzalez, L.; Acuna-Castroviejo, D. Changes in iNOS activity, oxidative stress and melatonin levels in hypertensive patients treated with lacidipine. *J. Hypertens.* **2004**, *22*, 629–635.

116. Escames, G.; Acuna-Castroviejo, D.; Lopez, L.C.; Tan, D.X.; Maldonado, M.D.; Sanchez-Hidalgo, M.; Leon, J.; Reiter, R.J. Pharmacological utility of melatonin in the treatment of septic shock: Experimental and clinical evidence. *J. Pharm. Pharmacol.* **2006**, *58*, 1153–1165.

117. Escames, G.; Lopez, L.C.; Tapias, V.; Utrilla, P.; Reiter, R.J.; Hitos, A.B.; Leon, J.; Rodriguez, M.I.; Acuna-Castroviejo, D. Melatonin counteracts inducible mitochondrial nitric oxide synthase-dependent mitochondrial dysfunction in skeletal muscle of septic mice. *J. Pineal Res.* **2006**, *40*, 71–78.

118. Lopez, L.C.; Escames, G.; Tapias, V.; Utrilla, P.; Leon, J.; Acuna-Castroviejo, D. Identification of an inducible nitric oxide synthase in diaphragm mitochondria from septic mice: Its relation with mitochondrial dysfunction and prevention by melatonin. *Int. J. Biochem. Cell Biol.* **2006**, *38*, 267–278.

119. Srinivasan, V.; Pandi-Perumal, S.R.; Spence, D.W.; Kato, H.; Cardinali, D.P. Melatonin in septic shock: Some recent concepts. *J. Crit. Care* **2010**, *25*, 656.e1–656.e6.

120. Lopez, L.C.; Escames, G.; Ortiz, F.; Ros, E.; Acuna-Castroviejo, D. Melatonin restores the mitochondrial production of ATP in septic mice. *Neuro Endocrinol. Lett.* **2006**, *27*, 623–630.

121. Escames, G.; Lopez, L.C.; Ortiz, F.; Lopez, A.; Garcia, J.A.; Ros, E.; Acuna-Castroviejo, D. Attenuation of cardiac mitochondrial dysfunction by melatonin in septic mice. *FEBS J.* **2007**, *274*, 2135–2147.

122. Pei, Z.; Cheung, R.T. Pretreatment with melatonin exerts anti-inflammatory effects against ischemia/reperfusion injury in a rat middle cerebral artery occlusion stroke model. *J. Pineal Res.* **2004**, *37*, 85–91.

123. Lee, M.Y.; Kuan, Y.H.; Chen, H.Y.; Chen, T.Y.; Chen, S.T.; Huang, C.C.; Yang, I.P.; Hsu, Y.S.; Wu, T.S.; Lee, E.J. Intravenous administration of melatonin reduces the intracerebral cellular inflammatory response following transient focal cerebral ischemia in rats. *J. Pineal Res.* **2007**, *42*, 297–309.

124. Koh, P.O. Melatonin regulates nitric oxide synthase expression in ischemic brain injury. *J. Vet. Med. Sci.* **2008**, *70*, 747–750.

125. Mohan, N.; Sadeghi, K.; Reiter, R.J.; Meltz, M.L. The neurohormone melatonin inhibits cytokine, mitogen and ionizing radiation induced NF-κB. *Biochem. Mol. Biol. Int.* **1995**, *37*, 1063–1070.

126. Reiter, R.J.; Calvo, J.R.; Karbownik, M.; Qi, W.; Tan, D.X. Melatonin and its relation to the immune system and inflammation. *Ann. N.Y. Acad. Sci.* **2000**, *917*, 376–386.

127. Hardeland, R. Antioxidative protection by melatonin: Multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine* **2005**, *27*, 119–130.

128. Kaur, C.; Sivakumar, V.; Lu, J.; Tang, F.R.; Ling, E.A. Melatonin attenuates hypoxia-induced ultrastructural changes and increased vascular permeability in the developing hippocampus. *Brain Pathol.* **2008**, *18*, 533–547.

129. Jan, J.E.; Wasdell, M.B.; Freeman, R.D.; Bax, M. Evidence supporting the use of melatonin in short gestation infants. *J. Pineal Res.* **2007**, *42*, 22–27.

130. Gitto, E.; Reiter, R.J.; Cordaro, S.P.; La Rosa, M.; Chiurazzi, P.; Trimarchi, G.; Gitto, P.; Calabro, M.P.; Barberi, I. Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: Beneficial effects of melatonin. *Am. J. Perinatol.* **2004**, *21*, 209–216.

131. Gitto, E.; Reiter, R.J.; Amodio, A.; Romeo, C.; Cuzzocrea, E.; Sabatino, G.; Buonocore, G.; Cordaro, V.; Trimarchi, G.; Barberi, I. Early indicators of chronic lung disease in preterm infants with respiratory distress syndrome and their inhibition by melatonin. *J. Pineal Res.* **2004**, *36*, 250–255.

132. Buonocore, G.; Groenendaal, F. Anti-oxidant strategies. *Semin. Fetal. Neonatal Med.* **2007**, *12*, 287–295.

133. Robertson, N.J.; Faulkner, S.; Fleiss, B.; Bainbridge, A.; Andorka, C.; Price, D.; Powell, E.; Lecky-Thompson, L.; Thei, L.; Chandrasekaran, M. *et al.* Melatonin augments hypothermic neuroprotection in a perinatal asphyxia model. *Brain* **2013**, *136*, 90–105.

134. Waldhauser, F.; Waldhauser, M.; Lieberman, H.R.; Deng, M.H.; Lynch, H.J.; Wurtman, R.J. Bioavailability of oral melatonin in humans. *Neuroendocrinology* **1984**, *39*, 307–313.

135. Aldhous, M.; Franey, C.; Wright, J.; Arendt, J. Plasma concentrations of melatonin in man following oral absorption of different preparations. *Br. J. Clin. Pharmacol.* **1985**, *19*, 517–521.

136. Lane, E.A.; Moss, H.B. Pharmacokinetics of melatonin in man: First pass hepatic metabolism. *J. Clin. Endocrinol. MeTable* **1985**, *61*, 1214–1216.

137. Merchant, N.M.; Azzopardi, D.V.; Hawwa, A.F.; McElnay, J.C.; Middleton, B.; Arendt, J.; Arichi, T.; Gressens, P.; Edwards, A.D. Pharmacokinetics of Melatonin in Preterm Infants. *Br. J. Clin. Pharmacol.* **2013**, doi:10.1111/bcp.12092.