β-Amyloid Treatment Sensitizes Mice to Amphetamine-Induced Locomotion but Reduces Response to Caffeine

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Key Words

Alzheimer's disease · Amphetamine · β-Amyloid ·
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Abstract

Background: Psychosis frequently occurs in Alzheimer's disease (AD), being associated with more severe cognitive decline, but the underlying mechanisms are unknown. **Objective:** To investigate the effect of centrally administered β-amyloid peptide, a model for AD, in the locomotor response to amphetamine, caffeine and MK-801, which are psychoactive drugs related to neurochemical changes occurring in psychosis. **Methods:** Mice were intracerebroventricularly injected with β-amyloid (25–35), and after 1 week they were tested in the passive avoidance, spontaneous alternation and locomotor tasks. **Results:** Besides impaired performance in inhibitory avoidance and spontaneous alternation tasks, β-amyloid-treated mice showed increased spontaneous locomotion, augmented response to amphetamine (1.5 mg/kg), blunted response to caffeine (30 mg/kg) and no difference in MK-801 (0.25 mg/kg)-induced locomotor activation when compared to its respective control. **Conclu-**

sion: These results are compatible with the hypothesis that β-amyloid peptide may predispose to psychotic symptoms of AD by increasing sensitivity of the dopaminergic system, possibly related to a decreased adenosinergic inhibitory tone.

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Introduction

Psychosis affects up to 30% of the Alzheimer's disease patients (AD), with delusions and hallucinations as the most frequently expressed psychotic symptoms [1]. Several studies have shown that psychotic patients have more rapid cognitive decline when compared to controls [2]. However, there is no consistent explanation for the pathophysiology of psychosis in AD patients in the few available studies, in which a small number of brain regions, and neuropathological and neurochemical parameters were investigated [1].

Animal models have been useful for studying the neurobiology of psychosis and AD. The behavioral effects of N-methyl-D aspartate (NMDA) [3] receptor antagonists, such as phencyclidine and MK-801, are currently ac-

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cepted pharmacological models of psychosis. NMDA antagonists produce positive, negative and disorganization symptoms in healthy subjects, which are mostly indistinguishable from those observed in schizophrenic patients [4]. Regarding the dopaminergic system, most antipsychotic drugs are dopamine D₂ receptor antagonists [5], and dopaminergic agonists such as amphetamine are classic models for psychosis [6]. Caffeine, a non-selective adenosine antagonist, is another psychostimulant, possibly by indirectly stimulating the dopaminergic system [7, 8]. In animal models, hyperlocomotor response produced by amphetamine and NMDA receptor antagonists is the most common behavioral parameter both to predict putative antipsychotics [9] and to evaluate predisposing conditions to psychotic states [10].

Neurofibrillary tangles and parenchymal deposition of β -amyloid in senile plaques are pathological hallmarks of AD, and their extension correlate with neuronal damage and cognitive deficits in the disease [11]. Central administration of the β -amyloid (25–35) fragment (β_{25-35}) in animal brain causes histological, biochemical and memory alterations, composing an animal model of AD [12]. There are currently few data concerning spontaneous and drug-induced locomotion in mice centrally treated with β_{25-35} . We investigated the effect of β_{25-35} administration on locomotor behavior by interfering with the glutamatergic, dopaminergic and adenosinergic systems.

Materials and Methods

Animals

Experiments were performed with male adult mice 3–4 months old (35–45 g; CF1 strain) maintained in our own animal facilities under controlled environment (23 $\pm$ 2 °C, 12-hour light/dark cycle, free access to food and water). All behavioral experiments were conducted between 10:00 a.m. and 2:00 p.m. and were in accordance with our institutional animal care protocols. Animals were used only once.

Drugs and Administration Procedure

MK-801, amphetamine, caffeine and β_{25-35} were purchased from Sigma (St. Louis, Mo., USA). The β_{25-35} peptide was dissolved in bidistilled water at a concentration of 2 mg/ml and stored at –20 °C until use. The peptide solutions were thawed and aggregated by incubation for 4 days at 37 °C in a 1 mg/ml solution in bidistilled water, providing the formation of birefringent fibril-like structures and amorphous globular aggregates. The β_{25-35} peptide was then administered intracerebroventricularly. Mice were anesthetized with 60 mg/kg of thiopental, and a 28-gauge 5-mm-long stainless-steel needle was inserted unilaterally 1 mm to the right of the midline point equidistant from each eye, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull (2.5 mm depth). Peptide or vehicle (3 μ l) was delivered within approximately 3 s intracerebro-

ventricularly. Administration had been confirmed by injecting methylene blue in preliminary experiments. Other drugs were injected intraperitoneally at a volume of 10 ml/kg.

Spontaneous Alternation

Spontaneous alternation performance was assessed 8 days after β -amyloid administration in the Y-maze. Each arm was 30 cm long, 20 cm in height and 6 cm wide, and converged to an equal angle. Each mouse was placed at the end of one arm and allowed to freely move through the maze during 8 min. The series of arm entries was recorded visually. An alternation was defined as entries in all three arms on consecutive occasions. The percentage of alternation was calculated (total alternation/total arm entries – 2).

Locomotor Activity

Locomotor activity was assessed 8 days after β -amyloid administration. Mice were randomly allocated to individual triangular boxes with rounded corners (800 cm²) placed on the floor of a soundproof and diffusely illuminated room. Motor activity of 8 mice was recorded simultaneously by a video-computerized system, with image analysis at 4 frames per second. The software tracked the animals by distinguishing their white color from the black background of the box floor, registering X and Y horizontal coordinates. The method was set to examine horizontal locomotor activity, ignoring small movements like breathing, head and tail actions, and tremors. Animals had not been previously habituated to the boxes and were observed for a total of 4 h, with data divided into 10-min blocks. MK-801 (0.25 mg/kg), amphetamine (1.5 mg/kg), caffeine (30 mg/kg) or saline was administered intraperitoneally to mice after a 1-hour habituation period. Pilot studies in our laboratory showed that these doses produce an increase in locomotion that is not in excess of the level of locomotion seen during the habituation period; therefore further enhancement of locomotion would not be masked by a ceiling effect.

Inhibitory Avoidance

Long-term memory was examined 9 days after β -amyloid administration using step-down inhibitory avoidance. The session apparatus is a 50 $\times$ 25 $\times$ 25 cm plastic box with a platform (2 cm high and 4 $\times$ 6 cm wide) at the center of the training apparatus. The floor of the apparatus was made of parallel 0.1-cm-caliber stainless steel bars spaced 1.0 cm apart. In training session, the animals were placed on the platform and the latency to step down the four paws on the grid was measured with a device; upon stepping down, mice received a 0.2-mA, 2-second scrambled foot shock. Test session step-down latency 24 h later was taken as measure of retention, to a ceiling time of 180 s. No foot shock was given in the test session.

Statistical Analysis

Locomotion and spontaneous alternation are expressed as means $\pm$ SEM and step-down latency is expressed in terms of medians and interquartile ranges. Spontaneous alternation was analyzed using one-way ANOVA. Step-down latency was analyzed using the Mann-Whitney test followed by the Kruskal-Wallis nonparametric test. Differences between time blocks in locomotor activity were assessed by one-way ANOVA. Comparisons between locomotor activities at different time points were analyzed using a general linear model with repeated measures (drug treatment vs. time) with time as the repeated measure. Duncan's post hoc test was used to determine differences among specific groups. A p value <0.05 was considered statistically significant.

Results

Confirming previous observations [13], central administration of β_{25-35} affected performance in the inhibitory avoidance test for long-term memory, as shown by the reduced step-down latency (fig. 1a) as well as spontaneous alternation performance in the Y-maze test (fig. 1b). There was no difference in total arm entries between β_{25-35} and sham-operated mice ($n = 16$ per group).

Mice injected with β_{25-35} showed higher spontaneous locomotion during the whole 4-hour experiment (fig. 2). Low-dose amphetamine (1.5 mg/kg) nonsignificantly increased spontaneous locomotion; however, β_{25-35} -treated mice presented a significantly higher locomotor response to amphetamine when compared to sham-operated mice [$F(1,12) = 9.125$; $p < 0.05$; fig. 2a, 3]. Caffeine (30 mg/kg) caused significant hyperlocomotion in control animals, whereas there was no increase in activity of mice treated with β_{25-35} [$F(1,14) = 6.140$; $p < 0.05$; fig. 2b, 3]. Both sham- and β_{25-35} -operated mice had a similar hyperlocomotion response to MK-801 (0.25 mg/kg) administration [$F(1,14) = 0.619$; $p > 0.05$] compared to its respective control group (fig. 2c, 3), but β_{25-35} treated mice showed nonsignificantly increased locomotion compared to the sham + MK-801 group ($n = 8$ per group).

Discussion

These results show a distinctive response of β_{25-35} -treated mice in a locomotor activity task, characterized by higher basal locomotor activity, enhanced response to low-dose amphetamine and reduced response to caffeine, whereas the response to MK-801 was minimally altered. This profile supports the notion that β -amyloid accumulation may underlie the clinical observation of psychotic symptoms in AD, which are possibly associated with a hyperdopaminergic state. Moreover, the blunted response to caffeine suggests a reduced striatal adenosinergic tone, which can contribute to the increased sensitivity to amphetamine due to adenosine-dopamine interactions [7].

Cerebral β_{25-35} microinjection produces both β -amyloid deposition and amnesia, which is considered a valid model for the neuropathology of AD [14], being mostly equivalent to complete β -amyloid (1–40) peptide while studying its neurotoxic effects and putative neuroprotective agents in vivo [15]. As previously described, intracerebroventricular microinjection of aggregated β_{25-35} affects the whole brain, particularly the frontoparietal cortex, hippocampal formation, corpus callosum, cingulum

Fig. 1. β -Amyloid impairs performance in memory tasks. Time latency in the inhibitory avoidance task (**a**) and spontaneous alternation in the Y-maze (**b**) of β -amyloid 25–35 (3 nmol)- or sham-operated mice are shown. * $p < 0.05$ (Kruskal-Wallis test for inhibitory avoidance, **a**, or one-way ANOVA for spontaneous alternation, **b**; $n = 16$ for both groups).

and striatum, with significant neuronal loss in the hippocampus CA3 region and cortex, both ipsi- and contralaterally [13]. This distribution correlates well with the diffuse and heterogeneous pattern encountered in AD patients, in whom the amount of β -amyloid, mainly in the entorhinal cortex and in the subiculum, correlates with cognitive decline [16]. The present results suggest that in addition to the cognitive symptoms, also the behavioral alterations found in AD patients (particularly psychotic symptoms and increased psychomotor activity) can be modeled by β_{25-35} microinjection. However, the lack of histopathological evaluation in our study is a limitation, not allowing a

Fig. 2. Effect of amphetamine, caffeine or MK-801 on locomotor activity in sham- and β -amyloid-treated mice. After 1 h of habituation, mice were treated with saline, amphetamine (1.5 mg/kg; **a**), caffeine (30 mg/kg; **b**) or MK-801 (0.25 mg/kg; **c**), and locomotor activity was recorded for 3 further hours. * $p < 0.05$ vs. the respective control; ** $p < 0.05$ vs. sham + amphetamine or caffeine group ($p < 0.05$) in the general linear model with repeated measures (drug treatment vs. time) with time as the repeated measure ($n = 8$ for all groups).

Fig. 3. Net locomotor activity of mice treated with amphetamine, caffeine and MK-801. Locomotor activity for 90 min after saline treatment in sham- and β -amyloid-treated mice is shown as total activity. These data were subtracted from the locomotor activity of the equivalent time period after treatment with amphetamine (1.5 mg/kg), caffeine (30 mg/kg) or MK-801 (0.25 mg/kg) in their respective groups. * $p < 0.05$ vs. their respective controls (one-way ANOVA; Duncan post-hoc test; $n = 8$ for all groups).

correlation between the behavioral responses to discrete brain regions.

The augmented spontaneous locomotion of mice treated with β_{25-35} can be explained by three different factors (occurring either alone or in combination): (i) an increased dopaminergic tone, as suggested by the increased response to amphetamine; (ii) a decreased adenosinergic tone, as suggested by the reduced response to caffeine, which blocks A1 and A2A receptors of the inhibitory neuromodulator adenosine [8], and (iii) increased anxiety response to mild stress associated with the new environment [17]. In contrast to our model, mice overexpressing β -amyloid precursor protein showed lower exploratory behavior in a 20-min open-field trial [18], and mice treated with β -amyloid 1–40 showed normal behavior in a similar 10-min task [14]. This discrepancy possibly derives from the short period of analysis in those works, since our results showed that higher locomotor activity is mostly expressed after 30 min. It is noteworthy that mice treated with β -amyloid did habituate to some degree to the novel environment, but maintained a higher locomotion throughout the whole 4-hour period, suggesting a genuine hyperactive state rather than a delayed habituation response secondary to spatial memory impairment.

There may be a relationship between the increased response to amphetamine, which induces dopamine release, and the blunted response to caffeine, which blocks adenosine receptors. Adenosine is an important inhibitory neuromodulator, inhibiting the release of several neurotransmitters, including glutamate, acetylcholine and

dopamine [7]. Adenosine receptors form heterodimers with dopamine receptors, exerting an inhibitory role [19]. Adenosine receptor agonists counteract the effect of several psychostimulants, including amphetamine, whereas adenosine receptor antagonists, such as caffeine, potentiate hyperlocomotion induced by dopamine receptor agonists [7]. Thus, the diminished effect of caffeine in β_{25-35} -treated mice could reflect a diminished influence of adenosine in the ventral striatum, resembling the situation of caffeine-tolerant mice [20].

As intracerebroventricular β_{25-35} microinjection causes significant hippocampal alterations in mice [13], our results can be compared to other lesion models. Hippocampal lesion with ibotenic acid in adult animals increased both spontaneous locomotion and the effects of amphetamine [10], with no effect on the response to MK-801 in adult animals [21], while aspirative hippocampal lesions also induced a higher response to amphetamine, mostly mediated by dopamine D2 receptors [22]. In contrast, when the ventral hippocampus is lesioned during the early neonatal period, which is a neurodevelopmental model for schizophrenia, adult animals show increased response to both amphetamine and MK-801 [23].

Further studies will be necessary to clarify if there is a similar response in transgenic models of AD with slow deposition of β -amyloid. At this point, our study showed that β -amyloid-treated mice showed memory impairment as well as locomotor alterations similar to other hippocampal-damage models, with higher spontaneous locomotion and response to amphetamine, possibly related to

a reduced adenosinergic tone. Since hyperlocomotion is a behavioral parameter thought to relate to psychotic symptoms in humans, these results are in line with the frequent manifestation of delusions and hallucinations as well as psychomotor agitation in AD patients.

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