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Brain, Behavior, and Immunity 23 (2009) 817–822

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Memory impairment induced by IL-1β is reversed by α-MSH through central melanocortin-4 receptors

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ARTICLE INFO

Article history:
Received 29 December 2008
Received in revised form 17 February 2009
Accepted 2 March 2009
Available online 9 March 2009

Keywords:
Interleukin-1beta
Melanocortins
Melanocortin receptors
Fear conditioning
Memory consolidation
Memory persistence
Hippocampus

ABSTRACT

Interleukin-1beta (IL-1β) significantly influences memory consolidation. Treatments that raise the level of IL-1β in the brain, given after training, impair contextual fear conditioning. The melanocortin α-MSH exerts potent anti-inflammatory actions by physiologically antagonizing the effect of pro-inflammatory cytokines. Five subtypes of melanocortin receptors (MC1R–MC5R) have been identified, with MC3R and MC4R predominating in the central nervous system. The present experiments show that injection of IL-1β (5 ng/0.25 μl) in dorsal hippocampus up to 15 min after training decreased freezing during the contextual fear test. The treatment with IL-1β (5 ng/0.25 μl) 12 h after conditioning cause amnesia when animals were tested 7 days post training. Thus, our results also demonstrated that IL-1β can influence persistence of long-term memory. We determined that animals previously injected with IL-1β can acquire a new contextual fear memory, demonstrating that the hippocampus was not damaged. Treatment with α-MSH (0.05 μg/0.25 μl) blocked the effect of IL-1β on contextual fear memory. Administration of the MC4 receptor antagonist HS014 (0.5 μg/0.25 μl) reversed the effect of α-MSH. However, treatment with γ-MSH (0.5 μg/0.25 μl), an MC3 agonist, did not affect IL-1β-induced impairment of memory consolidation. These results suggest that α-MSH, through central MC4R can inhibit the effect of IL-1β on memory consolidation.

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1. Introduction

Peripheral infection can produce severe and prolonged decline in cognitive functioning in individuals with neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Perry et al., 2003). Moreover, infection and the consequent activation of immune cells can even interfere with cognitive processes in healthy humans and animals (Reichenberg et al., 2001; Gibertini et al., 1995; Dantzer et al., 2008), although the effects in these subjects are smaller in magnitude and duration. Interestingly, the de novo induction of proinflammatory cytokines such as IL-1β within the brain is a key feature of the neural cascade induced by peripheral infection/immune activation (Layé et al., 1994; Nguyen et al., 1998). In this context, it is important that IL-1β can produce detrimental effects on memory at pathophysiological levels. These effects are specific for the consolidation of memories that depend on hippocampus, whereas hippocampal-independent memories seem not to be altered (Rachal Pugh et al., 2001). The particularly high expression of IL-1β, IL-1 receptor (IL-1R1) and proteins belonging to the IL-1 receptor family in the hippocampus may be underlying the effect of IL-1β within this structure (Loddick et al., 1998). IL-1β inhibits long-term potentiation (LTP) (Murray and Lynch, 1998; O'Connor and Coogan, 1999), a model system for the neural mechanism underlying memory in several hippocampal pathways (Martin et al., 2000). However, recent evidence suggests that in certain circumstances IL-1β may be required for normal physiological regulation of hippocampal plasticity and memory processes (Schneider et al., 1998; Goshen et al., 2007).

It is widely accepted that long-term memory (LTM) formation is dependent upon hippocampal de novo protein synthesis and brain-derived neurotrophic factor (BDNF) signalling early after acquisition (Bekinschtein et al., 2008). It has been demonstrated that BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1β administration (Barrientos et al., 2004). Also, a characteristic attribute of long-term memory is persistence. It has been demonstrated that 12 h after acquisition there is novel protein synthesis and BDNF-dependent phase in the rat hippocampus critical for the persistence of LTM storage (Bekinschtein et al., 2007). Although the effect of IL-1β on memory consolidation has been clearly demonstrated, the effect of intrahippocampal IL-1β administration on memory persistence has not yet been examined.

α-Melanocyte stimulating hormone (α-MSH) and other melanocortins (β-MSH, γ-MSH and ACTH) are members of a family of

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doi:10.1016/j.bbi.2009.03.001

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endogenous peptides derived from pro-opiomelanocortin (POMC). They exert their actions via five different G protein-coupled receptors (MC1/MC5), all of which couple positively via Gs to adenylate cyclase (Schiöth, 2001). MC4R is found throughout the limbic system structures including several nuclei of the amygdala, hippocampus and entorhinal cortex (Adan et al., 1994). Melanocortins exert multiple influences on the central nervous system, including immunomodulatory effects: they can modulate the production and action of proinflammatory cytokines (Ceriani et al., 1994; Macaluso et al., 1994; Robertson et al., 1988; Lasaga et al., 2008). Centrally administered α-MSH is a potent antipyretic in different species (Catania and Lipton, 1998) and can also prevent damage in brainstem ischemia and reperfusion injury (Giuliani et al., 2006). A variety of effects of central IL-1β administration are blocked by α-MSH (Cragnolini et al., 2004; Cragnolini et al., 2006a,b).

We examined the influence of melanocortins and the role of MC3/MC4 receptors on the effect of IL-1β on memory consolidation and persistence.

2. Methods

2.1. Animals

Adult male Wistar rats, weighing 270–300 g at the time of surgery, were used for these studies. All animals were housed in standard laboratory plastic cages in groups of three per cage. Food and water were available *ad libitum.* Animals were kept on a 12 h light/dark cycle (lights on 7:00–19:00 h) with a constant room temperature of 22 ± 1 °C. The experiments were performed according to the guidelines of the NIH Guide for the Care and Use of Laboratory Animals as approved by the School of Chemical Sciences, National University of Cordoba Animal Care and Use Committee. The number of animals used, as well as their suffering, was kept to the minimum possible needed to accomplish the goals of this study.

2.2. Surgery and drug infusion procedures

Rats were implanted bilaterally under ketamine hydrochloride (55 mg/kg) and xylazine (11 mg/kg) intraperitoneal anesthesia with 22-g guide cannulae in the dorsal CA1 region of the hippocampus on coordinates A: −3.3 mm; L: ±2.0 mm; V: −2.5 mm according to the atlas of Paxinos and Watson (1986). The cannulae were fixed to the skull with a screw and dental acrylic. After surgery, animals were gently handled every day, and were habituated to intrahippocampal injections throughout the recovery period. The behavioral tests commenced 7 days after surgery. To perform local infusions into the hippocampus, rats were hand-restrained, and drugs or vehicle were administered with infusion cannulae (30 gauge). Infusion cannulae fitted into and extended 1 mm beyond the guide cannulae. The infusion cannulae were connected, via polyethylene tubing (PE 10; Becton Dickinson, Sparks, MD), to 10 μl microsyringes (Hamilton, Reno, NV) mounted on a microinfusion pump (Harvard Apparatus, Holliston, MA). Each rat was injected with 0.25 μl/side at a flow rate of 0.25 μl/min. To allow diffusion of the drug, the infusion cannulae were kept in place for another minute.

2.3. Histological procedures

After behavioral tests, rats were killed by an overdose of chloral hydrate 16%, and their brains removed and immersion-fixed in a 4% formalin solution. Frontal sections were cut in a cryostat (Leica, Nussloch, Germany), the injection sites localized, and the extent of tissue damage caused by cannulation was examined under a light microscope. The injection sites were drawn on plates taken from a rat brain atlas (Paxinos and Watson, 1986). Only animals with proper cannula placements and tissue damage not exceeding the diameter of the cannulae were included in the study.

2.4. Drugs

The drugs used in these experiments were rrIL-1β (R&D Systems UK), α-MSH (MW 1664) (Peninsula Laboratories Inc.), γ-MSH (selective MC3R agonist, MW 1512.9) and HS024 (selective MC4R antagonist, MW 1678) (NeoMPS, France). The rrIL-1β was dissolved in sterile 0.9% saline containing 0.1% bovine serum albumin and stored in aliquots at −70 °C. Peptides were dissolved in sterile 0.9% saline and stored in aliquots at −20 °C. The rats received bilateral infusions of sterile saline (vehicle) or the different drugs in the following doses: IL-1β (5 ng/0.25 μl), α-MSH (0.05 μg/0.25 μl), γ-MSH (0.5 μg/0.25 μl), HS014 (0.5 μg/0.25 μl).

2.5. Apparatus

The conditioning environment was designated as context 1 (C1); made of transparent plastic (20 × 23 × 20 cm) with clear lid and the floor consisted of 10 parallel stainless steel grid bars, each measuring 4 mm in diameter and spaced 1.5 cm apart (center to center), enclosed in a sound attenuating chamber. The grid floor was attached to a scrambled shocker to provide footshock. Illumination was provided by a 2.5 W white light bulb. The chamber was cleaned with 50% ethanol in water before and after each use. Another chamber was made as different as possible from the context 1 and designated as context 2 (C2). This chamber consisted of a different Plexiglas chamber (60 × 20 × 20 cm) with orange transparent walls while the floor consisted of stainless steel rods separated by 1.0 cm (center to center). The chamber was illuminated by one light and was cleaned with 0.5% acetic acid before and after use. This chamber was located in a different room and used in experiment 3. Experiments were always performed between 10:00 and 14:00 h with the experimenters unaware of the treatment condition of the animals.

2.6. Conditioning

Training consisted in placing the rat in the chamber (C1) and allowing a 3 min adaptation period (pre-shock period). After this period, rats received three unsignaled footshocks (0.3 mA; 2.5 s duration; 30 s inter-shock interval). Animals remained in the chamber for an additional 2 min (post-shock period) and immediately afterwards were placed in their home cages and returned to the colony room.

Test session: Conditioning was assessed 24 h after training by placing the rats in the training environment for a 5 min period. Memory was assessed and expressed as the percentage of time rats spent freezing. This behavior, commonly used as an indicator of fear in rats (Blanchard and Blanchard, 1969), was observed during this exposure period. An animal was considered to be freezing when it was crouching without movement of the body or the head, except that associated with breathing. The total time spent freezing in each period was quantified (in seconds) using a stopwatch and expressed as the percentage of total time.

2.6.1. Experiment 1

This experiment was performed In order to determine the effect of IL-1β on the consolidation of a memory when administered at different times (0, 15 and 30 min) following conditioning. Another group of animals was injected with vehicle at the same times. Animals were tested in the conditioning chamber 24 h later (test 1). Freezing behavior was scored by a person who was blind in relation to the experimental condition of each animal.

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2.6.2. Experiment 2

To evaluate whether pre-treatment with IL-1β could affect new associative learning, rats from both groups (vehicle-IL-1β) were trained and tested as previously described in experiment 1 and 72 h following test 1, animals were exposed to a different conditioning chamber (C2) for 3 min (pre-shock period) and received one footshock (0.3 mA; 3 s duration). Animals remained in the chamber for an additional 1 min (post-shock period) and immediately afterwards they were returned to their home cages and the colony room. Twenty-four hours later rats were exposed to C2 for a 5 min period and the time they spent freezing was assessed (test 2). Yet another group of rats was infused with saline at the same time as other groups but was trained and tested only in C2.

2.6.3. Experiment 3

This experiment was designed to analyse whether IL-1β can also interfere with the process of persistence of LTM. The animals were trained as in experiment 1 and 12 h later they received infusions of different treatments. Freezing during test session was evaluated 24 h or 7 days after training by placing rats in the training environment for 5 min.

2.6.4. Experiment 4 (A and B)

To study the interaction between IL-1β and melanocortin (α-MSH or γ-MSH) peptides on the consolidation of a memory, animals were randomly assigned to different groups and injected immediately following conditioning as follow: (a) IL-1β (5 ng); (b) IL-1β (5 ng) and α-MSH (0.05 μg); (c) IL-1β (5 ng) and γ-MSH (0.5 μg); (d) IL-1β (5 ng) co-administered with HS014 (0.5 μg) and α−MSH (0.05 μg); (e) α-MSH (0.05 μg); (f) γ-MSH (0.5 μg) or (g) HS014 (0.5 μg). The melanocortins were injected 10 min after IL-1β. Doses were determined in dose-response experiments and in a previous work (Cragnolini et al., 2006a,b). The animals were re-exposed to C1 for 5 min 24 h later and the time they spent freezing was assessed (test 1).

2.7. Statistics

All data shown are means ± standard error of the mean (S.E.M.). Data were analyzed by one-way or two-way ANOVA. If any statistically significant difference was found, post-hoc analysis was performed using Student-Newman-Keuls multiple comparison test. Differences with a p value $\leqslant 0.05$ were considered statistically significant.

3. Results

3.1. Effect of intrahippocampal injection of IL-1β on the consolidation and persistence of a contextual fear memory

IL-1β impairs memory when administered shortly after the training session. Fig 1 shows rats that received intrahippocampal injection of IL-1β (5 ng/0.25 μl) displayed lower levels of freezing than animals injected with saline immediately after conditioning. Analysis revealed a significant treatment against time interaction ($F(2,34) = 4.44$, $p < 0.01$). The effect of IL-1β depends on the interval between conditioning and cytokine injection. Animals injected with IL-1β up to 15 min after conditioning showed significantly less freezing than those injected with saline (Fig 1). ANOVA revealed a significant effect of cytokine treatment ($F(1,34) = 15.09$, $p < 0.001$) and a significant effect of time of injection ($F(2,34) = 5.15$, $p < 0.01$) Subsequent post-hoc analysis revealed that the IL-1β-treated groups injected immediately or 15 min after conditioning were significantly different from saline and from IL-1β 30 min groups. These findings clearly show that the amnesic effect of IL-1β was effective up to 15 min after conditioning.

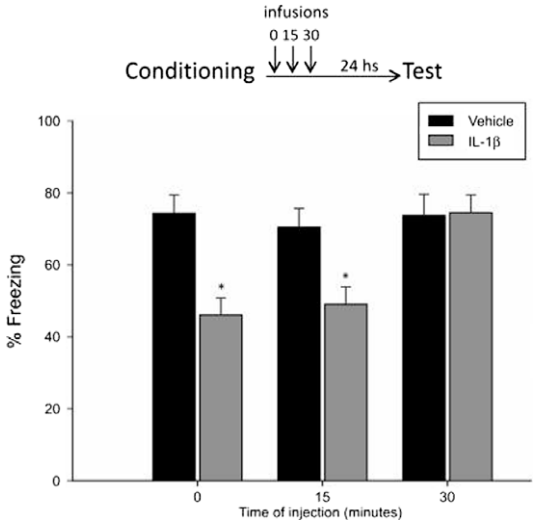

Fig. 1. Effect of IL-1β injected at different intervals of time after conditioning on a contextual fear memory. (a) Behavioural procedure used in this experiment (b) IL-1β injected up to 15 min post-conditioning cause a profound deficit in retention of learning. Data are the means ± S.E.M. percentage of time rats spent freezing during the 5 min exposure to the training context. ∗ Significantly different from vehicle group ($p < 0.001$) ANOVA follow by Newman Keuls test. n: 6–8 per group.

To test whether the hippocampus was permanently injured by the cytokine injection (or methodological manipulation), groups previously treated with IL-1β or vehicle were subjected to new conditioning in a different context (C2) 3 days afterwards. Results indicated that animals injected with IL-1β or vehicle normally acquired and retained the spatial memory associated with training in the new context, indicating that IL-1β did not cause permanent damage to the hippocampus. (Fig. 2) ($F(2,15) = 1.26$, $p = 0.3$). Animals injected with vehicle at the same time as the above groups, but that were only trained and tested in C2, displayed similar per-

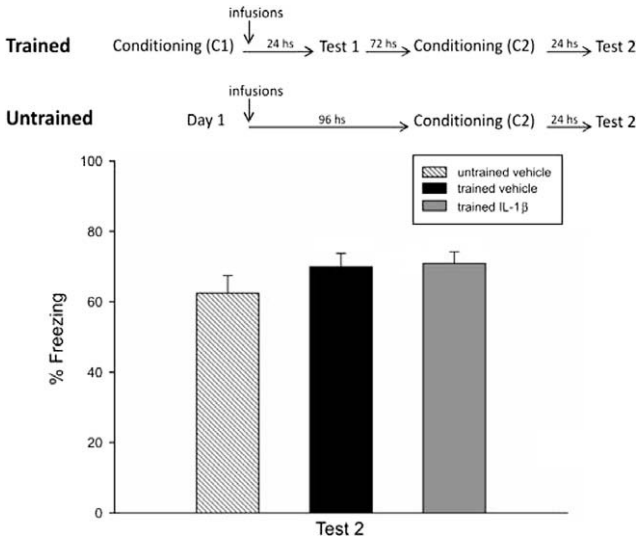

Fig. 2. IL-1β does not affect ability to learn new associative aversive learning. (a) Behavioural procedure used in this experiment. (b) Comparable levels of freezing during the second learning were observed between vehicle-naive animals and animals previously injected with saline or IL-1β. Data are the means ± S.E.M. percentage of time rats spent freezing during 5 min exposure to training context. n: 6 per group.

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centages of freezing (Fig. 2). There were no statistical differences between the three groups.

To study whether persistence of LTM storage was altered by IL-1β (5 ng/0.25 μl) it was administered 12 h after conditioning. This treatment did not cause amnesia when the test was carried out 1 day (24 h) after acquisition (Fig 3). In contrast, animals treated as mentioned above but tested for retention 7 days post training showed impaired long term memory. ($F(1,32) = 6.74$, $p < 0.01$). Contextual fear conditioning resulted in robust and long-lasting fear memory, since trained animals injected with vehicle showed highly significant freezing both 1 and 7 days post training (Fig. 3).

3.2. Influence of melanocortins on IL-1β-induced decrease in contextual fear memory

Our results are consistent with the literature indicating that IL-1β impairs memory of context. This effect is blocked by α-MSH injection into dorsal hippocampus. Thus, our results indicate that treatment of rats with α-MSH following IL-1β prevents the decrease in contextual fear memory. ANOVA revealed a significant effect of the treatments ($F(5,45) = 8.14$, $p < 0.001$). Addition of α-MSH (0.05 μg/0.25 μl) 10 min after IL-1β (5 ng/0.25 μl) completely antagonized the effect of the cytokine and the percentage of freezing was similar to that seen after saline (Fig 4A). However, co-administration of HS014 (0.5 μg/0.25 μl) and IL-1β (5 ng/0.25 μl) 10 min before administration of α-MSH (0.05 μg/0.25 μl) produced effects similar to treatment with IL-1β. Thus, treatment with HS014 impeded the α-MSH suppression of IL-1β-induced memory impairment (Fig 4A). Treatment with HS014 alone had no effect on conditioning. The injection of γ-MSH (0.5 μg/0.25 μl) 10 min after IL-1β did not attenuate the effect of the cytokine ($F(3,30) = 18.79$ $p < 0.001$) and the percentage of freezing was not significantly different from treatment with IL-1β alone (Fig 4B).

Interestingly, injection of α-MSH 12 h after conditioning and 10 min after treatment with IL-1β could not reverse the effect of IL-1β on persistence of the memory, measured 7 days after conditioning (% of freezing: vehicle: 76.96 ± 5.27; IL-1β: 56.64 ± 4.42; IL-1β+α-MSH: 55.53 ± 7.14). The above doses of α-MSH and γ-MSH administered alone did not produce any significant effect on consolidation of fear memory (Fig 4A and B).

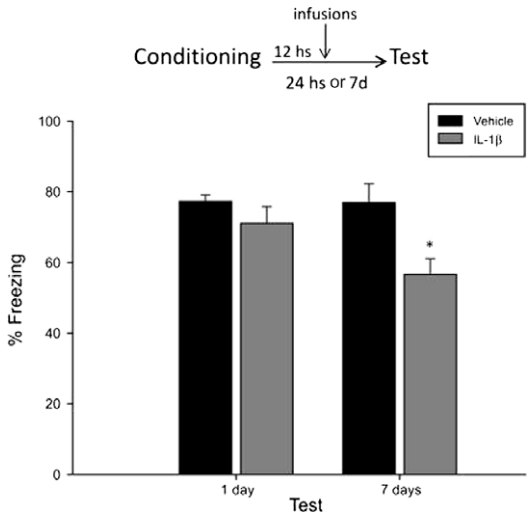

Fig. 3. Treatment with IL-1β in the rat hippocampus 12 h after training hinders persistence of long-term memory. (a) Procedure used in this experiment. (b) Levels of freezing 1 day and 7 days after conditioning. Data are the means ± S.E.M. percentage of time rats spent freezing during 5 min exposure to training context. ∗ Significantly different from vehicle group ($p < 0.01$) ANOVA followed by Newman Keuls test. *n*: 6–13 per group.

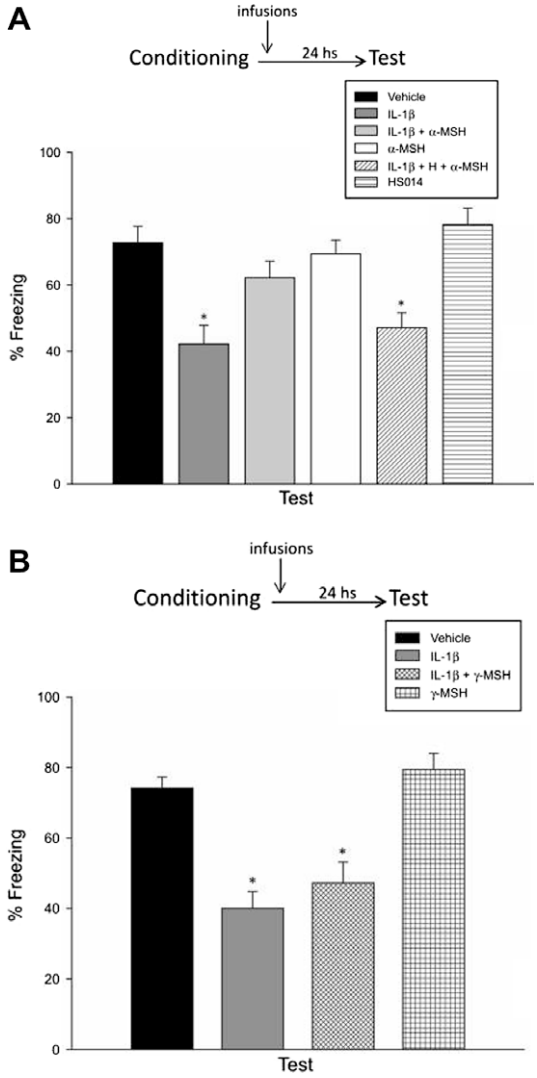

Fig. 4. Effect of melanocortins on IL-1β-induced impairment of memory consolidation. (A) (a) Behavioural procedure used in this experiment. (b) α-MSH blocked the inhibitory effect of IL-1β on memory consolidation. Administration of HS014, an MC4R antagonist, impeded the effect of α-MSH. (B) (a) Behavioural procedure used in this experiment. (b) Treatment with γ-MSH failed to modify the effect of IL-1β on fear memory consolidation. Data are the means ± S.E.M. percentage of time rats spent freezing during 5 min exposure to training context. ∗ Significantly different from vehicle group ($p < 0.001$) one way ANOVA follow by Newman Keuls test. *n*: 7–11 per group.

4. Discussion

An important recent advance in the biological basis of behaviour is the recognition of extensive communication between the central nervous system and the immune system. During an inflammatory or infectious process, innate immune cells produce pro-inflammatory cytokines that act on the brain to cause sickness behaviour. IL-1β is one of the main pro-inflammatory cytokines involved in sickness behaviour. Specifically, animals (or persons) show decreased motor activity, social withdrawal, reduced food and water intake, increased slow-wave sleep and altered cognition. As mentioned, during the sickness behaviour animals pay less attention to the environment. In general, sick or injected with pro-inflammatory cytokines animals stay in the corner of their home cage in a hunched posture and show little or no interest in their physical or social environment. As a consequence of their internal state, their appreciation of the surroundings presumably

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become of reduced importance. Therefore, elevated levels of IL-1β that occur during sickness behaviour impair the consolidation of contextual memories and more specifically the consolidation of memories dependent on the hippocampus (Rachal Pugh et al., 2001). This response should not be considered poor adaptation, but as a reorganization of the priorities of the organism, in order to fight the infection and preserve the energy (Dantzer et al., 2001; Konsman et al., 2002; Dantzer and Kelley, 2007).

However, extensive, prolonged or unregulated inflammation is highly detrimental. Thus, the study of the mechanism of action of cytokines in the central nervous system and possible antagonists to their effects became important.

Therefore, we studied the effect of IL-1β on contextual fear conditioning and the modulatory actions of melanocortins on this effect.

Our results demonstrated once more the effects of proinflammatory cytokine IL-1β on cognitive processes. Its injection significantly impairs the consolidation of a fear conditioning memory. We also demonstrated that the effect of IL-1β is specific rather than a consequence of a lesion in the hippocampus since the animals were able to perform new learning in a different context. In order to examine the time course of the effect of IL-1β on memory consolidation, the cytokine or vehicle were microinjected immediately, 15 min or 30 min after conditioning while testing was always conducted 24 h thereafter. The local infusions of IL-1β into the hippocampus up to 15 min after conditioning interferes with the processes responsible for storing the memory of contextual representation. Interestingly, IL-1β produced memory impairment 7 days after the acquisition of new learning when administered 12 h after conditioning. Thus, our results demonstrated that IL-1β can influence persistence of long-term memory. It might be noted that quite different memory processes occur at these intervals after the experience. Soon after the experience, early-phase LTP-related processes involving alterations in synaptic transmission are crucial, while after that, processes requiring protein synthesis become dominant.

Barrientos et al. (2002) had also studied the effect of intrahippocampal IL-1β administration on contextual memory. These authors demonstrated that IL-1β impaired the processes that support the storage of the contextual representation. They also studied the time course of IL-1β effect on memory consolidation. IL-1β administration in dorsal hippocampus immediately, 3 or 24 h after context exposure produces impairment of contextual fear conditioning. Although there are differences between both experimental models, it is tempting to discuss their and our results in relation to the time course of IL-1β effect in the same context.

Therefore, the effect of IL-1β we described in the first 15 min after learning could be related to the early phase LTP that involves glutamate release, NMDA receptors and AMPA receptors phosphorylation and MAPKs activation (among other processes) which had been proved to be altered by IL-1β (Vereker et al., 2000; Kelly et al., 2003; Viviani et al., 2003; Lai et al., 2006).

At around 3 h, the time interval studied by Barrientos et al. (2002), later phase processes requiring protein synthesis, such as neurotrophin brain derived neurotrophic factor (BDNF) become dominant and could also be altered by IL-1β.

We described that IL-1β injected 12 h after conditioning also altered the memory for the context, in a time point that coincide with a new BDNF synthesis in hippocampus (Bekinschtein et al., 2007).

Barrientos et al. also described that IL-1β produced memory impairment when administered 24 h after context pre-exposure.

In conclusion, intra-hippocampal IL-1β interferes with memory consolidation at different intervals and this will have implications concerning the mechanisms by which IL-1β acts on memory formation and storage.

As mentioned, BDNF is an interesting candidate as a potential mediator downstream from IL-1β. BDNF has been shown to be critical for middle- and late-phase events involved in synaptic plasticity. Thus, BDNF is critical for hippocampal-dependent memory consolidation. It is rapidly induced in the hippocampus during learning and memory tasks that require intact hippocampal function such as conditioning and is necessary for at least 4 h after training to form a long-term memory (Tyler et al., 2002). BDNF is also required for LTP induction (Pang et al., 2004). Consequently, reductions in hippocampal BDNF lead to both inhibition of LTP and hippocampal-dependent memory impairments. It has been demonstrated that intrahippocampal IL-1β administration following conditioning, a task known to increase BDNF mRNA in rats, blocked or reduced these increases in BDNF mRNA in different regions of hippocampus (CA1, CA2 and dentate gyrus), but had no effect on cortical regions (Barrientos et al., 2004). These data suggest that memory deficits induced by IL-1β may be mediated via BDNF. Recently, early infection was also demonstrated to influence induction of IL-1β and BDNF, which could influence memory impairments in adulthood (Bilbo et al., 2008). Another characteristic attribute of long-term memory is persistence. Recent findings indicate that a delay stabilization phase is specifically required for maintenance or persistence, but not formation, of the memory trace. These new approaches propose that memory formation and memory persistence share some of the same molecular mechanisms and that recurrent rounds of consolidation-like events take place in the hippocampus for maintenance of the memory trace. Consequently, this hypothesis predicts that there should be recurrent time windows of susceptibility in the consolidation processes over hours, days, or weeks. In this context it has been demonstrated that 12 h after acquisition there is novel protein synthesis and a BDNF-dependent phase in the rat hippocampus, critical for the persistence of long-term memory storage (Bekinschtein et al., 2007). Interestingly, treatment with IL-1β 12 h after acquisition, coincident with the delay wave of synthesis of BDNF, interferes with persistence of the long-term memory.

These results indicate that IL-1β can modulate different phases of memory and point again to BDNF as a possible mediator of the cytokine effect.

Here we demonstrated that the melanocortin α-MSH significantly protects against impairment in memory caused by IL-1β administration. This clear neuroprotective effect occurring at low doses is probably mediated by brain melanocortin MC4 receptors. Melanocortins are widely distributed in the CNS and MC4 receptors have been found in various brain areas including the hippocampus (Getting, 2002).

Administration of HS014, a selective MC4R antagonist, reversed the effect of α-MSH on the impairment of memory consolidation induced by IL-1β suggesting that α-MSH may exert this effect by activating central MC4R. In order to further investigate the melanocortin receptor involved, and considering that MC3R is also present in the limbic system, we administered γ-MSH, a selective MC3R agonist frequently used to distinguish between MC3R and MC4R, together with selective MC4R antagonists available. In the present study, at the dose tested, γ-MSH administration was unable to reverse IL-1β effect on memory consolidation, adding support to MC4R involvement. In the present study, α-MSH and γ-MSH administration had no effect on conditioning per se.

It has been demonstrated that the effect of IL-1β on LTP was mediated by p38 activation (Vereker et al., 2000). α-MSH was shown to inhibit activation of p38 MAPK in different experimental models. For example, α-MSH inhibited LPS-induced activation of p38 kinase in leukocytes (Yoon et al., 2003). The melanocortin analogue NDP-MSH also inhibited p38 MAPK activation in the brain after transient forebrain ischemia (Giuliani et al., 2006). Therefore, it could be suggested that the suppressant effect of α-MSH on IL-

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1β-induced memory impairment could be mediated by inhibition of p38 MAPK.

In conclusion, we confirm that IL-1β has a detrimental effect on consolidation of contextual memory. We also demonstrated that this cytokine can interfere with persistence of the memory and that α-MSH through activation of MC4R could reverse the effect of IL-1β on consolidation of a fear memory.

Acknowledgments

This work was supported by grants from SeCyT (Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and FONCYT. Dr. Mercedes Lasaga and Dr. Teresa N. Scimonelli are career investigators from CONICET. Patricia Gonzalez has a fellowship from CONICET.

We are grateful to Estela Salde for her technical assistance.

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