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Possible Role of Dynorphins in Alzheimer's Disease and Age-Related Cognitive Deficits

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Key Words
Dynorphin · Alzheimer's disease · Aging · Ionotropic glutamate receptor · AMPA receptor · NMDA receptor

Abstract
Background/Aims: Expression of dynorphin, an endogenous opioid peptide, increases with age and has been associated with cognitive deficits in rodents. Elevated dynorphin levels have been reported in postmortem samples from Alzheimer's disease (AD) patients, and prodynorphin (PDYN) gene polymorphisms might be linked to cognitive function in the elderly. Activation of κ-opioid receptors by dynorphins has been associated with stress-related memory impairments. Interestingly, these peptides can also modulate glutamate neurotransmission and may affect synaptic plasticity underlying memory formation. N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazol-propionate (AMPA) ionotropic glutamate receptor levels generally decrease with aging, and their function is impaired in AD. ***Methods:*** Here, we compared the impact of aging on ionotropic glutamate receptor levels in the hippocampal formation of wild-type (WT) and Pdyn knock-out (KO) mice. ***Results:*** We observed a significant reduction in GluR1 and GluR2 AMPA receptor subunits in the hippocampal formation of 18- to 25-month-old WT mice in comparison with 6-month-old mice. Conversely, the GluR1 protein level was maintained in old Pdyn KO mice, and the NMDA NR2B subunit level was increased by 42% when compared to old WT animals. ***Conclusions:*** These results suggest that elevated dynorphin expression occurring during aging and AD may mediate cognitive deficits by altering the glutamatergic system integrity.
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Introduction

Aging is generally characterized by slow progressive cognitive deficits. In Alzheimer's disease (AD), memory impairments are exacerbated by neuropathology development, and synaptic plasticity underlying learning and memory processes is irreversibly altered [1]. The neurotransmitter glutamate and its receptors have been closely linked to spatial and recognition memory [2–4]. Memantine, an antagonist of the N-methyl-D-aspartate (NMDA) glutamate receptor subtype, is currently used clinically to treat AD [5]. Impaired α-amino-3-hydroxy-5-methyl-4-isoxazol-propionate (AMPA) glutamate receptor trafficking has also been associated with AD mem-

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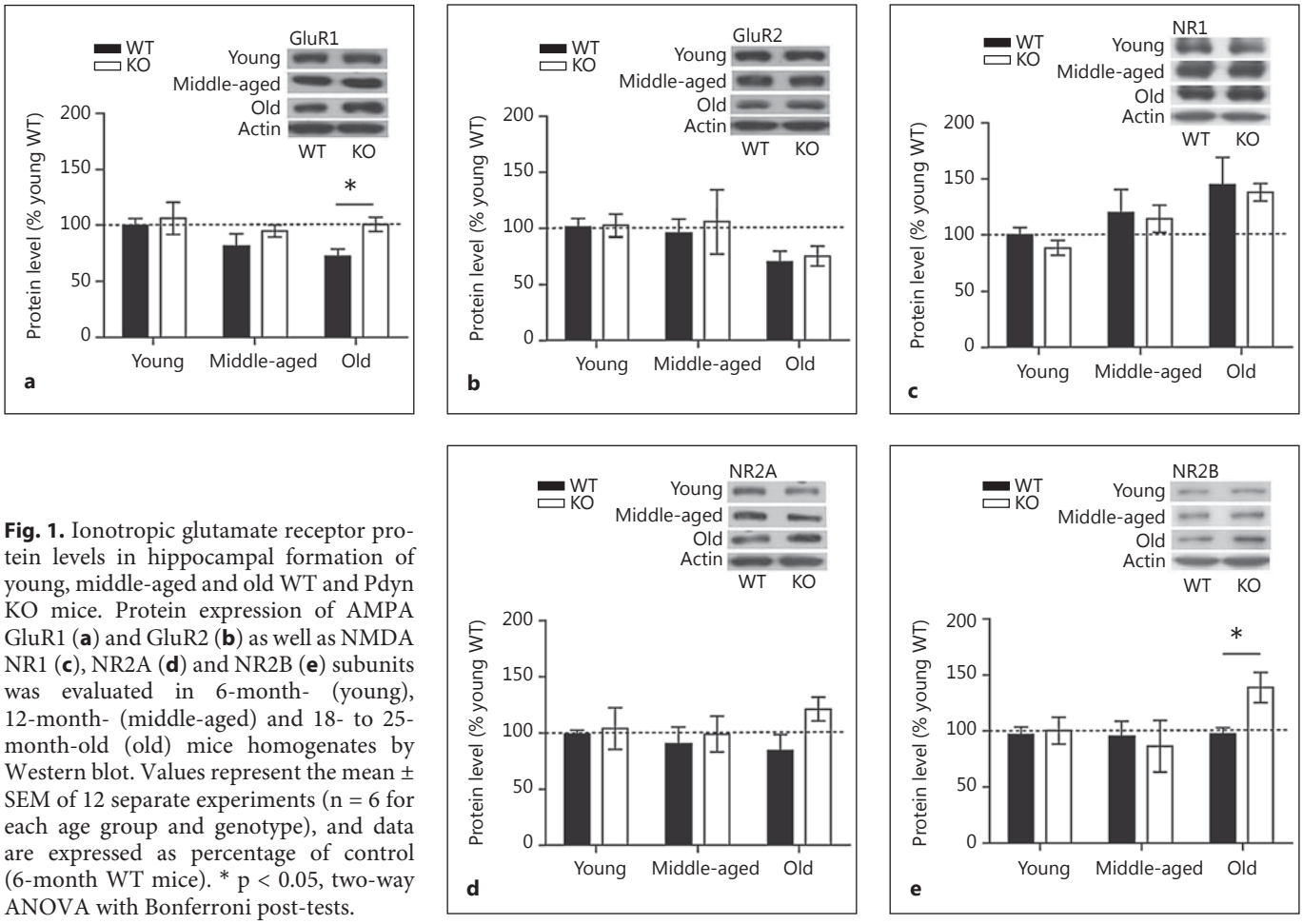

Fig. 1. Ionotropic glutamate receptor protein levels in hippocampal formation of young, middle-aged and old WT and Pdyn KO mice. Protein expression of AMPA GluR1 (**a**) and GluR2 (**b**) as well as NMDA NR1 (**c**), NR2A (**d**) and NR2B (**e**) subunits was evaluated in 6-month- (young), 12-month- (middle-aged) and 18- to 25-month-old (old) mice homogenates by Western blot. Values represent the mean ± SEM of 12 separate experiments (n = 6 for each age group and genotype), and data are expressed as percentage of control (6-month WT mice). * $p < 0.05$, two-way ANOVA with Bonferroni post-tests.

ory deficits [6]. However, the processes by which normal aging triggers age-related memory impairment and how this is altered in AD are still debated.

One possible mechanism for the observed progressive cognitive deficits occurring with aging may be related to rising dynorphin expression in the aged brain [7, 8]. The dynorphins, opioid peptides produced through prodynorphin (PDYN) gene expression, have been linked to learning, memory, emotional control and stress responses [9]. For example, enhanced dynorphin levels have been correlated with learning and memory impairments in aged rats [10, 11], and elevated levels of dynorphin A were reported in postmortem samples from AD subjects and correlated with neuritic plaque density [7]. Furthermore, κ-opioid receptor density, the main receptor of dynorphins, is increased in the amygdala, ventral putamen and cerebellar cortex of the AD brain [12, 13]. Activation of these receptors suppresses presynaptic glutamate release [14] and mediates stress-induced memory deficits [15].

Results

In this study, we evaluated the impact of Pdyn expression on ionotropic glutamate receptor levels in aging. We compared AMPA GluR1 and GluR2 as well as NMDA NR1, NR2A and NR2B subunit expression in the hippocampal formation of young (6 months), middle-aged (12 months) and old (18–25 months) C57Bl/6N wild-type (WT) and Pdyn KO mice [16]. The animals were sacrificed, and tissue containing the hippocampus, entorhinal, perirhinal and portions of adjacent neocortices were homogenized in a lysis buffer containing protease inhibitors. Protein concentration was determined using the bicinchoninic acid protein assay kit (Pierce, Rockford, Ill., USA). Western blot analysis was carried out on homogenates as described previously [17] with primary antibodies against GluR1, GluR2, NR2A, NR2B (dilution 1:1,000; Abcam, Cambridge, Mass., USA) and NR1 (dilution 1:2,000; Santa Cruz Biotechnology, Santa Cruz, Cal-

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if., USA). The actin level was used as a loading control, and 20 μg of proteins were loaded since this protein concentration was revealed as optimal for all the antibodies by serial dilutions of young WT reference samples (0, 3.125, 6.25, 12.5, 25, 50 and 100 μg; data not shown), done according to Halim et al. [18].

As shown in figure 1a, GluR1 protein level is significantly reduced in homogenates of older WT mice in comparison with Pdyn KO mice at the same age (genotype effect: two-way ANOVA, $F_{(1, 30)} = 4.86$, $p = 0.0353$). The impact of aging on GluR1 expression is significant between young and old WT mice (unpaired t test, $p = 0.0107$). In contrast, GluR2 protein levels decreased with age in both strains (fig. 1b). However, a significant variation was measured only for old WT (unpaired t test, $p = 0.0296$) in comparison with 6-month-old mice (unpaired t test, $p = 0.0718$ for KO mice). Interestingly, NMDA NR1 subunit protein levels were significantly increased in old mice of both strains (age effect: two-way ANOVA, $F_{(2, 30)} = 5.05$, $p = 0.0128$; fig. 1c) while no differences were observed for NR2A (fig. 1d). Finally, NR2B expression was significantly higher by 42% in homogenates of old Pdyn KO mice compared to old WT mice (fig. 1e, unpaired t test, $p = 0.0178$). Taken together, these results suggest that glutamatergic transmission in Pdyn KO mice is protected from the normally seen decline induced by aging.

Discussion

Modulation of AMPA and NMDA receptors has been proposed as a mechanism for the treatment of many neurological diseases including AD [5, 19]. As shown in this study, increased dynorphin expression as observed during normal aging [10, 11] and AD [7] might contribute to altered glutamatergic function associated with cognitive deficits. Lack of PDYN gene expression prevented age-related AMPA GluR1 protein decrease and enhanced the NMDA NR2B level in the hippocampal formation of old mice, suggesting an important role of dynorphin signaling in the control of AMPA and NMDA receptor expression. Both subunits are closely linked with cognition and performances in spatial memory tasks [20–23], a form of cognition specifically altered in human and AD mouse models [24, 25]. In line with our results, age-related cognitive deficits in the hippocampus-dependent Morris Water Maze spatial task are reduced in Pdyn KO mice [26]. Future studies will be necessary to determine the exact mechanism of the interactions of dynorphins with the glutamatergic system and to evaluate whether expression of these peptides correlated with memory impairments in AD mouse models and patients suffering from the disease. Blocking κ-opioid signaling might slow down the progression of cognitive decline generally occurring with aging and exacerbated in AD.

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Disclosure Statement

The authors have no conflict of interest.

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