Increased whole cerebellar serotonin in aged C57BL/6 mice

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Abstract

Mobility and locomotor impairments have high prevalence, morbidity, and significant mortality in older adult populations. Cerebellar functional changes have been implicated in the pathogenesis of these age-related mobility and gait deficits unrelated to stroke, Parkinson's disease, or degenerative joint disease. We thus examined total cerebellar glutamate, glutamine, GABA, glycine, dopamine, norepinephrine, tryptophan, serotonin, alanine, threonine, and asparagine content from male 2–3 month (young, n = 6) and 21–24 month old (aged, n = 6) C57BL/6 mice. Neurotransmitter and amino acid concentrations were determined by high-performance liquid chromatography followed with mass spectroscopy. We found a significant increase in cerebellar serotonin in aged versus young mice, but otherwise no significant phenotypic differences in measured neurotransmitter concentrations. Applying current thought about cerebellar aging and cerebellar serotonergic systems, we consider how this age-related increase in cerebellar serotonin may contribute to gait ataxia.

Introduction

It is essential to understand how aging affects mobility, and find interventions to prevent or delay mobility, impairments. Mobility impairments often accompany advanced age [1]. They are major causes of morbidity in the "oldest old," the most rapidly expanding demographic group in the United States [2] [3] [4]. Gait speed is a sensitive biomarker of overall functional status [5], as well as a powerful predictor of overall mortality [6] [7]. Cerebellar dysfunction may be a potential source of age related mobility impairments. In humans, overall cerebellar volume decreases with age as shown by voxel-based morphometry [8] and is associated with age related gait impairments including slow gait speed [9]. Aging is accompanied by a marked change in cerebellar gene expression patterns in both rodent models and humans [10] [11]. No age related decrease in cerebellar granule cell number or density has been noted in rodent models [12] [13]; small decreases have been described in humans [14]. Aging is also associated with a strain-specific increase in excitatory synaptic puncta in the internal granule cell layer; however, these synapses show deficits in excitatory amino acid neurotransmission [10]. Loss of visualized synaptic boutons on Purkinje cell dendritic networks also accompanies age-related Purkinje cell involution and loss [15]. Aging also evokes deficits in Purkinje cell function, including marked slowing of firing rates [16] and blunted responses to adrenergic stimulation [17]. These changes have been correlated with impairment in mobility-related behaviors, including walking on a runway and dynamic balance [18].

Objective

To further explore potential synaptic deficits underlying age related gait impairments, we assessed whole-cerebellum neurotransmitter and excitatory amino acid content in cohorts of young and aged C57BL/6 mice using HPLC mass spectroscopy (HPLC-MS). We report a novel finding of increased whole cerebellum serotonin in aged, compared to young, C57BL/6 mice.
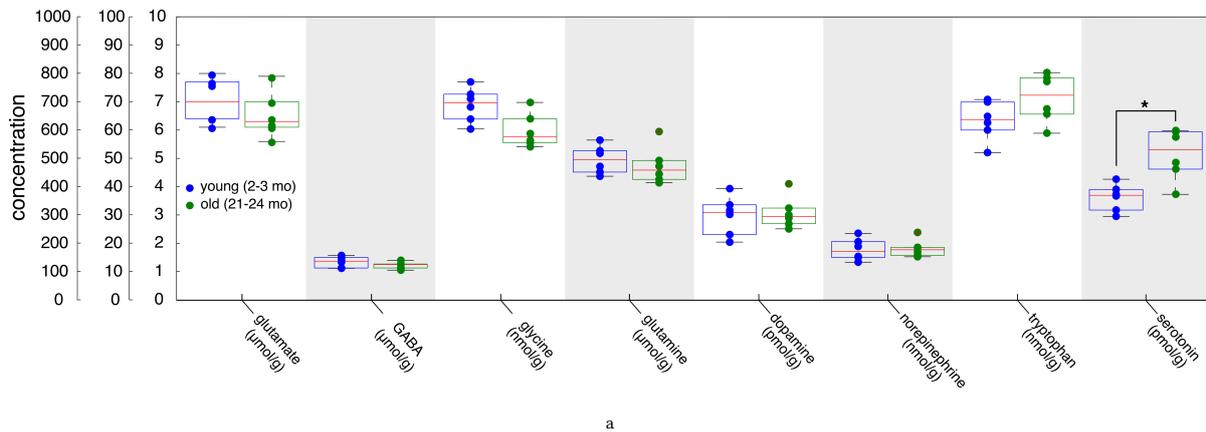
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Figure Legend

Figure 1. Age-evoked increase in whole cerebellar serotonin in C57BL/6 mice.
Boxplots provided for whole cerebellum neurotransmitter concentrations; data points superimposed upon boxplots. Glutamate, GABA, glutamine, tryptophan, and norepinephrine values correspond to the y-axis valued 0–10; dopamine values correspond to the y-axis labeled 0–100; glycine and serotonin values correspond to the y-axis labeled 0–1,000. Neurotransmitter concentration units provided on the x-axis. Blue dots depict measures from the young (2–3 months) cohort; green dots depict measures from the old (21–24 months) cohort.

For all comparisons, age df = 1, error df = 10, total df = 11. Respective F and p values are as follows: Glu $F_{1,10}$ = 1.13, p = 0.3131; GABA $F_{1,10}$ = 1.64, p = 0.229; Gln $F_{1,10}$ = 0.38, p = 0.5495; Gly $F_{1,10}$ = 6.85, p = 0.0257; Ala $F_{1,10}$ = 0.44, p = 0.52; Thr $F_{1,10}$ = 3.8, p = 0.0799; Trp $F_{1,10}$ = 3.13, p = 0.1073; Asn $F_{1,10}$ = 2.9. p = 0.1192; DA $F_{1,10}$ = 0.08, p = 0.7863; NE $F_{1,10}$ = 0.03, p = 0.8665; 5-HT (*) $F_{1,10}$ = 13.53, p = 0.0043. Critical p = 0.0063 to achieve α = 0.05 for Bonferroni correction over 8 comparisons.

The MATLAB program used to generate the figures as well as to run ANOVA statistics can be found in the supplementary data.

Results & Discussion

All samples were suitable for HPLC-MS determination of cerebellar neurotransmitter and amino acid levels: glutamate, GABA, glycine, glutamine, dopamine, norepinephrine, tryptophan, serotonin, alanine, threonine, and asparagine. Our results suggest a 1.4-fold statistically significant increase in whole-cerebellar serotonin in aged C57BL/6 mice compared to young conspecifics (Fig. 1A). We otherwise found no other differences in whole cerebellum neurotransmitter or amino acid concentrations between the young and old mouse cohorts.

Serotonin is an ancient regulator of cellular function [19], and has a coordinating role in the performance of movement behaviors across a wide variety of organisms [20] [21] [22] [23]. Aging has a clear impact on cerebellar serotonergic function. The activity of tryptophan hydroxylase, the enzyme catalyzing the rate-limiting conversion of tryptophan to serotonin, is significantly decreased in the midbrain and pons of aged rats [24] [25]. Concordantly, age-associated decreases in whole cerebellar serotonin have been reported in rats (assayed by a fluorometric reaction between serotonin and o-phthaldialdehyde [26]) and senescence-accelerated mice (assessed by ^{3}H-tryptophan injection [27]). However, no age-associated differences in cerebellar serotonin content were noted among cohorts of 6–24 month old BALB mice (assayed by HPLC [28]), and our study is the first to report an age-associated increase in cerebellar serotonin content in a mouse model. Multiple studies have also demonstrated a marked loss of serotonin reuptake transporter function in the cerebellum of older adults [29] [30].

Serotonin has its most profound effect on the Lugaro inhibitory interneurons of the internal granule cell layer. These cells are numerous, have strong inhibitory projections onto Golgi cell inhibitory interneurons, and are typically silent. Lugaro cells respond to

serotonin (likely through a 5-HT$_2$ subfamily receptor) with a marked increase in spiking activity leading to increased inhibitory postsynaptic potentials onto Golgi cell membranes [31] [32]. Lugaro cell activity also directly inhibits Purkinje neuron firing [33]. A single Lugaro cell makes inhibitory synaptic contact with about 150 nearby Golgi cells [31]; each Golgi cell makes inhibitory synaptic contact with about 3,000–5,000 granule cells [34]. Lugaro cell activity thus has the potential to reduce both the tonic and phasic components of Golgi cell inhibition onto large populations of granule cells. Serotonergic neurotransmission also contributes more subtle effects on cerebellar input signal processing. Serotonin attenuates parallel fiber input onto Purkinje cells (both by increasing activity of inhibitory basket and stellate interneurons and by presynaptic decreases of granule cell glutamate [35] [36]). Serotonin also modulates cerebellar deep nuclei function in a complex, incompletely understood manner [37].

Limitations

Our results suggest an age related 1.4-fold increase in whole cerebellar serotonin content. The methods we employed do not allow us to further determine if this difference reflects increased intravesicular serotonin within cerebellar serotonergic projections, and/or increased extracellular serotonin. Similarly, we cannot determine if this increase occurs over the cerebellum as a whole, or preferentially affects either the cerebellar input regions (internal granule cell, molecular, and Purkinje layers) or the cerebellar output nuclei; nor can we determine whether this finding localizes to specific folia.

The predicted outcome of increasing cerebellar serotonergic activity may thus be to increase granule cell input sensitivity to mossy fiber input, decrease Purkinje cell sensitivity to parallel fiber input, and increase inhibition of Purkinje cell firing. These effects would combine to expand mossy fiber input participating in center-surround inhibition [38] [39]. In other words, increased serotonergic activity may allow a larger set of afferent sensory inputs concurrent access to the Purkinje cell network while preserving overall Purkinje cell firing properties. Of note, in normal human volunteers, increased extracellular serotonin (through a single dose of a selective serotonin uptake inhibitor) evoked a significant increase in resting-state fMRI centrality measures across the cerebellum, consistent with the hypothesis that increased serotonergic activity enhances cerebellar functional connectivity [40].

It is thus interesting to note in this context that inhibition of serotonergic tone (by treatment with the partial 5-HT$_{1A}$R agonist buspirone) improved cerebellar tremor [41] [42]. Further, prominent cerebellar ataxia has been observed in aged (21–24 months old) compared to young (2–3 months old) C57BL/6 mice obtained from the same colony as the mice in this study [10]. Interestingly, this appeared to be strain-specific with no ataxia present in BALB mice of identical ages. Locomotor ataxia thus accompanies the increased cerebellar serotonin content observed in C57BL/6 mice, but not in BALB mice with no age related changes in cerebellar serotonin content [28]. Gait ataxia may potentially be a behavior evoked by serotonergic increases in cerebellar functional connectivity. Further studies to determine if age-associated increases in cerebellar serotonin content are reflected in extracellular serotonin concentrations, and to determine the specific serotonin receptor subtypes associated with Lugaro cell activation, will better define the mechanisms through which serotonergic activity may influence cerebellar tremor, as well as identify potential therapeutic targets.

Additional Information

Ethics Statement
Not applicable.

Citations

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