Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 477 (2003) 219–225
www.elsevier.com/locate/ejphar

Amnesia induced by β-amyloid fragments is counteracted by cannabinoid CB_1 receptor blockade

Carmen Mazzola, Vincenzo Micale, Filippo Drago *

Department of Experimental and Clinical Pharmacology, University of Catania Medical School, Viale A. Doria 6, 95125 Catania, Italy

Received 26 May 2003; received in revised form 8 August 2003; accepted 15 August 2003

Abstract

Administration of drugs activating cannabinoid CB_1 receptors in the brain induces memory deficit in rodents, and blockade of these receptors may restore memory capacity in these animals. Central administration of β-amyloid or β-amyloid fragments may also lead to memory disturbances. This study was undertaken to study the involvement of cannabinoid CB_1 receptors in amnesia induced by β-amyloid fragments in mice tested in a step-through passive avoidance paradigm. Pre-training intracerebroventricular (i.c.v.) injection of β-amyloid fragments, β-amyloid peptide-(25–35) (4, 8 or 16 nmol/mouse) or β-amyloid peptide-(1–42) (200, 400, 800 pmol/mouse) 7 days prior to the learning trial reduced in a dose-dependent manner the retention of passive avoidance response. This effect was observed in two retention tests, 1 and 7 days after the learning trial. The two β-amyloid fragments showed similar potency in reducing retention of passive avoidance behavior. This effect was counteracted by a single intraperitoneal (i.p.) injection of the cannabinoid CB_1 receptor antagonist, *N*-(piperidin-l-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A, 1 mg/kg), made 30 min prior to the second retention test. The injection of SR141716A per se did not affect memory capacity of mice. The i.c.v. administration of β-amyloid peptide-(25–35) (8 nmol/mouse) or of β-amyloid peptide-(1–42) (400 pmol/mouse) made 30 min prior to the learning trial failed to affect the retention capacity of mice as measured 1 and 7 days later. Also, the i.p. injection of SR 141716A (1 mg/kg) made 30 min prior to the learning trial did not influence the behavioral response of mice injected with β-amyloid peptide-(25–35) (8 nmol/mouse) or of β-amyloid peptide-(1–42) (400 pmol/mouse) 7 days prior to the learning trial. These results show that β-amyloid fragments induce a dose-dependent memory deficit. Their effect on memory retention depends upon the time of administration and seems to involve cannabinoid CB_1 receptors in the brain.

Keywords: Amnesia; β-Amyloid fragment; Cannabinoid CB_1 receptor; Cannabinoid; Avoidance retention

1. Introduction

The pathogenesis of Alzheimer's disease includes, among others, factors such as oxidative stress, inflammation and deficit of the brain cholinergic system (Whitehouse et al., 1981; Mountjoy et al., 1984; Yamamoto and Hirano, 1985; Behl, 1999; McGeer and McGeer, 1999; Lyness et al., 2003). There is evidence that intracerebroventricular (i.c.v.) infusion of β-amyloid causes brain dysfunctions similar to those of Alzheimer's disease, as evidenced by neurodegeneration and impairment of learning and memory in rodents (Kowall et al., 1991; Flood et al., 1994; Nitta et al., 1994, 1997; Giovannelli et al., 1995; Maurice et al., 1996; Yamada et al., 1998). However, the mechanisms of neurotoxic effects of β-amyloid in vivo are not fully understood yet (Clemens and Stephenson, 1992; Podlisny et al., 1993; Winkler et al., 1994; Fukuchi et al., 1996). Neuronal degeneration induced by β-amyloid affects subcortical nuclei modulating various physiological processes and behaviors, such as arousal and sleep, attention and vigilance, mood and aggression, pain and sensory reactivity, learning and memory (Lyness et al., 2003). Various neurotransmitters are involved in synaptic connections of these nuclei, including acetylcholine, norepinephrine, dopamine and serotonin (Curcio and Kemper, 1984; Yamamoto and Hirano, 1985). Neuronal degeneration, particularly of the cholinergic neuronal system, is usually accompanied by cognitive dysfunction (Whitehouse et al., 1981; Mountjoy et al., 1984).

Several drugs may induce memory alterations in rodents. Recent studies have demonstrated that marijuana or its active ingredient Δ^9-tetrahydrocannabinol impairs learning and memory processes in rats, mice (Heyser et al., 1993;

* Corresponding author. Tel.: +39-95-7384236; fax: +39-95-7384238.
E-mail address: fdrago@tin.it (F. Drago).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.08.026

Terranova et al., 1996; Brodkin and Moerschbaecher, 1997; Jentsch et al., 1997; Nava et al., 2000), non-human primates (Evans, 1992) and humans (Miller, 1983; Chait and Perry, 1994; Heishman et al., 1997). These effects seem to be mediated by cannabinoid CB_1 receptors. In fact, activation of cannabinoid CB_1 receptor by the selective agonist anandamide decreases acetylcholine cerebral levels and causes cognitive impairment (Lichtman et al., 1995; Lichtman and Martin, 1996; Mallet and Beninger, 1998). Administration of the selective cannabinoid CB_1 receptor antagonist SR141716A antagonizes the cognitive impairment induced by cannabinoids (Lichtman and Martin, 1996; Gessa et al., 1997; Carta et al., 1998; Nava et al., 2000). Thus, there is a connection between the amnesic effect induced by β-amyloid and the cannabinoid receptor system.

The present study was aimed at investigating in detail the amnesic effects of β-amyloid fragments and the possible involvement of cannabinoid CB_1 receptors in these effects. In particular, the influence of time of administration in relation to memory processes (consolidation and retrieval) has been evaluated.

2. Materials and methods

2.1. Animals

Male Swiss mice (40–50 g) were obtained from Morini (Italy). After arrival in the facilities, 10 animals were housed to a box and maintained at a constant temperature on a 12-h light–dark cycle (lights on between 0800 and 2000 h) with food and water ad libitum. After at least 1 week of habituation in the facilities, animals were admitted to the experimental procedures.

All experiments were carried out according to the European Community Council Directive 86/609/EEC and efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

β-Amyloid peptide-(25–35) (Sigma, USA) was dissolved in sterile double-distilled water in a concentration of 1 mg/ml and stored at −20 °C. The substance was aggregated by incubation in sterile distilled water at 37 °C for 4 days, as described by Maurice et al. (1996). Sterile distilled water was injected into control mice.

β-Amyloid peptide-(1–42) (Sigma) was prepared as stock solutions in sterile 0.1 M phosphate-buffered saline (pH 7.4). The proper volume was freshly prepared and used. Sterile 0.1 M phosphate-buffered saline was injected into control animals.

The aggregated form of β-amyloid fragments, β-amyloid peptide-(25–35) (4, 8 or 16 nmol) or β-amyloid peptide-(1–42) (200, 400 or 800 pmol) was administered i.c.v. using a microsyringe with a 28-gauge stainless-steel needle 3.0-mm-long (Hamilton). In brief, the 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 (Maurice et al., 1996). β-Amyloid fragments or vehicle for peptide solution (2 μl) were delivered gradually within 3 s. Mice exhibited normal behavior within 1 min after injection. At the end of experimental procedures, all animals were killed by decapitation and the correct insertion of the needle into the lateral ventricle has been checked by histological examination.

The cannabinoid CB_1 receptor selective antagonist/inverse agonist, *N*-(piperidin-l-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A, Sanofi-Synthelabo, France), was suspended in 20% Tween-80. This preparation was made just before use and injected in a total volume of 0.1 ml/100 g animal. The same volume of 20% Tween-80 suspension was used for control mice.

2.3. Passive avoidance test

2.3.1. Apparatus

The apparatus for the step-through passive-avoidance test was an automated shuttle-box (Cat. 7551 Passive Avoidance Controller and Cat. 7553 Passive Avoidance Mouse Cage, Basile, Italy), divided into an illuminated compartment and a dark compartment of the same size by a wall with a guillotine door.

2.3.2. Adaptation, training trial, and retention test

In the experimental session, each mouse was trained to adapt to the step-through passive avoidance apparatus (Venault et al., 1986). The animal was put into the illuminated compartment, facing away from the dark compartment. After 10 s, the door between these two boxes was opened and the mouse was allowed to move into the dark compartment freely. The latency to leave the illuminated compartment was recorded.

Two hours after the adaptation trial, the mouse was again put into the illuminated compartment. The learning trial was similar to the adaptation trial except that the door was closed automatically as soon as the mouse stepped into the dark compartment and an inescapable foot-shock (0.2 mA, 2 s) was delivered through the grid floor. The retention of passive avoidance response was measured 1 and 7 days after the learning trial. Each animal was again put into the illuminated compartment and the latency to re-enter the dark compartment was recorded. No foot-shock was delivered while the retention test was performed. The maximum cut-off time for step-through latency was 300 s (Venault et al., 1986).

2.4. Experimental procedures

In the first experiment, 7 days after the i.c.v. injection of β-amyloid fragments, β-amyloid peptide-(25–35) (4, 8 or 16 nmol) or β-amyloid peptide-(1–42) (200, 400 or 800

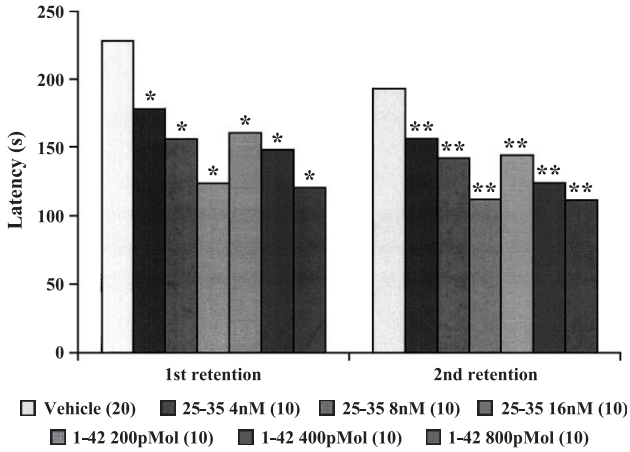

Fig. 1. Values are expressed as median. Mice were injected i.c.v. with β-amyloid fragments, β-amyloid peptide-(25–35) or β-amyloid peptide-(1–42) (indicated as only 25–35 and 1–42, respectively) 7 days prior to the learning trial. The assessment of retention capacity was made in two retention tests, 1 and 7 days after the learning trial. As no difference was observed between the two groups of control animals receiving the vehicle used for the solution of the β-amyloid fragments, these data were conglomerated. *Significant difference vs. control group injected with vehicle and tested in the first retention test ($P<0.05$, Mann–Whitney *U*-test). **Significant difference vs. control group injected with vehicle and tested in the second retention test ($P<0.05$, Mann–Whitney *U*-test).

pmol), mice were tested in the passive avoidance task, as described in Section 2.3.2 (Maurice et al., 1996). Control animals were treated with the vehicle used for the peptide preparation, and subjected to the same behavioral procedure. Their memory retention was assessed 1 and 7 days after the learning trial.

In the second experiment, an effective dose of β-amyloid fragments was selected, namely β-amyloid peptide-(25–35) 8 nmol and β-amyloid peptide-(1–42) 400 pmol. In order to assess the possible anti-amnesic effect of the selective cannabinoid CB_1 receptor antagonist, SR141716A, a dose of 1 mg/kg of this drug was administered i.p. 30 min prior to the second retention test (7 days after the learning trial) to mice treated with β-amyloid fragments or to the control group. Another group of animals without treatment with β-amyloid fragments, but injected i.c.v. with the vehicle alone 7 days prior to the learning trial, was given i.p. SR141716A 30 min prior to the second retention test and the latency to re-enter the dark box was measured in these animals.

In addition, β-amyloid fragments were injected i.c.v. into a group of mice 30 min prior to the learning trial and their ability to retain the behavioral response was assessed 1 and 7 days after the learning trial. The selective cannabinoid CB_1 receptor antagonist, SR141716A (1 mg/kg) was administered i.p. 30 min prior to the second retention test (7 days after the learning trial) to mice treated with β-amyloid fragments or to the control group.

Finally, SR141716A was also injected i.p. 30 min prior to the learning trial into mice that were given i.c.v. β-amyloid fragments, β-amyloid peptide-(25–35) 8 nmol or β-amyloid peptide(1–42) 400 pmol 7 days before it. Their ability to retain the behavioral response was assessed 1 and 7 days after the learning trial.

2.5. Data analysis

The results (for re-entering the dark box in the first or second retention test) were expressed as median. Data from control animals receiving the vehicle used for the solution of the β-amyloid fragments were conglomerated, when possible. The data were analyzed using the Mann–Whitney *U*-test for non-parametric data. A level of $P<0.05$ was considered as indicative of statistical significance.

3. Results

As no difference was observed between the two groups of control animals receiving the vehicle used for the solution of the β-amyloid fragments, these data were conglomerated when possible. The i.c.v. injection of β-amyloid peptide-(25–35) (4, 8 or 16 nmol/mouse) or β-amyloid peptide-(1–42) (200, 400, 800 pmol/mouse) 7 days prior to the learning trial induced a reduction of latency to re-enter the dark box in a dose-dependent manner (Fig. 1). This effect was observed in both retention tests, made 1 and 7 days after the learning trial. The two β-amyloid fragments showed similar effects in reducing retention of passive avoidance response in mice, as compared to controls.

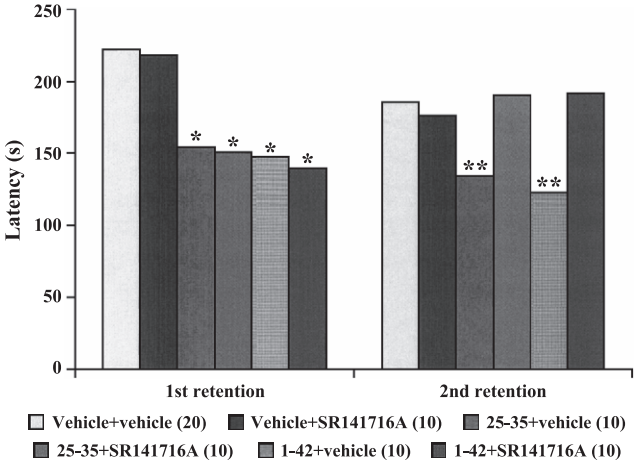

Fig. 2. Values are expressed as median. Mice were injected i.c.v. with β-amyloid fragments, β-amyloid peptide-(25–35) or β-amyloid peptide-(1–42) (indicated as only 25–35 and 1–42, respectively) 7 days prior to the learning trial, and with the cannabinoid CB_1 receptor antagonist, SR141716A 30 min prior to the second retention test. The assessment of retention capacity was made in two retention tests, 1 and 7 days after the learning trial. As no difference was observed between the two groups of control animals receiving the vehicle used for the solution of the β-amyloid fragments, these data were conglomerated. *Significant difference vs. control group injected with vehicle and tested in the first retention test ($P<0.05$, Mann–Whitney *U*-test). **Significant difference vs. control group injected with vehicle and tested in the second retention test ($P<0.05$, Mann–Whitney *U*-test).

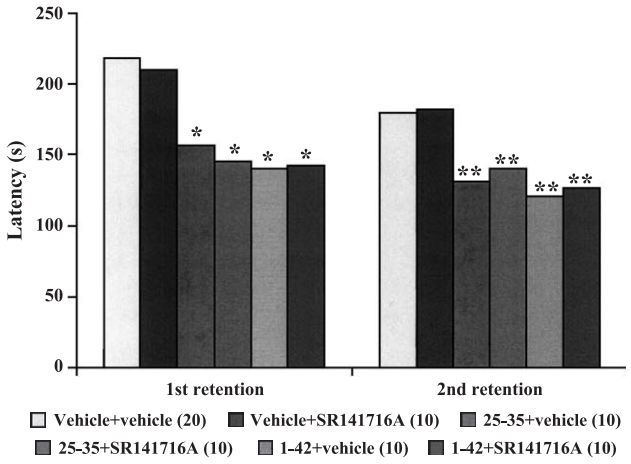

Fig. 3. Values are expressed as median. Mice were injected i.c.v. with β-amyloid fragments, β-amyloid peptide-(25–35) or β-amyloid peptide-(1–42) (indicated as only 25–35 and 1–42, respectively) 7 days prior to the learning trial, and with the cannabinoid CB_1 receptor antagonist, SR141716A 30 min prior to the learning trial. The assessment of retention capacity was made in two retention tests, 1 and 7 days after the learning trial. As no difference was observed between the two groups of control animals receiving the vehicle used for the solution of the β-amyloid fragments, these data were conglomerated. *Significant difference vs. control group injected with vehicle and tested in the first retention test ($P<0.05$, Mann–Whitney *U*-test). **Significant difference vs. control group injected with vehicle and tested in the second retention test ($P<0.05$, Mann–Whitney *U*-test).

The i.p. injection of the cannabinoid CB_1 receptor antagonist, SR141716A (1 mg/kg), made 30 min prior to the second retention test inhibited the amnesic affect of β-amyloid fragments. However, the injection of SR141716A per se did not affect memory capacity of mice tested in the passive avoidance paradigm (Fig. 2).

The i.c.v. administration of β-amyloid peptide-(25–35) (8 nmol/mouse) or of β-amyloid peptide-(1–42) (400 pmol/mouse) made 30 min prior to the learning trial failed to affect the retention capacity of mice as measured 1 and 7 days later. In fact, retention of passive avoidance response of these animals was similar to that of controls (data omitted). No effect was observed of the i.p. injection of SR141716A made in these animals 30 min prior to the second retention test.

The i.p. injection of SR 141716A (1 mg/kg) made 30 min prior to the learning trial did not influence the behavioral response of mice injected with β-amyloid peptide-(25–35) (8 nmol/mouse) or of β-amyloid peptide-(1–42) (400 pmol/mouse) 7 days prior to the learning trial (Fig. 3). These mice, in fact, showed a reduction of latency to re-enter the dark box as measured 1 and 7 days after the learning trial, as compared to control animals.

4. Discussion

Plenty of evidence demonstrates that β-amyloid may induce amnesia in rodents. This effect can be mimicked by i.c.v. administration of β-amyloid fragments (Hiramatsu et al., 2000; Chen et al., 2000; Wang et al., 2001), especially β-amyloid peptide-(1–42) (Yamada et al., 1999; Janus et al., 2000; Nakamura et al., 2001; Yan et al., 2001). Here, we show that both β-amyloid fragments, β-amyloid peptide-(25–35) and β-amyloid peptide-(1–42) are active in reducing the capacity of memory retention in mice trained in a step-through passive avoidance paradigm. The analysis of ED50 values for the two fragments revealed that similar effects were produced by 8 nmol of β-amyloid peptide-(25–35) and by 400 pmol of β-amyloid peptide-(1–42), the latter being roughly fivefold more potent than the former. However, since the molecular weight of β-amyloid peptide-(25–35) is about fivefold lower than that β-amyloid peptide-(1–42), the two fragments seem to be equipotent in reducing the capacity of memory retention in mice.

Brain cannabinoid CB_1 receptors were found to be involved in memory processes (Lichtman and Martin, 1996; Reibaud et al., 1999). Activation of these receptors by their agonists reduces cerebral acetylcholine levels and induces amnesia (Lichtman et al., 1995; Lichtman and Martin, 1996; Mallet and Beninger, 1998). Administration of Δ^9-tetrahydrocannabinol is followed by a reduction of hippocampal acetylcholine levels in vitro (Gifford and Ashby, 1996; Gifford et al., 2000). It has been suggested that pre-synaptic cannabinoid CB_1 receptors modulate cerebral acetylcholine release (Pertwee and Ross, 2002), a neurotransmitter that is deeply implicated in attention (Perry et al., 1998) and memory (Aigner, 1995). Furthermore, several studies have demonstrated that cannabinoid CB_1 receptors are especially localized in the hippocampus (Herkenham, 1995; Pertwee, 1997), where they participate in memory and learning processes (Vizi and Kiss, 1998). Consistent with this hypothesis, the present data show that blockade of brain cannabinoid CB_1 receptors by the selective antagonist, SR141716A, improves amnesia induced by β-amyloid fragments in mice, suggesting that endogenous cannabinoids may be involved in cognitive impairment induced by these fragments.

The present findings suggest that both β-amyloid fragments do not block memory retention directly, but rather produce some other change that ultimately affects the function of brain cannabinoid CB_1 receptors in a way that results in the expression of amnesia, and that the block of these receptors reverses this delayed alteration of function but not the preceding action of β-amyloid fragments that lead up to it. Between the initial action of β-amyloid and the dysfunction of cannabinoid CB_1 receptors, various factors might have intervened. For instance, β-amyloid fragments are known to activate glutamate-induced neurotoxicity and to inhibit cellular redox activity (Nitta et al., 1997; Yamada et al., 1999; Chen et al., 2000; Nakamura et al., 2001; Yan et al., 2001).

Intra-hippocampal injections of Δ^9-tetrahydrocannabinol reduce spatial memory (Lichtman et al., 1995) and cerebral acetylcholine concentration (Carta et al., 1998; Gessa et al.,

1998b; Nava et al., 2000; Gifford et al., 2000). These data suggest that hippocampal cholinergic activity mediates cannabinoid effects on memory.

Here we show that injection of SR141716A alone does not cause any significant change in the capacity of mice to retain passive avoidance response. However, the administration of this compound antagonizes the amnesic effect induced by β-amyloid peptide-(25–35) or β-amyloid peptide-(1–42). Indeed, SR141716A may counteract the behavioral changes induced by agonists of the cannabinoid CB_1 receptors. Thus, the effects induced by SR141716A may be observed only when cannabinoid CB_1 receptors are activated by their agonists. In fact, SR141716A, administered prior to the treatment with Δ^9-tetrahydrocannabinol, improves mice performance in the T-maze test and increases hippocampal acetylcholine levels in vitro, but it is ineffective when administered alone (Lichtman and Martin, 1996; Gessa et al., 1997; Carta et al., 1998; Nava et al., 2000).

SR141716A has been reported also to act, under particular conditions, as an inverse agonist of cannabinoid CB_1 receptors (Hurst et al., 2002). However, this seems to be irrelevant to the present results as this drug was unable to modify cognitive performance of mice when administered alone (as an inverse agonist would have been done). Furthermore, no partial agonistic activity has been reported for SR141716A on cannabinoid CB_1 receptors, thus the behavioral results observed after its administration cannot be due to a mild activating effect capable of reversing the behavior of animals induced by a non-cannabinoid antagonist action.

Besides the hypothesis that blockade of brain cannabinoid CB_1 receptors improves memory deficit induced by β-amyloid fragments through an increase in hippocampal acetylcholine release, the other possibility cannot be ruled out that SR141716A may act directly on cannabinoid neuronal circuits responsible for memory capacity. In this case, it should be assumed that β-amyloid fragments induce a deficit of memory capacity interfering directly with the brain cannabinoid system. However, no data are yet available supporting such a hypothesis.

The present study also shows that timing of administration of β-amyloid fragments or SR141716A is important for the behavioral effects of these substances observed in mice. In fact, β-amyloid fragments reduce passive avoidance retention when injected 7 days prior to learning trial and are ineffective when the administration was made 30 min prior to the learning trial. In contrast, the cannabinoid CB_1 receptor antagonist is totally unable to attenuate amnesia induced by β-amyloid fragments when administered 30 min prior to learning trial and is fully active when injected 30 min prior to the second retention test. Thus, pharmacological manipulation of brain mechanisms involved in memory consolidation during the learning trial is essential for the manifestation of behavioral effects of these substances. It should be recalled that in rodents, memory consolidation takes place immediately after the aversive stimulus. Thus, effects of drugs on consolidation processes can be studied if they are administered at this time (De Wied, 1993). The effects of drugs on retrieval processes can be studied after consolidation has taken place, 24 h or later after the learning trial. Avoidance latency during the retention test is a measure of consolidation when the drug is given at the time of the learning trial and a measure of the retrieval when the drug is given prior to the retention test. Thus, it can be concluded that administration of β-amyloid fragments seems to affect the consolidation processes of memory only when injected 7 days prior to learning trial, while blockade of cannabinoid CB_1 receptors may selectively influence the retrieval processes of memory as SR141716A was ineffective when administered at the time of learning trial. This phenomenon is similar to that found for some neuropeptides affecting memory processes when administered only at a critical time (De Wied et al., 1988).

The present findings showing that cannabinoid CB_1 receptors play a role in the amnesic effects of β-amyloid fragments may strengthen the possible use of antagonists of these receptors in the therapy of cognitive disturbances in Alzheimer's disease. However, the reader should be cautioned about the limited interpretations of the present findings. In fact, only one model of amnesia has been tested and only one of the several neurotransmitters that are involved in the acquisition and retention of memory has been studied here. For instance, activation of brain cannabinoid receptors may induce an activation of dopamine neurons in areas involved in cognitive functions (Gessa et al., 1998a).

References

Aigner, T.G., 1995. Pharmacology of memory: cholinergic–glutamatergic interactions. Curr. Opin. Neurobiol. 5, 155–160.

Behl, C., 1999. Alzheimer's disease and oxidative stress: implication for novel therapeutic approaches. Prog. Neurobiol. 57, 301–323.

Brodkin, J., Moerschbaecher, J.M., 1997. SR141716A antagonizes the disruptive effects of cannabinoid ligands on learning in rats. J. Pharmacol. Exp. Ther. 282, 1526–1532.

Carta, G., Nava, F., Gessa, G.L., 1998. Inhibition of hippocampal acetylcholine release after acute and repeated Δ^9-tetrahydrocannabinol in rats. Brain Res. 809, 1–4.

Chait, L.D., Perry, J.L., 1994. Acute and residual effects of alcohol and marijuana, alone and in combination, on mood and performance. Psycopharmacology 115, 340–349.

Chen, G., Chen, K., Knox, J., Inglis, J., Bernard, A., Martin, S.J., Justice, A., McConlogue, L., Games, D., Freedman, S.B., Morris, R.G.M., 2000. A learning deficit related to age and β-amyloid plaques in a mouse model of Alzheimer's disease. Nature 408, 975–979.

Clemens, J.A., Stephenson, D.T., 1992. Implants containing beta-amyloid protein are not neurotoxic to young and old rat brain. Neurobiol. Aging 13, 581–586.

Curcio, C.A., Kemper, T., 1984. Nucleus raphe dorsalis in the dementia of the Alzheimer-type: neurofibrillary changes and neuronal packing density. J. Neuropathol. Exp. Neurol. 43, 359–368.

De Wied, D., 1993. From stress hormones to neuropeptides. In: Burbach, J.P.H., De Wied, D. (Eds.), Brain Functions of Neuropeptides. The Partenon Publishing Group, Lancs, pp. 65–84.

De Wied, D., Joëls, M., Burbach, J.P.H., De Jong, W., De Kloet, E.R., Gaffori, E.J.W., Urban, I.J.A., Van Ree, J.M., Van Wimersma Greidanus, Tj.B., Veldhuis, H.D., Versteeg, D.H.G., Wiegant, V.M., 1988. Vasopressin effects of the central nervous system. In: Negro-Villar, A., Conn, P.M. (Eds.), Peptide Hormones: Effects and Mechanisms of Action. CRC Press, Boca Raton, pp. 97–140.

Evans, S., 1992. Effects of drug abuse on acquisition of behavioural chain in squirrel monkeys. Psycopharmacology 107, 55–60.

Flood, J.F., Morley, J.E., Roberts, E., 1994. An amyloid β-protein fragment, Aβ[12–28] equipotently impairs post-training memory processing when injected into different limbic structures. Brain Res. 663, 271–276.

Fukuchi, K., Ho, L., Younkin, S.G., Kunkel, D.D., Ogburn, C.E., Le Bouef, R.C., Furlong, C.E., Deeb, S.S., Nochlin, D., Wegiel, J., Wisniewski, H.M., Martin, G.M., 1996. High levels of circulating β-amyloid peptide do not cause cerebral β-amyloidosis in transgenic mice. Am. J. Pathol. 149, 219–227.

Gessa, G.L., Mascia, M.S., Casu, M.A., Carta, G., 1997. Inhibition of hippocampal acetylcholine release by cannabinoids: reversal by SR141716A. Eur. J. Pharmacol. 327, R1–R2.

Gessa, G.L., Melis, M., Muntoni, A.L., Diana, M., 1998a. Cannabinoids activate dopamine neurons by an action on cannabinoid CB1 receptors. Eur. J. Pharmacol. 341, 39–44.

Gessa, G.L., Casu, M.A., Carta, G., Mascia, M.S., 1998b. Cannabinoids decrease acetylcholine release in the medial prefrontal cortex and hippocampus, reversal by SR141716A. Eur. J. Pharmacol. 355, 119–124.

Gifford, A.N., Ashby Jr., C.R., 1996. Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, SR141716A. J. Pharmacol. Exp. Ther. 227, 1431–1436.

Gifford, A.N., Bruneus, M., Gatley, S.J., Volkow, N.D., 2000. Cannabinoid receptor-mediated inhibition of acetylcholine release from hippocampal and cortical synaptosomes. Br. J. Pharmacol. 131, 645–650.

Giovannelli, L., Casamenti, F., Scali, C., Bartolini, L., Pepeu, G., 1995. Differential effects of amyloid peptides β-(1–40) and β-(25–35) injections into the rat nucleus basalis. Neuroscience 66, 781–791.

Heishman, S.J., Arastesh, K., Stitzer, M.L., 1997. Comparative effects of alcohol and marijuana on mood, memory and performance. Pharmacol. Biochem. Behav. 58, 93–101.

Herkenham, M., 1995. Localization of cannabinoid receptors in brain and periphery. In: Pertwee, R.G. (Ed.), Cannabinoid Receptors. Academic Press, New York, NY, pp. 145–166.

Heyser, C.J., Hampson, R.E., Deadwyler, A., 1993. Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. J. Pharmacol. Exp. Ther. 264, 294–307.

Hiramatsu, M., Inoue, K., Kameyama, T., 2000. Dynorphin A-(1–13) and (2–13) improve β-amyloid peptide-induced amnesia in mice. NeuroReport 11, 431–435.

Hurst, D.P., Lynch, D.L., Barnett-Norris, J., Hyatt, S.M., Seltzman, H.H., Zhong, M., Song, Z.H., Nie, J., Lewis, D., Reggio, P.H., 2002. *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR141716A) interaction with LYS 3.28(192) is crucial for its inverse agonism at the cannabinoid CB1 receptor. Mol. Pharmacol. 62, 1274–1287.

Janus, C., Pearson, J., McLaurin, J., Mathews, T., Jiang, Y., Schmidt, S., Chishti, M., Horne, P., Heslin, D., French, J., Mount, H.T.J., Nixon, R.A., Mercken, M., Bergeron, C., Fraser, P., St. George-Hyslop, P., Westaway, D., 2000. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 408, 979–982.

Jentsch, J.D., Andrusiak, E., Tran, A., Browers, M.B., Roth, R.H., 1997. Delta-9-tetrahydrocannabinol increases prefrontal cortical catecholaminergic utilization and impairs spatial working memory in the rats: blockade of dopaminergic effects with HA966. Neuropsychopharmacology 16, 426–432.

Kowall, W.N., Beal, F.M., Busciglio, J., Duffy, K.L., Yankner, B.A., 1991. An in vivo model for the neurodegenerative effects of β-amyloid and protection by substance P. Proc. Natl. Acad. Sci. U. S. A. 88, 7247–7251.

Lichtman, A.H., Dimen, K.R., Martin, B.R., 1995. Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psycopharmacology 119, 282–290.

Lichtman, A.H., Martin, B.R., 1996. Delta-9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psycopharmacology 126, 125–131.

Lyness, A.S., Zarow, C., Chui, H.C., 2003. Neuron loss in key cholinergic and aminergic nuclei in Alzheimer disease: a meta-analysis. Neurobiol. Aging 24, 1–23.

Mallet, P.E., Beninger, R.J., 1998. The cannabinoid CB1 receptor antagonist SR141716A attenuates the memory impairment produced by delta-9-tetrahydrocannabinol or anandamide. Psycopharmacology 140, 11–19.

Maurice, T., Lockart, B.P., Private, A., 1996. Amnesia induced by centrally administered β-amyloid peptides involves cholinergic dysfunction. Brain Res. 706, 181–193.

McGeer, P.L., McGeer, E.G., 1999. Inflammation of the brain in Alzheimer's disease: implications for therapy. J. Leukoc. Biol. 65, 409–415.

Miller, L.L., Branconnier, R.J., 1983. Cannabis: effects on memory and the cholinergic limbic system. Psychol. Bull. 93, 441–456.

Mountjoy, C.Q., Rossor, M.N., Iversen, L.L., Roth, M., 1984. Correlation of cortical cholinergic and GABA deficit with quantitative neuropathological findings in senile dementia. Brain 107, 507–518.

Nakamura, S., Murayama, N., Noshita, T., Annoura, H., Ohno, T., 2001. Progressive brain dysfunction following intracerebroventricular infusion of beta$_{1-42}$-amyloid peptide. Brain Res. 912, 128–136.

Nava, F., Carta, G., Battasi, A.M., Gessa, G.L., 2000. D2 dopamine receptor enables delta-9-tetrahydrocannabinol induced memory impairment and reduction of hippocampal extracellular acetylcholine concentration. Br. J. Pharmacol. 131, 1201–1210.

Nitta, A., Itoh, A., Hasegawa, T., Nabshima, T., 1994. β-Amyloid protein induced Alzheimer's disease animal model. Neurosci. Lett. 170, 63–66.

Nitta, A., Fukuta, T., Hasegawa, T., Nabshima, T., 1997. Continuous infusion of β-amyloid protein into cerebral ventricle induces learning impairment and neuronal and morphological degeneration. Jpn. J. Pharmacol. 73, 51–57.

Perry, E., Walker, M., Grace, J., Perry, R., 1998. Acetylcholine in mind: a neurotransmitter correlate of consciousness? Trends Neurosci. 22, 273–280.

Pertwee, R.G., 1997. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol. Ther. 74, 129–180.

Pertwee, R.G., Ross, R.A., 2002. Cannabioid receptors and their ligands. Prostaglandins Leukot. Essent. Fat. Acids 66, 101–121.

Podlisny, M.B., Stephenson, D.T., Frosch, M.P., Tolan, D.R., Lieberburg, I., Clemens, J.A., Selkoe, D.J., 1993. Microinjection of synthetic amyloid β-protein in monkey cerebral cortex fails to produce acute neurotoxicity. Am. J. Pathol. 142, 17–24.

Reibaud, M., Obinu, M.C., Ledent, C., Parmentier, M., Bohme, G.A., Imperato, A., 1999. Enhancement of memory in cannabinoid receptor CB1, receptor knock-out mice. Eur. J. Pharmacol. 379, R1–R2.

Terranova, J.P., Storme, J.J., Lafon, N., Perio, A., Rinaldi-Carmona, M., Le Fur, G., Soubrie, P., 1996. Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR141716A. Psychopharmacology 126, 165–172.

Venault, P., Chapouthier, G., de Carvalho, L.P., Simiand, J., Morre, M., Dodd, R.H., Rossier, J., 1986. Benzodiazepine impairs and beta-carboline enhances performance in learning and memory tasks. Nature 321, 864–866.

Vizi, E.S., Kiss, J.P., 1998. Neurochemistry and pharmacology of the major hippocampal transmitter systems: synaptic and non-synaptic interactions. Hippocampus 8, 566–607.

Wang, H., Chou, C., Liao, J., Chen, C., 2001. Dehydroevodiamine attenuates β-amyloid peptide-induced amnesia in mice. Eur. J. Pharmacol. 413, 221–225.

Whitehouse, P.J., Price, D.L., Clarke, A.W., Coyle, J.T., DeLong, M.R., 1981. Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann. Neurol. 10, 122–126.

Winkler, J., Conoer, D.J., Frautschy, S.A., Behl, C., White, J.J., Cole, G.M., Thal, L.J., 1994. Lack of long-term effects after β-amyloid protein injections in rat brain. Neurobiol. Aging 15, 601–607.

Yamada, K., Tanaka, T., Sensaki, K., Kameyama, T., Nabeshima, T., 1998. Propentofylline improves learning and memory deficits in rats induced by β-amyloid protein-(1–40). Eur. J. Pharmacol. 349, 15–22.

Yamada, K., Tanaka, T., Han, D., Senzaki, K., Kameyama, T., 1999. Protective effects of idebenone and α-tocopherol on β-amyloid-(1–42)-induced learning and memory deficits in rats: implication of oxidative stress in β-amyloid-induced neurotoxicity in vivo. Eur. J. Neurosci. 11, 83–90.

Yamamoto, T., Hirano, A., 1985. Nucleus raphe dorsalis in Alzheimer's disease: neurofibrillary tangles and loss of large neurons. Ann. Neurol. 17, 573–577.

Yan, J.J., Cho, J.Y., Kim, H.S., Kim, K.L., Jung, J.S., Huh, S.O., Suh, H.W., Kim, Y.H., Song, D.K., 2001. Protection against β-amyloid peptide toxicity in vivo with long-term administration of ferulic acid. Br. J. Pharmacol. 133, 89–96.