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Polyaminergic agents modulate the reconsolidation of conditioned fear

Daniela Aymone Ribeiro [a], Carlos Fernando Mello [b,1], Cristiane Signor [a], Maribel Antonello Rubin [a,*,1]

[a] Department of Chemistry, Center of Exact and Natural Sciences, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil
[b] Department of Physiology and Pharmacology, Center of Health Sciences, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil

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ABSTRACT

When consolidated memories are reactivated, they become labile and, to persist, must undergo a new stabilization process called reconsolidation. During reactivation, memory is susceptible to pharmacological interventions that may improve or impair it. Spermidine (SPD) is an endogenous polyamine that physiologically modulates the N-methyl-D-aspartate (NMDA) receptor in mammals by binding on the polyamine-binding site at the NMDA receptor. While polyamine agonists and antagonists of the polyamine binding site on the NMDA receptor respectively improve and impair early consolidation, it has not been defined whether these agents alter memory reconsolidation. Male Wistar rats were trained in a fear conditioning apparatus using a 0.4 mA footshock as unconditioned stimulus. Twenty four hours after training, animals were re-exposed to the apparatus in the absence of shock (reactivation session). Immediately after the reactivation session, SPD (1–30 mg/kg, i.p.) or the antagonist of the polyamine-binding site at the NMDA receptor, arcaine (0.1–10 mg/kg, i.p.), were injected, and the animals were tested in the same apparatus 24 h later. Freezing scores at testing were considered a measure of memory. While SPD (3 and 10 mg/kg) improved, arcaine (1 and 10 mg/kg) impaired memory reconsolidation. These drugs had no effect on memory if they were administered in the absence of reactivation, or 6 h after reactivation session. Arcaine (0.1 mg/kg, i.p.) prevented SPD (3 mg/kg)-induced improvement of memory reconsolidation. Accordingly, SPD (1 mg/kg) prevented arcaine (10 mg/kg)-induced impairment of memory reconsolidation. The amnesic effect of arcaine was not reversed by arcaine administration prior to test, ruling out state dependence in this effect. These results suggest that systemic administration of polyamine binding site ligands modulate memory reconsolidation.

1. Introduction

Memories are not formed instantaneously. After learning (acquisition), a series of molecular and cellular changes occur in different brain regions, such as hippocampus, striatum and amygdala, that lead to a progressive memory stabilization (McGaugh, 2000). This process, which initiates immediately after learning and is time-dependent, has been named "consolidation" (Dudai, 2004; McGaugh, 1966, 2000). During consolidation, memories are labile and susceptible to positive or negative modulation by different means, including pharmacological agents (McGaugh, 1966, 2000). For decades, prevailed the general belief that consolidated memories could not be modified (McGaugh, 2000). Several studies, however, have shown that reactivating or retrieving consolidated memories renders them labile again, thus requiring a new stabilization process, called reconsolidation (Dudai, 2004; Nader, Schafe, & Le Doux, 2000; Sara, 2000).

Different neurotransmitter systems have been implicated in memory reconsolidation (Nader & Hardt, 2009; Sara, 2000; Tronson & Taylor, 2007). Notwithstanding, convincing pharmacological and neurochemical evidence supports that glutamate NMDA receptors, particularly those containing a NR2B subunit, play a major role in this process (Wang, de Oliveira Alvares, & Nader, 2009). Accordingly, while NMDA receptor antagonists MK-801, AP5 and ifenprodil disrupt, the NMDA receptor agonist D-cycloserine improves fear memory reconsolidation in mice and rats (Ben Mamou, Gamache, & Nader, 2006; Lee, Milton, & Everitt, 2006; Przybyslawski & Sara, 1997; Suzuki et al., 2004). In this regard, it is worth noticing that spermidine (SPD) and spermine, endogenous polyamines that bind to and modulate NR2B subunit activity, have been implicated in memory acquisition and consolidation (Johnson, 1996; Shimada, Spangler, London, & Ingram, 1994; Williams, 1997; Williams, Romano, Dichter, & Molinoff, 1991).

Current evidence suggests that polyamines modulate consolidation by interacting with the polyamine-binding site on the NMDA receptor (Kishi, Ohno, & Watanabe, 1998; Rubin et al., 2004, 2000, 2001; Shimada et al., 1994). In line with this view, the systemic (Camera, Mello, Ceretta, & Rubin, 2007), intrahippocampal (Berlese

* Corresponding author. Fax: +55 55 3220 8978.
E-mail address: maribel.rubin@gmail.com (M.A. Rubin).
[1] These authors contributed equally to this work.

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et al., 2005; Gomes et al., 2010; Guerra et al., 2006; Rubin et al., 2000), and intra-amygdalar (Rubin et al., 2004, 2001) administration of SPD improves the memory of different tasks in rats. SPD-induced memory facilitation of the inhibitory avoidance task involves the sequential activation of PKC and PKA/CREB pathways in the hippocampus (Guerra et al., 2011, 2012).

Although the involvement of NR2B-containing NMDA receptors in memory reconsolidation has been shown, no study has addressed whether polyamines, endogenous agonists of these receptors, modulate memory reconsolidation. Therefore, in the current study we investigated whether SPD and arcaine, respectively an agonist and an antagonist of the polyamine binding site on the NMDA receptors, alter fear memory reconsolidation.

2. Materials and methods

2.1. Animals

Experimentally naive male Wistar rats (260–360 g), from the animal house of the Federal University of Santa Maria were used. The animals were housed four to a cage on a 12-h day/night cycle (lights on at 7:00 a.m.) at a temperature of 21 °C with water and standard laboratory chow (Guabi, Santa Maria, Rio Grande do Sul, Brazil) *ad libitum*. All experimental procedures were conducted during the light phase of the cycle (from 11:00 a.m. to 4:00 p.m.). All experimental procedures were conducted in accordance with the policies on the use of animals and humans in neuroscience research, revised and approved by the Society for Neuroscience Research in January 1995 and with the institutional and national regulations for animal research (process 068/2011).

2.2. Drugs

Animals were injected with saline (0.9% NaCl), 1,4-diguanidinobutane sulfate (arcaine; Pfaltz & Bauer, Waterbury, CT, USA), or N-(3- aminopropyl)-1.4-butanediamine trihydrochloride (spermidine; Sigma, St. Louis, MO). All drugs solutions were prepared daily in saline and injections were performed intraperitoneally (i.p.) in a 1 ml/kg injection volume. Doses were selected based on previous studies (Camera et al., 2007) and pilot experiments.

2.3. Conditioning apparatus

Contextual fear conditioning training, reactivation and test took place in a fear conditioning chamber (30 × 25 × 25 cm), located in a well-lit room. The front wall and ceiling of the chamber were made of clear acrylic plastic, whereas the lateral and rear walls were made of opaque plastic. The floor of the chamber consisted of 32 stainless steel rods (3 mm diameter), spaced 1 cm apart and wired to a shock generator. The chamber was cleaned with 30% ethylic alcohol before and after each rat occupied it.

2.4. Behavioral procedure

2.4.1. Contextual fear conditioning

In the conditioning trial each animal was subjected to a single fear-conditioning training session, as described by Rubin et al. (2004), with some modifications. In brief, the rat was placed in the conditioning chamber (conditioned stimulus, CS) and habituated to the apparatus (CS) for 3 min. Immediately after habituation, three 1 s, 0.4 mA footshocks (unconditioned stimulus, US) were delivered. The shocks were 40 s apart. After the last CS/US pairing, rats were allowed to stay in the chamber for additional 60 s before returning to their home cages.

2.4.2. Reactivation session

Twenty four hours after the conditioning session, the rats were re-exposed to the conditioning apparatus for 3 min, but no footshocks were delivered. During this time, the rat was observed every 4 s to assess whether it was in freezing, or not, by a trained observer who was unaware of the experimental treatment conditions. Behavior was judged as freezing if there was an absence of any visible movement, except for that required for breathing. Data were converted to the percentage of samples scored as freezing.

2.4.3. Test session

Twenty-four hours after reactivation, each rat was placed back in the conditioning chamber and a 6-min test was performed. During this time, the rat was observed every 4 s to assess whether it was in freezing, or not, as described above, and data were converted to the percentage of samples scores as freezing.

2.5. Experimental groups

2.5.1. Experiment 1

This experiment was designed to investigate the effect of SPD on memory reconsolidation. Animals were trained in the fear conditioning apparatus, as described above. Twenty-four hours later, the animals were subjected to the reactivation session. Immediately after reactivation session, the animals were injected with saline or SPD (1, 3, 10 or 30 mg/kg) and, 24 h later, tested in the fear conditioning apparatus where their freezing responses were scored, as described above.

2.5.2. Experiment 2

This experiment was designed to investigate the effect of arcaine on memory reconsolidation. Animals were trained in the fear conditioning apparatus, as described above. Twenty-four after training, the animals were subjected to the reactivation session. Immediately after reactivation session, the animals were injected with saline or arcaine (0.1, 1 or 10 mg/kg) and, 24 h later, tested in the fear conditioning apparatus where their freezing responses were scored, as described above.

2.5.3. Experiment 3

To evaluate whether the systemic administration of SPD and arcaine are specific for reconsolidation of contextual fear memories two control experiments were performed. In the first, animals were trained in the fear conditioning apparatus, as described above, but were not subjected to the memory reactivation session 24 h later (“no reactivation” control). The animals were injected with saline, SPD (3 mg/kg) or arcaine (10 mg/kg) 24 h after training and, 24 h later, were tested in the fear conditioning apparatus and had their freezing responses scored, as described above. The doses of spermidine and arcaine used in this experiment were chosen based on the dose-response curve experiments (Experiments 1 and 2), which determined fully effective and non-effective doses for both compounds.

In order to confirm the specificity of enhanced or disrupted reconsolidation by SPD and arcaine, respectively, a second control experiment was performed. The animals were trained in the fear conditioning apparatus and 24 h later they were subjected to the reactivation session, as described above. Six hours after reactivation session (“delayed infusion” control), the animals were injected with saline, SPD (3 mg/kg) or arcaine (10 mg/kg). Twenty-four hours after reactivation, the animals were tested in the fear conditioning apparatus and their freezing responses were scored, as described above.