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N-Acetylserotonin: Neuroprotection, Neurogenesis, and the Sleepy Brain

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Abstract

N-Acetylserotonin (NAS) is a naturally occurring chemical intermediate in biosynthesis of melatonin. Previous studies have shown that NAS has different brain distribution patterns from those of serotonin and melatonin suggesting that NAS might have functions other than as a precursor or metabolite of melatonin. Indeed several studies have now shown that NAS may play an important role in mood regulation and may have antidepressant activity. Additional studies have shown that NAS stimulates proliferation of neuroprogenitor cells and prevents some of the negative effects of sleep deprivation. It is believed that the antidepressant and neurotrophic actions of NAS are due, at least in part, to the capability on this molecule to activate the TrkB receptor in a BDNF-independent manner. Emerging evidence also indicates that NAS and its derivatives have neuroprotective properties and protect retinal photoreceptor cells from light-induced degeneration. In this review we will discuss the literature about this exciting and underappreciated molecule.

Keywords

N-Acetylserotonin; Melatonin; Trk B; Sleep; Neuroprotection; Circadian Rhythms; Photoreceptors

N-Acetylserotonin (NAS) is synthesized in the mammalian pineal gland and retina. Until very recently, NAS was believed to be just an intermediate in the biosynthetic pathway of melatonin with no or very limited biological function of its own. NAS is produced from serotonin by the enzyme arylalkylamine *N*-acetyltransferase (AANAT) and is converted to melatonin by acetylserotonin *O*-methyltransferase (ASMT; or hydroxyindole *O*-methyltransferase, HIOMT) (Figure 1). Although NAS is predominantly synthesized in the pineal gland a series of studies have also provided experimental evidence that NAS and AANAT activity are present in the hippocampus, the olfactory bulb, the spinal cord and the cerebellum (Paul et al., 1974; Psarakis et al., 1982; Gaudet 1991; Chae et al., 1999). These earlier findings have been supported by more recent studies using RT-PCR to analyze *Aanat* mRNA expression (Uz et al., 2002). The experimental observation that NAS may reside in the specific brain areas separate from melatonin and serotonin suggests that NAS may have a role in the central nervous system distinct from that of being a precursor for melatonin.

As with melatonin, NAS shows a clear daily/circadian variation in its level. In the pineal gland the levels of NAS start to surge 3–4 hrs after the onset of darkness at night and begin to decrease before the onset of light in the morning (Chattoraj et al., 2009). The rhythmic

synthesis in the pineal gland is controlled by the circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus. At night, the SCN sends signals to the pineal gland via sympathetic neurons that release norepinephrine (NE), thus activating adrenergic receptors to increase intracellular Ca^{2+} and cAMP with the consequent phosphorylation of the cAMP response element (CRE) binding protein (CREB) [Klein et al., 1997]. Phosphorylated CREB activates *Aanat* gene expression via CREs in the *Aanat* promoter (Baler et al., 1997, 1999; Figure 2). cAMP also stimulates the phosphorylation of AANAT protein, which promotes its association with 14-3-3 proteins, activating the enzyme and protecting it from degradation (Ganguly et al., 2001; Pozdeyev et al., 2006; Figure 2). The changes in the activity of AANAT are responsible for the variation in NAS (and melatonin) levels (Klein et al., 1997). The levels of NAS are tightly controlled by light at the level of AANAT activity. Exposure to light rapidly (within minutes) reduces AANAT activity by reducing cAMP, resulting in dephosphorylation and proteasomal proteolysis of the AANAT protein (Klein and Weller, 1972; Klein et al., 1978; Gastel et al., 1998; Fukuhara et al., 2001; Pozdeyev et al., 2006). The rapid destruction of the AANAT protein results in an almost immediate decrease in pineal levels of NAS and melatonin (Figure 2).

An important aspect of NAS biology is the identification of the receptors that are involved in modulation of its biological functions. Previous studies have shown that NAS has some activity on the membrane G-protein-coupled melatonin receptors (MT_1 and MT_2) (Nonno et al., 1999), but the affinity is several orders of magnitude lower than that for melatonin. Other studies have suggested that the putative *MT3* binding site has a higher affinity for NAS than for melatonin. Thus, it has been suggested that *MT3* may act as an NAS receptor (Nosjean et al., 2000). A recent study has shown that NAS may be a ligand for TrkB receptor, the cognate receptor for brain-derived neurotrophic factor (BDNF). NAS robustly activates the TrkB receptor in a BDNF- and *MT3* receptor-independent manner (Jang et al., 2010).

NAS Displays Antidepressant-like Activity

A number of early studies suggested that NAS may be an endogenous antidepressant molecule. For example, exogenous administration of NAS decreases immobility in the mouse tail suspension test (Prakhie and Oxenkrug, 1998) and chronic administration (three weeks) of the antidepressant fluoxetine induces a five-fold increase in the levels of *Aanat* mRNA and, presumably, NAS in the hippocampus (Uz and Manev, 1999). Additionally, clorgyline, a selective monoamine oxidase A (MAO-A) inhibitor with antidepressant-like activity, increases (5-fold) rat pineal melatonin and NAS content, and decreases 5-HIAA (MAO-oxidized metabolite) level by 80%; whereas deprenyl, a selective MAO-B inhibitor, does not change the content of melatonin or other pineal indoles (Oxenkrug et al., 1985). As we have previously discussed, NAS activates TrkB receptors (Jang et al., 2010), and several investigations have indicated that activation of TrkB receptors may be a common mechanism of antidepressant drug action (e.g., Rantamaki et al., 2007). The activation of TrkB by acute administration of some but not all antidepressant drugs may involve NAS. For example, the selective serotonin reuptake inhibitors (SSRI) fluoxetine and citalopram and the tricyclic antidepressant desipramine robustly stimulate TrkB activation in hippocampus of mice that synthesize NAS (C3H/ $f^{+/+}$ mice) as well as in C57BL/6 mice (Jang et al., 2010), which have a mutation in AANAT that prevents the synthesis of appreciable amounts of NAS or melatonin (Roseboom et al., 1998). In contrast, the MAO-A inhibitor clorgyline, which increases serotonin levels, stimulates TrkB activation in the C3H/ $f^{+/+}$ mice but not in the C57BL/6 mice (Jang et al., 2010). Interestingly, clorgyline only activates TrkB in C3H/ $f^{+/+}$ mice when administered at night in the dark, when AANAT is active; in contrast, clorgyline is ineffective at activating TrkB when administered to mice exposed to light, which inactivates AANAT. These findings suggest that clorgyline-induced

TrkB activation is attributable to increased levels serotonin and the subsequent conversion to NAS in darkness. Because deprenyl, an MAO-B inhibitor does not stimulate serotonin or NAS levels, it is unable to trigger TrkB activation whether the light is on or off. However, the SSRI and tricyclic antidepressants markedly activate TrkB regardless of dark or light. This result combined with the observation that these drugs stimulate TrkB phosphorylation in hippocampus of C57BL/6J mice with defective AANAT indicates that NAS is not a major effector in TrkB activation by acute administration of these agents. Clorgyline increases both melatonin and NAS levels in rat pineal glands (Oxenkrug et al., 1985). Further experiments have shown that intraperitoneal administration of NAS 1 h before testing significantly decreases the duration of immobility in the forced swim test as compared to saline control (Jang et al., 2010). By contrast, melatonin has little effect. This finding is consistent with a previous report that NAS, but not melatonin, reduces duration of immobility in the tail-suspension test (Prakhie and Oxenkrug, 1998). Interestingly, pretreatment with a specific inhibitor of TrkB abolished the antidepressant-like behavioral effect of NAS, suggesting that the antidepressant-like effect of NAS is mediated through TrkB activation (Jang et al., 2010).

A growing body of evidence suggests that BDNF-mediated TrkB signaling is both sufficient and necessary for antidepressant-like behaviors in rodents. However, it appears that the action of NAS is independent from BDNF since administration of exogenous NAS, but not of melatonin, activates TrkB in mice lacking BDNF (Jang et al., 2010). However, these results do not exclude the possibility that NAS and BDNF might additively or synergistically regulate each other's neurotrophic activity on TrkB. Interestingly, it has been observed that TrkB activation in C3H/f^{+/+} mice follow a daily/circadian pattern that is consistent with the oscillation of endogenous NAS, and this effect is absent in C57BL/6J mice that lack endogenous NAS. Furthermore, the observations that TrkB is activated by clorgyline in C3H/f^{+/+} mice, but not in C57BL/6J mice, and is prevented by light exposure, which inhibits NAS synthesis, further indicate that the synthesis of endogenous NAS induced by clorgyline accounts for this effect (Jang et al., 2010). The results provide compelling evidence that the molecular mechanism underlying the antidepressant role of NAS involves activation of TrkB receptors and suggest that the chronic treatment with fluoxetine, which increases *Aanat* transcription, leads to an increase in NAS, activation of TrkB, enhanced synaptic plasticity, neurogenesis, and synaptogenesis (Li et al., 2008). In conclusion, activation of TrkB by NAS appears to provide a molecular mechanism for the antidepressant-like action of the melatonin precursor (Jang et al., 2010).

Agglomerating, which is structurally homologous to melatonin, is a novel antidepressant with agonist activity at melatonin receptors (MT₁ and MT₂), and antagonistic effects at the 5HT_{2c} serotonin receptor (San and Arrant, 2008). Agglomerating produces strong effects on circadian sleep phase disturbances, improving time to sleep onset and quality of sleep (Spading et al., 2011). It has been shown to be superior to placebo and similar to existing antidepressants, as demonstrated by short-term clinical trials and one relapse prevention trial (Vickie and Rogers, 2011). Agglomerating appears to be well tolerated, without sexual or cardiac adverse effects, weight gain or discontinuation syndromes that are normally occurred to the monoaminergic antidepressants (Demyttenaere, 2011). Since melatonin itself does not provoke TrkB receptor activation in primary neurons or animals, conceivably, agomelatine might exert its antidepressant effect through its demethylated metabolite that may mimic NAS and activate TrkB receptors in the brain.

NAS and Neuroprotection

NAS has antioxidant properties and it has been suggested that it may useful in protection from oxidative stress-related disorders (Oxenkrug, 2005), such as Alzheimer's disease,

Parkinsonism, and age-related macular degeneration. The antioxidant effects of NAS may involve both direct chemical interactions and receptor-mediated mechanisms. NAS appears to be protective against excitotoxic neuronal injury by activating TrkB receptors. In TrkB F616A knockin mice, administration of kainic acid induces apoptosis in brain neurons, as indicated by activation of caspase 3 (Jang et al., 2010). Pretreatment with NAS but not melatonin inhibits caspase 3 activation by kainic acid, and this effect is blocked by the specific TrkB F616A inhibitor 1NMMPP1.

Several derivatives of NAS have recently been developed (Shen et al., 2012). One of these, *N*-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboximide (HIOC) selectively activates TrkB with greater potency than NAS and has a significantly longer biological half-life than NAS after systemic administration. The compound can pass the blood-brain and blood-retinal barriers when administered systemically and reduces kainic acid-induced neuronal cell death in a TrkB-dependent manner.

Brain-derived neurotrophic factor (BDNF), an endogenous ligand of TrkB, has neuroprotective effects in the retina, decreasing ganglion cell loss from optic nerve damage (Mansour-Robaey *et al.* 1994) and photoreceptor loss in bright light-induced retinal degeneration (LaVail *et al.* 1998). However, BDNF must be injected directly into the eye to be effective, as it does not cross the blood-retinal barrier. Interestingly, systemic administration of HIOC mitigates bright light-induced photoreceptor degeneration (Shen et al., 2012), suggesting that this compound may be useful in the treatment of retinal degenerative diseases.

NAS may be an endogenous neuroprotectant. C57BL/6 mice, which are genetically deficient in NAS, lose ~50% of their retinal ganglion cells by 18 months of age (Daniai et al., 2003). In contrast, C3H/f^{+/+} mice, which make NAS, show no age-related decline in retinal ganglion cells (Baba et al., 2009). While it is tempting to attribute this difference in cell survival to NAS, caution is warranted as many genetic differences exist between these two strains of mice.

Sleep, Neurogenesis and NAS

Sleep is a naturally recurring state characterized by a reduced sensory activity and is divided into two main types: rapid eye movement (REM) and non-rapid eye movement (NREM) sleep. The purposes and mechanisms of sleep are only partially understood and are the subject of intense research. In humans, as well as in laboratory rodents, the temporal organization of sleep during the 24-hour daily cycle ultimately results from the activity of two interacting time-keeping mechanisms in the central nervous system: endogenous circadian rhythmicity and sleep-wake homeostasis. Sleep-wake homeostasis refers to the increase in propensity to fall asleep and increased sleep duration and intensity that occur following extended waking. The sleep “homeostat”, also referred to as “process S”, is often represented as an hourglass mechanism relating the amount and intensity of sleep to the duration of prior wakefulness (Figure 3; Borbely and Achermann, 1999). In both humans and rodents, slow-wave activity (SWA or “delta power”, i.e., EEG power density in the low frequency range <4Hz), an index which quantifies the depth of non-REM sleep or “sleep intensity”, is the primary marker of sleep-wake homeostasis (Borbely and Achermann, 1999). Chronic partial sleep loss in humans appears to have cumulative effects on process S (Carskadon and Dement 1979; Dinges et al., 1997).

Chronic partial sleep loss, whether due to voluntary sleep curtailment, sleep disorders or medical illnesses is pervasive throughout our society. “Normal” sleep duration has decreased from approximately 9 hrs in 1910 to an average of 7 hrs in 2000 (Jonhson, 2000). Today, many individuals are in bed only 5–6 hours per night on a chronic basis (Jean-Louis

et al., 2000). Until a few years ago, the dogma was that sleep is only important for brain function (Benington and Heller, 1995). Now, accumulating experimental evidence indicates that sleep loss induces significant alterations in metabolism, cardiovascular function, mood and cognitive performance (Neckelmann et al., 2007; Spiegel et al., 2009).

Neurogenesis is the process by which neurons are generated during the development of the central nervous system. Until very recently it was believed that neurogenesis in mammals could only occur during early development; today we know that neurogenesis can continue in a few brain regions of adults (Ming and Song 2005). Neuronal progenitor cell (NPC) proliferation is an early event of neurogenesis, a process by which new neurons are continuously generated and incorporated into the nervous system. New neurons are continually generated in the dentate gyrus of the hippocampus and in the subventricular zone of the adult brain (Erickson et al., 1998, Gould et al., 1999). It had been shown that aging and stress can suppress neurogenesis but enriched environmental conditions, voluntary exercise such as running, as well as antidepressants can positively modulate hippocampal neurogenesis (Ming and Song, 2005; Lledo et al., 2006; Zhao et al., 2008; Ma et al., 2009).

Mounting evidence also suggests that sleep may contribute to hippocampal functions by promoting neurogenesis. Total sleep deprivation for 96 hrs reduces cell proliferation and neurogenesis in the dentate gyrus of the hippocampus in adult rats (Guzman-Marin et al., 2005) and sleep deprivation impairs hippocampus-dependent learning and abolishes learning-induced neurogenesis (Hairston et al., 2005). Initially, it was proposed that stress and glucocorticoids were the major factor in reduction of neurogenesis (Mirescu et al., 2006). However, additional study reveals that adrenalectomized animals also show a significant reduction (more than 50%) in the number of proliferating cells with respect to control, thus suggesting that sleep deprivation reduces hippocampal neurogenesis, at least in part, by a glucocorticoid-independent mechanism (Guzman-Marin et al., 2007; Mueller et al., 2011).

Interestingly, the effect of sleep seems to be associated with changes in REM sleep (Guzman-Marin et al., 2008). The reduction in neurogenesis induced by sleep fragmentation is likely to underlie the delayed changes in cognitive function often observed after sleep deprivation (Sportiche et al., 2010). Finally, contrary to what has been reported from extended sleep deprivation, acute sleep deprivation (12 hrs) may upregulate hippocampal neurogenesis (Grassi-Zucconi et al., 2006; Junek et al., 2010). The mechanisms by which sleep deprivation affects neurogenesis are still unknown.

Our recent study suggests that NAS is critical for promoting an early event of neurogenesis in dentate gyrus (Sompol et al., 2011). Mice treated with NAS showed a significant increase (about 30 %) in NPCs that correlates with an increase in TrkB phosphorylation in the hippocampus (Sompol et al., 2011). The increase in NPCs in the dentate gyrus was inhibited by blockage of the TrkB receptors (Jang et al., 2010; Sompol et al., 2011).

A series of investigations have suggested that alterations of circadian rhythms may affect neurogenesis since many aspects of hippocampal physiology show significant circadian fluctuation (Chaudhury and Colwell, 2002; Chaudhury et al., 2005). Moreover, it has been reported that clock gene expression or clock-controlled gene expression is rhythmic in the hippocampus (Fukuhara et al., 2004; Jilg et al., 2010) and that the clock gene *Period 2* is expressed in NPC (Borgs et al., 2009). Additional studies indicate that cell proliferation may vary with the time of day (Guzman-Marin et al., 2007; Tamai et al., 2008; Gilhooley et al., 2011). Our data indicate that the effect of NAS on NPC is independent from the time of day since exogenous NAS was equally effective in inducing NPC proliferation in both the active and sleeping phases in mice (Sompol et al., 2011). Interestingly, sleep deprivation

significantly diminishes NPC proliferation in C57BL/6 mice but not in C3H mice, suggesting that in C3H mice endogenously generated NAS may reduce the negative consequences of sleep deprivation (Sompol et al., 2011).

Recently, it has been suggested that melatonin may contribute to neurogenesis (Rennie et al., 2009; Ramirez-Rodriguez et al., 2009; 2011; Manda and Reiter 2010). Melatonin has been reported to promote sleep in many animal models and in humans (Brzezinski et al., 2005; Buscemi et al., 2006; Ochoa-Sanchez et al., 2011) and administration of exogenous melatonin increases neurogenesis in the hippocampus (Rennie et al., 2009; Ramirez-Rodriguez et al., 2009; 2011; Manda and Reiter 2010). Our recent studies indicate that administration of exogenous melatonin does not increase the number of proliferating NPCs even with 3 weeks of treatment (Jang et al., 2010; Sompol et al., 2011). However, it is possible that melatonin may still promote neuronal survival once NPC proliferation has occurred (Ramirez-Rodriguez et al., 2011).

Nevertheless, the data available in the literature suggest that NAS and melatonin have different physiological functions, and NAS acts as a potent neuroprotective agent through the neurotrophic receptor TrkB, whereas melatonin exerts its biological effects through melatonin receptors or as an antioxidant. Further studies using melatonin receptor knock-out mice are required to fully understand the specific roles played by melatonin and NAS in neuroprotection and neurogenesis.

An important aspect of the action of NAS is the mechanisms by which NAS can induce neurogenesis. A large amount of published data indicates that BDNF modulates neuronal survival and may also affect neurogenesis via TrkB signaling. We have shown that NAS can activate TrkB and its associated signaling cascades and this is the likely mechanism whereby NAS promotes neurogenesis and neuronal survival (Jang et al., 2010, Figure 4).

Sleep deprivation is a common characteristic of modern society and therefore understanding the negative effects of sleep loss on health and well being is an important step to prevent disease states associate with it and to develop effective treatment that may prevent the negative consequences of sleep deprivation. Emerging experimental evidence suggests that NAS, and its derivatives, may represent a new treatment that may prevent the decrease in cognitive function often observed in sleep-deprived individuals.

Is NAS involved in the Regulation of Circadian Rhythms?

In mammals, circadian rhythms are driven by a master pacemaker located in the SCN of the hypothalamus. The SCN is necessary for expression of most sustained circadian rhythms. Destruction of these cells eliminates most circadian rhythms of physiology and behavior, demonstrating the SCN's ability to dictate circadian periodicity (Herzog and Tosini, 2004). The SCN is also responsible for the photic entrainment of circadian rhythms by receiving a direct projection from a specialized class of retinal ganglion cells (Paul et al., 2010). Previous studies have also implicated TrkB in the photic regulation of SCN function; TrkB receptors are expressed in the SCN (Liang et al., 1998; Allen and Earnest 2005) and blockade of TrkB signaling in the SCN inhibits the photic phase-shift (Liang et al., 2000). Finally, it has been shown that heterozygous TrkB mutant mice have significantly smaller phase shifts in response to light at night, compared to wild type controls (Allen et al., 2005). Our new data demonstrating that NAS can activate TrkB receptors (Jang et al., 2010) together with observation that NAS may be synthesized within the SCN (Hamada et al., 1999) raises the intriguing hypothesis that NAS, together or independently from BDNF, may also play a role in modulating the entrainment of circadian rhythms. Finally, it is also possible to speculate that NAS may serve an endogenous neuroprotective role in the SCN in

order to provide an extra protection for the neurons located in this important key brain structure.

Conclusions

NAS has recently been found to play unexpected roles in neuronal cell biology by activating TrkB signaling. NAS may contribute to neurogenesis and neuronal survival, circadian rhythms and sleep, affective function, and hippocampus-dependent cognitive function. NAS derivatives may be useful as neuroprotectant drugs for the treatment of retinal degenerations and, perhaps, other neurodegenerative disorders.

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Figure 1.

The levels of *N*-Acetylserotonin are high during the night and low during the day and the rhythm is controlled by circadian clocks. The circadian clock controls the transcription of the *Aanat* gene and thus the enzymatic activity of AANAT and NAS levels (see text for details).

Figure 2.

Regulation of NAS biosynthesis and its suppression by light. At night in darkness cAMP levels are elevated, activating PKA, which induces *Aanat* gene transcription and phosphorylates AANAT protein. Phosphorylated AANAT (pAANAT) associates with 14-3-3 proteins, which activate and stabilize the enzyme resulting in increased conversion of serotonin to N-acetylserotonin. Light exposure decreases cAMP levels resulting in dephosphorylation of AANAT and its subsequent degradation by proteasomal degradation.

Figure 3.

The current model for sleep regulation involves two processes: a sleep-dependent process (Process S, blue line) and a circadian process (Process C, red line). Sleep propensity (Process S) increases during the wake time and rapidly decrease during sleep. The circadian clock (Process C) opposes sleep propensity by sending an alerting signal that begins to rise before the awakening and continues to increase into the late part of the wake period. Therefore, the sleep and wake cycle is determined by the coincident and opposing actions of these two processes (adapted from Borbely and Ackermann, 1999).

Figure 4.

BDNF interacts with TrkB receptors activating pathways that promote neuronal survival and neurogenesis. NAS also activates TrkB and its downstream signaling pathways, but it is unclear whether NAS directly interacts with the TrkB receptor to promote its activation or triggers activation through unknown molecular effectors.