available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

AM251, a selective antagonist of the CB1 receptor, inhibits the induction of long-term potentiation and induces retrograde amnesia in rats

Lucas de Oliveira Alvares[a,c], *Bruna Pasqualini Genro*[a], *Ricardo Vaz Breda*[b], *Michele Franzen Pedroso*[b,c], *Jaderson Costa Da Costa*[b,c], *Jorge Alberto Quillfeldt*[a,c,*]

[a]*Laboratório de Psicobiologia e Neurocomputação, Departamento de Biofísica, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Prédio 43422, room 208, CEP 91.501-970-Porto Alegre, RS, Brazil*
[b]*Laboratório de Neurociências, Instituto de Pesquisas Biomédicas, Pontifícia Universidade Católica do Rio Grande do Sul- Porto Alegre, RS, Brazil*
[c]*Programa de Pós-Graduação em Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul-Porto Alegre, RS, Brazil*

ARTICLE INFO

Article history:
Accepted 21 November 2005
Available online 7 February 2006

Keywords:
Memory
LTP
AM251
CB1 cannabinoid receptor
Hippocampus

ABSTRACT

Long-term potentiation (LTP) has a long history as putative mechanism of memory formation, specially in the hippocampus, a structure essential for memory formation. Endocannabinoids are one of the endogenous systems that modulate this plasticity event: the activation of hippocampal CB1 receptors may inhibit local GABA release. Here, we have studied both (1) the role of the selective CB1 antagonist AM251 upon LTP induction in a hippocampal slice preparation, and (2) the effect of its intrahippocampal administration in the step-down inhibitory avoidance (IA) and the open field habituation tasks (OF). Standard extracellular electrophysiology techniques were used to record field excitatory postsynaptic potentials from the dendritic region of CA1 neurons in response to a high frequency stimulation of Schaffer's collaterals; a micropipette ejected 0.2 μM of AM251 (in DMSO/PBS) 2 min before the stimulus: LTP was induced and lasted more than 30 min in the control, but not in the AM251-treated group. Immediately after training, either in IA (footshock, 0.5 mA) or OF, animals received a bilateral infusion of 0.55 or 5.5 ng/side of AM251 or its vehicle in the CA1 region, and test was performed 24 h later. AM251 has caused a significative decrease in the test step-down latency when compared to the control group, but no differences were detected in the OF task, including the number of crossings, i.e., there were no motor effects. The LTP supression could be caused by AM251 acting over GABAergic interneurons that modulate the LTP-bearing glutamatergic neurons. Endocanabinoids would then be the natural dis-inhibitors of local plasticity in the dorsal hippocampus, and the amnestic action of AM251 would be due to a disruption of this endogenous modulatory system.

* *Corresponding author*. Laboratório de Psicobiologia e Neurocomputação, Departamento de Biofísica, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Prédio 43422, room 208, CEP 91.501-970-Porto Alegre, RS, Brazil. Fax: +55 51 3316 7003.
E-mail address: quillfe@ufrgs.br. (J.A. Quillfeldt).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.11.101

1. Introduction

Cannabinoid receptors CB1 are widely distributed in the CNS, mainly in the hippocampus, cortex, basal ganglia, and cerebellum (Davies et al., 2002; Wilson and Nicoll, 2002). Being one of the most abundant class of metabotropic receptors in the brain, it is specially proeminent in the hippocampus (Ameri, 1999; Hampson and Deadwyler, 1999), a structure both essential for memory formation (Izquierdo and Medina, 1995; Squire, 1992) and extensively studied in LTP experiments (Bliss and Collingridge, 1993).

CB1 receptors couple to $G_{i/o}$ in order to inhibit cAMP formation, decrease Ca^{++} conductance (specially through N-type voltage-gated calcium channels) and increase both K^+ conductance and MAPK activity (Ameri, 1999; Davies et al., 2002; Mackie et al., 1995; Pertwee and Ross, 2002; Wilson and Nicoll, 2002). In the hippocampus, CB1 receptors are located in the presynaptic portion of GABAergic axon terminals (Herkenham et al., 1991), upon which endocannabinoids may be acting in order to inhibit the release of GABA (Katona et al., 1999), leading to a facilitation of any subsequent glutamatergic plasticity event.

Many studies have shown that the administration of CB1 agonists impairs memory (Davies et al., 2002; Hampson and Deadwyler, 1999; Hernandez-Tristan et al., 2000; Lichtman et al., 1995); antagonists otherwise, may improve it (Lichtman, 2000; Takahashi et al., 2005; Terranova et al., 1996; Wolff and Leander, 2003) or simply have no effect (Da Silva and Takahashi, 2002; Davies et al., 2002). Since most of these studies have investigated only the systemic effect, the ubiquity of CB1 receptors in the CNS may explain the diversity of cognitive effects (Alvares et al., 2005). Accordingly, we have previously reported a memory deficit with the direct intrahippocampal infusion on the selective CB1 antagonist AM251 (Alvares et al., 2005), a result not found elsewhere and contrasting with only two previous reports (Egashira et al., 2002; Lichtman et al., 1995), that (also different from us) investigated distinct types of memory or employed different pharmacological tools.

The local, intrahippocampal amnestic effect described by us was consistent with three facts: (1) in the hippocampus, CB1 receptors seem to be located basically in the presynaptic portions of the GABAergic axon terminals, mostly on CCK-releasing basket cells, which should explain the inhibition of GABA release by CB1 agonists (Katona et al., 1999; Wilson and Nicoll, 2002); (2) in DSI (depolarization-induced suppression of inhibition), endocannabinoids may be acting as retrograde messengers mediating down-regulation of GABA release in the hippocampus (Kreitzer and Regehr, 2001; Ohno-Shosaku and Kano, 2001; Wilson and Nicoll, 2001, 2002); (3) LTP, a phenomenon itself reinforced by DSI, was shown to be indirectly modulated by endocannabinoids that reduce presynaptic neurotransmiter release, suppressing the postsynaptic membrane depolarization necessary to activate NMDA receptors (Carlson et al., 2002; Wilson and Nicoll, 2002).

Long-term potentiation (LTP) has a long history as putative mechanism of memory formation, but even if it is not exactly "the" mechanism, its close scrutiny has brought us a great load of knowledge about synaptic plasticity, the phenomenon that may explain the engram register into brain neural networks (Bliss and Collingridge, 1993; Frankland and Bontempi, 2005; Izquierdo and Medina, 1995; Lamprecht and LeDoux, 2004). With one exception (Carlson et al., 2002), most studies show that cannabinomimetics inhibit the induction of LTP (Collins et al., 1995; Davies et al., 2002; Terranova et al., 1995), and there is evidence that mice lacking cannabinoid CB1 receptors exhibit an enhanced long-term potentiation (Bohme et al., 2000). Consistently, cannabinoids acting upon CB1 receptors have been shown to inhibit the release of glutamate in hippocampal preparations (Davies et al., 2002).

In this work, we have studied both (1) the role of the selective CB1 receptor antagonist AM251 upon LTP induction in a hippocampal slice preparation, and (2) the effect of its intrahippocampal administration in the step-down inhibitory avoidance and the open field habituation task.

2. Results

2.1. *Electrophysiological effects: long-term potentiation*

Slices from 7 out of 10 dissected animals were used to record fEPSPs. Data from pretetanic (–10, –5 and 0 min, or HFS) and posttetanic (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 min) moments were analyzed by Repeated Measure ANOVA to compare recordings from AM251 (0.2 μM) and vehicle (8% DMSO) groups: there was a significant between-subjects Drug effect ($F_{1,7}$ = 14.039, P = 0.010) and a within-subjects Time ($F_{1,7}$ = 9.827, P = 0.000), and Time vs. Drug interaction ($F_{1,7}$ = 9.303, P = 0.000).

Since the DMSO-treated (or control) group develops (1) a fEPSP potentiation response of 207.4 ± 4.9% that (2) lasts more than 30 min, the Δ-curve seen in Fig. 1 can be considered to be a long-term potentiation response (Bliss and Collingridge, 1993). Except for the short posttetanic potential peak, the AM251 treatment (◇) seems to have prevented the onset of any long-lasting potentiation, as confirmed by the near-baseline 103.3 ± 1.1% response.

Fig. 2 shows the percentage change of fEPSP% amplitude in pretetanic (<$t_{-9-0\ min}$>), and two posttetanic time windows (<$t_{26-30\ min}$> and <$t_{56-50\ min}$>). The average fEPSPs for each posttetanic group in each of the two posttetanic time windows (<$t_{26-30\ min}$> and <$t_{56-50\ min}$>) were significantly different (both with a P = 0.000, Student's t test). To confirm that the recording was being performed on hippocampal CA1 neurons, some cells were filled with biocytin, as shown in Fig. 2B.

2.2. *Behavioral effects: step-down inhibitory avoidance*

Behavioral results are shown in Figs. 3 and 4. In Inhibitory Avoidance task (Fig. 3, n = 10 in each group), comparisons among test latencies were possible since there was no statistically significant difference among the training session latencies (P = 0.155; Kruskal–Wallis ANOVA); test latencies, however, exhibited a difference (P = 0.020; Kruskal–Wallis ANOVA). Post hoc Dunn's All Pairwise Multiple Comparison Procedure has shown that only the 5.5 ng/side of AM251 is significantly smaller than the control group ($P < 0.05$), the other groups being not different among themselves ($P > 0.050$). Each

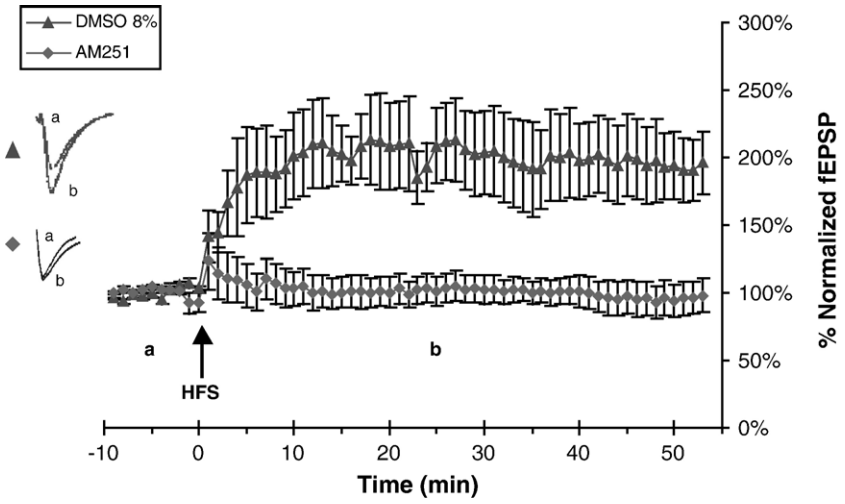

Fig. 1 – Effect of AM251 upon LTP. Time course of changes in field EPSPs (a) before and (b) after application of brief episode of HFS (arrow) to a hippocampal slice from treated (♦, *n* = 5) and control (▲, *n* = 4) groups. Each point represents average ± SEM of three different fEPSP measures normalized with respect to baseline. Inset shows a representative recording of each group at times a and b.

of the three experimental groups, respectively, vehicle, 0.55 and 5.5 ng/side-treated groups, has shown a significant difference between training and test session latencies (P = 0.005, 0.005 and 0.022, respectively, Wilcoxon signed ranks test), i.e., all groups learned the task.

In the Open Field Habituation task (Fig. 4, A and B, n = 15 in each group), the One-way ANOVA test showed no statistically significant differences among the groups' means, either for the training session (rearings, P = 0.472, crossings, P = 0.477), or for the test session (rearings, P = 0.707, crossings, P = 0.981). Each of the three experimental groups, respectively, vehicle, 0.55 and 5.5 ng/side-treated groups, exhibits significant differences between training and test session rearings (P = 0.000 in all groups, Paired samples t test) and crossings ($P < 0.001$ in all groups, Paired samples t test), i.e., all groups learned the task. The fact that crossings did not differ among groups suggests that AM251 did not cause any motor performance interference in the treated animals.

3. Discussion

3.1. *Electrophysiological results*

Our results show that 0.2 μM of AM251 was able to suppress LTP in a hippocampal slice preparation. Although with slight differences in our electrophysiological preparations and stimulation protocols, this result agrees with previous findings with the same CB1 antagonist (Carlson et al., 2002; Chevaleyre and Castillo, 2003). The most important difference is that the 0.2 μM concentration of AM251 is 10 times smaller than the concentration used by the above cited authors (2 μM), a decision we have taken due to our infusion protocol that does not bath the slice in a medium plus the drug, but, instead, ejects a smaller amount of the drug solution directly above the recording point (see Section 4.1.1). The selected concentration is consistent with the small doses found effective by us in recent behavioral experiments (Alvares et al., 2005).

It has been shown that CB1 receptors are basically located in the presynaptic portions of the CCK-immunoreactive basket cells (GABAergic axon terminals) and some of the calretinin-containing cells (also GABAergic interneurons) of the hippocampus (Katona et al., 1999; Wilson and Nicoll, 2002), a fact that explains the inhibition of GABA release (or the supression of evoked IPSCs) by CB1 agonists. Hájos and Freund (2002) have determined that CB1 agonist WIN 55,212-2 blocks evoked inhibitory postsynaptic currents (IPSCs) with an EC_{50} value 10 times smaller than the one necessary to suppress the evoked excitatory postsynaptic currents (EPSCs) in rat hippocampal slices. They have also found that AM251 blocks the WIN55,212-2-induced inhibition of evoked IPSCs (GABAegic), but not of EPSCs (glutamatergic), providing evidence for a fine CB1-selectivity; SR141716A, otherwise, was able to block the inhibition also of evoked EPSCs, even in CB1–/– knockout mice (Hájos et al., 2001), that proves to have some affinity for the glutamatergic neuron cannabinoid receptor suggested to be of a 'non-CB1, non-CB2' class (termed by some as "CB3") (Breivogel et al., 2001; Hájos and Freund, 2002; Hájos et al., 2001).

In accordance with these considerations, and specially taking into account the AM251 specificity of action, we interpret the LTP supression here obtained as caused by the action of AM251 over GABAergic interneurons that (indirectly) modulate the glutamatergic, LTP-bearing pyramidal cells.

3.2. *Behavioral results*

Fig. 3 shows that AM251, when administered into the rat hippocampus immediately after training, disrupts memory consolidation of the Inhibitory Avoidance task, with both doses causing a reduction in the test latency, but only the 5.5 ng/side one being statistically significant. The absence of any effect in the Open Field Habituation task (Fig. 4) shows that

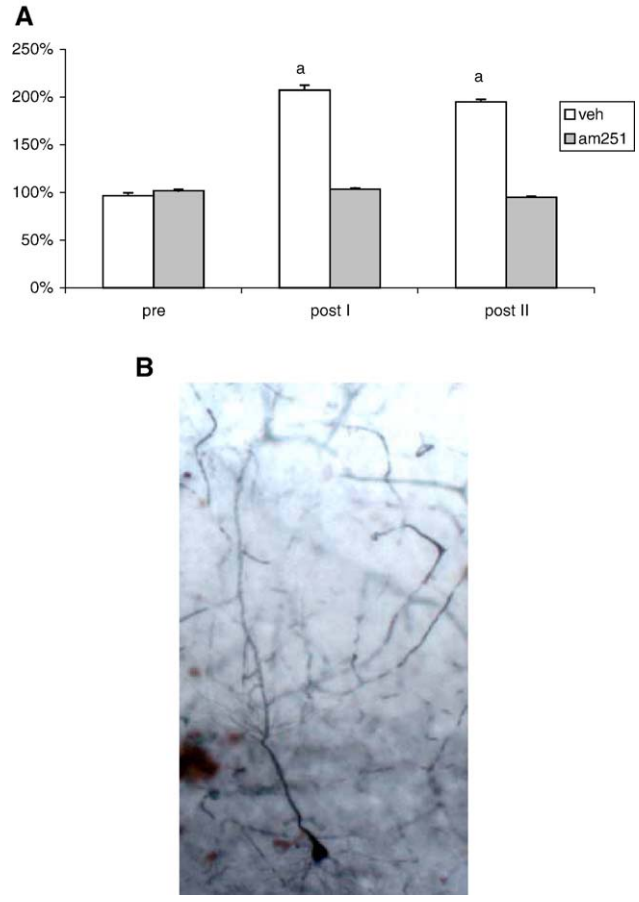

Fig. 2 – (A) Percentage change of fEPSP% amplitude in pretetanic (average – 9 to 0 min), and two posttetanic time windows (average 26–30 min and 56–60 min). (a) Difference from control in their respect time ($P = 0.000$, Student's t test). (B) Representative photomicrograph showing a pyramidal neuron from the CA1 region of the rat hippocampus filled with biocytin (0.5% w/v).

memory of this non-aversive task was unaffected by the CB1 antagonist (in the same doses studied in the aversive task); as the number of crossings was also unaffected by any of the administered doses, we may conclude that AM251 induces no motor performance effects that would interfere with the IA result. Therefore, AM251 amnestic effect in IA appears to be essentially cognitive, interfering specifically with the memory consolidation process, since the drug was infused after training, when acquisition had already finished (McGaugh, 1966).

Our findings agree with a previous study recently published by our group (Alvares et al., 2005). It is important to add that there appears to be no opposition between our (amnestic-with-antagonist) result with reports of a facilitatory effect of CB1 antagonists, or a disruptive effect of agonists, as advanced by several authors (Ameri, 1999; Davies et al., 2002; Hernandez-Tristan et al., 2000; Lichtman, 2000): most of these studies deal only with systemic effects (for instance, Da Silva and Takahashi, 2002; Davies et al., 2002; Takahashi et al., 2005; Wolff and Leander, 2003) and the observed results cannot be a priori related to any specific brain structure. This "multitarget" nature of systemic treatments could explain the quite contradictory reports with the CB1 antagonist SR141716A being either facilitatory (Lichtman, 2000; Takahashi et al., 2005; Terranova et al., 1996; Wolff and Leander, 2003) or having no effect at all (Da Silva and Takahashi, 2002; Davies et al., 2002). The fact that SR141716A is, as mentioned above, less selective than AM251, only further complicates the interpretation of the non-locally injected experiments. Interestingly, however, Marsicano et al. (2002) have found that systemic SR141716A has disrupted the extinction of aversive memories in mice.

There is one report of a spatial memory impairment with an intrahippocampal injection of the agonist Δ^9-THC (Egashira et al., 2002), and Lichtman et al. (1995) have found memory deficits following intrahippocampal injection of one of two different agonists (CP55,940 and anandamide, respectively); these findings would be in great contrast to ours if were not the case that (1) both have dealt only with agonists (with different affinities), and (2) have employed different behavioral tasks (8-arm maze) or memory paradigms (working memory).

Furthermore, the possibility that AM251 might be acting as an inverse agonist – its structure is very similar to SR141716A (Gatley et al., 1996), a known inverse agonist (Landsman et al., 1997), and both chemicals were shown to inhibit G-protein activity under certain circumstances (Savinainen et al., 2003) – would not change much of our conclusion, since (a) it has a real competitive advantage over both endocannabinoids due to its much higher affinity for the CB1 receptors (Gatley et al., 1996), and (b) even if acting like this (as an inverse agonist), its hypothetical excitatory effect would also favor GABA release (Katona et al., 1999), and, consequently, promote a downstream inhibition that would explain the amnestic effect observed the same way.

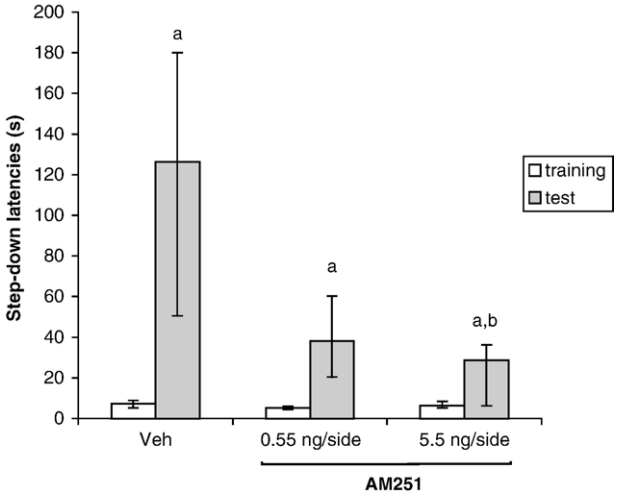

Fig. 3 – Effect of AM251, administered immediately after the training session, in the step-down inhibitory avoidance task. Data expressed as median [interquartile range] of step-down latencies. Kruskal–Wallis Test has shown no significant difference among training session latencies. (a) Significant difference between training and test session latencies ($P < 0.05$, Wilcoxon t test); (b) different from the control group value ($P < 0.05$, Dunn's post hoc test).

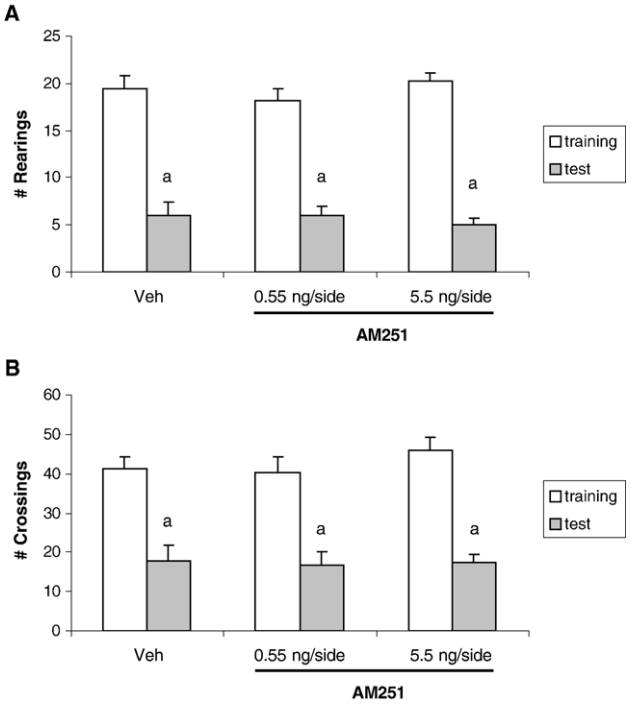

Fig. 4 – Effect of AM251, administered immediately after the training session, in the open field habituation task. Data expressed as means ± SEM of the number of crossings (B) and rearings (A). There were no statistically significant differences among groups' means, for both variables (crossings or rearings) either for the training session, or for the test session (One-way ANOVA test). (a) Significant difference between training and test session ($P < 0.001$, Paired samples *t* test).

The amnestic effect observed by us in the aversive (IA), but not in the non-aversive (OF) task, suggests that some degree of emotion (aversiveness) would be required in order to recruit the CB1-sensitive response. In the spirit of the hippocampal circuitry scenario described above, we may suppose that endogenous ligands to the CB1 receptors – such as the endocannabinoids anandamide and 2-AG (Pertwee and Ross, 2002) – may be released in that brain structure in response to the aversive components of the IA task, i.e., the shock: in fact, the release of endocanabinoids in response to stressful factors has been demonstrated in the periacqueductal grey substance (Hohmann et al., 2005) and even in the amygdala (Marsicano et al., 2002). This stress-induced endocannabinoid mediation may be the main differential between the consolidation mechanisms of the two studied memory tasks, adding to the well-known modulatory role of released stress hormones (Cahill and McGaugh, 1998).

3.3. *Further considerations*

The hypothesis emerging here is that increased levels of endogenous cannabinoids immediately after the training session would contribute to facilite memory consolidation in the hippocampus because the agonization of CB1 receptors would block GABAergic interneurons (Alger and Pitler, 1995; Katona et al., 1999; Mackie et al., 1995; Ohno-Shosaku and Kano, 2001; Pertwee and Ross, 2002), that restrain their target glutamatergic pyramidal neurons (Bliss and Collingridge, 1993; Carlson et al., 2002; Davies et al., 2002; Izquierdo et al., 1992; Izquierdo and Medina, 1995; Lamprecht and LeDoux, 2004; Marsicano et al., 2002; Wilson and Nicoll, 2001; Wilson and Nicoll, 2002): endocanabinoids would then be the natural dis-inhibitors of local plasticity in the dorsal hippocampus (Bohme et al., 2000; Carlson et al., 2002; Chevaleyre and Castillo, 2003; Katona et al., 1999), and the amnestic action of AM251 would be due to a disruption of this endogenous modulatory system.

This scenario is furthermore fully consistent with the endocannabinoid-mediated facilitation of hippocampal LTP through a retrograde inhibition of presynaptic GABA release (Alger and Pitler, 1995; Carlson et al., 2002), a situation broadly compatible with the hypothesis that LTP – or any similar or derived glutamatergic plasticity phenomenon – is the physical substrate (or part of the mechanism) involved in the memory formation process (Bliss and Collingridge, 1993; Frankland and Bontempi, 2005; Izquierdo and Medina, 1995; Lamprecht and LeDoux, 2004). Our electrophysiological data, despite the uncertainty about the real AM251 concentration around the target CB1 receptors, agree with all this, and may be considered an additional "positive" report. Nevertheless, with the present data, it is not possible to assure any causal connection between the CB1-sensitive hippocampal LTP and the IA-activated memory consolidation process, being only clear that both processes are disrupted by the local infusion of AM251, i.e., they are both CB1-dependent.

Finally, if it is true that a concomitantly activated, CB1-sensitive hippocampal LTP appears to be necessary for the consolidation of the aversive (IA) memory, it is also true that the same electrophysiological phenomenon is clearly NOT necessary for the memory consolidation of the OF habituation task. The engram of this less aversive task was equally well retained, with no need of such a blatant putative mechanism. Other subjacent plastic mechanisms may be responsible for the OF memory, that is, moreover, CB1-insensitive.

4. Experimental procedures

4.1. *Animals and experimental groups*

Ninety-eight (98) male Wistar rats (age 2–3 months, weight 210–300 g) from our breeding colony were used in this experiment. All procedures were in strict accordance with protocols approved by our institutional ethics committee, which complies to Brazilian national legislation (Law no. 6.638/1979) and to the European Communities Council Directive of 24 November 1986 (86/609/EEC), and all efforts were made to reduce the number of animals used. Animals were housed in plastic cages, 4–5 to a cage, under a 12 h light/dark cycle and at a constant temperature of 24 ± 1 °C, with water and food ad libitum. Animals were divided in two experimental groups, electrophysiology ($n = 10$), and the other for the behavioral tasks ($n = 75$ out of 88 operated rats; see groups in Section 4.1.2).

4.1.1. *Electrophysiology*

Animals were anesthetized with thiopental (50 mg/kg, i.p.) and killed by decapitation immediately after disappearance of the

pinch reflex. Their brains were rapidly removed from the skull and cooled in a dissection plate with artificial cerebrospinal fluid (ACSF) containing, in mM: 130 NaCl, 3.5 KCl, 1.3 NaH_2PO_4, 5 Mg + 2, 0.2 $CaCl_2$, 10 D-glucose and 24 $NaHCO_3$, previously gassed with a 95% O_2–5% CO_2 mixture to attain a pH value of 7.3–7.4. Transverse slices (400 μm) containing the hippocampus were obtained using a vibratome (Vibroslice 725 M, Campden Instruments, USA). Each slice was allowed to recover for at least 1 h (at room temperature) from dissection trauma in normal ACSF (composition in mM): 130 NaCl, 3.5 KCl, 1.3 NaH_2PO_4, 2 Mg + 2, 2 $CaCl_2$, 10 D-glucose and 24 $NaHCO_3$, pH 7.3–7.4, at room temperature and gased with 95% O_2 and 5% CO_2. A maximum of two slices per rat were used.

A micropipette containing AM251, 0.2 μM, diluted in DMSO/saline 8% was placed next of the stratum radiatum, and the substance, or its diluent (control group), ejected by a pneumatic pump (PV830 Pneumatic Pico Pump WPI) (as in Salamoni et al., 2005) 2 min before the tetanic stimulus.

Standard extracellular electrophysiology techniques were used to record field excitatory postsynaptic potentials (fEPSPs) from the dendritic region of the CA1 neurons (stratum radiatum) in response to stimulation of the Schaffer's collaterals afferent pathway, using square-wave current pulses (Master 8, A.M.P.I., Israel). Stimulation electrode consisted of a twister bipolar pair of 75 um platinum–iridium wires (A–M Systems, USA). The recording electrode was pulled on a horizontal micropipette puller (Sutter P-87, Sutter Instrument, USA) from borosilicate glass capillaries filled with 0.9% NaCl (electrode resistance 0.5–10 MΩ). At the start of each experiment, an input–output (I/O) relation of EPSP amplitude to stimulus intensity was recorded, after which intensity was adjusted to evoke the baseline EPSP amplitude at 67% of the maximum EPSP amplitude obtained during I/O curve (Dalbem et al., 2005).

Field potentials were evoked by a current stimulus (60–120 μA). Baseline responses to 0.05 Hz single-pulse stimuli (0.2 ms) were monitored for 20–40 min. After a stable baseline-evoked response was observed, high-frequency stimulation (HFS) protocol was applied (four trains of 1 s duration at 100 Hz, pulse duration of 0.2 ms, with an intertrain interval of 20 s). Field potentials were monitored for at least 60 min after the HFS.

Sample cells in the recording field were labeled in order to identify neuronal morphology; this was done by an intracellular electrode with higher resistance (~100 MΩ) and filled with biocytin in 0.05 M Tris and 0.9% NaCl.

Electrophysiological data were amplified 1000× and low-pass filtered at 0.6 kHz (CyberAmp 320, Axon Instruments, USA), digitized (Digidata, Axon Instruments, USA) and recorded (Axo-Clamp 2B, Axon Instruments, USA). Data were monitored and recorded on a personal computer via the Axoscope software (Axon Instruments, USA).

A Repeated Measure ANOVA (General Linear Model) of fEPSP recordings collected every 5 min, before and after the high-frequency stimulus (HSF), was used to analyze and compare data from two different groups (AM251 and its vehicle, i.e., DMSO 8% in PBS). The average fEPSPs for two posttetanic 5-min time windows ($\langle t_{26-30\ min}\rangle$ and $\langle t_{56-50\ min}\rangle$) were also compared by Student's t test. The last 10 min prior the tetanic stimulus was averaged and defined as "baseline response"; in order to emphasize the LTP criteria here used—a minimum of 40% potentiation in relation to the baseline-graphic presentation of data was in percental form.

4.1.2. *Behavioral procedures*

All animals were anesthetized by a mixture of Ketamine and Xilazine (i.p., 75 and 10 mg/kg, respectively) and bilaterally implanted with a 27-gauge guide cannulae aimed at AP – 4.2 mm (from bregma), LL ±3.0 mm, DV 1.8 mm, just 1.0 mm above area CA1 of the dorsal hippocampus (according to Paxinos and Watson, 1998). Once recovered from surgery (48 h), the animals were submitted to a training session in the one trial step-down inhibitory avoidance (IA) or the open-field habituation task (OF); 24 h later, they were tested (Izquierdo et al., 1992; Rosat et al., 1992). At the time of infusion, immediately after the training session, 30-gauge cannulae were fitted into the guide cannulae. The tip of the infusion cannulae protruded 1.0 mm beyond that of the guide cannulae and was, therefore, aimed at the pyramidal cell layer of CA1 in the dorsal hippocampus (Fig. 5), with a 0.5 μl volume being administered at a 20 μl/h rate. For each behavioral task, there were three groups, two infused with the doses 0.55 and 5.5 ng/side of AM251, and other with its vehicle (8% DMSO in phosphate-buffered saline).

The IA task was carried out in an automatically operated, brightly illuminated box, in which the left extreme of the grid (42.0 × 25.0 cm grid of parallel 0.1 cm caliber stainless steel bars spaced 1.0 cm apart) was covered by a 7.0 cm wide, 5.0 cm high formica-covered platform. Animals were placed on the platform and their latency to step-down placing their four paws on the grid was measured. In the training session, immediately upon stepping down, the animals received a 0.5 mA, 3.0 s scrambled footshock. In the test session, no footshock was given, and a ceiling of 180 s was imposed on the step-down latency (Izquierdo et al., 1992).

Open Field Habituation was studied using a 50 cm high, 60 × 40 cm plywood box with a frontal glass wall and a linoleum floor divided in 12 equal rectangles. Animals were left there for 3 min both in the training and the test session, and the number of rearings and crossings between sectors were counted each time. The difference in the number of rearings, or of crossings between rectangles, between the two sessions was considered a measure of retention of habituation to the open field: if the animals had habituated to the field during the first session, they should recognize it as familiar, and, in consequence, the number of rearings and crossings should be significantly smaller in the second session (Rosat et al., 1992). The number of crossings in the

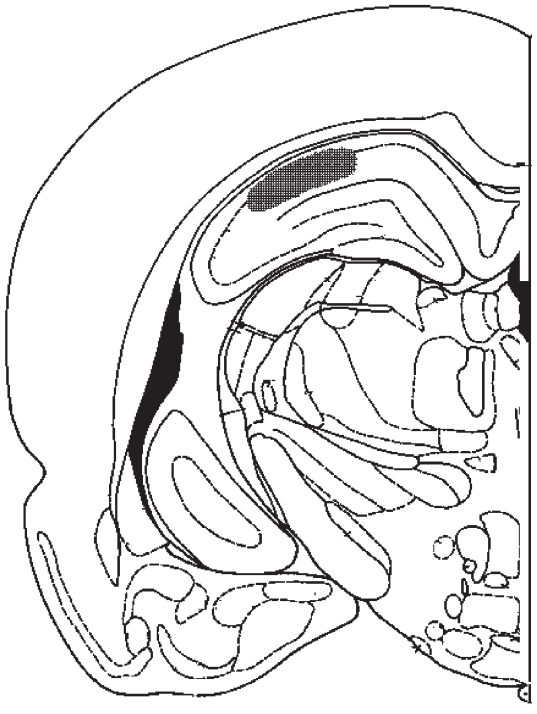

Fig. 5 – Drawing representing AP plane – 4.3 mm adapted from the atlas of Paxinos and Watson (1998) showing the extent of the area reached by our infusions in the rat dorsal hippocampus (stippled areas represent typical regions of accepted animals, as labeled by 2% methylene blue in 0.5 μl saline infused through the same cannulae).

test session may also be used as a control for the possible motor and general performance effects of the drug administered 24 h before.

Statistical analysis of the behavioral data (latencies to step-down in IA and number of rearings and crossings in OF) was limited to the animals with correct cannula placements (Fig. 5)—75 out of 88 operated rats, as described in Izquierdo et al. (1992) (for each group, $n = 10$ in the IA task and $n = 15$ in the OF task). Since (1) the step-down latencies have not passed a normality test ($P < 0.001$ for test latencies, Kolmogorov–Smirnov test with Lilliefors' correction), and (2) because a 180 s "ceiling" time was imposed to the test session, differences among groups were evaluated by a non-parametric Kruskal–Wallis One-way ANOVA on Ranks Test (with a Dunn's All Pairwise Multiple Comparison Procedure as a post hoc test, when suitable); training vs. test latencies were correspondingly compared by the Wilcoxon Signed Ranks Test. In the OF task, as both measures were normally distributed ($P > 0.05$, Kolmogorov–Smirnov test with Lilliefors' correction), groups were compared by a One-way ANOVA, and the differences ordered by Tukey's pairwise HSD post hoc test, when suitable; training vs. test latencies were correspondingly compared by the paired t test.

Acknowledgments

The authors would like to thank the LPBNC and Neurolab colleagues and staff for technical support, and, in particular, Andréa Alencar Tavares, for assistance with the biocytin labeling procedure. This research was supported by fellowships from the National Research Council of Brazil (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and resources from Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS).

REFERENCES

Alger, B.E., Pitler, T.A., 1995. Retrograde signaling at $GABA_A$-receptor synapses in the mammalian CNS. Trends Neurosci. 18, 333–340.

Alvares, L.O., Oliveira, L.F., Camboim, C., Diehl, F., Genro, B.P., Lanziotti, V.B., Quillfeldt, J.A., 2005. Amnestic effect of intrahippocampal AM251, a CB1-selective blocker, in the inhibitory avoidance, but not in the open field habituation task, in rats. Neurobiol. Learn. Mem. 83 (2), 119–124.

Ameri, A., 1999. The effects of cannabinoids on the brain. Prog. Neurobiol. 58, 315–348.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Bohme, G.A., Laville, M., Ledent, C., Parmentier, M., Imperator, A., 2000. Enhanced long-term potentiation in mice lacking cannabinoid CB1 receptors. Neuroscience 95, 5–7.

Breivogel, C.S., Griffin, G., di marzo, V., Martin, B.R., 2001. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Mol. Pharmacol. 60, 155–163.

Cahill, L., McGaugh, J.L., 1998. Mechanisms of emotional arousal and lasting declarative memory. Trends Neurosci. 21, 294–299.

Carlson, G., Wang, Y., Alger, B.E., 2002. Endocannabinoids facilitate the induction of LTP in the hippocampus. Nat. Neurosci. 5 (8), 723–724.

Chevaleyre, V., Castillo, P.E., 2003. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron 38, 461–472.

Collins, D.R., Pertwee, R.G., Davies, S.N., 1995. Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. Br. J. Pharmacol. 115, 869–870.

Dalbem, A., Silveira, C.V., Pedroso, M.F., Breda, R.V., Baes, C.V.W., Bartmann, A.P., Da Costa, J.C., 2005. Altered distribution of striatal activity-dependent synaptic plasticity in the 3-nitropropionic acid model of Huntington's disease. Brain Res. 1047 (2), 148–158.

Da Silva, G.E., Takahashi, R.N., 2002. SR 141716A prevents delta 9-tetrahydrocannabinol-induced spatial learning deficit in a morris-type water maze in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 26 (2), 321–325.

Davies, S.N., Pertwee, R.G., Riedel, G., 2002. Functions of cannabinoid receptors in the hippocampus. Neuropharmacology 42, 993–1007.

Egashira, N., Mishima, K., Iwasaki, K., Fujiwara, M., 2002. Intracerebral microinjections of delta 9-tetrahydrocannabinol: search for the impairment of spatial memory in the eight-arm radial maze in rats. Brain Res. 952, 239–245.

Frankland, P.W., Bontempi, B., 2005. The organization of recent and remote memories. Nat. Rev., Neurosci. 6, 119–130.

Gatley, S.J., Gifford, A.N., Volkow, N.D., Lan, R., Makriyannis, A., 1996. ^{123}I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors. Eur. J. Pharmacol. 307, 331–338.

Hájos, N., Freund, T.F., 2002. Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. Neuropharmacology 4 (4), 503–510.

Hájos, N., Ledent, C., Freund, T.F., 2001. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. Neuroscience 106 (1–3), 1–4.

Hampson, R.E., Deadwyler, S.A., 1999. Cannabinoids, hippocampal function and memory. Life Sci. 65, 715–723.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., De Costa, B.R., Rice, K.C., 1991. Characterization and localization of cannabinoid receptors in the rat brain: a quantitative in vitro autoradiographic study. J. Neurosci. 11, 563–583.

Hernandez-Tristan, R., Arevalo, C., Canals, S., Leret, M.L., 2000. The effect of acute treatment with delta9-THC on exploratory behaviour and memory in the rat. J. Physiol. Biochem. 56 (1), 17–24.

Hohmann, A.G., Suplita, R.L., Bolton, N.M., Neely, M.H., Fegley, D., Mangieri, R., Krey, J.F., Walker, J.M., Holmes, P.V., Crystal, J.D., Duranti, A., Tontini, A., Mor, M., Tarzia, G., Piomelli, D., 2005. An endocannabinoid mechanism for stress-induced analgesia. Nature 435, 1108–1112.

Izquierdo, I., Medina, J.H., 1995. Correlation between the pharmacology of long-term potentiation and the pharmacology of memory. Neurobiol. Learn. Mem. 63, 19–32.

Izquierdo, I., Da Cunha, C., Rosat, R., Jerusalinsky, D., Ferreira, M.B.C., Medina, J.H., 1992. Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum and hippocampus of the rat. Behav. Neural Biol. 58, 16–26.

Katona, I., Sperlagh, B., Sik, A., Kafalvi, A., Vizi, E.S., Mackie, K., Freund, T.F., 1999. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axonal Terminals of specific hippocampal interneurons. J. Neurosci. 19, 4544–4558.

Kreitzer, A.C., Regehr, W.G., 2001. Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. J. Neurosci. 21 (20), RC174.

Lamprecht, R., LeDoux, J., 2004. Structural plasticity and memory. Nat. Rev., Neurosci. 5, 45–54.

Landsman, R.S., Burkey, T.H., Consroe, P., Roeske, W.R., Yamamura, H.I., 1997. SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. Eur. J. Pharmacol. 334, R1–R2.

Lichtman, A.H., 2000. SR141716A enhances spatial memory as assessed in a radial-arm maze task in rats. Eur. J. Pharmacol. 404 (1–2), 175–179.
Lichtman, A.H., Dimen, K.R., Martin, B.R., 1995. Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharmacology 119, 282–290.
Mackie, K., Lai, Y., Westenbroek, R., Mitchell, R., 1995. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in At T20 cells transfected with rat brain cannabinoid receptor. J. Neurosci. 15, 6552–6561.
Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Zieglgänsberger, W., Di Marzo, V., Lutz, B., 2002. The endogenous cannabinoid system controls extinction of aversive memory. Nature 418, 530–534.
McGaugh, J.L., 1966. Time-dependent processes in memory storage. Science 153, 1351–1358.
Ohno-Shosaku, T., Kano, M., 2001. Endogenous cannabinoids mediate retrograde signals from depolarised postsynaptic neurons to presynaptic terminals. Neuron 29, 729–738.
Paxinos, G., Watson, C., 1998. The Rat Brain In Stereotaxic Coordinates, 4th ed. Academic Press, San Diego.
Pertwee, R.G., Ross, R.A., 2002. Cannabinoid receptors and their ligands. Prostaglandins Leukot. Essent. Fat. Acids 66, 101–121.
Rosat, R., Da-Silva, R.C., Zanatta, M.S., Medina, J.H., Izquierdo, I., 1992. Memory consolidation of a habituation task: role of *N*-methyl-D-aspartate, cholinergic muscarinic and GABA-A receptors in different brain regions. Braz. J. Med. Biol. Res. 25, 267–273.
Salamoni, S.D., Da Costa, J.C., Palma, M.S., Konno, K., Nihei, K., Tavares, A.A., Abreu, D.S., Venturin, G.T., Cunha, F.B., Oliveira, R.M., Breda, R.V., 2005. Antiepileptic effect of acylpolyaminetoxin JSTX-3 on rat hippocampal CA1 neurons in vitro. Brain Res. 1048 (1–2), 170–176.
Savinainen, J.R., Saario, S.M., Niemi, R., Jarvinen, T., Laitinen, J.T., 2003. An optimized approach to study endocannabinoid signaling: evidence against constitutive activity of rat brain adenosine A1 and cannabinoid CB1 receptors. Br. J. Pharmacol. 140, 1451–1459.
Squire, L.R., 1992. Memory and the hippocampus: a synthesis from findings with rats, monkeys and humans. Psychol. Rev. 99, 195–231.
Takahashi, R.N., Pamplona, F.A., Fernandes, M.S., 2005. The cannabinoid antagonist SR141716A facilitates memory acquisition and consolidation in the mouse elevated T-maze. Neurosci. Lett. 380 (3), 270–275.
Terranova, J.P., Michaud, J.C., Le Fur, G., Soubrie, P., 1995. Inhibition of long-term potentiation in rat hippocampal slices by anadamide and WIN55,212-2: reversal by SR141716A, a selective antagonist of CB1 cannabinoid receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 576–579.
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, SR 141716. Psychopharmacology (Berlin) 126, 165–172.
Wilson, R.I., Nicoll, R.A., 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410, 588–592.
Wilson, R.I., Nicoll, R.A., 2002. Endocannabinoid signaling in the brain. Science 296, 678–682.
Wolff, M.C., Leander, J.D., 2003. SR141716A, a cannabinoid CB1 receptor antagonist, improves memory in a delayed radial maze task. Eur. J. Pharmacol. 477, 213–217.