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Short communication

In vitro aggregation facilitates β-amyloid peptide-(25–35)-induced amnesia in the rat

Stéphanie Delobette, Alain Privat, Tangui Maurice *

I.N.S.E.R.M. Unité 336, Développement, Plasticité et Vieillissement du Système Nerveux, Ecole Nationale Supérieure de Chimie, 8, rue de l'Ecole Normale, 34296 Montpellier Cedex 5, France

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Abstract

The β-amyloid peptide-(25–35) fragment, but not β-amyloid peptide-(1–28), shares with β-amyloid protein-(1–42) the ability to self-aggregate and to induce neurotoxicity in vitro. This study examined the induction of amnesia in rats given intracerebroventricularly soluble or aggregated β-amyloid peptide-(25–35) (5–45 nmol), or β-amyloid peptide-(1–28) (15 nmol). Memory deficit in the water-maze test, examined 14 days after aggregated β-amyloid peptide-(25–35) injection, was more pronounced than with soluble β-amyloid peptide-(25–35). β-Amyloid peptide-(1–28) only affected retention. These results confirm the direct amnesic properties of β-amyloid peptides in the rat brain and showed that prior peptide aggregation markedly facilitates the appearance of amnesia.

Keywords: β-Amyloid peptide-(25–35); Amyloid-type amnesia; Water-maze test

1. Introduction

Alzheimer's disease is a neurodegenerative pathology, characterized by extracellular deposits, made up primarily of β-amyloid proteins (Selkoe, 1991). Different forms of a 40–43-amino-acid-residue β-amyloid protein are generated from the amyloid precursor protein, pathologically overexpressed in certain cases of familial Alzheimer's disease. Intraneuronal neurofibrillary tangles and neuronal losses constitute the other pathological hallmarks of Alzheimer's disease. Cognitive and neurochemical deficits correspond to these characteristic histological alterations (Selkoe, 1991). The presence of dystrophic neurites and neuronal degeneration surrounding and infiltrating the amyloid plaques suggests a direct neurotoxic effect of β-amyloid proteins (Selkoe, 1991). Indeed, several in vitro studies showed that peptides containing the 11 amino acids (25–35) are sufficient to induce neurotoxicity in neuronal cultures and share with β-amyloid protein-(1–42) the capacity to self-aggregate (Yankner et al., 1990; Pike et al., 1991, 1993; Cotman and Anderson, 1995). Pike et al. (1991, 1993) reported that β-amyloid peptide-(25–35) induced a concentration-dependent neurotoxicity in rat hippocampal cultures, whereas β-amyloid peptide-(1–28) was ineffective. In vivo, we previously examined the effects of central administration of these peptides in mice (Maurice et al., 1996). Both fragments were found amnesic in a passive avoidance test, but apparent moderate neuronal loss and Congo red-stained amyloid deposits were observed only after β-amyloid peptide-(25–35) administration. Herein, we report on the effect of intracerebroventricular (i.c.v.) administration of β-amyloid peptide-(25–35) in the rat compared with that of β-amyloid peptide-(1–28), and focus on the importance of soluble vs. aggregated forms of amyloid peptide in the appearance of memory deficits.

2. Materials and methods

2.1. Animals and drug treatments

Male Wistar rats (Iffa-Crédo, L'Arbresle, France), weighing 250–300 g at the start of the experiments, were housed individually, with free access to food and water except during the experiments, and were kept in a regulated environment (23 ± 1°C, 50 ± 5% humidity, 12-h light/dark cycle). The β-amyloid peptide-(25–35) and β-amyloid peptide-(1–28) (Neosystems, Strasbourg, France) were dissolved in sterile distilled water (vehicle) at

* Corresponding author. Tel.: (33-4) 6714-7217; Fax: (33-4) 6754-0610; e-mail: maurice@cit.enscm.fr

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a concentration of 3 μg/μl or 1 μg/μl (soluble form), and stored at −20°C. The β-amyloid peptide-(25–35) was aggregated by in vitro incubation at 37°C for 4 days (aggregated form). Light-microscopic observation indicated that incubation of β-amyloid peptide-(25–35), but not β-amyloid peptide-(1–28), yielded two types of insoluble precipitate, birefringent fibril-like structures and globular amorphous aggregates (not shown). Peptides (5 μl) were administered into the right ventricle, with stereotaxic coordinates from the bregma being, in mm, A −0.8, L −1.5, V 3.5 (Paxinos and Watson, 1986).

2.2. Behavioral observations

The ability of rats to perform spatial learning tasks was examined using the water-maze test, 14 days after the central administration of β-amyloid peptide. The maze was a circular pool, 150 cm in diameter, 40 cm in height, arbitrarily divided into 4 quadrants. The water temperature (25 ± 2°C), light intensity, external cues in the room, and water opacity, obtained by suspension of lime carbonate, were rigorously reproduced. A transparent Plexiglas platform, 10 cm in diameter, was immersed 2 cm under the water surface at the centre of one quadrant. This quadrant was termed the Training, and the others Opposite, Adjacent Right, and Adjacent Left quadrants. Swimming was recorded using a CCD camera connected to a computer, trajectories being analyzed in terms of latencies and distances by means of the Videotrack 2.0 software (Viewpoint, Lyon, France). Training consisted of 4 swims per day for 5 days. Start positions, set at each limit between quadrants, were randomly selected for each animal. Each rat was allowed a 90-s swim to find the platform and was left for a further 30 s on the platform. Rats failing to find the platform were placed on it manually. Retention was examined 48 h after the last training session. The platform was removed, and each rat was allowed a free 60-s swim. The percentage of time spent in each quadrant was determined.

2.3. Data analysis

The results are expressed as mean ± S.E.M. Swimming speeds were analyzed over trials using a repeated measures analysis of variance (ANOVA) and over groups using a two-way ANOVA, followed by Dunnett's test. Latencies were analyzed using the non-parametric Friedman repeated measures test (F values), comparisons between groups being made using Dunn's test. Data from the retention sessions were analyzed using Dunn's test after a Kruskal-Wallis ANOVA.

3. Results

Rats were trained in the water maze 14 days after the i.c.v. administration of β-amyloid peptides (Fig. 1A,B).

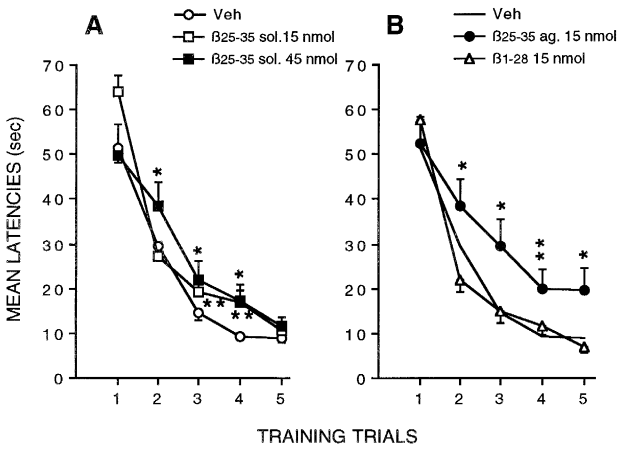

Fig. 1. Performance of β-amyloid peptide-(25–35)- or β-amyloid peptide-(1–28)-treated rats during training sessions in the water-maze test. (A) Veh, vehicle ($n = 20$); β25–35 sol., soluble β-amyloid peptide-(25–35) 15 nmol ($n = 23$) and 45 nmol ($n = 9$); (B) β25–35 ag., aggregated β-amyloid peptide-(25–35) 15 nmol ($n = 14$); β1–28, β-amyloid peptide-(1–28) 15 nmol ($n = 13$). Peptides were administered i.c.v. 14 days before the first training session. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle-treated group during the same training session (Dunn's test).

For the vehicle-treated group, latencies to finding the platform decreased over the course of acquisition training: $F(5,100) = 67.5$, $P < 0.001$ (Fig. 1A). Between trials, there was a significant decline in latencies from trial 1 to trials 3–5 ($P < 0.01$). Several treatments with the β-amyloid peptides were tested, and significant decreases in latencies were observed for all groups during training: $F(5,115) = 64.9$, $P < 0.001$ for soluble β-amyloid peptide-(25–35) at 15 nmol (Fig. 1A); $F(5,45) = 26.5$, $P < 0.001$ for soluble β-amyloid peptide-(25–35) at 45 nmol (Fig. 1A); $F(5,70) = 32.4$, $P < 0.001$ for aggregated β-amyloid peptide-(25–35) at 15 nmol (Fig. 1B); and $F(5,65) = 35.4$, $P < 0.001$ for β-amyloid peptide-(1–28) at 15 nmol (Fig. 1B). Particularly between trials, the latencies measured during the last two trials in each group were significantly lower than the latency measured in the first trial. However, differences from the control profile appeared among peptide-treated groups. Animals treated with soluble β-amyloid peptide-(25–35), 15 nmol, showed significantly higher latencies during trials 3 and 4, but not 5 (Fig. 1A). The group treated with the highest dose exhibited a similar, although more pronounced, profile (Fig. 1A). However, when the peptide was administered in its aggregated form, profiles developed differently, since latencies from trials 3–5 were significantly higher than that of the control group, with no further decrease between the last two training trials (Fig. 1B). No difference from the vehicle-treated group was observed with the β-amyloid peptide-(1–28)-treated group (Fig. 1B). It must be noted that the mean speed of the control group decreased continuously between the first and fifth trial, from 0.182 m/s to 0.153 m/s ($F(5,99) = 4.4$, $P = 0.003$). The different treatments did not significantly

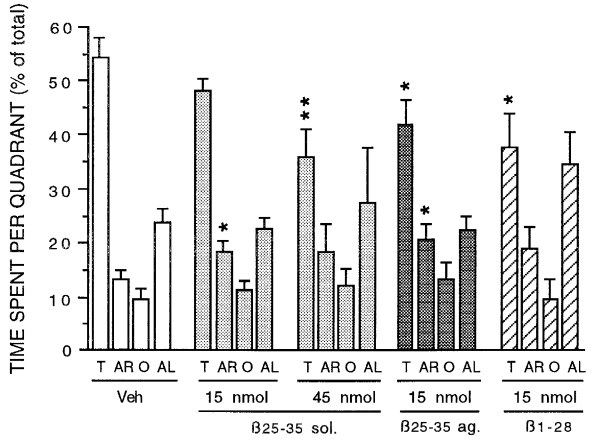

Fig. 2. Performance of β-amyloid peptide-(25–35) or β-amyloid peptide-(1–28)-treated rats during retention in the water-maze test. Results are expressed as percentages of time spent in each quadrant, during the 60-s swim. Quadrants: T, training; AR, adjacent right; O, opposite; AL, adjacent left. Veh: vehicle, sol.: soluble, ag.: aggregated. Peptides were administered i.c.v. 14 days before the first training session. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle-treated group (Dunn's test).

modify this decrease. During the retention test, all groups exhibited a clear tendency to swim preferentially in the Training quadrant (Fig. 2). However, significant diminutions of the percentages of time spent in this quadrant were measured for all peptide-treated groups, except the group treated with soluble β-amyloid peptide-(25–35), 15 nmol (Fig. 2).

The effect of soluble β-amyloid peptide-(25–35), 15 nmol, was also examined on the learning capacity in the water maze, 28 days after i.c.v. administration (not shown). Significant decreases in latencies were observed during training: $F(5,65) = 34.6$, $P < 0.001$, for the β-amyloid peptide-(25–35)-treated group ($n = 13$); $F(5,50) = 21.3$, $P < 0.001$ for the vehicle-treated group ($n = 10$). In particular between trials, latencies measured during the third to fifth trials were significantly lower than the latency measured in the first trial. A higher latency during the second trial was the only significant difference from the control profiles. Furthermore, animals performed similarly during the retention test.

4. Discussion

These results showed that, in the rat, i.c.v. administration of aggregated β-amyloid peptide-(25–35) induced a significant learning disturbance in the water-maze test. Soluble β-amyloid peptide-(25–35) induced a significant but marginal impairment, while β-amyloid peptide-(1–28) did not impair acquisition. All peptides impaired retention to some degree. Several previous studies had shown that central administration of β-amyloid peptides in the rat induced amnesia. First, chronic infusion of β-amyloid protein-(1–40) led to marked learning deficits in the water maze, that could be correlated with a decreased acetylcholinesterase activity (Nitta et al., 1994). Furthermore, acute administration of β-amyloid protein-(1–40) was reported to impair memory consolidation selectively, since behaviors well learned were not affected (McDonald et al., 1994). Dornan et al. (1993) failed to show any histological lesions or significant modification of water-maze acquisition, with either aggregated or soluble β-amyloid peptide-(25–35) when it was administered alone into the hippocampus. On the other hand, lesions and focal amyloid deposits, combined with disruption of learning, were observed when β-amyloid peptide-(25–35) was co-administered with non-toxic doses of ibotenic acid. It was also reported that administration of β-amyloid peptide-(1–28), β-amyloid peptide-(12–28), β-amyloid peptide-(18–28), β-amyloid peptide-(12–20), or (Gln^{11})β-amyloid peptide-(1–28) impairs learning in a footshock active avoidance test in mice (Flood et al., 1991). Study of the structure/activity relationship with these peptides showed that the impairment was dependent on the presence of the configuration Val-Phe-Phe (VFF), in position (18–20) (Flood et al., 1994). We did not observe any effect of β-amyloid peptide-(1–28) on water-maze acquisition in the present study. However, Abe et al. (1994) reported that acetylcholine outflow is differentially affected by β-amyloid protein-(1–40), β-amyloid peptide-(12–28), or β-amyloid peptide-(25–35), 7–21 days after administration. The amnesic effect induced by β-amyloid peptide-(12–28), or β-amyloid peptide-(1–28), i.e., the VFF-containing peptides, seems to be limited to a few days after injection, contrary to β-amyloid protein-(1–40) or β-amyloid peptide-(25–35). We designed our experiments, using 14-day pretraining peptide administration, with the time parameters for altered acetylcholine efflux described by Abe et al. (1994). This protocol permitted the demonstration of impairment effects for β-amyloid peptide-(25–35), but not for β-amyloid peptide-(1–28).

The important point of this study was the influence of the physical state of the peptide, aggregated or soluble, at the time of administration. The in vitro incubation of β-amyloid peptide-(25–35), as well as β-amyloid protein-(1–40), leads to the formation of stable oligomeric aggregates which contain an increased proportion of β-sheet structure, that appears to be an important feature of the β-amyloid-induced neurotoxicity (Pike et al., 1991, 1993; Lockhart et al., 1994; Simmons et al., 1994). This aggregation mimics in vitro the slow aging process which in vivo leads to the formation of the senile plaques. It must be noted that administration of peptide aggregates i.c.v. must affect its ability to penetrate the brain structures. However, Congo red-stained amyloid-like deposits could be observed in the mouse brain after administration of aggregated β-amyloid peptide-(25–35) (Maurice et al., 1996). Thus, aggregation appears as an important factor of β-amyloid peptide-(25–35)-induced neurotoxicity (Pike et al., 1991,

1993; Lockhart et al., 1994; Cotman and Anderson, 1995), and our behavioral results using the water-maze test demonstrate that prior aggregation of the peptide also markedly facilitated the appearance of learning deficits. Similarly, solubilization of β-amyloid protein-(1–40) in solvents that prevent aggregation, or use of the non-aggregated β-amyloid protein-(1–40), resulted in amnesia but only after chronic i.c.v. infusion for at least 14 days (Nitta et al., 1994).

It must be noted however that the exact availability of the soluble or aggregated peptide in the brain, before it is presumably broken down, remains to be determined. We only examined the impairing effect of peptide administration 14–28 days after administration, in relation with the neurotoxic effect of the peptide. The effects of soluble peptide injected a shorter time prior to training, or injected at higher doses and with a longer time prior to training, remains be examined, keeping in mind that β-amyloid protein-(1–42) administered into the hippocampus or the lateral ventricles, 15 months prior to training, failed to induce long-term behavioral, biochemical, or histological effects (Winkler et al., 1994).

Finally, these results, and previous observations in mice (Maurice et al., 1996), clearly evidence that distinct mechanisms are involved in the appearance of amnesia after β-amyloid peptide-(25–35) or β-amyloid peptide-(1–28) administration. With the β-amyloid peptide-(25–35), amnesia is markedly facilitated when the peptide is administered as aggregates and is likely to involve the neurotoxic properties, whereas β-amyloid peptide-(1–28), and its derivatives containing the VFF motif, impair learning through a more direct effect on cholinergic systems. The more endogenous β-amyloid protein-(1–40), β-amyloid protein-(1–42), or β-amyloid protein-(1–43) may in fact combine these different modes of action.

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