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Cerebrolysin Ameliorates Performance Deficits, and Neuronal Damage in Apolipoprotein E-Deficient Mice

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MASLIAH, E., F. ARMASOLO, I. VEINBERGS, M. MALLORY AND W. SAMUEL. *Cerebrolysin ameliorates performance deficits, and neuronal damage in apolipoprotein E-deficient mice.* PHARMACOL BIOCHEM BEHAV **62**(2) 239–245, 1999.—Recent studies suggest that Cerebrolysin improves behavioral performance by affecting synaptic transmission in the hippocampus. The main objective of this study was to determine if Cerebrolysin administration ameliorates the neurodegenerative and performance deficits in aged apolipoprotein E (apoE)-deficient mice. ApoE-deficient mice treated with Cerebrolysin showed a significant improved performance in the Morris water maze, compared to saline-treated apoE-deficient mice. Although the improved performance in the Cerebrolysin-treated apoE-deficient mice was associated with restoration of the neuronal structure, the poor learning ability of saline-treated apoE-deficient mice was related to the a disrupted synaptodendritic structure. This study supports the contention that Cerebrolysin might have a neurotrophic effect in vivo. © 1999 Elsevier Science Inc.

Cerebrolysin apoE Behavior Water maze

RECENT in vivo studies have shown that endogenous low molecular-weight neurotrophic factors are capable of ameliorating the behavioral alterations in aged rodents (6). It is then possible that brain extracts containing these factors might ameliorate cognitive alterations in neurodegenerative diseases (3). Cerebrolysin (Cerebrolysin™, EBEWE Pharmaceuticals, Austria) is a commercially available brain-derived peptide preparation, which is produced by biotechnological procedures, using a standardized controlled enzymatic breakdown of highly purified porcine brain proteins. Because of this method of production, it consists of a fraction of free amino acids and a fraction of low molecular-weight, biologically active peptides (19). Recent studies have shown that Cerebrolysin affects passive avoidance behavior in rats (9,17) and improves memory alterations in patients with mild to moderate cognitive impairment (20). Furthermore, Cerebrolysin induces a dose-dependent suppression of the synaptic response followed by a small rebound increase in the CA1 (3). This inhibition is mediated via $GABA_B$ receptors in the CA1 area of the hippocampus (24). In addition, this inhibition is presynaptic and can be reduced by low doses of a specific blocker of adenosine A1 receptors, 8-cyclopentyltheophylline (25). This suggests that the behavioral changes observed after Cerebrolysin administration might be related to its ability to modify synaptic transmission.

Recent studies have shown that in in aged apolipoprotein E (apoE)-deficient (knock-out) mice there is synaptic and dendritic damage in the neocortex and limbic system, accompanied by disruption of the microtubular cytoskeleton (12–14). Moreover, apoE-deficient mice display significant cholinergic (8) and memory deficits (8,15,16). These studies suggest that apoE might play a neurotrophic/neuroprotective function within the central nervous system (CNS), and that altered function of this molecule could result in neurodegeneration. These findings in the apoE-deficient model are potentially relevant to Alzheimer's disease (AD), because greater sus-

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ceptibility for this neurodegenerative disorder has been associated with the presence of the allele e4 of APOE (21). Because Cerebrolysin appears to display neurotrophic capabilities, it is possible that this compound might ameliorate the neurodegenerative and behavioral alterations associated with apoE deficiency in the knock-out mice. In this context, the main objectives of the present study were to determine if the neurodegenerative and cognitive alterations in apoE-deficient mice are reversed by Cerebrolysin administration.

METHOD

Colony Breeding and Characterization

As previously described (14), the homozygous apoE-deficient mice (C57BL/6J) were obtained by crossbreeding heterozygous mutants that were generously provided by Dr. J. Breslow (Rockefeller University, New York). Additional aged homozygous apoE-deficient (C57BL/6J) and wild-type mice (C57BL/6J) were provided by Drs. Palinsky and Steinberg (University of California at San Diego, Department of Medicine, La Jolla, CA). A total of 19 apoE-deficient homozygous (8 months old) and 17 wild-type (8 months old) mice were used for the present study. The experiment was performed in two phases. For the first phase, a group of 10 apoE-deficient and 8 wild-type control mice were analyzed. For the second phase, a group of nine apoE-deficient and nine wild-type controls were analyzed. Both groups of mice were tested, treated, and analyzed identically to assure reproducibility of results. At the end of both experiments, results were averaged for the cohort as a whole. Mouse genotype was confirmed by PCR, as previously described (14).

Behavioral Water Maze Testing and Cerebrolysin Treatment

ApoE-deficient homozygous and wild-type mice were trained to find a 10 × 10 cm escape platform in a Morris water maze, as previously described (15). The maze consisted of a dark-blue vinyl circular tank (176 cm in diameter) filled with room-temperature water to a depth of 30 cm and subdivided into four equal quadrants by two imaginary lines intersecting at right angles in the middle of the pool. An on-line video computerized tracking system (San Diego Instruments, San Diego, CA) was used to quantify the distance traveled (m) and the time spent (s) to reach the platform. At the beginning of a trial (days 1 to 3), the visible platform was placed in the northeast quadrant and the animals were placed in the water at the east pole 20 cm from the platform (Near Vis trials). When the mouse located the visible platform it was allowed to remain there for 20 s, followed immediately by another trial (three trials per animal per day, distance and duration data averaged for each day). On days 4 to 6 the visible platform was retained, but the mice were placed in the water at a distant start point, alternating between south and west poles (Far Vis trials). On days 7 to 12 the platform was submerged and the animals were placed in the water at the far start points (Far Invis trials). Following this test (day 13), the visible platform was again placed in the tank and the mice were given two additional trials at the far start points (Vis Test trials). A summary of the experimental paradigm is presented in Fig. 1.

Two days after this first phase of training in the water maze was completed, apoE-deficient and wild-type mice were randomly divided into two groups. In one group, mice received a daily intraperitoneal injection of Cerebrolysin (5 ml/kg, EBEWE Pharmaceuticals, Austria) for 28 days. One milliliter of Cerebrolysin contains 215.2 mg of concentrate. In the second group, the mice received saline alone. To evaluate the functional effects of treatment, 28 days after the treatment started the animals were again trained in the water maze, beginning with 6 days of exposure to the visible platform. They were then tested with the invisible platform for 6 days followed by two Vis Test trials on the last day. During the course of the maze testing, mice were treated daily with either Cerebrolysin or saline. After this second round of water maze training, the animals were sacrificed for subsequent analysis of the synaptodendritic integrity of the brain. All experiments described were approved by the animal subjects committee at the University of California at San Diego and were performed according to NIH recommendations for animal use.

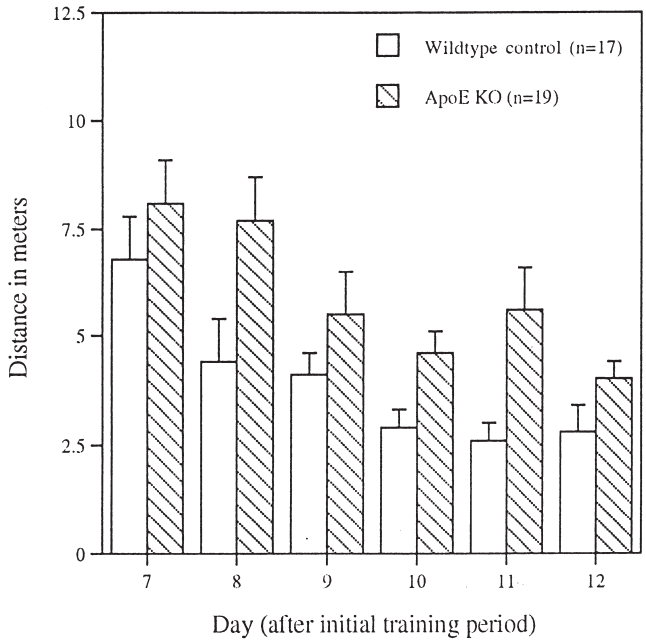

FIG. 1. Behavioral performance in the Morris water maze before treatment. Mice were trained for an initial period of 6 days in the water maze and they were tested with an invisible platform (not shown). This was followed by 6 days of testing (days 7–12), which are presented in the graph. ApoE-deficient mice showed significant impairment when compared to wild-type controls in a two-way ANOVA with one repeated measure, $F(1, 34) = 13.85$, $p < 0.008$, *Two-way ANOVA, [$F(1, 34) = 13.85$, $p < 0.008$].

Evaluation of Neurodegenerative Alterations

Animals were perfused with cold saline and the brains removed. The left hemibrain was frozen with isopentane and cooled in a Histobath (Shandon Lipshaw, Pittsburgh, PA), and the right hemibrain was immersion-fixed in 4% paraformaldehyde in pH 7.4 phosphate-buffered saline (PBS). The cortex and hippocampus from the frozen samples were dissected, homogenized, and fractionated as previously described for subsequent Western blot and dot blot analysis (14). Fixed hemibrains were serially sectioned at 40 μm thickness with a Leica Vibratome 1000E (Vienna, Austria) for subsequent routine analysis with the cresyl violet stain and immunocytochemical/computer aided image analysis.

To evaluate the integrity of the synaptic and dendritic system, blind-coded sections were immunolabeled with the mouse monoclonal antimicrotubule associated protein 2 (MAP2,

5 μg/ml, dendritic marker, Boehringer-Mannheim, Indianapolis, IN), mouse monoclonal antisynaptophysin (SYN, 1 μg/ml, synaptic marker, Boehringer), mouse monoclonal anti-growth-associated protein 43 (GAP43, Sigma), mouse monoclonal antiglial fibrillary acidic protein (GFAP, Boehringer), and mouse monoclonal antibody to polymerized β tubulin (SMI62, Sternberger Monoclonals, Inc., Baltimore, MD), as previously described (11,14). After overnight incubation with anti-SYN or MAP2, sections were incubated with FITC-conjugated horse antimouse IgG secondary antibody (1:75, Vector Laboratories, Burlingame, CA). The immunolabeled sections were transferred to SuperFrost slides (Fisher Scientific, Tustin, CA) and mounted under glass coverslips with antifading media (Vector). All sections were processed under the same standardized conditions that have previously been shown to yield reproducible results in experimental models of dennervation and reinnervation (11). The immunolabeled blind-coded sections were analyzed, as previously described, with the laser scanning confocal microscope (MRC1000, Bio-Rad) (14). From each case, three fields (2730 sq μm each field) within the frontal, hippocampus, and basal ganglia were imaged. The aperture, contrast, and gain levels were initially adjusted manually to obtain images with a pixel intensity within a linear range. Quantification of the % area of the neuropil covered by SYN-immunoreactive presynaptic terminals and MAP2-immunoreactive dendrites was performed with the IMAGE software, as previously described (11,14). This approach has been validated in experimental models of dennervation and reinnervation (11,23). Sections stained with anti-GFAP, GAP43, and SMI62, were reacted with diaminobenzidine and analyzed with the Quantimet 570C densitometer, as previously described (14).

Statistical Analysis

After the results were obtained the code was broken and sets of data were assigned to their corresponding groups (apoE-deficient homozygous or wild type, with or without treatment). Statistical analyses of the results were performed using the STAT VIEW II and SUPERANOVA software packages (Abacus Concepts) running on a Macintosh personal computer. Statistical comparisons among the groups was done by the one- or two-factor ANOVA. Additional comparisons were done by using the post hoc Scheffe test, as indicated. For comparisons between the apoE-deficient and wild-type mice, results from the water maze training phase (days 1–6) and the testing phase (days 7–12) were averaged and compared using one- and two-factor ANOVA. Performance in the Morris water maze during training and testing phases was expressed as mean distance swum in meters (m). All values were expressed as mean ± SEM. For comparisons between groups on a day-to-day basis, repeated measures ANOVA was used. All values were expressed as mean ± SEM.

RESULTS

Cognitive Impairment in apoE-Deficient Mice Is Reversed by Cerebrolysin Treatment

During 6 days of training with a visible platform (days 1–6), two-way ANOVA with one repeated measure showed no significant differences between the apoE deficient (1.8 ± 0.1; $n = 19$) and wild-type control mice (2.1 ± 0.2; $n = 17$), [$F(1, 34) = < 1$, NS], indicating that the groups were approximately equivalent in their motor and visual abilities. Numbers in parentheses represent mean distance swum in meters ± SEM.

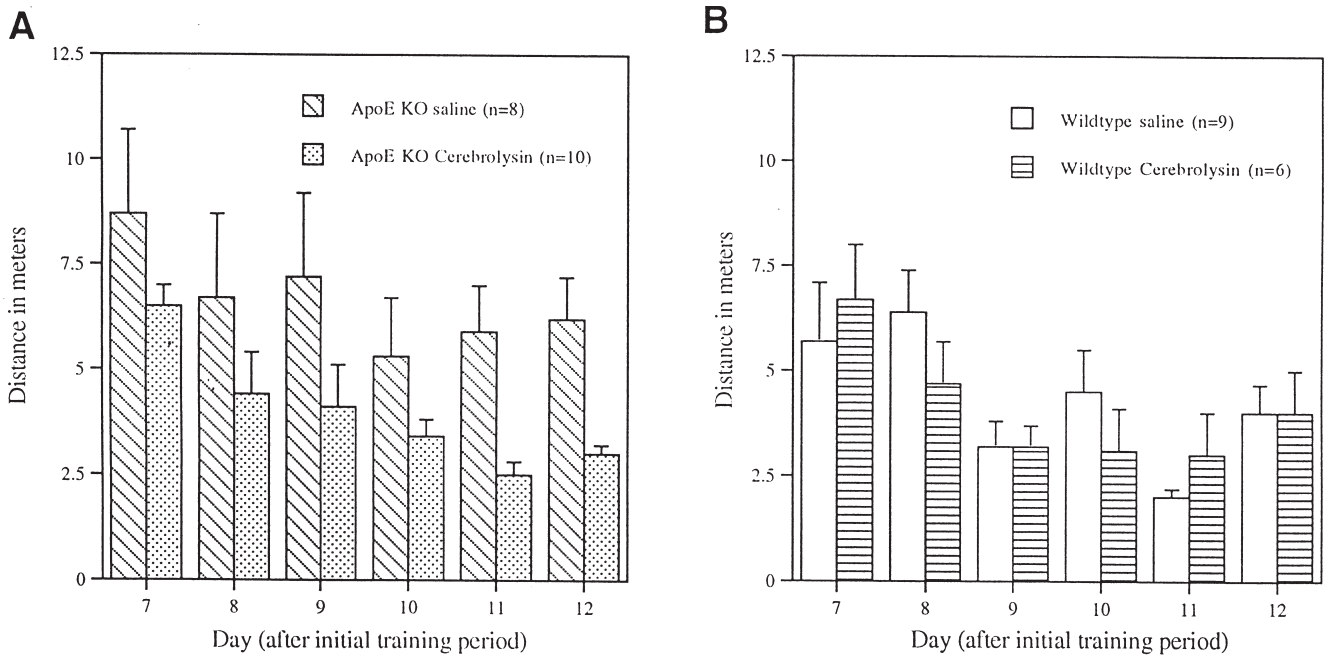

FIG. 2. Behavioral performance after treatment. Mice were treated for 4 weeks with saline or Cerebrolysin and tested in the water maze after an initial training period of 6 days (A). Saline-treated apoE-deficient mice continued to show impairment in performance in contrast to Cerebrolysin-treated mice mice that performed at the same levels as the wild-type control mice (B). Three-way ANOVA with one repeated measure showed a significant interaction between genotype and treatment, [$F(1, 29) = 7.76$, $p < 0.01$]. *Two-way ANOVA, [$F(1, 29) = 3.83$, $p < 0.05$].

Consistent with previous studies (15), when the platform was submerged (Far Invis, days 7–12), the apoE-deficient mice had a longer mean swim distance (5.9 ± 0.4) when compared to wild-type controls (3.9 ± 0.35) in a two-way ANOVA with one repeated measure, [$F(1, 34) = 13.85$, $p < 0.008$] (Fig. 1). The interaction term was not significant in this analysis, indicating that the performance impairment of the apoE-deficient mice was fairly uniform across days of testing. This learning impairment was not due to motor or visual disability, since when the platform was again made visible on day 13 (Vis Test), the apoE-deficient (2.1 ± 0.41) and wild-type (1.5 ± 1.37) mice did not significantly differ in the distance swum swam similar distances to find the target, [$F(1, 34) = 2.19$, NS].

After 4 weeks of intraperitoneal injection with either Cerebrolysin or saline treatment, mice were again trained in the Morris water maze to assess the effects of the treatment on learning. Across the initial 6 days of training with the visible platform (days 1–6), no significant differences were observed on repeated measures ANOVA between wild-type saline (1.05 ± 0.06; $n = 9$), wild-type Cerebrolysin-treated (1.04 ± 0.06; $n = 6$), apoE-deficient saline (1.8 ± 0.5; $n = 8$) and apoE-deficient Cerebrolysin mice (1.06 ± 0.07; $n = 10$) [main effect and interaction F-values ≤; 2.27, $df = 1,29$]. After this training phase, mice were tested in the water maze for 6 days using the Far Invis procedure (days 7–12). Three-way ANOVA with one repeated measure showed a significant interaction between genotype and treatment, [$F(1, 29) = 7.76$, $p < 0.01$]. The components of the significant interaction term were analyzed using post hoc comparisons between means by the Newman–Keuls procedure. Among apoE-deficient mice (Fig. 2A), those injected with saline continued to swim a long distance to reach the platform (6.7 ± 0.67, averaged across 6 days), whereas those injected with Cerebrolysin had a significantly ($p < 0.05$) shorter mean swim distance (3.9 ± 0.28). Among wild-type control mice (Fig. 2B), those injected with saline (4.4 ± 0.4) had a mean swim distance that was not significantly different from that of mice injected with Cerebrolysin (4.5 ± 0.43). These data indicate imply that Cerebrolysin treatment significantly improved the learning ability performance in the water maze of apoE-deficient mice, but had little effect on control animals. There were no significant interactions with the repeated measure in the three-way ANOVA (all F-values ≤ 1.07), which suggests that the relatively greater swim distance observed for saline-injected apoE-deficient mice was fairly consistent across each of the 6 testing days.

Following completion of the Far Invis trials, the platform was again made visible for 1 day of testing (Vis Test, day 13) and mice in all groups showed a decrease in their swim distances. There were no longer any significant differences among apoE-deficient (1.5 ± 0.3) or wild-type (1.6 ± 0.14) saline-injected animals, nor between apoE-deficient (1.4 ± 0.2) or wild-type (1.3 ± 0.2) Cerebrolysin-injected mice (main effect and interaction F-values ≤; 1.30, $df = 1,29$). This further corroborated, $F(1, 29) = 0.149$, NS), further corroborating that the impaired poor performance of the apoE-deficient, saline-injected animals on the Far Invis trials was due to a with the submerged platform was the result of spatial learning deficits and not to visual or motor impairments.

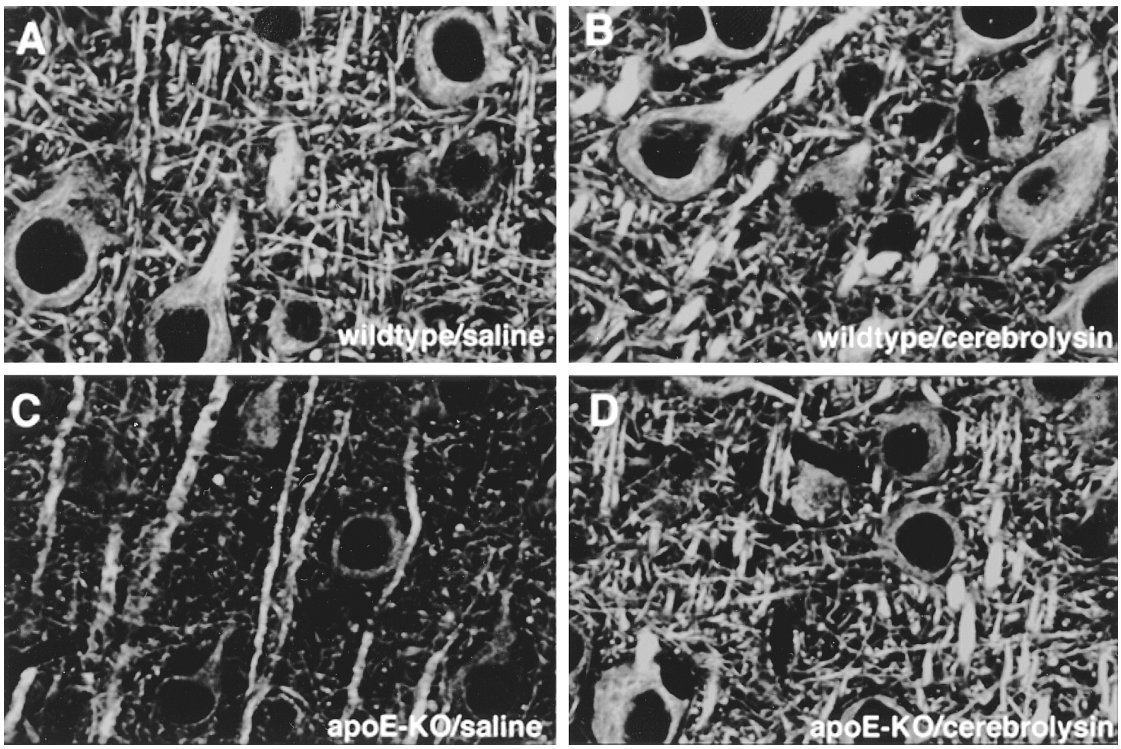

FIG. 3. Neurotrophic effects of Cerebrolysin in apoE-deficient mice. Images were obtained from the frontal cortex of MAP2-immunolabeled sections imaged with the laser scanning confocal microscope. (A) A wild-type saline-treated mouse shows normal neuronal organization. (B) A wild-type Cerebrolysin-treated mouse displays a slight enhancement of the dendritic architecture. (C) An ApoE-deficient saline-treated mouse presents widespread neuronal degeneration. (D) An ApoE-deficient Cerebrolysin-treated mouse shows recovery of the neuronal cytoarchitecture.

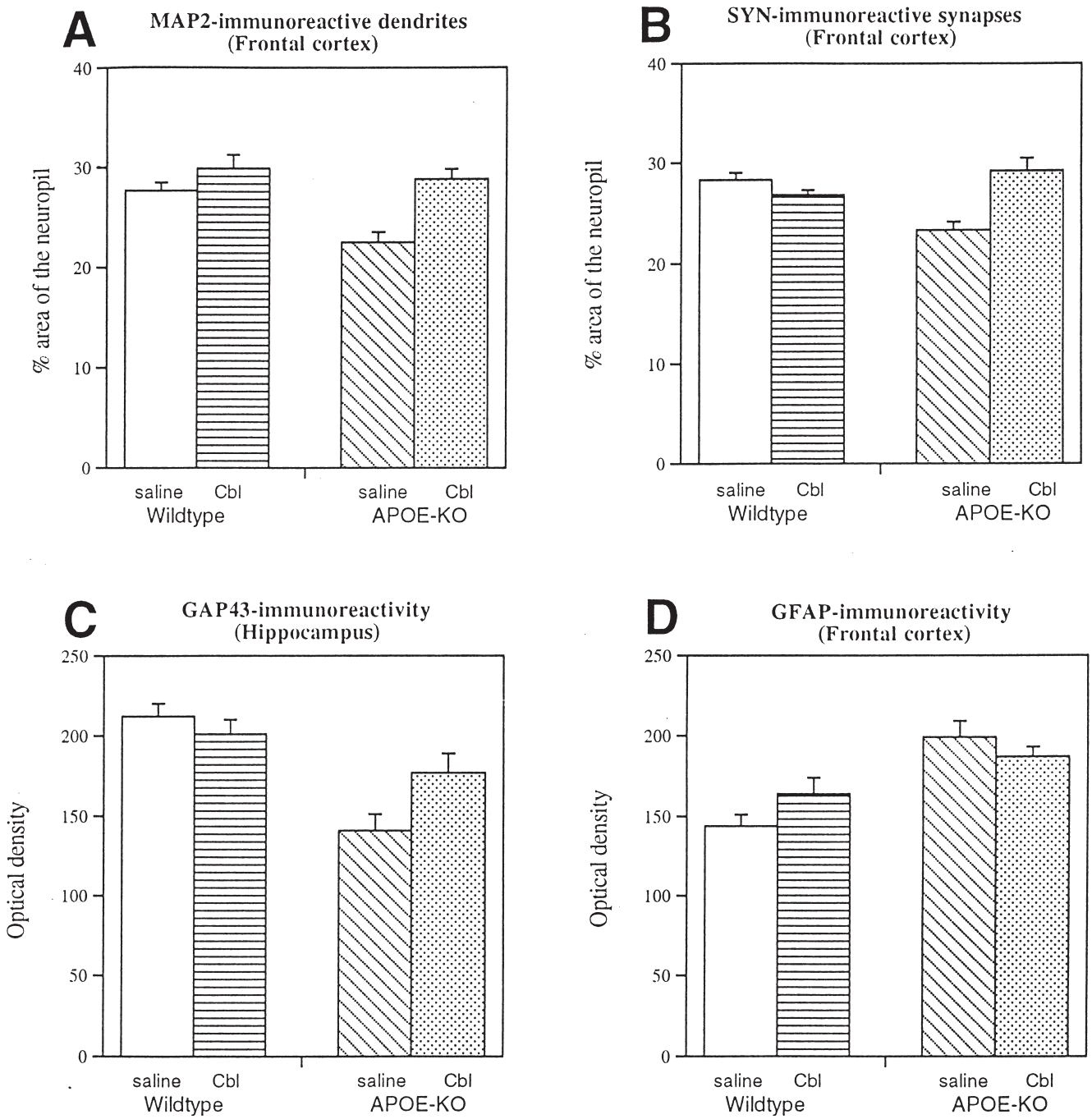

FIG. 4. Quantitative analysis of Cerebrolysin neurotrophic effects. ApoE-deficient mice treated with Cerebrolysin displayed a significant recovery in the area covered by: (A) MAP2-immunolabeled dendrites [one-way ANOVA, $F(3, 31) = 10.93$, $p < 0.0002$], and (B) SYN-immunoreactive presynaptic terminals [one-way ANOVA, $F(3, 31) = 7.99$, $p < 0.0005$], when compared to apoE-deficient mice treated with saline. No significant differences were observed among wild-type mice. (C) The recovery observed in the apoE-deficient mice treated with Cerebrolysin was accompanied by a significant increase in GAP43-immunoreactivity (marker of regeneration) in the hippocampus [one-way ANOVA, $F(3, 31) = 9.71$, $p < 0.0002$], however. (D) No no effect with Cerebrolysin was observed in the levels of reactive astrogliosis, as evidenced by GFAP-immunolabeling.

Neurodegenerative Alterations in apoE-Deficient Mice Are Reversed After Cerebrolysin Treatment

Analysis of the mouse brains after the behavioral testing showed that, when compared to wild-type controls (Fig. 3A and B), apoE-deficient mice treated with saline (Fig. 3C) displayed significant neurodegeneration characterized by dendritic damage (Fig. 3A). In contrast, apoE-deficient Cerebrolysin-treated mice showed substantial recovery of the dendritic

structure (Fig. 3D). Because there was no repeated measures factor to consider, analysis of the neuropathological data was simplified by entering scores for the four treatment groups into a one-way ANOVA, followed by post hoc pairwise comparisons of all group means using the Tukey–Kramer test. One-factor ANOVA of the qualitative analysis of MAP2 reactivity [$F(3, 31) = 10.93$, $p < 0.0002$]. Post hoc comparisons showed that the apoE-deficient saline group scored significantly below each of the other three groups ($p < 0.05$). One-way ANOVA also revealed that the four groups differed significantly in the numbers of SYN-immunoreactive terminals (Fig. 4B) [$F(3, 31) = 7.99$, $p < 0.0005$]. Post hoc Tukey–Kramer tests showed that differences between apoE-deficient saline and apoE-deficient Cerebrolysin-treated mice or wild-type saline-treated mice were significant ($p < 0.05$). No significant difference was observed between apoE-deficient Cerebrolysin or wild-type saline- or Cerebrolysin-treated mice. An impaired sprouting reaction in the perforant pathway as represented by a reduction in GAP43 immunoreactivity in the molecular layer of the hippocampus differed similarly between groups (Fig. 4C), [$F(3, 31) = 9.71$, $p < 0.0002$]. Post hoc Tukey–Kramer test showed that differences between apoE-deficient saline-treated mice and apoE-deficient Cerebrolysin or wild-type saline-treated mice were significant ($p < 0.05$). No significant difference was observed between apoE-deficient Cerebrolysin or wild-type saline-treated mice. Finally, the groups also differed significantly in GFAP immunoreactivity reflecting an increased astroglial reaction (Fig. 4D) [$F(3, 31) = 14.53$, $p < 0.0002$]. Post hoc Tukey–Kramer tests showed significant differences between wild-type saline-treated mice and apoE-deficient saline- or apoE-deficient Cerebrolysin-treated mice ($p < 0.05$). No significant difference was observed between apoE-deficient saline- or apoE-deficient Cerebrolysin-treated mice.

DISCUSSION

The present study showed that Cerebrolysin treatment improves the synaptodendritic pathology in apoE-deficient mice and indicates that these alterations are reversible. This is consistent with a previous study showing that Cerebrolysin has a neurotrophic effect on septal cholinergic neurons after transection of the fimbria-fornix in the rat brain (1). Furthermore, Cerebrolysin mimics the effects of NGF (but not FGF) in terms of recovering the impaired phenotype after fimbria-fornix lesion (7). Consistent with this concept, the present study also showed that Cerebrolysin treatment in the apoE-knockout mice resulted in an increased GAP43 immunoreactivity in the dentate gyrus of the hippocampus, indicating that Cerebrolysin exerted an NGF-like neurotrophic activity. This latter assumption is based on previous studies showing that NGF stimulation induces upregulation and translocation of GAP43 (22). The mechanisms responsible for the trophic effects of Cerebrolysin are not known. However, recent studies have shown that Cerebrolysin is able to modulate the expression of the blood brain barrier (BBB)-GLUT1 glucose transporter gene by increasing the mRNA transcript stability (4,5). Because it has been shown that this transporter is regulated by neurotrophic factors, the effects of Cerebrolysin on the BBB-GLUT1 model might support this as a possible target for its putative trophic activity (4).

In addition, the present study showed that the trophic effects of Cerebrolysin in the apoE-deficient mice were associated with amelioration of performance deficits in the Morris water maze. This is consistent with previous studies in aged rats showing that trophic factors that alter the synaptodendritic structure correlate with functional response in learning paradigms in the water maze (6,18). Furthermore, our results are also consistent with studies showing that Cerebrolysin affected passive avoidance behavior in neonatal (17) and aged (9) rats, and orientation in patients suffered mild to moderate cognitive impairment (10,20). Supporting the possibility that the improved behavioral performance promoted by Cerebrolysin might be related to synaptic changes, the present study showed a significant inverse correlation between distance spent in the water maze task and dendritic and synaptic content. Further supporting the possibility that Cerebrolysin affects synaptic function, recent studies have shown that Cerebrolysin induces a dose-dependent suppression of the synaptic response followed by a small rebound increase in the CA1 (3,24). Taken together, these studies suggest that the behavioral changes observed after Cerebrolysin administration might be related to its ability to modify synaptic functioning and structure. In conclusion, the fact that the behavioral and neurodegenerative alterations in the apoE-deficient mice were reversed by Cerebrolysin suggests that this compound has a neurotrophic potential that might be of therapeutic potential in human neurodegenerative disorders.

ACKNOWLEDGEMENTS

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REFERENCES

1. Akai, F.; Hiruma, S.; Sato, T.; Iwamoto, N.; Fujimoto, M.; Ioku, M.; Hashimoto, S.: Neurotrophic factor like effect of FPF1070 on septal cholinergic neurones after transection of fimbria-fornix in the rat brain. Histol. Histopathol. 7:213–221; 1992.
2. Allain, C. C.; Poon, L. S.; Chan, C. S. G.; Richmond, W.; Fu, P. C.: Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470–475; 1974.
3. Baskys, A.; Wojtowicz, J. M.: Effects of brain tissue hydrolysate on synaptic transmission in the hippocampus. Pharmacol. Biochem. Behav. 49:1105–1107; 1994.
4. Boado, R. J.: Brain-derived peptides regulate the steady state levels and increase stability of the blood–brain barrier GLUT1 glucose transporter mRNA. Neurosci. Lett. 197:179–182; 1995.
5. Boado, R. J.: Brain-derived peptides increase the expression of a blood–brain barrier GLUT1 glucose transporter reporter gene. Neurosci. Lett. 220:53–56; 1996.
6. Chen, K. S.; Masliah, E.; Mallory, M.; Gage, F. H.: Synaptic loss in cognitively impaired rats is ameliorated by chronic NGF infusion. Neuroscience 68:19–27; 1995.
7. Francis-Turner, L.; Valouskova, V.: Nerve growth factor and nootropic drug Cerebrolysin but not fibroblast growth factor can reduce spatial memory impairment elicited by fimbria-fornix transection: Short-term study. Neurosci. Lett. 202:1–4; 1996.
8. Gordon, I.; Grauer, E.; Genis, I.; Sehayek, E.; Michaelson, D. M.: Memory deficits and cholinergic impairments in apolipoprotein E-deficient mice. Neurosci. Lett. 199:1–4; 1995.
9. Hutter-Paier, B.; Eggenreich, U.; Windisch, M.: Effects of two protein-free peptide derivatives on passive avoidance behaviour of 24-months-old rats. Arzneimittelforsch./Drug Res. 46:237–241; 1996.
10. Kofler, B.; Erhard, C.; Erhard, P.; Harrer, G.: A multidementional approach in testing nootropic drug effects (cerebrolysin). Arch. Gerontol. Geriatr. 10:129–140; 1990.

11. Masliah, E.; Fagan, A. M.; Terry, RD.; DeTeresa, R.; Mallory, M.; Gage, F. H.: Reactive synaptogenesis assessed by synaptophysin immunoreactivity is associated with GAP-43 in the dentate gyrus of the adult rat. Exp. Neurol. 113:131–142; 1991.
12. Masliah, E.; Mallory, M.; Alford, M.; Ge, N.; Mucke, L.: Abnormal synaptic regeneration in hAPP695 transgenic and APOE knockout mice. In: Iqbal, K.; Mortimer, J. A.; Winblad, B.; Wisniewski, H. M., eds. Research advances in Alzheimer's disease and related disorders. New York: John Wiley & Sons Ltd.; 1995:405–414.
13. Masliah, E.; Mallory, M.; Alford, M.; Veinbergs, I.; Roses, A. D.: Apolipoprotein E role in maintaining the integrity of the aging central nervous system. In: Christen, Y.; Roses, A. D., eds. Apolipoprotein E and Alzheimer's disease. New York: Springer Verlag; 1995:59–73.
14. Masliah, E.; Mallory, M.; Ge, N.; Alford, M.; Veinbergs, I.; Roses, A. D.: Neurodegeneration in the CNS of apoE-deficient mice. Exp. Neurol. 136:107–122; 1995.
15. Masliah, E.; Samuel, W.; Veinbergs, I.; Mallory, M.; Mante, M.; Saitoh, T.: Neurodegeneration and cognitive impairment in apoE-deficient mice is ameliorated by infusion of recombinant apoE. Brain Res. 751:307–314; 1997.
16. Oitzl, M. S.; Mulder, M.; Lucassen, P. J.; Havekes, L. M.; Grootendorst, J.; de Kloet, E. R.: Severe learning deficits in apolipoprotein E-knockout mice in a water maze task. Brain Res. 752:189–196; 1997.
17. Paier, B.; Windisch, M.; Eggenreich, U.: Postnatal administration of two peptide solutions affects passive avoidance behaviour of young rats. Brain Res. 51:23–28; 1992.
18. Roch, J.-M.; Masliah, E.; Roch-Levecq, A.-C.; Sundsmo, M. P.; Otero, D. A. C.; Veinbergs, I.; Saitoh, T.: Increase of synaptic density and memory retention by a peptide representing the trophic domain of the amyloid β/A4 protein precursor. Proc. Natl. Acad. Sci. USA 91:7650–7654; 1994.
19. Ruether, E.; Ritter, R.; Apecechea, M.; Freitag, S.; Windisch, M.: Efficacy of Cerebrolysin in Alzheimer's disease. In: Jellinger, K. A.; Ladurner, G.; Windisch, M., eds. New trends in the diagnosis and therapy of Alzheimer's disease. Vienna: Springer Verlag; 1994:131–141.
20. Ruther, E.; Ritter, R.; Apecechea, M.; Freytag, S.; Windisch, M.: Efficacy of the peptidergic nootropic drug Cerebrolysin in patients with senile dementia of the Alzheimer's type (SDAT). Pharmacopsychiatry 27:32–40; 1994.
21. Saunders, A. M.; Strittmatter, W. J.; Schmechel, D.; St. George-Hyslop, P. H.; Pericak-Vance, M. A.; Joo, S. H.; Rosi, B. L.; Gusella, J. F.; Crapper-MacLachlan, D. R.; Alberts, M. J.; Hulette, C.; Crain, B.; Goldgaber, D.; Roses, A. D.: Association of apolipoprotein E allele E4 with late-onset familial and sporadic Alzheimer's disease. Neurology 43:1467–1472; 1993.
22. Van Hooff, C. O.; Holthuis, J. C.; Oestreicher, A. B.; Boonstra, J.; De Graan, P. N.; Gispen, W. H.: Nerve growth factor-induced changes in the intracellular localization of the protein kinase C substrate B-50 in pheochromocytoma PC12 cells. J. Cell Biol. 108:1115–1125; 1989.
23. Walaas, S. I.; Jahn, R.; Greengard, P.: Quantitation of nerve terminal populations: Synaptic vesicle-associated proteins on markers of synaptic density in the rat neostriatum. Synapse 2:516–520; 1988.
24. Xiong, H.; Baskys, A.; Wojtowicz, J. M.: Brain-derived peptides inhibit synaptic transmission via presynaptic GABAB receptors in CA1 area of rat hippocampal slices. Brain Res. 737:188–194; 1996.
25. Xiong, H.; Wojtowicz, J. M.; Baskys, A.: Brain tissue hydrolysate acts on presynaptic adenosine receptors in the rat hippocampus. Can. J. Physiol. Pharmacol. 73:1194–1197; 1995.