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Research report

Administration of *N*-acetylserotonin and melatonin alleviate chronic ketamine-induced behavioural phenotype accompanying BDNF-independent and dependent converging cytoprotective mechanisms in the hippocampus

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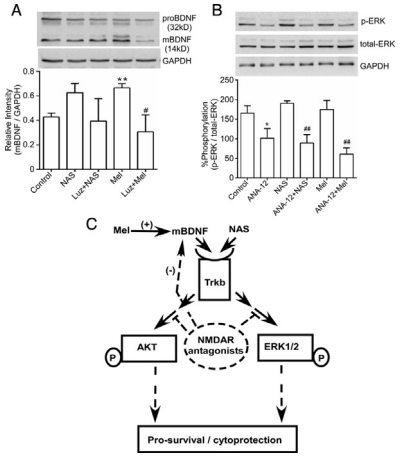
HIGHLIGHTS

- Melatonin and NAS block NMDAR antagonist-induced immobility in forced swimming.
- Melatonin induces BDNF protein via melatonin receptor(s) in the hippocampus.
- NAS induces hippocampal pro-survival mechanisms independent of BDNF.
- NAS and melatonin effects associate BDNF/Trkb-mediated downstream mechanisms.
- Converging role of serotonin metabolites i.e. NAS and melatonin in psychiatric disorder.

GRAPHICAL ABSTRACT



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ABSTRACT

Though growing evidence implicates both melatonin (MLT) and its immediate precursor *N*-acetylserotonin (NAS) in the regulation of hippocampal neurogenesis, their comparative mechanistic relationship with core behavioural correlates of psychiatric disorders is largely unknown. To address this issue, we investigated the ability of these indoleamines to mitigate the behavioral phenotypes associated with NMDA-receptor (NMDAR) hypofunction in mice. We demonstrated that exogenous MLT and NAS treatments attenuated the NMDAR antagonist (ketamine) induced immobility in the forced swim test (FST) but not the classical striatum-related hyperlocomotor activity phenotype. The MLT/NAS-mediated protection of the phenotype in FST could be correlated to the ability of these indoleamines to counteract

Abbreviations: NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA-receptor; NAS, *N*-acetylserotonin; MLT, melatonin; mBDNF, mature brain-derived neurotrophic factor; FST, forced swim test; TrkB, tropomyosin related kinase B.

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the deleterious effects of chronic ketamine on pro-survival molecular events by restoring the activities in MEK-ERK and PI3K-AKT pathways in the hippocampus. MLT seems to modulate these pathways by promoting accumulation of the mature form of BDNF above the control (vehicle-treated) levels, perhaps via MLT receptor-dependent mechanisms and in the process overcoming the ketamine-induced down-regulation of BDNF. In contrast, NAS appears to partly restore the ketamine-induced decrease of BDNF to the control levels. In spite of this fundamental difference in modulating BDNF levels in the upstream events, both MLT and NAS seem to overlap in the TrkB-induced downstream pro-survival mechanisms in the hippocampus, providing protection against NMDAR-hypofunction related cellular events. Perhaps, this also signifies the physiological importance of robust MLT synthesizing machinery that converts serotonin to MLT, in ensuring positive impact on hippocampus-related symptoms in psychiatric disorders.

1. Introduction

Daily rhythm in melatonin (MLT) biosynthesis, with dramatically high values at night and low levels during the daytime, is a remarkably consistent feature across varieties of species in the animal kingdom [1]. The rhythm in MLT production is largely regulated by 'master clock' in the suprachiasmatic nucleus (SCN) leading to the activation of the serotonin (5HT) acetylating enzyme, arylalkylamine *N*-acetyltransferase (AANAT or serotonin *N*-acetyltransferase) in the pineal gland producing high levels of *N*-acetylserotonin (NAS) at night. NAS is eventually methylated to form MLT. MLT has been implicated in large array of physiological functions that range from neuroprotective, and antioxidant properties to even anti-inflammatory and antitumor effects [2,3]. However, melatonergic mode of action has received renewed attention with a major shift in the traditional monoamine hypothesis for the disease pharmacology [4]. Several lines of evidence also suggest involvement of MLT, MLT receptors and the related circadian dysfunction in psychiatric illnesses [5,6].

Like MLT, NAS has also been lately highlighted in regulating hippocampal neurogenesis and is envisaged to play critical role in disease related pathologies [7,8,4,9]. As demonstrated in C57BL/6J mouse strains, NAS perhaps unlike MLT, seemed to act via direct binding and activation of TrkB receptor in the hippocampus, reminiscent of the brain-derived neurotrophic factor (BDNF) mediated cellular pathways [10]. Surprisingly, in C57BL/6J and C3H strains of mice, exogenous NAS only but not MLT, showed significant antidepressant-like behavioural properties [10,11]. Thus, critical gaps exist in understanding of the mechanistic paradigm of these two major pineal-derived indoleamines in the non-pineal tissues of the brain, specifically in the hippocampus.

Here, we used a ketamine-induced model for experimental psychosis in mouse to test and compare the efficacy of the pineal indoleamines, MLT and *N*-acetylserotonin (NAS) in attenuating the behavioural deficits that are believed to be associated with *N*-methyl-D-aspartate (NMDA)–receptor (NMDAR) hypofunction and related cellular events. The experimental approach was designed such that it would address the mechanistic and phenotypic correlates involving MLT and NAS modes of action in the hippocampus. Though low (<10 mg/kg of body weight) threshold doses of ketamine are implicated in anti-depressant effects [12,13] at higher sub-anaesthetic doses (30–100 mg/kg) its effect is reversed and it acts as a psychotomimetic agent [14]. We had previously determined that chronic ketamine administered to Swiss mice strain, in contrast to a single-dose of ketamine or acute treatment, induced a gamut of behavioural phenotypes including the despair-like symptom characterized by increased immobility in the forced swim test (FST), which could be blocked by atypical antipsychotics like clozapine [15,16]. This behavioural phenotype is correlated with atrophy in the limbic structures, particularly in the core hippocampal-area [17,18]. Using this chronic ketamine-induced mouse model and FST as an end-point assay, we determined the comparative mechanistic roles MLT and NAS might play in confronting the NMDAR hypofunction-induced deleterious events in the hippocampus.

2. Methods

2.1. Animals

Swiss albino mice and C57BL/6 mice strains, weighing 20–25 g, were used in the study. Mice were randomly selected and distributed into groups of 5–8 animals each, housed at constant temperature (27 ± 2 °C) and 12 h light/12 h dark cycle. Food was provided in the form of dry pellets and water was given ad libitum. All the experimental procedures used in this work were approved by the Institutional Animal Ethical Committee which follows the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), India and the National Institutes of Health guide for the care and use of laboratory animals. All efforts were made to minimise animal suffering and to reduce the number of animals used.

2.2. Drugs and treatment schedule

Ketamine obtained from Ranbaxy, India was used at a dose of 100 mg/kg (Swiss strain) or 70 mg/kg (C57BL/6 strain) in saline. MLT (Sigma, India) and NAS (Sigma, India) were dissolved in 1% ethanol. Final doses of MLT (1 mg/kg) and NAS (20 mg/kg) were used as published [10]. The compounds were administered intraperitonially (i.p) in a volume of 0.1 ml/25 g mouse. Luzindole (Sigma) was a generous gift from Dr. David Klein, NIH and ANA-12 was obtained from Sigma.

Drug administration for the entire study was done in the morning hours between Zeitgeber time (ZT) 3 (morning) to ZT 6 (midday) and behaviour experiments were completed by ZT 9 (early afternoon). For all experiments involving co-administration of MLT or NAS with the ketamine, MLT or NAS were injected 30 min prior to ketamine administration.

2.3. Experimental designs

2.3.1. Phenotype rescue experiment

Animal groups to test the reversal of chronic ketamine-induced phenotype by acute (one time administration) MLT and NAS administration—(i) Cont (control) group with vehicle (1% ethanol and saline to mimic ketamine and indoleamine i.p injections, respectively), treated for 10 days; (ii) Ket group, where ketamine administered for 10 days continuously followed by vehicle (1% ethanol) on 11th day; (iii) Ket + MLT group, where ketamine was