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Short communication

Blockade of adenosine A_1 receptors in the posterior cingulate cortex facilitates memory in rats

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Abstract

Male Wistar rats were bilaterally implanted with indwelling cannulae in the caudal region of the posterior cingulate cortex. After recovery, animals were trained in a step-down inhibitory avoidance task (3.0-s, 0.4-mA foot shock) and received, immediately after training, a 0.5-μl infusion of the adenosine A_1 receptor agonist N6-cyclopentyladenosine (CPA; 1, 50 or 100 nM) or of the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1, 25 or 50 nM). Animals were tested twice, 1.5 h and, again, 24 h after training, in order to examine the effects of these agents on short- and long-term memory, respectively. Only 50-nM DPCPX was effective in altering memory, promoting a facilitation. These results suggest that adenosine A_1 receptors in the posterior cingulate cortex inhibit memory consolidation in a way that their blockade facilitates memory for inhibitory avoidance in rats.

Keywords: Adenosine A_1 receptor; Cortex, posterior cingulate; Short-term memory; Long-term memory

1. Introduction

Adenosine is considered to have an important role in the modulation of synaptic transmission and neuronal excitability (Sebastião and Ribeiro, 2000). Adenosine mediates its effects through four types of receptors, A_1, A_{2A}, A_{2B}, and A_3, to which G proteins are coupled (Hauber and Bareiβ, 2001). Adenosine A_1 receptors are the most prevalent and have the highest affinity among the adenosine receptors in the central nervous system. Adenosine A_1 receptors inhibit neurotransmitter release (Cunha, 2001) and affect activity-dependent synaptic plasticity in the hippocampus, attenuating long-term depression and inhibiting long-term potentiation (De Mendonça and Ribeiro, 1997).

Several adenosine A_1 receptor agonists and antagonists administered systemically have been reported to alter inhibitory avoidance learning (Normile and Barraco, 1991; Von Lubitz et al., 1993; Zarrindast and Shafaghi, 1994; Ohno and Watanabe, 1996; Kopf et al., 1999). Most of the available data demonstrate a modulatory role of adenosine in memory in the hippocampus (De Mendonç a and Ribeiro, 2000). However, cortical structures other than the hippocampus are also involved in the consolidation of memory for the step-down inhibitory avoidance task (Mello e Souza et al., 1999). In fact, there is increasing evidence to indicate that the posterior cingulate cortex is important for memory processes such as those involved in step-down inhibitory avoidance (Mello e Souza et al., 1999; Souza et al., in press). Interestingly, binding of [^{3}H] 8-cyclopentyl-1,3-dipropylxanthine ([^{3}H] DPCPX) to adenosine A_1 receptors is higher in the more inferior part of the posterior cingulate cortex (Svenningsson et al., 1997), where synaptic plasticity is observed (Hedberg and Stanton, 1995).

Since there are no studies providing support for an involvement of adenosine A_1 receptors in the posterior cingulate cortex in memory, the aim of the present work was to evaluate the effect of immediate post-training infusions into the posterior cingulate cortex of the adenosine A_1

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receptor agonist N6-cyclopentyladenosine (CPA) and of the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on short- and long-term memory for inhibitory avoidance.

2. Material and methods

A total of one-hundred thirty male Wistar rats (age 60 to 90 days) from our breeding colony was used. The animals were housed five to a cage with food and water ad libitum. The animal house was on a 12-h light/dark cycle (lights on at 0700 h) at a temperature of 23±1 °C. Procedures for the care and use of animals were according to the regulation of the Brazilian Society for Neuroscience and Behavior.

The animals were bilaterally implanted under thionembutal anesthesia (30 mg/kg, i.p.) with 27-gauge guide cannulae. After at least 48 h, all animals were trained in a step-down inhibitory avoidance task (Izquierdo et al., 1997). Latency to step-down placing the four paws on the grid was measured. In the training session, immediately upon stepping down, the animals received a 3.0-s, 0.4-mA foot shock. Animals were tested twice, 1.5 h and, again, 24 h after training, in order to measure short- and long-term memory, respectively (Izquierdo et al., 1998). Test sessions were procedurally identical to the training session except that no foot shock was given and the step-down latency was cut off at 180 s, i.e., test session values higher than 180 s were counted as 180 s. Retention test performances were taken as measurements of retention.

At the time of infusion, 30-g cannulae were fitted into the guide cannulae (Izquierdo et al., 1997). Animals received, immediately after training, a bilateral infusion of 0.5 μl of the adenosine A_1 receptor agonist N6-cyclopentyladenosine (CPA; 1, 50 or 100 nM) in phosphate buffer saline (saline; pH 7.4; 1 mM $CaCl_2$), of the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1, 25 or 50 nM) in dimethyl sulphoxide 20% (DMSO; vehicle), or of either saline or vehicle. Infusion site was chosen using coordinates from bregma and dura obtained from the Atlas of Paxinos and Watson (1986), as follows (units in cm): *A*, −0.58; *L*, ± 0.10; *V*, 0.28.

The histological localisation of infusion sites was confirmed as explained elsewhere (Izquierdo et al., 1997). Only animals with correct cannulae locations were included in the final statistical analysis.

Parametric statistics was used when comparing groups in the training session, where latencies may be presumed to have a normal distribution. In this case, there was no difference among groups in the training session [one-way analysis of variance (ANOVA): $F(7,107)=1.637$; $P=0.133$; overall mean, 6.1 s; S.E.M., 0.4 s]. However, the assumption of normal distributions in the test sessions was not taken into consideration because of the use of a 180-s ceiling and the simultaneous presence of high interquartile values of DCPCX at 1 and 50 nM. Thus, retention test data are reported as medians (interquartile range) of retention scores of all test sessions (Fig. 1) and nonparametric statistics was applied (Kruskal–Wallis ANOVA followed by individual Mann–Whitney *U* test, two-tailed).

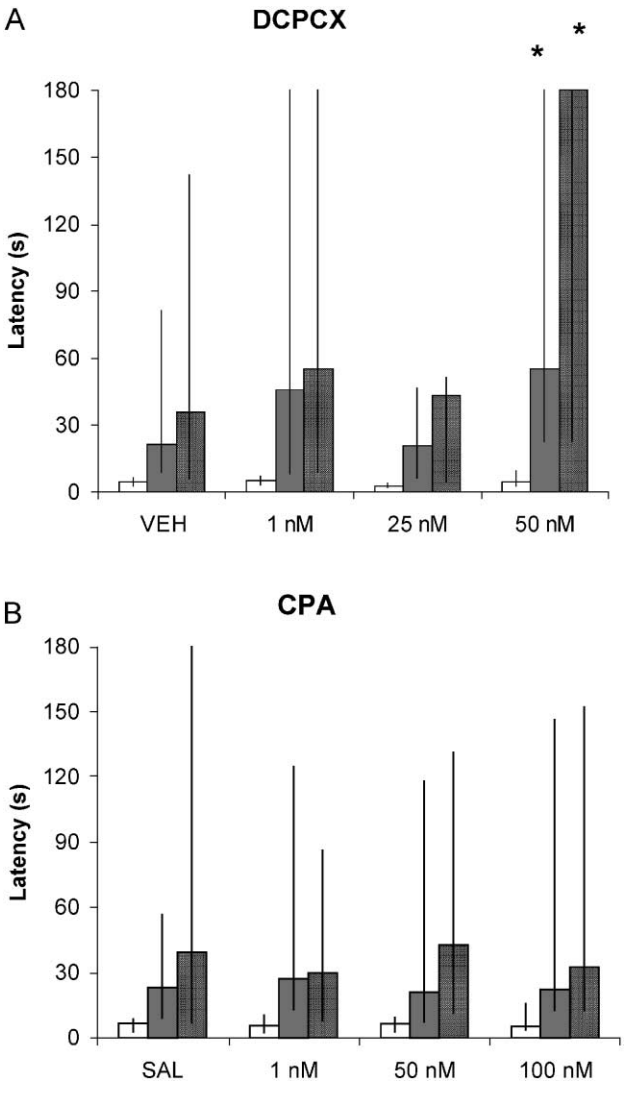

Fig. 1. Medians (interquartile range) of retention scores of training (white bars) and test sessions for short-term memory (gray bars) and long-term memory (hatched bars) in groups bilaterally infused into the posterior cingulate cortex, immediately after training, with (A) DPCPX (1, 25 or 50 nM) in DMSO 20% (vehicle) or vehicle, or (B) CPA (1, 50 or 100 nM) in phosphate-buffered saline (saline; pH 7.4; 1 mM $CaCl_2$) or saline. Asterisk indicates statistical significance in the Mann–Whitney *U* test, two-tailed, at $P<0.05$, respectively, from the respective control group (in this case, vehicle). $N=22$ and 18 in saline and vehicle, respectively.

3. Results

DPCPX at 50 nM ($n=16$) significantly increased the retention test performance for short-term memory ($P=0.031$) and long-term memory ($P=0.011$). DPCPX at lower doses (1 and 25 nM; $n=11$ and $n=10$, respectively) did not alter

either short-term memory ($P=0.355$ and $P=0.745$, respectively) or long-term memory ($P=0.244$ and $P=0.935$, respectively) (Mann–Whitney U test; Fig. 1A; $n=22$ for vehicle). Our results show that memory is facilitated when adenosine A_1 receptors in the posterior cingulate cortex are strongly inhibited immediately after training. Since infusions were given post-training, our results also show that this facilitation was due to an alteration of memory consolidation rather than acquisition, indicating that memory consolidation of inhibitory avoidance is under a modulatory inhibition promoted by adenosine A_1 receptors in the posterior cingulate cortex. It is worth pointing out that DPCPX was not used at doses sufficient to affect adenosine A_2 receptors (Ki = 260–529 nM and Ki = 1440 nM for adenosine A_{2A} and A_{2B} receptors, respectively), indicating that this drug was used at specific doses for adenosine A_1 receptors (Jarvis et al., 1989; Kirk and Richardson, 1995).

CPA at 1 nM ($n=14$), 50 nM ($n=10$), or 100 nM ($n=8$) did not alter either short-term memory ($P=0.437$, $P=0.899$ and $P=0.750$, respectively) or long-term memory ($P=0.759$, $P=0.797$ and $P=0.729$, respectively) (Mann–Whitney U test; Fig. 1B; $n=18$ for saline). This indicates that the neuromodulatory inhibition mediated by the adenosine A_1 receptors is in its upper limit because they are already saturated by endogenous adenosine in the posterior cingulate cortex due to inhibitory avoidance training and/or these receptors have a limited power of inhibiting memory consolidation.

4. Discussion

Previous studies demonstrated that adenosine A_1 receptors modulate aversive memory (Normile and Barraco, 1991; Ohno and Watanabe, 1996; Zarrindast and Safaghi, 1994), showing that their agonists can impair memory. However, in all of these studies, the drugs were administered systematically, suggesting that regions other than the posterior cingulate cortex (e.g., hippocampus) were affected and may have contributed to the amnesiac effect. Interestingly, the adenosine antagonists were shown to facilitate the retention or prevent the amnesiac effects of scopolamine on passive avoidance task (Pitsikas and Borsini, 1997).

There is increasing evidence to indicate that the posterior cingulate cortex is important in mediating memory processes (Mello e Souza et al., 1999; Pereira et al., 2002; Souza et al., in press). The posterior cingulate cortex may perform its functions and might store information by mechanisms of activity-dependent synaptic plasticity such as long-term potentiation and long-term depression (Hedberg and Stanton, 1995). Adenosine modulates long-term potentiation in a way that adenosine A_1 receptor agonists and antagonists, respectively, impair and enhance long-term potentiation in hippocampus (De Mendonça and Ribeiro, 1990). In fact, DPCPX enhances hippocampal long-term potentiation by more than 100% (De Mendonça and Ribeiro, 2000), probably because it blocks adenosine A_1 receptor-mediated inhibition of excitatory neurotransmitter release and/or of protein kinase A activity (Costenla et al., 1999). Therefore, DPCPX may facilitate memory of inhibitory avoidance by means of facilitating long-term potentiation-like plasticity in the posterior cingulate cortex. Interestingly, immediate post-training inhibition of protein kinase A activity in the posterior cingulate cortex is amnesiac for both short- and long-term memory for inhibitory avoidance (Souza et al., in press). Furthermore, DPCPX might also facilitate memory consolidation via an interaction between adenosine A_1 and A_2 receptors. In the hippocampus, for instance, the excitatory responses of adenosine A_2 receptors are increased in the presence of adenosine A_1 receptor antagonists (Correia-de-Sá and Ribeiro, 1994).

It is worth pointing out that there is an increase in ectonucleotidase activities in the posterior cingulate cortex after inhibitory avoidance training (Pereira et al., 2002), which is an indirect, but not conclusive, evidence that adenosine levels are increased at this time. In fact, adenosine may be released to protect the neurons against an excitotoxic damage due to the high neuronal activity that occurs during stressing events, as pointed out by Minor et al. (2001). Further studies are necessary to clarify the mechanisms by which DPCPX facilitates retention and their relevance to the understanding of memory processing.

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