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Review

N-Acetylserotonin and Aging-Associated Cognitive Impairment and Depression

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ABSTRACT: Normal brain aging is associated with depression and cognitive decline. One of the mechanisms of aging-associated emotional and cognitive impairment might be the down-regulation of biosynthesis of N-acetylserotonin (NAS), one of the methoxyindole derivatives of tryptophan (TRP). Aging is associated with decreased NAS production, largely resulting from the down-regulation of beta 1 adrenoreceptors that activate serotonin *N*-acetyltransferase, the enzyme catalyzing formation of NAS from serotonin. NAS exerts antidepressant-like and cognition-enhancing effects. The NAS role in cognition supported by the discovery that scotophobin, decapeptide extracted from brain and associated with cognition improvement, inhibits NAS conversion into melatonin. Furthermore, NAS (and its derivatives) attenuated cognitive impairment induced by cholinergic neurotoxin and protected against beta-amyloid neurotoxicity. Considering that NAS (but not serotonin or melatonin) is a potent agonist to high-affinity BDNF tyrosine kinase (TrkB) receptors, antidepressant and cognition-enhancing effect of NAS might be mediated by activation of TrkB receptors. NAS and TRkB gradually decreased from 1 postnatal week becoming undetectable in the brains of old rats. Additional mechanisms might include non-receptor mediated anti-inflammatory and anti-oxidative effects of NAS. Therapeutic antidepressant and cognition-improving interventions might include administration of NAS and its analogs; inhibition of tryptophan - kynurenine metabolism to increase serotonin availability as a substrate for NAS biosynthesis; up-regulation of NAS formation from serotonin and down-regulation of NAS conversion into melatonin.

Key words: N-acetylserotonin, aging, memory, scotophobin, BDNF, TrkB

N-acetylserotonin (NAS) was considered only as an intermediate product (and immediate precursor) of melatonin biosynthesis from serotonin. The finding that the memory-enhancing (in the model of dark avoidance) effect of NAS (but not melatonin) and that the brain decapeptide (scotophobin) caused dark avoidance due to inhibition of NAS conversion into melatonin [1] was completely underappreciated [2]. G. Brown and his team were the first to suggest that NAS may have a role in the central nervous system distinct from that of being a precursor for melatonin. This hypothesis was based on the immunohistochemically identified presence of NAS in specific brain areas separate from melatonin and serotonin (e.g., brain stem, cerebellum and hippocampus, and within the reticular formation nuclei and motor nuclei of the brain stem); on the inhibitory action of NAS on glutamate induced firing of pyramidal cells; and on its analgesic effect (separate from melatonin and serotonin) [3-6]. They wrote "If this hypothesis is correct it would suggest that indoleamines have certain similarities to catecholamines. Thus for the catecholamines, dopamine, norepinephrine and

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epinephrine form a synthetic sequence and yet have independent roles as neurotransmitters and/or hormones. The three indoleamines serotonin, NAS and melatonin also form a synthetic sequence and these three substances may also have independent roles as neurotransmitters and/or hormones [3].

Our support of the "NAS hypothesis" was based on our observation of NAS effects (cognition-enhancing, antidepressant-like, antihypertensive, prolongation of life span, prevention of pathological opening of the mitochondrial permeability transition pores, protection from beta-amyloid toxicity inhibition of lipid peroxidation) that were independent from melatonin or much stronger than similar effects of melatonin) [7-10]. Recent discovery that NAS (but not melatonin or serotonin) is an agonist to brain derived neurotropic factor (BDNF), tyrosine kinase (TrkB) receptors [11,12] provides further and crucial support for the hypothesis that NAS has a role in the central nervous system distinct from that of being a precursor for melatonin.

The present paper offers a review of the literature and author's data on NAS role in aging and aging-associated depression and cognitive impairment.

Regulation of N-acetylserotonin biosynthesis

N-acetylserotonin (NAS) was initially indentified in the urine of rats and rabbits as an additional (to oxidative deamination and re-uptake) pathway of serotonin metabolism [13]. NAS production occurs mostly in the pineal gland and retina [14]. However, NAS was also found in the hypothalamus, cortex, cerebellum, olfactory bulb, spinal cord and peripheral tissues [3,6,15]. Conversion of serotonin (formed from tryptophan) into NAS is catalyzed by serotonin-*N*-acetyl transferase (SNAT) [16,17]. The next step in NAS metabolism is methylation of NAS into melatonin catalyzed by hydroxyindole-*O*-methyl transferase (HIOMT) [14]. About 15% of melatonin is converted "back" into NAS in humans [18] and in rats [19] (Fig.1).

Figure 1. N-acetylserotonin biosynthesis.
NAS – N-acetylserotonin

Under physiological conditions, the rate-limiting factors of NAS biosynthesis from serotonin are 1) availability of serotonin as an immediate substrate for NAS biosynthesis, and 2) up-regulation of beta-adrenoceptors that mediate noradrenalin-induced stimulation of SNAT [17]. The circadian rhythm of SNAT is generated within the hypothalamic suprachiasmatic nuclei (SCN), site of the circadian clock. Environmental light signal transmitts from the retina to the pineal gland primarily via the retinohypothalamic tract and superior cervical ganglion (SCG) [20].

Increased sympathetic tone (e.g., in spontaneously hypertensive rats) resulted in higher (than in normotensive rats) elevation of pineal NAS in response to stimulation by increased production of NA-induced by cold immobilization stress or clorgyline [21].

Pinealocyte's beta-adrenoceptors develop supersensitivity (increase in Bmax) during the light phase of light: dark cycle. With the beginning of dark, NA, released from nerves originating in SCG, stimulates up-regulated beta-receptors, and, consequently, triggers the *de novo* synthesis of SNAT. Regulation of the circadian rhythm of NAS biosynthesis varies between species, e.g., pineal beta-adrenoceptor density of the Wistar rat did not decline as rats age [22]. Therefore, it was important to evaluate NAS biosynthesis in the human pineal. We studied 24-hour cycle of serotonin, NAS, melatonin, and beta adrenoceptor density in human pineal glands obtained from 77 post-mortem sources from various times of death. Density (Bmax) of pineal beta adrenoceptors (but not receptors affinity that remained in narrow ranges near 58 pM) was relatively constant between midnight and 18.00 h and became significantly higher between 18.00 and 20.00 h as measured by ligand saturation binding experiments using (125-I) iodocyanopindolol. The up-regulation of receptors coincided with an increase in the concentration of serotonin that began to rise between 16.00 and 20.00 h and became maximal between 20.00 and midnight. NAS was at maximal levels between 20.00 h and midnight. Both serotonin and NAS began declining after midnight, and this change corresponded to the maximal pineal gland concentration of melatonin between midnight and 4.00 h. It is therefore suggested that the up-regulation of beta adrenoceptors noted during the late afternoon and early evening hours corresponds to the increased synthesis of serotonin and the subsequent conversion to NAS [23]. Aging-associated reduced density of adrenergic receptors [24] might contribute to aging-associated decline of NAS biosynthesis (see below).

NAS and Aging

NAS biosynthesis and aging

Ontogenetically, pineal NAS appears earlier than melatonin. Rat pineal SNAT is active while HIOMT is absent during first week of neonatal life. Our *in vivo* and

in vitro studies reveal that clorgyline stimulates pineal NAS biosynthesis from day five onward with a marked increase between day 14 and day 21. In contrast, MEL is not increased until day 21, with a sharp rise thereafter [25].

Aging is associated with the declined content [26-29] and flattened circadian rhythm of NAS in normotensive [30] and spontaneously hypertensive rats [7,8]. The expression of pineal and hippocampus SNAT mRNA was lower in old (24 months) than in young (2 months) rats [31].

Aging affects two major rate-limiting factors of NAS biosynthesis: 1) up-regulation of beta-adrenoceptors, and 2) availability of serotonin.

Beta-receptors

Aging is associated with a reduced density of adrenergic receptors and reduced adaptation of these receptors to changing neuronal input, e.g., up-regulation in response to light–induced suppression of NA release [24]. We reported that clorgyline, monoamine oxidase type A (MAO-A) inhibitor, increased NAS content in both 3 and 12 months old male Sprague-Dawley rats kept under regular (12:12 h) light: dark schedule, suggesting the preservation of the enzymatic machinery for NAS biosynthesis in aged rats. However, only young but not middle-aged rats responded by additional elevation of pineal NAS when exposed to light for 24 h before administration of clorgyline, or serotonin precursor, 5-hydroxytryptophan [32,33]. In the same vein, administration of isoproterenol, beta-adrenoceptor agonist, caused lower elevation of pineal NAS content in older than in younger spontaneously hypertensive rats [7,8,10]. These observations suggested that aging-associated reduced density of pineal beta-adrenergic receptors contributed to aging-associated decline of NAS biosynthesis.

Availability of serotonin

Decreased availability of serotonin as a substrate for NAS biosynthesis might the consequence of aging-associated activation of TRP conversion into kynurenine (KYN) that shifts TRP metabolism from serotonin to KYN production [34].

It is noteworthy that genetic or pharmacological inhibition of KYN formation from TRP (that is supposed to increase the availability of serotonin as a substrate for NAS biosynthesis) prolongs life span in Drosophila melanogaster [35-37].

Calcification of pineal gland

Aging-associated decline of NAS biosynthesis might be a consequence of calcification of human (but not rat) pineal gland. Human pineal gland, the major site of NAS biosynthesis, undergoes increased calcification during the life cycle [38]. Alzheimer's disease patients had more severe pineal calcification than age-matched control [39]. Our postmortem study of 33 subjects (age range 3 months to 65 years) calcium deposits (evaluated by atomic absorption spectrometry) revealed a positive correlation with age in both day and night samples (day: $r = 0.56$, $P < 0.05$; night: $r = 0.818$, $P < 0.001$) [40].

Neuronal regulation of NAS biosynthesis.

Degeneration of the retina-SCN-pineal axis may underlie disrupted NAS production and rhythms in aging and in the very first preclinical stages of Alzheimer's Disease. Our studies revealed that chemical [41] or surgical [42] sympathectomy; or lesions of SCN [43] attenuated or abolished NA-induced stimulation of rat pineal NAS biosynthesis.

Aging and jetlag

Circadian rhythms (i.e, nighttime increase of pineal SNAT and NAS) were abolished for three nights after 8 hrs advance of the beginning of the light phase of light: dark cycle (experimental model of jetlag associated with eastward flights) [47,48]. Reappearance of the circadian rhythms of NAS biosynthesis was delayed in aged rats in comparison to the young animals [48].

Pharmacological down-regulation of NAS biosynthesis

The use of beta-blockers and benzodiazepines (BZ) is increased in elderly. Both medications negatively affect cognitive functions and NAS biosynthesis. Our *in vivo* and *in vitro* studies revealed that administration of the central BZ agonist, clonazepam, or the mixed agonist-antagonist, diazepam, inhibited nocturnal elevation of SNAT [49].

NAS and life span

NAS administered in drinking water at 2.5 mg/kg/day starting at 4 weeks of age delayed the occurrence of first death from 11 to 15-th month of age, and prolonged the life span of C3H male mice by more than 20% [50]. To the best of our knowledge this was the first report of a prolongation of life span by NAS.

NAS might contribute to life span extension effect of irreversible MAO-B type inhibitor, deprenyl. Night-(but not day-time) pineal NAS levels were increased after six months of subcutaneous administration of deprenyl (0.25 mg/kg) to retired female breeders Fisher 344N rats [51,52].

NAS and cognition

NAS and "memory peptide", scotophobin

The idea that memories could be transferred from one organism to another by administration of a "trained" donor brain to a naive recipient seized both scientific and public attention in the 1960's and early 1970's [2]. One of the "memory peptide" candidates was identified in the brain extracts of rats trained with shock to avoid dark (hence, "scotophobin"), leading to synthesis of its analogs [53]. Scotophobin induced avoidance behavior and heightened emotionality as measured by defecation rate when mice were locked in the dark box, but not when they were locked in a white or transparent box [54]. Scotophobin was found to be an effective inhibitor (KI50, 6 x 10(-7) M) of purified bovine HIOMT, the enzyme, converting NAS in to melatonin. The finding that scotophobin inhibits HIOMT suggested that NAS is a mediator of the memory-enhancing effect of scotophobin. Further experiments confirmed that scotophobin action required intact pineal, and that NAS induced dark avoidance in goldfish, as did S-adenosyl-homocysteine, another HIOMT inhibitor [1].

Cognition-enhancing and neuroprotective effects of NAS

We found that three weeks of NAS treatment reversed the impairment of performance in active avoidance (learning and retention paradigms) and water maze tests induced in Wistar rats by cholinergic neurotoxin ethylcholine aziridinium (single intracerebroventricular injection of 3 nmol) [55].

Possible mechanisms of NAS effect on memory

NAS and aging-associated oxidative stress

Aging is associated with increased production of reactive oxygen species and oxidation-induced damage to intracellular structures and membranes. We found an age-associated increase of lipid peroxidation (malonaldehyde+4-hydroxyalkenals, MDA+HAE) in the liver (from 3- to 6- to 12-months old), and kidney (from 3- to 6-month without further increase in 12–months old animals) of genetically deficient for NAS formation C57Bl/6j male mice. MDA+4HAE brain levels did not differ between 3-, 6- and 12-months old animals, leaving the possibility that lipid peroxidation has already reached it peak at 3-month of age [56].

We observed that subcutaneous injections of NAS (four weeks, 30 mg/kg/day) decreases brain (2.2 fold) and kidney MDA+HAE in 11-month old male C57BL/6J mice. While control animals had large areas of baldness, NAS-treated animals had the healthy and luxuriant fur, and lost about 10% of their initial body weight in comparison with no weight loss in control group [50].

The cognition enhancing effect of NAS might be further supported by our observations of NAS protective effect against ß-amyloid toxicity in cultured rat cerebellar granulate cells [55] and MPP+ and ß-amyloid–induced pathological activation of mitochondrial permeability transition pores [57].

NAS attenuated lipopolysaccharide- , iron- and TNF-alpha-induced lipid peroxidation [58-60]. Other mechanisms of the antioxidant effects of NAS might include stimulation of glutathione peroxidase; suppression of phospholipase A2; and inhibition of sepiapterin reductase, the key enzyme of biosynthesis of tetrahydrobiopterin, the essential cofactor of nitric oxide synthase [9].

NAS and* N-methyl-d-aspartate (*NMDA)

Aging- and depression-associated up-regulation of TRP – KYN metabolism (see above) lead to increased production of endogenous NMDA receptors antagonist, kynurenic acid (KYNA), one of the KYN derivatives [61] (Fig.2). Exogenous NMDA receptors antagonist, MK-801, inhibits SNAT (and, presumably, NAS biosynthesis) [62]. Therefore, increased production of endogenous KYNA might (similar to exogenous antagonist, MK-801) inhibit NAS biosynthesis and contribute to KYNA-induced cognitive impairment associated with aging and depression ([62]. On the other hand, the inhibitory effect of NAS on glutamate-induced firing of pyramidal cells [4] and glutamate-induced lipid peroxidation in retinal homogenates [63] might contribute to the cognition-enhancing effect NAS.

NAS and neurogenesis

NAS might contribute to its cognition-enhancing and antidepressant effects by promoting hippocampal neuroprogenitor cell proliferation (64) and increasing cell proliferation through upregulation of BDNF [65].

NAS and aging-association depression.

NAS and depression

Aging is associated with the increased prevalence of late onset depression, one of the components of aging-associated medical and psychiatric disorders, including metabolic syndrome [66-68]. One of the mechanisms of aging-associated depression might be the shift of TRP metabolism from formation of NAS (and other methoxyindoles, such as serotonin and melatonin) to production of kynurenines (Fig.2) [69,70]. The other consequence of up-regulated TRY – KYN metabolism is the increased formation of neuroactive KYN derivatives, the endogenous NMDA agonists and antagonist [71].

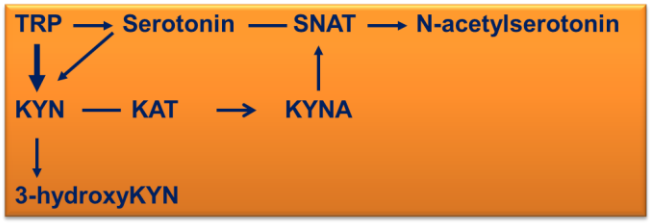

Figure 2. Tryptophan shift from Serotonin to Kynurenine formation.
TRP – tryptophan; SNAT – serotonin-N-acetyltransferase; KYN – kynurenine; KAT– KYN aminotransferase; KYNA – kynurenic acid.

NAS and antidepressant effect

The antidepressant-like effect of NAS was reported in a mice tail-suspension test [72] and confirmed in a forced swim test [11]. Selective tricyclic antidepressants and serotonin uptake inhibitors increased SNAT mRNA expression [73]. Our *in vivo* and *in vitro* experiments revealed that acute administration of irreversible (clorgyline) and reversible (brofaromine, beflaxotone, moclobemide) selective monoamine oxidase (MAO) type A inhibitors and high doses (or chronic administration of low doses) of relatively selective MAO-B inhibitors (but not of highly selective MAO-B inhibitors) suppressed MAO-A activity and stimulated pineal NAS biosynthesis. The effect of MAO-A inhibitors is strain (spontaneously hypertensive rats > Fisher344N > Wistar Kyoto > Sprague-Dawley) and gender (male > female) dependent [74,75]. Considering that MAO-A but not MAO-B inhibitors exert a clinically antidepressant effect, our data suggested that stimulation of NAS biosynthesis contributes to the antidepressant effect of MAO-A inhibitors. Antidepressant and selective MAO-A inhibitor, clorgyline, stimulates rat pineal NAS biosynthesis by preserving MAO-A substrates (serotonin and NA) from deamination [76]. Reversible MAO-A inhibitors, brofaromine and beflaxotone, stimulated pineal NAS production as well [8]. We reported that antidepressant, methylene blue, and other blue dyes, the selective MAO-A inhibitors [77] stimulate pineal NAS production [78]. Acute administration of MAO-B type inhibitor, deprenyl (not exerting an antidepressant effect), did not stimulate rat pineal NAS biosynthesis. However, chronic (6 months) injections of low dose of deprenyl, an irreversible MAO-B inhibitor, inhibits MAO-A and stimulated pineal NAS production but only during the dark phase [51,52].

The bioprecursor amino acid (MDL 72394) is converted into the irreversible selective MAO-A inhibitor (MDL 72392) by aromatic L-amino acid decarboxylase (AADC). Pretreatment with carbidopa, peripheral AADC inhibitor, which does not penetrate blood-brain-barrier (BBB), prevents the liberation of the MAO-A inhibitor outside the BBB and results in exclusive inhibition of brain MAO-A. We found that systemic administration of MDL 72394 (0.5 mg/kg) stimulated rat pineal melatonin biosynthesis. Carbidopa, in a dose-dependent manner, attenuated or completely prevented MDL-induced stimulation of melatonin biosynthesis in the pineal gland confirming the location of the pineal gland outside of BBB (although in the brain) [79].

Possible mechanisms of the antidepressant effect of NAS

NAS and QR2/MT3

Besides non-receptor mediated anti-oxidant (see above) effects of NAS, some receptors-mediated effects of NAS might contribute to it antidepressant action. The melatonin 3 type (MT-3) receptor has higher affinity to NAS than to melatonin, and was identified as the same protein as quinone reductase 2 (QR2) detoxifying and antioxidant enzyme [80]. We found that QR2/MT3 agonist 5-methoxycarbonylamino-*N*-acetyltryptamine (5MCA-NAT) decreased, while the QR2/MT3 antagonist prazosin increased the duration of immobility in the tail suspension test in C57BL/6 mice. Prazosin, in a dose that did not affect the duration of immobility, attenuated the antidepressant-like effect of NAS and 5MCA-NAT [81]. It is noteworthy that QR2/MT3 mediated effects appeared to be specific for antidepressant-like action since 5MCA-NAT did not affect NAS-induced protection against LPS toxicity [58].

5MCA-NAT NAS as agonist to TrkB receptors

The most important step in establishing NAS biological activity independent from serotonin and melatonin is a recent discovery that NAS (but not serotonin or melatonin) is an agonist to tyrosine kinase B(TrkB) (but not TrkA or TrkC) receptors of brain derived neurotrophic factor (BDNF) [11]. Recent review indicates that activation of TrkB by NAS might contribute to cognition-enhancing, neuroprotective and antidepressant effects of NAS (and MAO-A inhibitors) [12]. Discovery of the TrkB-mediated antidepressant effect of NAS is in line with the hypothesis that loss of BDNF is directly involved in the pathophysiology of depression, and that its restoration may underlie the therapeutic efficacy of antidepressant treatment [82]. NAS-induced TrkB stimulation might be especially important for the treatment of age-associated depression and cognitive decline considering the age-associated decrease in expression of BDNF (mRNAs and proteins) and its neuronal and glial (TrkB) receptors [83].

Conclusion

Current review strongly suggests that NAS has an important role in mechanisms of aging-associated depression and impairment of cognition. NAS and its derivative might serve as new tools in prevention and treatment of aging-associated emotional and cognitive decline; and for protection against oxidative stress- and inflammation-related conditions (cell death, mutagenesis, aging) and diseases (sepsis, cancer, postischemic trauma, Alzheimer's disease, Parkinsonism and retinal degeneration). Therapeutic antidepressant and cognition-improving interventions might include administration of NAS and its analogs; inhibition of tryptophan-kynurenine metabolism to increase serotonin availability as a substrate for NAS biosynthesis; up-regulation of NAS formation from serotonin and down-regulation of NAS conversion into melatonin.

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This review is dedicated to the memory of dear friend and colleague, Prof Arthur Yuwiler (1927 -2012).

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