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The intrahippocampal infusion of crotamine from *Crotalus durissus terrificus* venom enhances memory persistence in rats

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ABSTRACT

Previous research has shown that crotamine, a toxin isolated from the venom of *Crotalus durissus terrificus*, induces the release of acetylcholine and dopamine in the central nervous system of rats. Particularly, these neurotransmitters are important modulators of memory processes. Therefore, in this study we investigated the effects of crotamine infusion on persistence of memory in rats. We verified that the intrahippocampal infusion of crotamine (1 μg/μl; 1 μl/side) improved the persistence of object recognition and aversive memory. By other side, the intrahippocampal infusion of the toxin did not alter locomotor and exploratory activities, anxiety or pain threshold. These results demonstrate a future prospect of using crotamine as potential pharmacological tool to treat diseases involving memory impairment, although it is still necessary more researches to better elucidate the crotamine effects on hippocampus and memory.

1. Introduction

Snake venoms are natural biological resources that contain several components of potential therapeutic value (Koh et al., 2006). The venom of the South American rattlesnake *Crotalus durissus terrificus* (Cdt) has long been known to induce neurotoxicity at both central (Habermann and Cheng-Raude, 1975; Mello and Cavalheiro, 1989) and peripheral nervous system (Dal Belo et al., 2013), in which crotamine plays an important role (Dal Belo et al., 2013). Crotamine is a non-enzymatic polypeptide myotoxin, composed by 42 amino acid residues with a molecular weight of 4880 Da (Mancin et al., 1998). At mioneural regions, crotamine induces depolarization of skeletal muscle membrane by increasing the permeability to sodium ions (Na^+), suggesting that it binds to voltage-dependent Na^+ channels in the sarcolemma (Ruff and Lennon, 1998). Crotamine is also thought a cell-penetrating peptide-mediated delivery drug and an anticancer agent (Kerkis et al., 2010). Recently, it has been demonstrated the beneficial of crotamine in the treatment of myastenic rats (Hernandez-Oliveira e Silva et al., 2013), reinforcing the potential therapeutic application of this toxin. Other activities ascribed to crotamine include an increase in the cytosolic Ca^{2+} through activation of ryanodine-sensitive

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Ca^{2+} channels and an improvement in the basal release of acetylcholine and dopamine in the rat central nervous system (CNS) (Camillo et al., 2001).

Acetylcholine is a neurotransmitter strikingly involved in learning, memory and attention processes, participating in the encoding of new information (Doralp and Leung, 2008; Robinson et al., 2011). The dopaminergic system also plays a critical role in the modulation of neuronal activity being involved in different forms of learning and memory (Jay, 2003; Rossato et al., 2013) and may alter the ability of learning and of store information (Adriani et al., 1998). In the cognitive functioning of rodents, the combined effect of both cholinergic and dopaminergic systems is well documented (Wahlstrom et al., 2010; Klinkenberg et al., 2011; Newman et al., 2012). Behavioral studies have shown that dopamine can promote memory consolidation in the hippocampal system by facilitating cholinergic function (Levin and Rose, 1991; Hersi et al., 1995), being also important to promote memory persistence (Rossato et al., 2009). Furthermore, it is known that changes in these neuronal systems affect negatively the persistence of memory, which is somewhat impaired in some diseases such as, Alzheimer's and Parkinson's (Xu et al., 2012).

Considering the importance of these neurotransmitters in mnemonic processes, the impairment of these two systems in the view of several cognitive disorders of the CNS and the scarcity of the current therapeutic arsenal to treat them, it becomes important to study new potential substances that are suitable to modulate positively these processes. In the present study we demonstrate that intrahippocampal administration of crotamine promotes the persistence of aversive and object recognition memory in rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (3 months old) were bought from a registered vivarium. They were housed four per cage and maintained under controlled light and environmental conditions (12 h light/12 h dark cycle at a temperature of 23 ± 2 °C and humidity of 50 ± 10%) with food and water *ad libitum*. All experiments were conducted in accordance with the "*Principles of laboratory animal care*" (NIH publication n° 80-23, revised 1996) and with the guidelines established by the Institutional Animal Care and Use Committee of the Local Institution (IRB #0442012), ensuring that animal numbers and suffering were kept to a minimum.

To study the effects of crotamine on memory persistence, 40 rats were implanted with chronic bilateral guide cannulas in CA1 region of hippocampus and divided into two groups: controls ($n = 20$), which received 1 μl/side of vehicle (saline), and crotamine ($n = 20$), which received 1 μl/side of crotamine infusion (1 μg/μl) after training in the behavioral procedures described hereafter (Fig. 1). CA1 region of hippocampus was chosen considering its important role in memory processes (Harooni et al., 2009). Retention tests were conducted 24 h after training and persistence tests 7, 14 and 21 days after training. Afterward, animals from all groups were euthanized for postmortem verification of cannulas' placement.

2.2. Drugs

Ketamine and xylazine were supplied by Sigma Aldrich Brazil (São Paulo, SP, Brazil). Crotamine isolated from Cdt venom was a reminiscent stock and was kindly donated by Dr. Sérgio Marangoni from Laboratory of Protein Chemistry (LAQUIP) (UNICAMP, Campinas-SP, Brazil) and was prepared daily by dilution in saline immediately before use. The crotamine dose was chosen based on previous in vitro experiments (Camillo et al., 2001), followed by pilot experiments.

2.3. Surgery and drug infusion procedures

In order to implant the rats with indwelling cannulas, they were deeply anesthetized with ketamine and xylazine

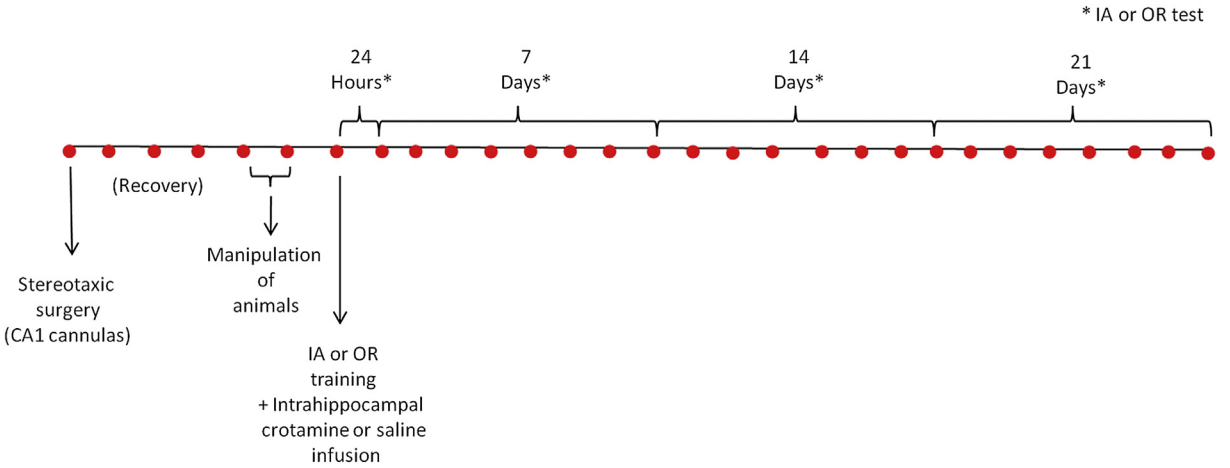

Fig. 1. Schematic drawing of the behavioral experimental design. The rats were submitted to a stereotaxic surgery to implant cannulas on CA1 region of hippocampus. After 4 days of recovery, they are manipulated for 2 days. Then, one group of rats was training in Inhibitory Avoidance (IA; $n = 20$) and other in Object Recognition (OR; $n = 20$) memory task. Immediately after training, the rats received an intrahippocampal infusion of crotamine or saline ($n = 10$/group for IA and 10/group for OR). In the followed days they are submitted to test sessions: 24 h, 7, 14 and 21 days after training. In the figure, each point represents one day.