Understanding the mechanism of learning enhancement: NMDA and GABA receptor expression

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OBJECTIVE: The administration of neurotrophic peptides NAPVSIPQ (NAP) + SALLRSIPA (SAL) to aged mice resulted in significant learning enhancement. N-methyl-D-aspartate (NMDA) and gamma-aminobutyric acid (GABA) receptors are fundamental for learning because they are the major modulators of the long-term potentiation, the electrophysiologic mechanism for learning. Also, these receptors have been shown to be involved in NAP + SAL prevention of learning deficit in a mouse model for fetal alcohol syndrome, when administered prenatally during development. Our objective was to test whether NMDA and GABA receptors contribute to the learning enhancement that is induced by the peptides after adult administration.

STUDY DESIGN: Aged (14.5 months) male mice were treated for 10 consecutive days with placebo or D-NAP + D-SAL (20 μg, by gavage). At the end of the treatment, brains were harvested. Calibrator-normalized relative real-time polymerase chain reaction was performed with primers for GABA-$_A\beta$3, GABA-$_A\alpha$5, and the NMDA receptor subunits NR2A and NR2B, with GAPDH standardization. Statistical analysis included analysis of variance, with a probability value that was considered significant at $<.05$.

RESULTS: Five control brains and 6 brains from animals that were treated with NAP + SAL were collected. There was no difference in GABA-$_A\beta$3, GABA-$_A\alpha$5, NR2A, and NR2B subunits after adult administration of NAP + SAL, as compared with the controls ($P > .05$).

CONCLUSION: Postnatal treatment with NAP + SAL induced learning enhancement in aged mice with a mechanism that does not involve alteration in NMDA and GABA receptor expression. Thus, the mechanism of learning enhancement might be different for a developing fetus than an adult or in the absence of a perturbing agent.

Key words: activity-dependent neurotrophic factor (ADNF), activity-dependent neuroprotective protein (ADNP), gamma-aminobutyric acid (GABA), learning, N-methyl-D-aspartate (NMDA)

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Many investigations have focused on looking for strategies to prevent mental retardation and developmental disabilities in different pathologic conditions. In our previous work, we demonstrated that prenatal or postnatal treatment with 2 synthetic peptides that are derived from neuroprotective proteins can enhance learning in healthy adults and aged mice.[1,2] These small 8 and 9 amino acid peptides, SALLRSIPA (SAL) from activity-dependent neurotrophic factor (ADNF) and NAPVSIPQ (NAP) from activity-dependent neuroprotective protein (ADNP) mimic the activity of their parent proteins. These parent proteins (ADNF and ADNP) are released by glial cells and are regulated by vasoactive intestinal peptide, which is a central nervous system neurotransmitter and neuromodulator with neurotrophic properties.

Numerous studies have demonstrated the neurotrophic activity of the peptides NAP and SAL. Prenatal or postnatal administration of NAP + SAL prevented alcohol-induced learning deficit in a model for fetal alcohol syndrome (FAS).[2,3] In a model for FAS that was based on chronic alcohol exposure, treatment with SAL prevented alcohol-induced brain growth compromise and anatomic alterations.[4] Intranasal administration of NAP + SAL prevented learning deficit induced by a blocker of choline-uptake.[5] Daily injections of NAP + SAL to newborn Apo-lipoprotein E-deficient mice, a model for Alzheimer disease, accelerated the acquisition of developmental reflexes and long-term memory deficits.[6,7] In a mouse model for Down syndrome, prenatal treatment with NAP + SAL prevented developmental deficits in newborn pups.[8]

Understanding the mechanism of action of these peptides is key in the development of a potential treatment for conditions that include developmental delay and learning deficits. In a model for FAS, the peptides have been shown to act through the prevention of changes in the expression of N-methyl-D-aspartate (NMDA) and gamma-aminobutyric acid (GABA) receptor subunits.[9,10] The GABA and the NMDA receptors for glutamate are pentameric and tetrameric ion channels, respectively, the function of which is determined by their subunit

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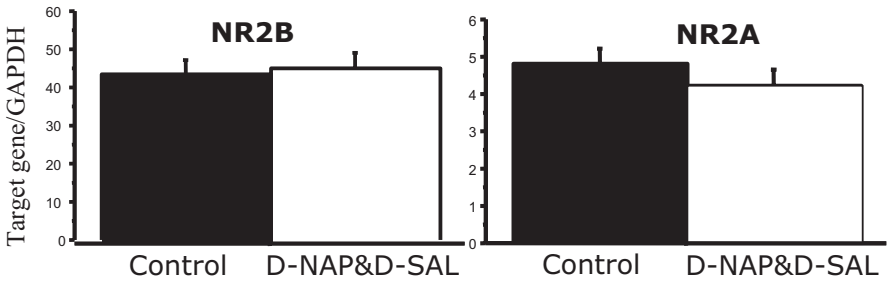
FIGURE 1
Postnatal treatment with the peptides D-NAP & D-SAL did not result in a significant difference in aged mice brains in NR2A and NR2B expression ($P > .05$)



The *bar* represents the ratio of NR2A-B/GAPDH messenger RNA expression ± SEM.
Toso. Understanding the mechanism of learning enhancement: NMDA and GABA receptor expression. AJOG 2007.

composition.[11] These receptors are key components of a brain circuit that leads to learning.[12] Specifically, the GABA receptor subunits $GABA_A\beta 3$ and $GABA_A\alpha 5$, and the NMDA receptor subunits NR2A and NR2B, play an important role in the learning process.

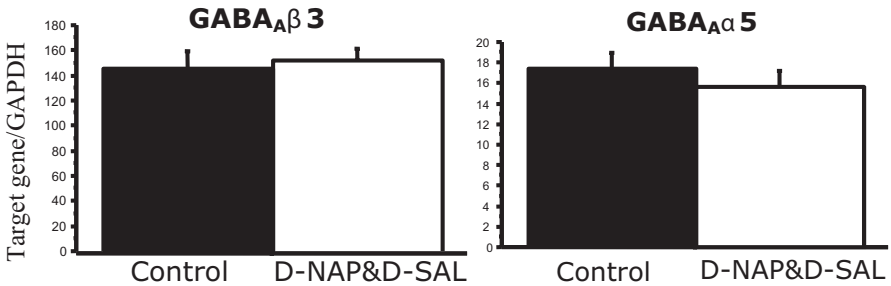
Our objective was to see whether learning enhancement of aged mice with NAP + SAL also acts through modification of the NMDA and GABA receptor subunits.

Materials and Methods

Fourteen-and-a-half month old C57Bl6/J male mice (The Jackson Laboratory, Bar Harbor, ME) were kept in a 12-hour light/12-hour dark regimen, with food and water available at all times. Mice received humane animal care in compliance with the National Institutes of Health Guidelines for Care and Use of experimental animals. The protocol was approved by the National Institute of Child Health and Human Development Animal Care and Use Committee. Following previously published methods,[1] mice were withheld food and water for 3 hours before treatment every day to avoid interference with drug absorption. For 10 consecutive days mice were treated by gavage with either D-NAP (20 μg) + D-SAL (20 μg; SynPep, Dublin, CA; n = 6 from 4 litters) or placebo (n = 5 from 3 litters). The all-D amino acid configurations of the peptides were used that allow for oral administration. D-NAP was diluted in 50 μL of dimethyl sulfoxide and diluted in 2.5 mL of filtered Dulbecco's phosphate-buffered saline solution; D-SAL was dissolved in the same solution. On day 11, brains from NAP + SAL and control groups were collected with microdissection and immediately frozen in dry ice.

RNA was isolated; complementary DNA was synthesized with reverse transcriptase reaction; real-time polymerase chain reaction (PCR) was performed for gene expression, and relative quantification was performed with calibrator-normalized data with efficiency correction. The data represent the delta-delta crossing point (CT) values; each sample was run in duplicate. Results are presented as the normalized ratio of NR2B, NR2A, $GABA_A\alpha 5$, or $GABA_A\beta 3$ to GAPDH. Real-time PCR comparisons were made between the NAP + SAL and control groups. Statistical analysis included analysis of variance, with a probability value of $<.05$ considered significant (StatView software, version 5.0.1; SAS Institute Inc, Cary, NC). A detailed description of the methods are provided in the Appendix.

FIGURE 2
Postnatal treatment with the peptides D-NAP + D-SAL did not result in a significant difference in aged mice brains in $GABA_A\alpha 5$ and $GABA_A\beta 3$ expression ($P > .05$)



The *bar* represents the ratio of NR2A-B/GAPDH messenger RNA expression ± SEM.
Toso. Understanding the mechanism of learning enhancement: NMDA and GABA receptor expression. AJOG 2007.

Results

Here we show that, in the brains from aged male mice, a 10-day treatment with the peptides D-NAP + D-SAL did not result in a modification in the expression of the NMDA receptor subunits NR2A and NR2B ($P > .05$; Figure 1). Similarly, the $GABA_A$ receptor subunits $GABA_A\alpha 5$ and $GABA_A\beta 3$ expression was not modified by treatment with the peptides ($P > .05$; Figure 2; control, 6 pups from 4 litters) and D-NAP + D-SAL (n = 5 from 3 litters). As previously reported, the animals treated with the peptides learned better than the controls in the Morris water maze, a model of spatial learning.[1]

Comment

Postnatal oral treatment with the peptides NAP + SAL resulted in learning enhancement in aged mice that is not mediated through the NMDA and GABA receptor subunits NR2A, NR2B, $GABA_A\alpha 5$ and $GABA_A\beta 3$. This is in contrast with findings of the administration of the peptides during development in a model for FAS, in which alterations in these subunits were found in prevention of learning deficit.[9,10]

Long-term potentiation (LTP) is the electrophysiologic mechanism of learn-