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Activation of adenosine receptors in the posterior cingulate cortex impairs memory retrieval in the rat

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Abstract

Adenosine A_1 and A_{2A} receptor agonists and antagonists have been reported to alter learning and memory. The aim of our study was to investigate the involvement of adenosinergic system in memory retrieval into posterior cingulate cortex (PCC) of Wistar rats. To clarify this question, we tested specifics agonist and antagonists of adenosine A_1 and A_{2A} receptors in rats submitted to a one-trial inhibitory avoidance task. The stimulation of adenosine A_1 and A_{2A} receptors by CPA and CGS21680, respectively, impaired memory retrieval for inhibitory avoidance task, into PCC. These findings provide behavioral evidence for the role of adenosinergic system in the memory retrieval into PCC.

Keywords: Adenosine A_1 receptor; Adenosine A_{2A} receptor; Retrieval; Memory; Posterior cingulate cortex; Inhibitory avoidance task

1. Introduction

Adenosine is a neuromodulator (Dunwiddie & Masino, 2001), which acts through four types of receptor: A_1, A_{2A}, A_{2B}, and A_3. A_1 and A_3 receptors inhibit neuronal activity through G_i and G_0 proteins, while A_{2A} and A_{2B} act through stimulation of neuronal activity via G_s protein (Klinger, Freissmuth, & Nanoff, 2002). Endogenous adenosine modulates long-term synaptic plasticity phenomena, such as long-term potentiation (LTP), long-term depression (LTD) and depotentiation (de Mendonça, Costenla, & Ribeiro, 2002). Adenosine A_1 receptor is highly expressed in brain cortex and hippocampus, while A_{2A} receptor is found in striato-pallidal GABAergic neurons, being expressed in lower levels in other brain regions (Ribeiro, Sebastião, & de Mendonça, 2003). Several studies have reported that adenosine A_1 and A_{2A} receptor agonists and antagonists alter learning and memory (Corodimas & Tomita, 2001; Khavandgar, Homayoun, Torkaman-Boutorabi, & Zarrindast, 2001; Kopf, Melani, Pedata, & Pepeu, 1999; Normile & Barraco, 1991; Ohno & Watanabe, 1996).

The posterior cingulate cortex (PCC) is a neocortical region that projects to the parahippocampal formation and has reciprocal connections with the prefrontal cortex and the anterior thalamic nuclei (Maddock, 1999). Therefore, PCC is strategically located to mediate signals between these areas and may participate in hippocampal functions. PCC might also have a role in implementing emotional memory prioritization at an

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earlier processing stage (Maddock, 1999). Kubota and Gabriel (1995) postulated that the Papez circuit may have a comparator function, where incoming data are compared with stored data: if they are in accordance, already planned behavioral programs are executed; if not, outputs are generated that heighten attention and inhibit planned actions. Studies from our laboratory has been showed that memory consolidation in PCC is modulated by glutamatergic, GABAergic and adenosinergic system (Mello e Souza et al., 1999; Pereira et al., 2002; Souza et al., 2002).

Retrieval of one-trial inhibitory avoidance memory (IA) involves the participation of a large network of cortical regions including the entorhinal, posterior parietal, and anterior cingulate cortices (Barros et al., 2000). Pharmacological findings have shown that retrieval of IA memory requires glutamate receptors, cAMP-dependent protein quinase (PKA), and mitogen-activated protein kinases (MAPK) in the above-mentioned regions (Barros et al., 2000). Most of studies on the pharmacology of retrieval have been carried out in the hippocampus, which is involved in most declarative and episodic memories (Izquierdo & Medina, 1997; Izquierdo & McGaugh, 2000). There are many studies demonstrating functional interconnections between the hippocampus and the amygdale (Suzuki, Wang, Edge, Mimaki, & Walson, 1999), entorhinal cortex and perirhinal cortex (Hyman, Van Hoesen, & Damasio, 1990) and many other areas of the cortex, including sensory and associative areas, the anterior and posterior cingulate cortex (Van Hoesen, 1985).

Since the role of PCC into memory retrieval remains unknown, and in view of the influence of adenosine receptors on memory consolidation, the present experiments were designs to identify the role of A_1 and A_{2A} receptors in inhibitory avoidance memory retrieval in PCC.

2. Materials and methods

2.1. Animals

Male Wistar rats (3 months of age, 250–280 g of weight) from our own breeding stock were used. The animals were housed into plastic cages under a 12 h light/dark cycle (lights on at 7:00 AM), with water and Purina lab chow freely available and at a constant temperature of 23 °C. To deliver the pharmacological agents to be tested, rats were bilaterally implanted under deep thionembutal anesthesia with 27-gauge guides aimed to the posterior cingulate cortex in accordance to coordinates (A −5.8 L ± 1.0, V 2.8) taken from the atlas of Paxinos and Watson (Paxinos & Watson, 1986). Animals were allowed to recover for 4 days before submitting them to any other procedure. In all experiments the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1996) were strictly followed.

2.2. Inhibitory avoidance task

After recovery from surgery, rats were trained in a one trial, step-down, inhibitory avoidance task (IA), a hippocampal-dependent, fear motivated learning paradigm much used for the biochemical analysis of memory formation (Bevilaqua et al., 1999; Cammarota, Bevilaqua, Kerr, Medina, & Izquierdo, 2003). In order to do that, animals were gently put on a 2.5 cm high, 7.0 cm wide wood platform placed inside and at the leftmost extreme of a 50 × 25 × 25 cm acrylic training box whose floor was made of a grid of parallel bronze bars spaced 1 cm apart. At the very moment the animal stepped down from the platform and put its four paws on the grid, it received a 0.5 mA, 2 s scrambled footshock. After that, it was immediately removed from the training box.

At the time of drug delivery, a 30-gauge cannula was tightly fitted into the implanted guide and linked by an acrylic tube to a microsyringe. Infusions (0.5 μl/side) were carried out over 30 s, first on the right and then on the left posterior cingulate cortex; the 30-gauge cannula was left in place for 15 additional seconds to minimize backflow. For experiments involving co-infusion of drugs, the final volume of the infusion was 0.5 μl/side. To evaluate memory retention, latency to step down onto the grid during the training session was compared to that obtained in a test session performed 24 h later. In the test session the procedure was identical to that used during training except that the electric foot-shock was omitted. Cannula placement was verified postmortem as described previously (Bonini, Rodrigues, Kerr, Bevilaqua, & Cammarotta, 2003). Briefly, 2–4 h after the behavioral test, 0.5 μl of a 4% methylene-blue solution were infused as described above and the extension of the dye 30 min thereafter was taken as indicative of the presumable diffusion of the vehicle or drug previously given to each animal. Only data from animals with correct cannula implants were included in statistical analyses.

2.3. Open field and plus maze

To analyze exploratory and locomotor activities, animals were placed on the left rear quadrant of a 50 × 50 × 39 cm open field with black plywood walls and a brown floor divided into 12 equal squares. The number of line crossings and the number of rearings were measured over 5 min and taken as an indicative of locomotor and exploratory activities. To evaluate their anxiety state, rats were exposed to an elevated plus maze exactly as detailed in (Pellow, Chopin, File, & Briley, 1985). The total number of entries into the four arms, the number of entries and the time spent into the open arms were recorded over a 5 min session. It has been repeatedly

reported that confinement to the closed arms of an elevated plus maze is associated with the observation of significantly more anxiety-related behaviors than confinement to the open arms; moreover, anxiogenic drugs significantly reduce the percentage of entries into, and time spent on, the open arms (Pellow et al., 1985). Ten minutes before exposure to the open field or the plus maze, animals received bilateral 0.5 μl infusions of vehicle or of the drug under scrutiny into the posterior cingulate cortex.

2.4. Drugs

N6-cyclopentyladenosine (CPA) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) were purchased from Sigma, St. Louis, MO, USA. 4-(2-[7-Amino-2-[2-furyl]-[1,2,4] triazolo[2,3-α]{1,3,5}triazin-5-yl-amino]ethyl) phenol(ZM241385) and 2-[*p*-(2-carbonyl-ethyl)-phenyl-ethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21 680) were obtained from Tocris, Ballwin, MO, USA. All drugs were dissolved in saline containing 2% DMSO.

2.5. Statistical analysis

Data are reported as medians (interquartile range) of the step-down latencies during training and test sessions and were analyzed by Kruskal–Wallis or Mann–Whitney *U* tests as required.

Parametric statistics (ANOVA followed by post hoc Duncan multiple range test) were applied to plus maze measures and open field crossing or rearing values.

3. Results

To analyze the role of A_1 receptors in the expression of the long-term memory (LTM) for the IA task, rats were trained in the mentioned paradigm and, 10 min before testing received bilateral 0.5 μl infusions of vehicle, the A_1 receptor antagonist, DPCPX (Bruns et al., 1987), or the A_1 receptor agonist, CPA (Lohse et al., 1988), into the posterior cingulate cortex (PCC) (Fig. 1). DPCPX did not affect IA memory expression when given at a dose of 1, 50, and 100 nM (Fig. 2). Conversely, CPA significantly decreased test step-down latencies when infused at 1 nM ($p < .001$), 50 nM ($p < .001$), and 100 nM ($p < .05$) (Fig. 3). To study the participation of A_{2A} receptors within PCC in IA memory retrieval, the A_{2A} receptor antagonist, ZM241385 (Poucher et al., 1995) or the A_{2A} receptor agonist, CGS21680 (Jarvis & Williams, 1989) were employed. ZM241385 did not alter IA memory retrieval at any dose (1, 50, and 100 nM) analyzed (Fig. 4). On the contrary, the intra-PCC infusion of CGS21680 induced an amnesic effect in all doses tested 1 nM ($p < .05$), 50 nM ($p < .05$), and 100 nM ($p < .01$) (Fig. 5).

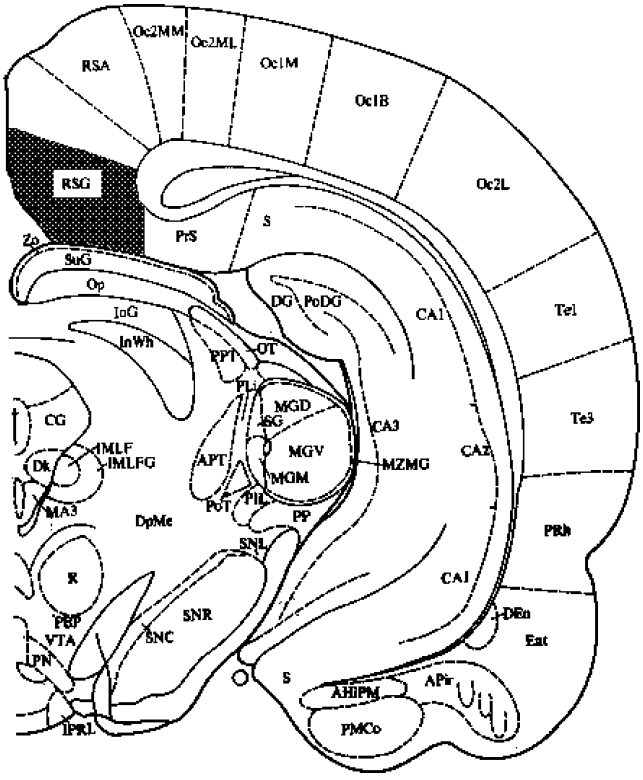

Fig. 1. Schematic drawing of the rat brain section at coronal plane A −0.58 cm from the Atlas of Paxinos and Watson (1986) showing (stippled) the extension of the area reached by infusions into posterior cingulate cortex. The maximum extension reached by any individual infusion was less than 1 mm^3 in the animals with correct infusion placements.

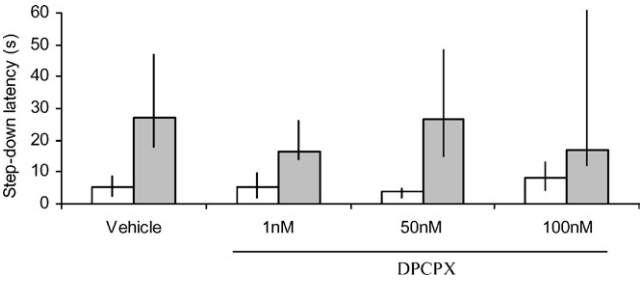

Fig. 2. Infusion of DPCPX into posterior cingulate cortex does not affect retrieval of inhibitory avoidance long-term memory. Rats bilaterally implanted with indwelling cannulae into PCC were trained in IA and tested for retention 24 h later. Ten minutes before that they received 0.5 μl bilateral infusions of vehicle (2% DMSO in saline) or DPCPX (1, 50 or 100 nM). Data represent median (interquartile range) of the step-down latency time (i.e., the time spent on the training box platform before stepping down to the grid) during training (white bars) and test (gray bars) sessions and were analyzed using Kruskal–Wallis non-parametric test; $n = 10$–14 per group.

To rule out the possibility that the amnesic effect of intra-PCC CGS21680 was due to its action on A_1 rather than A_{2A} receptors, we performed the co-administration intra-PCC of CGS21680 and DPCPX at a higher dose (100 nM). We also co-infused CPA and DPCPX into PCC in order to confirm the action of DPCPX on A_1 receptors. DPCPX was unable to block the amnesic

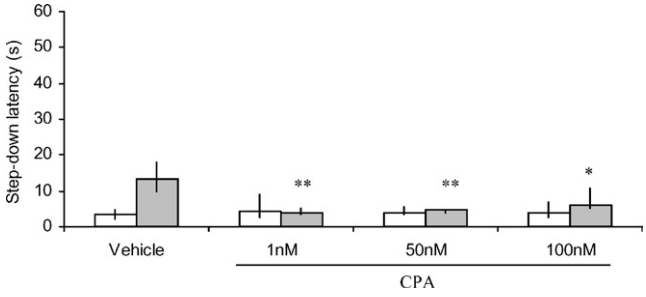

Fig. 3. Infusion of CPA into posterior cingulate cortex blocks retrieval of inhibitory avoidance long-term memory. Rats bilaterally implanted with indwelling cannulae into PCC were trained in IA and tested for retention 24 h later. Ten minutes before that they received 0.5 μl bilateral infusions of vehicle (2% DMSO in saline) or CPA (1, 50 or 100 nM). Data represent median (interquartile range) of the step-down latency time (i.e., the time spent on the training box platform before stepping down to the grid) during training (white bars) and test (gray bars) sessions and were analyzed using Kruskal–Wallis non-parametric test; $n = 10$–14 per group. *$p < .05$ and **$p < .001$ with respect to vehicle (2% DMSO) in Mann–Whitney U test, two-tailed; $n = 10$–14 per group.

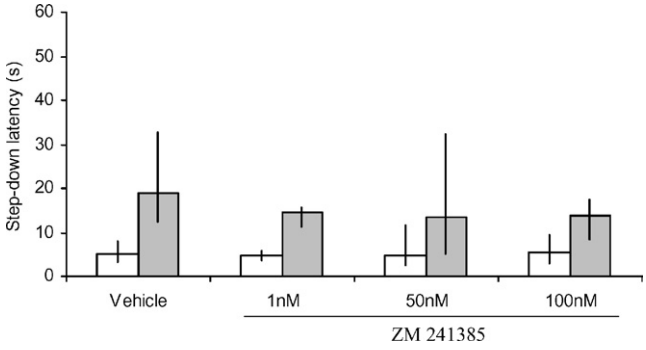

Fig. 4. Infusion of ZM241385 into posterior cingulate cortex does not affect retrieval of inhibitory avoidance long-term memory. Rats bilaterally implanted with indwelling cannulae into PCC were trained in IA and tested for retention 24 h later. Ten minutes before that they received 0.5 μl bilateral infusions of vehicle (2% DMSO in saline) or ZM241385 (1, 50 or 100 nM). Data represent median (interquartile range) of the step-down latency time (i.e., the time spent on the training box platform before stepping down to the grid) during training (white bars) and test (gray bars) sessions and were analyzed using Kruskal–Wallis non-parametric test; $n = 10$–14 per group.

effect of CGS21680, demonstrating that the effect of CGS21680 was due to its action on A_{2A} receptor rather than A_1 receptor. In contrast, DPCPX blocked the amnesic effect of CPA ($p < .05$) (Fig. 6).

To further confirm the involvement of A_{2A} receptors on memory IA expression, we tested whether the A_{2A} receptor antagonist ZM241385 was able to counteract the retrieval deficit induced by the intra-PCC infusion of CGS21680. As can be seen in Fig. 7 when the ZM241385 (100 nM) was co-infused with CGS21680 (100 nM) 10 min before a memory retention session it completely reverse the amnesic effect of the A_{2A} agonist. Conversely, ZM241385 (100 nM) did not block the amnesic effect of CPA (100 nM; Fig. 7).

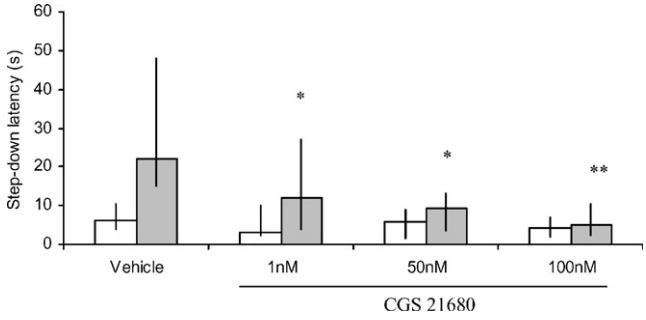

Fig. 5. Infusion of CGS21680 into posterior cingulate cortex blocks retrieval of inhibitory avoidance long-term memory. Rats bilaterally implanted with indwelling cannulae into PCC were trained in IA and tested for retention 24 h later. Ten minutes before that they received 0.5 μl bilateral infusions of vehicle (2% DMSO in saline) or CGS21680 (1, 50 or 100 nM). Data represent median (interquartile range) of the step-down latency time (i.e., the time spent on the training box platform before stepping down to the grid) during training (white bars) and test (gray bars) sessions and were analyzed using Kruskal–Wallis non-parametric test; $n = 10$–14 per group. *$p < .05$ and **$p < .001$ with respect to vehicle (2% DMSO) in Mann–Whitney U test, two-tailed; $n = 10$–14 per group.

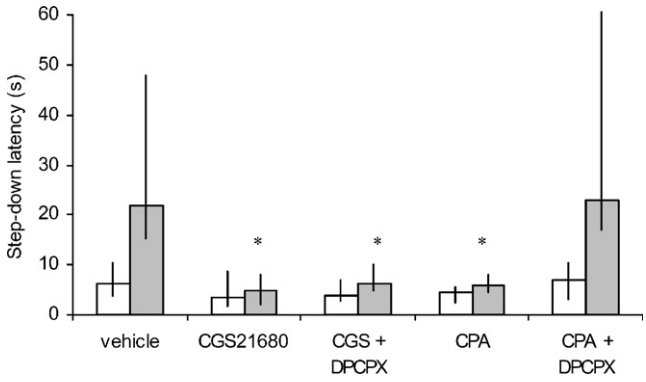

Fig. 6. Infusion of DPCPX into posterior cingulate cortex blocks the amnesic effect induced by CPA. Rats bilaterally implanted with indwelling cannulae into PCC were trained in IA and tested for retention 24 h later. Ten minutes before that they received 0.5 μl bilateral infusions of vehicle (2% DMSO in saline), CGS21680 (100 nM), CGS21680 (100 nM) + DPCPX (100 nM), CPA (100 nM) or CPA (100 nM) + DPCPX (100 nM). Data represent median (interquartile range) of the step-down latency time (i.e., the time spent on the training box platform before stepping down to the grid) during training (white bars) and test (gray bars) sessions and were analyzed using Kruskal–Wallis non-parametric test; $n = 10$–14 per group. *$p < .05$ with respect to vehicle (2% DMSO) in Mann–Whitney U test, two-tailed; $n = 10$–14 per group.

To evaluate whether CPA and CGS21680 have any consequence on locomotor activity or anxiety state when infused into PCC, we analyzed the effect of these drugs in the open field and elevated plus maze behavioral tasks. When infused into PCC 10 min before the behavioral session neither CPA (100 nM) nor CGS21680 (100 nM) modified the number of crossings [$F(2,17) = 1.059$, $p = .369$] and rearings [$F(2,17) = 1.206$, $p = .324$] in the open field or the number of entries into the open arms [$F(2,15) = 0.646$, $p = .538$], into the closed arms [$F(2,15) = 2.019$, $p = .167$] or the time spent into the

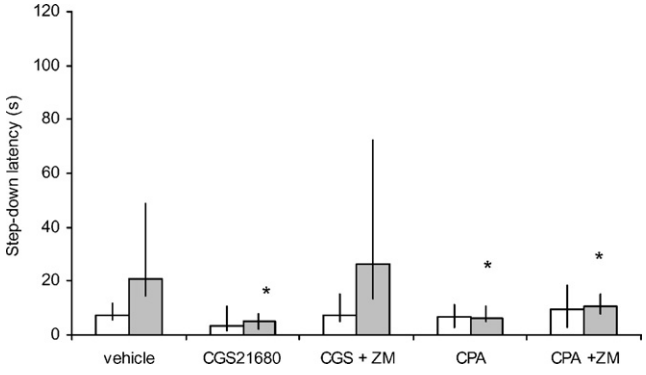

Fig. 7. Infusion of ZM241385 into posterior cingulate cortex blocks the amnesic effect induced by CGS21680. Rats bilaterally implanted with indwelling cannulae into PCC were trained in IA and tested for retention 24 h later. Ten minutes before that they received 0.5 μl bilateral infusions of vehicle (2% DMSO in saline), CGS21680 (100 nM), CGS21680 (100 nM) + ZM21680 (100 nM), CPA (100 nM) or CPA (100 nM) + ZM241385 (100 nM). Data represent median (interquartile range) of the step-down latency time (i.e., the time spent on the training box platform before stepping down to the grid) during training (white bars) and test (gray bars) sessions and were analyzed using Kruskal–Wallis non-parametric test; $n = 10$–14 per group. $^*p < .05$ with respect to vehicle (2% DMSO) in Mann–Whitney U test, two-tailed; $n = 10$–14 per group.

open and closed arms [$F(2,15) = 1.032$, $p = .380$] in an elevated plus maze.

4. Discussion

Our data show that stimulation of adenosine A_1 and A_{2A} receptors by CPA and CGS21680, respectively, impaired memory retrieval for inhibitory avoidance task in posterior cingulate cortex (PCC). Furthermore, the amnesic effect of CGS21680 in PCC was due to its action on A_{2A} receptors rather than A_1 receptors.

In memory consolidation of IA task, CPA did not promote changes when infused into PCC (Pereira et al., 2002). However, in this study, CPA infusion promoted impairment of the retrieval. There are many studies demonstrating the role of adenosine A_1 receptor in cognitive processes. CPA, administered intraperitoneally, disrupted the acquisition in two distinct tasks, contextual fear conditioning (Corodimas & Tomita, 2001) and passive avoidance (Normile & Barraco, 1991). The highly selective A_1 receptor agonist, CHA, increased the number of errors in a working memory task (Ohno & Watanabe, 1996) and showed additive effects in restoring morphine-induced amnesia of passive avoidance (Khavandgar et al., 2001). In fact, the biochemical changes underlying consolidation are not identical to those of retrieval, although both processes might involve the same synapses (Barros, Izquierdo, Medina, & Izquierdo, 2003). If activation of A_1 receptors at the time of consolidation was ineffective, why this activation impaired retrieval? Studies from our laboratory demonstrated that AP5, a glutamate-NMDA (*N*-methyl-D-aspartate) receptor antagonist, infused into the parietal or anterior cingulate cortex inhibit retrieval (Barros et al., 2000). Then, it is reasonable to suggest that NMDA receptor integrity is required to retrieval. The activation of A_1 receptors promotes decrease of glutamate release (Dunwiddie & Haas, 1985) reduces NMDA receptor currents by a postsynaptic action (de Mendonça, Sebastião, & Ribeiro, 1995) and would, consequently, impair the memory retrieval. The effect of adenosine analogs administration such as, CPA, suggests that adenosine A_1 receptors may modulate the memory retrieval when activated. However, it is important to observe that the infusion of DPCPX into PCC did not alter memory retrieval, demonstrating that, at least in basal condition, adenosine A_1 receptors are not essential to retrieval in PCC.

Adenosine A_{2A} receptors are highly expressed in striatal medium-sized spiny neurons (Fink et al., 1992; Schiffmann, Libert, Vassart, & Vanderhaeghen, 1991) and play an important role in the control of motor behavior (Barraco, 1993; Brockwell & Beninger, 1996). Studies demonstrate that i.p. administration of adenosine A_{2A} receptor antagonist, SCH58261, facilitate retention of passive avoidance task when administered immediately but not 180 min latter (Kopf et al., 1999). CGS21680 is 140 times more active at A_{2A} than at A1 receptors and exhibits very low activity at cloned A_{2B} and A_3 receptors (Hutchison et al., 1989). There are several reports that CGS21680 exerts biological activity in brain structures outside the basal ganglia (Cunha, Johansson, Constantino, Sebastião, & Fredholm, 1996). In cortex and hippocampus and possibly other structures, CGS21680 very likely binds mainly to site different from the A_{2A} receptor (Johansson & Fredholm, 1989). In order to exclude the participation of A1 receptor in amnesic effects promoted by CGS21680 we tested the co-administration of CGS21680 and DPCPX. Our results demonstrated that the effect of CGS21680 is due to its action in A_{2A} more than A_1. It has been shown that binding of [^{3}H]CGS21680 to rat cerebral cortex can be detected with autoradiography and that this binding shows somewhat different pharmacological characteristics than the binding of the drug to the striatum (Johansson, Georgiev, Parkinson, & Fredholm, 1993). Therefore, despite the low expression of A_{2A} receptors in cortical areas, it is possible to suggest that the activation of these receptors into PCC could be able to promote amnesic effect. Further studies are necessary to investigate if the participation of A_{2A} receptors is required in IA memory consolidation.

Molecular pharmacological data showed that biochemical changes underlying consolidation are similar to those of retrieval, but not all of the mechanisms involved in LTM consolidation are also crucial for retrieval (Barros et al., 2000, 2003; Izquierdo et al., 1997). Considering

that there are differences between consolidation and retrieval in hippocampus, entorhinal, parietal, and anterior cingulate cortices, it is acceptable that these distinctions exist into PCC. Imaging of activity-dependent genes revealed an involvement of parietal and retrosplenial cortices during consolidation of remote memory. Long-term memory storage was accompanied by synaptogenesis and laminar reorganization within some of these neocortical regions, concomitant with functional disengagement of the hippocampus and posterior cingulate cortices (Maviel, Durkin, Menzagui, & Bontempi, 2004).

Although, some of these points remain to be proven experimentally, it is hoped that the pharmacological findings presented in this study can provide a framework for development of hypotheses and strategies for future studies on the role of PCC and adenosinergic system in modulating memory retrieval.

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