ELSEVIER

European Journal of Pharmacology 379 (1999) R1–R2

ejp

www.elsevier.nl/locate/ejphar

Rapid communication

Enhancement of memory in cannabinoid CB_1 receptor knock-out mice

Michel Reibaud [a,*], Maria C. Obinu [a], Catherine Ledent [b], Marc Parmentier [b], Georg A. Böhme [a], Assunta Imperato [a]

[a] *CNS Department, Research Center of Vitry-Alfortville, Rhône-Poulenc Rorer, 13, quai Jules Guesde, BP 14, 94403 Vitry-sur-Seine Cedex, France*
[b] *IRIBHN, Université libre de Bruxelles, B-1070 Brussels, Belgium*

Received 5 July 1999; accepted 7 July 1999

Abstract

We have used cannabinoid CB_1 knock-out mice in a two-trial object recognition test to assess the role of cannabinoid CB_1 receptors in memory. Cannabinoid CB_1 knock-out mice are able to retain memory for at least 48 h after the first trial whereas the wild-type controls lose their capacity to retain memory after 24 h. These results suggest that endogenous cannabinoid CB_1 receptors play a crucial role in the process of memory storage and retrieval.

Keywords: Cannabinoid receptor; Cannabinoid CB_1 knock-out mouse; Memory

Cannabinoids exert their actions in the brain of humans and animals through cannabinoid CB_1 receptors (Howlett et al., 1990) which have recently been cloned (Matsuda et al., 1990). Although several studies have been performed to investigate the physiological role of endogenous cannabinoids and cannabinoid CB_1 receptors, this still remains to be fully elucidated.

One of the central behavioral effects of Δ^9-tetrahydrocannabinol in several animal species, including humans (Schwartz et al., 1989; Heyser et al., 1993), and of synthetic agonists in rodents (Lichtman et al., 1995), is the impairment of memory. Recently, cannabinoid CB_1 receptor knock-out (KO) mice, which do not respond Δ^9-tetrahydrocannabinol (Ledent et al., 1999), have been generated.

Cannabinoid CB_1-KO mice ($CB_1^{-/-}$) and wild-type (WT) mice ($CB_1^{+/+}$), 11–12 weeks old, were generated as described (Ledent et al., 1999) and provided by Transgenic Alliance (France). Animals were housed four per cage ($n = 11–12$ animals per group) with free access to food and water. Room temperature was 22 ± 2°C and the light–dark cycle was 12:12 h (lights on at 0600 h). Experiments were performed in the morning.

Memory was assessed by using the two-trial object recognition test for the assessment of memory, as previously described (Dodart et al., 1997). In brief, on day one mice were trained to explore an empty open field for 50 min. During this session the locomotor activity was analysed for 15 min using a video analyser system (Videotrack®, View Point, France). On day two mice are allowed to explore the same open field in the presence of an object A (a plastic figure, 2.5 cm high), for 10 min. The time spent exploring this object was recorded using a system for the collection and analysis of observational data (Observer, Noldus, the Netherlands). At 3, 24 and 48 h after the exploration trial of object A, the retention performance was evaluated by allowing the same animal to re-explore the open field for 10 min in the presence of two objects: the familiar object A and a novel object B (a plastic flag, 3.5 cm high). The time spent exploring each object (time *A* and time *B*) were recorded. Recognition memory was assessed by comparing the time spent exploring each object during the second trial. A recognition index was calculated for each animal and expressed as a ratio ((time $B \times 100$)/time A + time B). The recognition index is around 50% when animals do not remember and time *A* and time *B* are equivalent but recognition index is more than 50% when time *B* is superior to time *A*, indicating that they have remembered the familiar object. Non-parametric Kruskall and Mann–Whitney *U*-tests were

* Corresponding author: Tel.: +33-1-55-71-80-59; fax: +33-1-55-71-36-85; E-mail: michel.reibaud@rp-rorer.fr

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00496-3

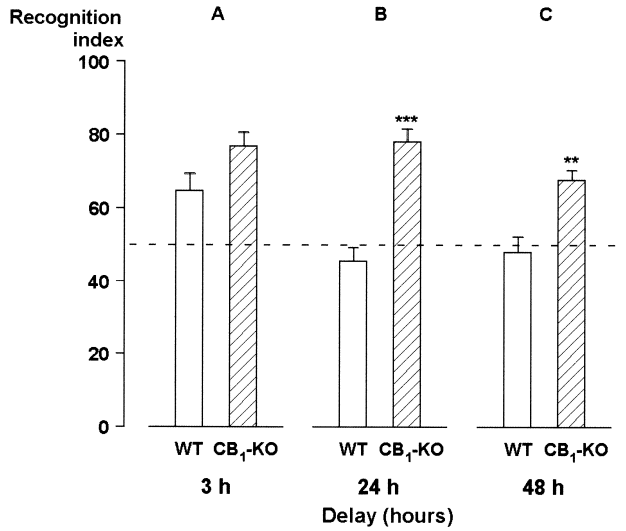

Fig. 1. Enhancement of memory in cannabinoid CB_1-KO mice compared to WT mice. Recognition index was measured at 3 h (A), 24 h (B) and 48 h (C) after the training session. Values are means ± S.E.M. Significantly different from wild-type mice **: $P < 0.01$ and ***: $P < 0.001$.

used to compare the recognition indexes between groups. Time *A* and time *B* were compared by using a within-group analysis (Wilcoxon matched pairs signed-ranks test).

Fig. 1 shows that cannabinoid CB_1-KO mice and the WT controls initially have a similar high recognition index, as measured 3 h after the exploration training of object A. This suggests that both types of animals have a comparable ability for recognition. When the memory test is performed 24 or 48 h after the training session the recognition index in the WT animals declines as demonstrated by their inability to recognise the familiar object. By contrast cannabinoid CB_1-KO mice at 24 and 48 h after training, still have an recognition index above 50%, suggesting that they are able to recognise the familiar object, and that they spend more time investigating the new one.

The reduction in the time spent in exploration is not due to a decrease in locomotor activity, as measured through a video analyzer system (data not shown). This strengthens the hypothesis that the difference observed between two groups is due to differences in memory retention.

Our results confirm and extend previous pharmacological evidence, obtained Δ^9-tetrahydrocannabinol, synthetic analogues and cannabinoid CB_1 antagonists (Schwartz et al., 1989; Heyser et al., 1993; Terranova et al., 1996), demonstrating that modulation of cannabinoid CB_1 receptors affects memory. The present results in cannabinoid CB_1-KO mice further suggest that an endogenous cannabinoid tonus is present in physiological conditions and is involved in the modulation of memory.

References

Dodart, J.C., Mathis, C., Ungerer, A., 1997. Scopolamine-induced deficits in a two-trial object recognition task in mice. NeuroReport 8, 1173–1178.

Heyser, C.J., Hampson, R.E., Deadwyler, S.A., 1993. Effects of delta-9-tetrahydrocannabinol on delayed matching 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, 194–307.

Howlett, A.C., Bidaut-Russel, M., Devanne, W.A., Melvin, L.S., Johnson, M.R., Herkenham, M., 1990. The cannabinoid receptor: biochemical, anatomical and behavioral characterization. Trends. Neurosci. 13, 420–423.

Ledent, C., Valverde, O., Cossu, G., Petitet, F., Aubert, J.-F., Baslet, F., Böhme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., Vassart, G., Fratta, W., Parmentier, M., 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knock-out mice. Science 283, 401–404.

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

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Schwartz, R.H., Gruenewald, P.J., Klitzner, M., Fedio, P., 1989. Short-term memory impairment in cannabis-dependent adolescents. Am. J. Dis. Child. 143, 1214–1219.

Terranova, J.P., Storme, J.J., Lafon, N., Pério, R.-C.M., LeFur, G., Soubrié, P., 1996. Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716. Psychopharmacology 126, 165–172.