Differential involvement of glutamatergic and catecholaminergic activity within the amygdala during taste aversion retrieval on memory expression and updating

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Abstract

During memory retrieval, consolidated memories are expressed and destabilized in order to maintain or update information through a memory reconsolidation process. Despite the key role of the amygdala during memory acquistion and consolidation, the participation of neurotransmitter signals in mem-ory retrieval is poorly understood. Hence, we used conditioned taste aversion and in vivo microdialysis to evaluate changes in glutamate, norepinephrine and dopamine concentrations within the amygdala during memory retrieval. We observed that exposure to an aversive-conditioned stimulus induced an augmentation in glutamate, norepinephrine and dopamine levels within the amygdala, while exposure to a familiar and safe stimulus did not induce changes in these neurotransmitters levels. Also, we evaluated the amygdalar blockade of -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-d-aspartate (NMDA), -adrenergic and dopamine D1 receptors in memory retrieval and updating. Results showed that during retrieval, behavioural expression was impaired by intra-amygdalar blockade of AMPA and -adrenergic receptors, whereas NMDA, D1 and -adrenergic receptors blockade hindered memory updating. In summary, during conditioned taste aversion retrieval there was an increase in the extracel-lular levels of glutamate, norepinephrine and dopamine within the amygdala, and their receptors activity were differentially involved in the behavioural expression and memory updating during retrieval.

Keywords:Retrieval; Glutamate; Dopamine; Norepinephrine; Memory updating; Microdialysis

INTRODUCTION

In conditioned taste aversion (CTA), animals associate a novel taste with gastric malaise; this association produces taste aver-sion measured as a decrease in the consumption of the taste in further presentations [3]. The amygdala is highly involved in the acquisition, consolidation and retrieval of CTA (see Ref. [18]. We have demonstrated that during the acquisition of CTA, exposure to a novel taste stimulus induces an increase in the extracellu-lar levels of norepinephrine (Guzmán-Ramos et al. [9], whereas induction of gastric malaise promotes an augmentation of extracel-lular levels of glutamate and norepinephrine within the amygdala [22,13,9]. Although, there is scarce information about amygdala neurotrasmitters relase during CTA retrieval, it has been shown that exposure to aversive-conditioned saccharin induces an augmenta-tion of glutamate within the amygdala [22]. However, it remains unknown whether catecholamines could follow similar extracellu-lar augmentations as glutamate during memory retrieval.

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Memory is consolidated over time through a protein synthesis-dependent process. Furthermore, during memory retrieval, consolidated memories become labile and require similar protein-synthesis processes to maintain or update memories, a process named memory reconsolidation [16,5]. However, it has been shown that inhibition of protein synthesis in the amygdala has no effect on CTA memory reconsolidation when a single acquisition trial is used [1]. Thus, we have proposed that multi-trials protocol induces strong CTA memory and its extinction is delayed on subsequent presentations, facilitating the observation of memory reconsoli-dation process and updating [20,5]. Using this model, we have demostrated that AMPA receptor antagonists impair behavioural expression during retrieval without affecting memory reconsoli-dation [20,6]. Whereas infusions of protein synthesis inhibitors or an NMDA receptor antagonist in the amygdala disrupt CTA mem-ory reconsolidation without affecting behavioural expression on retrieval [20,6]. In regard of catecholaminergic activity within the amygdala, there is scarce information about memory retrieval. Nevertheless it has been demonstrated that norepinephrine antag-onists impaired reconsolidation of aversive memories induced by morphine [23], and dopaminergic activity seems to be required for taste-rewarded memory reconsolidation [12].

In this study, we used in vivo microdialysis in order to evaluate changes in glutamate, norepinephrine and dopamine levels within the amygdala during memory retrieval. Moreover, to analyse the functional role of NMDA, AMPA, -adrenergic and D1 receptors in CTA memory retrieval and updating, we used selective antagonists in the amygdala before a second CTA acquistion trial.

Adult male Wistar rats from the Instituto de Fisiología Celular were anesthetized with a ketamine–xylazine mixture (100–10 mg/kg) and a unilateral guide cannula (CMA Microdial-ysis. Stockholm, SE) aiming at the amygdala was implanted using standard stereotaxic procedures, right an left hemispheres were counterbalanced. The guide cannula was implanted with coordi-nates from Bregma (AP—2.8 mm; L 4.8 mm; DV—7.5 mm) [17], and was fixed to the skull using two screws and dental acrylic cement. All procedures were approved by the institutional committee for the care and use of laboratory animals of the Instituto de Fisiología Celular (FBR25-14) which is based on National Institutes of Health Guide for the Care and Use of Laboratory Animals. Behavioural scheme began 6 days after surgery allowing the animals to recover. Rats were then water-deprived 24 h prior behavioural scheme. Ani-mals were habituated in a microdialysis chamber once a day for 1 h and were allowed to drink 30 mL of tap water from a graded bottle during 15 min, water baseline consumption intake was established over 6 days. A second drinking session in the afternoon served to prevent dehydration. On the seventh day, rats were separated in two groups, an aversively conditioned group (Aversive, n = 10), which was exposed for 15 min to 30 mL of a 0.1% (wt/vol) sodium saccharin solution (Sigma-Aldrich. Missouri, US) and fifteen min-utes later rats received an i.p. LiCl (Baker. New Jersey, US) injection (0.2 M, 7.5 mL/kg, this concentration induces a robust CTA, see Ref.[19]. In the control group (Non-Aversive, n = 8), rats drank the same saccharin solution, paired with an i.p. NaCl (Sigma-Aldrich. Missouri, US) injection (0.2 M, 7.5 mL/kg). NaCl does not cause gas-tric malaise and therefore no taste aversion would be developed. Three days after training, microdialysis procedure was performed in both groups by the insertion of 1 mm length membrane dialy-sis probe (CMA 12 MD Probe, CMA Microdialysis. Stockholm, SE) connected to the micro-infusion pump system. (CMA Microdialy-sis. Stockholm, SE). The pump perfused the probe continuously at a rate of 0.8 L/min with Ringer solution (NaCl 118 mM, KCl 4.7 mM, $NaH_2PO_4H_2O$ 1.2 mM, $MgSO_47H_2O$ 1.2 mM, $NaHCO_3$ 19 mM, $CaCl_2$ 2.5 mM, glucose 3.3 mM). After probe insertion, the first hour of sampling was discarded due to fluid stabilization; samples were collected every 5 min (4 in vials containing 1 of antioxidant mixture (0.25 mM ascorbic acid, Na2EDTA 0.27 mM, 0.1 M acetic acid). The first three samples were used to calculate the basal concentration of extracellular neurotransmitters; afterwards, a graded bottle with 0.1% (wt/vol) sodium saccharin solution was placed in the microdialysis chamber for 15 min and consumption intake was measured.

Neurotransmitter concentrations were determined by capillary electrophoresis as described in Ref. [8]. Capillary electrophoresis-based separations with laser induced fluorescence detection were used for the analysis (Beckman-Coulter PACE/ MDQ, Glycopro-tein System). In order to identify glutamate, norepinephrine and dopamine, we matched the obtained electropherograms with a spiked sample. Samples were corrected by relating the area under the curve of the unknown sample with the area under the curve of the internal standard. Analyses were performed with the Karat System Gold software (Beckman Coulter. California, US). Results are expressed as percentage of baseline concentration (% Baseline concentration = analyte concentration × 100/mean of the three first samples).

A repeated measures ANOVA indicated a significant interaction between time and group ($F(7,98) = 2.171$ $p < 0.05$) in the extracellular levels of glutamate. The Non-aversive group remained without changes among time in glutamate levels within the amygdala (p = NS), whereas the Aversive group showed significant differences when the conditioned stimulus was present at the 25 min fraction ($p < 0.01$). Exposure to the aversive-conditioned gustatory stimulus induced a significant augmentation of glutamate within the amyg-dala at the 25 min fraction between Aversive and Non-Aversive group ($p < 0.05$).

During CTA retrieval, a repeated measures ANOVA showed a significant interaction between time and group ($F(7,63) = 2.121$ $p < 0.05$) in the concentration of norepinephrine (Fig. 1B) within the amyg-dala. Post hoc tests indicated that in the Aversive group, there were differences in norepinephrine levels compared to baseline extra-cellular levels ($p < 0.05$) and also there were differences between groups ($p < 0.05$) at minutes 20 and 25 when the conditioned stim-ulus is present.

We also observed a significant main effect of the group on the extracellular levels of dopamine (Fig. 1C) within the amyg-dala during CTA retrieval ($F(1118) = 4.172$ $p < 0.05$). Furthermore, we did not observe changes in extracellular levels of dopamine between time ($F(7118) = 0.838$ p = NS), neither a time-group inter-action ($F(7118) = 1.222$ p = NS). The post hoc test revealed that the Aversive group showed changes in dopamine levels compared to baseline concentration ($p < 0.01$) and also compared to Non-Aversive groups at minute 25. Student's non-paired statistical analysis showed that the Aversive group produced a significant and clear taste aversion, while the Non-Aversive group failed to elicit reliable CTA ($t = 4.343$, $p < 0.01$; Fig. 1D).

According to these results, when animals are exposed to saccha-rin previously paired with gastric malaise, there is a reduction in saccharin consumption and there is an augmentation in the extra-cellular levels of glutamate, norepinephrine and dopamine in the amygdala compared to Non-Aversive group animals.

In order to evaluate the functional role of NMDA, AMPA, -adrenergic or D1 receptors in the behavioural expression and memory updating during CTA memory retrieval, rats were implanted bilaterally with 12 mm long stainless-steel guide cannu-lae (23 gauges) directed to the amygdala (AP—2.8 mm, L ± 4.8 mm, DV—6.5 mm relative to Bregma). Animals were deprived of water for 24 h and baseline water consumption intake was established over 6 days. Animals were handled for 3 min a day until the infusion day to diminish handling-associated stress. During CTA acquisition, rats were exposed to a 0.1% (wt/vol) saccharin solution for 15 min, and 15 min later they received a 0.2 M LiCl i.p. injection (7.5 mL/kg). Seventy-two hours after training, rats were divided in 5 groups counterbalanced according to their average saccharin consumption intake during CTA acquisition. All drugs were dissolved in saline solution (0.9% wt/vol). A volume of 1 (0.5 L/min) was injected per hemisphere in the amygdala; the injector was left for another minute to allow diffusion into the tis-sue. All microinjections were given 20 min before a second CTA acquisition trial. Memory was tested 24 h after the second CTA acquisition, in the test probe the animals were allowed to drink 30 mL of 0.1% (wt/vol) saccharin solution and the consumption intake was measured. Experimental groups according to drugs infusions were separated as follows: saline solution (SS, n = 10); dl-2-amino-5-phosphonovaleric acid (APV 10 n = 9); 6-cyano-7-nitroquinoxaline-2,3-dione disodium salt hydrate (CNQX 1 n = 9); Propranolol (PROP 5 , n = 9); and SCH-23390 (SCH 2 n = 11). Saccharin consumption intake during first CTA acquisition; second CTA acquisition and memory test are reported as percentage of mean consumption intake during the first CTA acquistion (% CTA 1 = saccharin solution intake ×100/mean saccharin intake during first CTA acquistion).

A repeated measures ANOVA was conducted to compare sac-charin consumption intake. There was a significant interaction between days and treatment ($F(8129) = 3.079$ $p < 0.01$). Saccharin consumption intake during the first CTA acquisition trial was similar in all groups (p = NS). Pharmacological treatments given 20 min before CTA2 acquisition trial, showed that blockade of AMPA (Fig. 2B; $p < 0.01$), and □-adrenergic (Fig. 2D; $p < 0.01$) receptors in the amygdala, hindered behavioural expression during retrieval compared to SS group. Conversely, infusions of APV (Fig. 2A) or SCH (Fig. 2C) within the amygdala did not affect behavioural expression in CTA2 (p = NS). During memory test, the CNQX group showed a reliable CTA similar to the SS group (p = NS). Contrarily, block-ade of NMDA ($p < 0.05$, Fig. 2A), □-adrenergic ($p < 0.05$, Fig. 2D) or dopamine D1 receptors ($p < 0.05$, Fig. 2C) impaired memory updat-ing.

After experiments, all animals were sacrificed and brains were removed. Coronal sections (40 □m) were obtained and stained with cresyl violet. We observed the placement of the microdialysis probes for the Aversive group and the Non-Aversive group in the amygdala region (Fig. 3A). Location sites for the injector tips were verified (Fig. 3B); despite the fact that in some cases the injection tip is located only in the central or basolateral amygdala, the volume of drug infused (1 □L) is sufficient to reach both nuclei.

These data support the idea that AMPA receptors in the amyg-dala play a key role on behavioural expression but are independent of memory updating [20,6]. Also, NMDA and dopamine D1 recep-tors are not necessary for behavioural expression but are involved in memory updating. Meanwhile, □-adrenergic receptors play a key role in the behavioural expression and CTA memory updating during retrieval.

According to the reconsolidation theory, memory retrieval has been considered an important process where stored information is susceptible to be strengthened in a multi trial protocol by infor-mation updating [5]. The exposure to a novel taste stimulus does not modify glutamate concentrations within the amygdala [13,7,9], even when the animals are exposed to a naturally aversive taste stimulus (quinine 0.005% w/v) [7]. In this work, exposure to an aversive-learned stimulus, elicits an augmentation in the extra-cellular levels of glutamate in the amygdala, similar to a previous report [22]. Thus, glutamate might be associated to the aversive learned value of the stimulus not observed during acquisition where the stimulus is only perceived as novel.

The behavioural expression of aversive tasks during retrieval has been associated with AMPA receptors activity in the amygdala [24,6], whereas NMDA receptors seem to be involved in memory consolidation and reconsolidation [8,6]. Accordingly, we observe that blockade of AMPA receptors within amygdala reduces the aversion displayed in retrieval while memory updating remains intact. On the other hand, an antagonist of NMDA receptors has no effect on behavioural expression but interrupts memory updating. In accordance, the AMPA antagonist disrupt behavioural expression without affecting updating, as previously reported García-Delatorre et al. [6]. However, we did not observe NMDA effects in memory reconsolidation as described by García-Delatorre and coworkers but in the consolidation of the second CTA acquis-tion (updating) instead. These differences could be explained due to the fact that the microinjections were done between central and basolateral nuclei of the amygdala, affecting both nuclei. Blockade of NMDA receptors in the central [6] and basolateral [21] amygdala had a strong effect in the updating of a second trial acquisition. As reported, when NMDA antagonists were infused in the basolateral amygdala a disruption of reconsolidation was observed, while the same manipulation in the central amygdala only hindered memory updating [6]

Exposure to a novel taste stimulus induces an augmentation of norepinephrine within the amygdala [9]. Here during CTA retrieval, there is an increase of norepinephrine within the amygdala only when animals are exposed to an aversive-conditioned stimulus. It is known that aversive stimuli elicit norepinephrine release in the amygdala [11,14]. In CTA retrieval, exposure to the aversive-stimulus is a stressful event because taste has been previously associated with a threat to the organism's health, a fact that is consistent with our observation of increased norepinephrine con-centration within the amygdala.

Involvement of receptors in memory retrieval and reconsolidation has remained poorly understood. Already it has been demonstrated that infusions of receptors antagonists hindered expression of fear memories in, amygdala, entorhinal, parietal and anterior cingulate cortices [2]. Here, our data show that infusions of propranolol in the amygdala before a second CTA acquisition impairs behavioural expression of CTA during retrieval and hinders memory updating. Similarly, involvement of Ç-adrenergic receptors of the basolateral amygdala has been demonstrated during reconsolidation of aversive memories induced by morphine [23]. It is clear from the results presented herein that norepinephrine is involved in the behavioural expres-sion and updating of taste aversive memories. In this regard, new and aversively gustatory stimuli are signaled by norepinephrine within the amygdala [9] and it would be possible that blockade of ß-adrenergic activity impedes taste recognition affecting retrieval and a subsequent memory strengthening.

During retrieval of an aversive stimulus, we observe that there is an increase in dopamine extracellular levels within the amyg-dala. Interestingly, when the stimulus is familiar and non-aversive, we do not observe changes in dopamine levels. In this regard, previous exposure to a flavor decreases dopamine changes in the nucleus accumbens when the flavor is re-exposed [4], suggesting that salience has diminished. Our data suggest that presentation of an aversive stimulus is very salient information as a result of life-threatening situations increasing dopamine levels within the amygdala.

In this study, intra-amygdalar infusions of a dopamine D1 receptor antagonist do not impair behavioural expression during memory retrieval. However, it has been reported that D1 [10] and D2 [15] receptors are involved in the retrieval of fear memories. These differences could be explained by different drug concen-trations and the behavioural task used. Although behavioural expression of CTA remains intact, D1 receptors are necessary for memory updating. There is plenty evidence indicating that D1 receptors potentiate the memory stablishment processes in con

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