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A role for ERK2 in reconsolidation of fear memories in mice

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Abstract

Recent studies have shown that consolidated fear memories, when reactivated, return to a labile state that requires a new protein synthesis for reconsolidation. Post-retrieval infusion of an inhibitor of protein synthesis blocks memory reconsolidation processes. In a previous research, the role of MAPKs in memory consolidation has been shown in emotional tasks, such as passive and active avoidance. In particular, mice knockout for ERK1 had a better performance in comparison to wild type mice in both passive and active avoidance tasks. In the present study, in order to investigate the involvement of MAPKs in memory reconsolidation processes we administered immediately after retrieval, different doses of SL327 (an inhibitor of MEK, a kinase that activates both ERK1 and ERK2) both in C57BL/6 (C57) mice and ERK1 mutant mice tested in a fear conditioning task. Systemic administration of SL327 dose-dependently reduced the memory reconsolidation of fear memories in C57 mice. Moreover, SL327 administration impaired memory reconsolidation also in ERK1 mutant mice. Altogether, these results clearly indicate a central role for ERK2 protein in memory reconsolidation processes in mice.

Keywords: MEK inhibitor; SL327; Ras/MAPK cascade; Cued fear conditioning; C57BL/6 strain; ERK1 knockout mice; Memory reconsolidation; Memory consolidation

1. Introduction

New information is stored in long-term memory (LTM) through a consolidation process (Muller & Pilzeccker, 1900) involving molecular and structural modification of neurons (Abel & Lattal, 2001; McGaugh, 1966, 2000; Miller & Matzel, 2000). Post-training administration of drugs, such as receptor agonists and protein synthesis inhibitors, modulate consolidation in rodents and indicates that LTM formation depends on new protein synthesis (Castellano, Cabib, & Puglisi-Allegra, 1996, for a review; Meiri & Rosenblum, 1998; Schafe, Nadel, Sullivan, Harris, & LeDoux, 2004; Schafe & LeDoux, 2000; Schafe et al., 1999).

It has recently been observed that consolidated memory traces can return to a labile state after retrieval and that a new process is required to reconsolidate previously learned information (Debiec, LeDoux, & Nader, 2002; Duvarci & Nader, 2004; Nader, 2003; Nader, Schafe, & LeDoux, 2000). A debate is ongoing as to whether memory consolidation and reconsolidation share the same neural mechanisms.

On the one hand, protein synthesis required for memory reconsolidation has a temporal window that parallels that of consolidation (Duvarci & Nader, 2004; Duvarci, Nader, & LeDoux, 2005; Igaz, Vianna, Medina, & Izquierdo, 2002; Inda, Delgado-Garcia, & Carrion, 2005; Nader et al., 2000). Moreover, the administration of different NMDA receptor antagonists disrupts memory consolidation (Castellano, Cestari, & Ciamei, 2001; Suzuki et al., 2004) as well as memory reconsolidation (Pedreira, Perez-Cuesta, & Maldonado, 2002; Przybyslawski & Sara, 1997; Suzuki et al., 2004). Further, mice transgenic for a repressor of CREB show a deficit both in memory consolidation and reconsolidation of a fear conditioning task (Kida et al., 2002), suggesting that the two processes involve similar neural mechanisms.

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On the other hand, intra-amygdala infusion of anisomycin affects consolidation and extinction, but not reconsolidation, of a conditioning taste aversion task in rats, indicating that different neural mechanisms might underlie these processes (Bahar, Dorfman, & Dudai, 2004). Moreover, Lee, Everitt, and Thomas (2004) administered either BDNF or Zif268 antisense oligodeoxynucleotides in the hippocampus of rats submitted to a fear conditioning task and their data suggest that the two processes require independent cellular pathways. Further, the inhibition of C/EBPβ in the rat hippocampus impaired consolidation but not reconsolidation in an inhibitory avoidance task (Taubenfeld, Milekic, Monti, & Alberini, 2001). However, since in rodents the performance in this task requires not only the hippocampus but also other structures, such as the amygdala (Bonini, Cammarota, Kerr, Bevilaqua, & Izquierdo, 2005; Brambilla et al., 1997; Cestari, Mele, Oliverio, & Castellano, 1996; Liang et al., 1982; Parent & McGaugh, 1994; Roozendaal & McGaugh, 1996), the possibility remains that, in brain areas other than the hippocampus, the same intracellular pathways are involved in both processes.

For the Ras/MAPK pathway, that has a central role in learning and memory processes (Adams & Sweatt, 2002; Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998; Brambilla et al., 1997; Chen et al., 2006; Mazzucchelli et al., 2002; Orban, Chapman, & Brambilla, 1999; Selcher, Atkins, Trzaskos, Paylor, & Sweatt, 1999; Selcher, Nekrasova, Paylor, Landreth, & Sweatt, 2001; Walz, Roesler, Barros, et al., 1999; Walz, Roesler, Quevedo, et al., 1999; Walz et al., 2000), there are reports indicating a possible involvement both in consolidation and reconsolidation. Administration of MEK inhibitors impaired both consolidation and/or reconsolidation of emotional memories (Atkins et al., 1998; Duvarci et al., 2005; Schafe et al., 2000; Selcher et al., 1999; Walz, Roesler, Barros, et al., 1999; Walz, Roesler, Quevedo, et al., 1999; Walz et al., 2000) and of recognition memories (Kelly, Laroche, & Davis, 2003) in rats. However, the studies with pre- or post-training MEK inhibitor administration failed to differentiate the specific role of the two ERK proteins in both processes.

As concerns this point, it is very important to note that the induction of a null mutation for the ERK1 protein produces an up-regulation in the activity of ERK2 in the brain of ERK1 knockout mice. This biochemical profile correlates with an increase in the retention level of avoidance tasks in ERK1 knockout mice, suggesting a crucial role for ERK2 in learning and memory (Mazzucchelli et al., 2002).

Moreover, it has been observed that the retention level of ERK1 knockout mice, on a 98% of 129SvImJ background, submitted to a fear conditioning task did not differ from that of littermate control mice, suggesting that ERK2 plays a predominant role in the plastic changes accompanying emotional learning and that the ERK1 null mutation is not involved in the consolidation processes (Selcher et al., 2001).

On the basis of the above reported results, it seems worth of interest to further assess the role of each of the two ERK proteins (ERK1 and ERK2) in memory reconsolidation processes.

In the present study, we firstly investigated the effect of the post-reactivation systemic administration of the MEK inhibitor SL327 on memory reconsolidation processes in C57BL/6 (C57) mice submitted to a cued fear conditioning paradigm. Further, in order to better clarify the role of the ERK proteins in memory reconsolidation processes, we have combined the behavioural analysis of a line of null mutant mice for ERK1, on a C57BL/6 background, together with a pharmacological approach using the administration of SL327. In this context, different groups of ERK1 mutant mice have been submitted to the experimental procedure used with C57 mice.

2. Materials and methods

2.1. Subject

Male C57BL/6 (C57) (Charles River Labs, Como, Italy) aged 8 weeks and weighing 25–30 g were used in the first and second experiments. Upon arrival in the laboratory mice were housed in groups of four in standard breeding cages (21 × 21 × 12 cm).

In the third experiment, ERK1 mutant mice were used, generated as F2 littermates of a strain backcrossed in C57BL/6 background for five generations. The animal genotypes were assessed by PCR.

All mice were kept in a 12-h light/12-h dark cycle (light were on from 07:00 to 19:00) at constant temperature of 21 °C, given food and water ad lib and tested during the second half of the light period (between 14:00 and 17:00 h). The research was carried out according to Italian National laws and regulations on the use of animals in research and NIH guidelines on animal care.

2.2. Drugs

The α-[amino[(4-aminophenyl)thio]methylene]-2-(trifluoromethyl)- benzeneacetonitrile (SL327) (Tocris Cookson Ltd., UK), a selective MEK inhibitor, was administered intraperitoneally (i.p.). The drug was dissolved in dimethyl sulfoxide (DMSO) in a volume of 2 ml/kg and injected at different doses (0, 5, 10, and 50 mg/kg).

2.3. Apparatus

All experiments were carried out in two different chambers.

2.3.1. Chamber A

All mice were trained in the conditioning chamber A (26 × 22 × 18 cm) made of transparent Plexiglas with a grid metal floor and located in a sound-insulated box lighted by a tensor lamp (60 W). A micro-video camera was mounted at the top of the box and the animals were videotaped during the test.

2.3.2. Chamber B

In both retrieval and test sessions, all mice were placed in chamber B. This chamber had a black Plexiglas floor of triangular shape (26 × 22 × 34 cm) and the walls (high 18 cm) were made of the same material of the floor. A blue tensor lamp (60 W) illuminated the sound-insulated box in which the conditioning chamber was located. Moreover, a vanilla essence odour was placed inside the conditioning chamber to further modify the environment.

2.4. Experimental procedures

All mice were trained and tested for auditory fear conditioning assessed by freezing. Mice were considered to be freezing when they