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Intra-amygdala spermidine administration improves inhibitory avoidance performance in rats

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Abstract

In the present study, we investigated the effect of immediate post-training bilateral infusion of spermidine, a polyamine agonist, into the amygdala on inhibitory avoidance learning of rats. Bilateral microinjection of spermidine (0.02–20 nmol) caused an increase in test step-down latencies at high concentrations. Administration of arcaine (0.002–0.2 nmol), an antagonist of the NMDA receptor polyamine binding site, decreased test step-down latencies. On the other hand, co-administration of arcaine and spermidine completely reversed the spermidine-induced increase of test step-down latencies. These results provide evidence that polyamines may be involved in learning and memory modulation in the amygdala.

Keywords: Spermidine; Arcaine; Polyamine; Amygdala; Learning; Memory

1. Introduction

Spermine and spermidine are ubiquitous, naturally occurring polyamines required for cell growth and differentiation, which are present at high concentrations in the brain (Anderson et al., 1975; Williams et al., 1991; Shimada et al., 1994; Johnson, 1996; Williams, 1997). The central actions of polyamines are complex and seem to involve the dual modulation of the NMDA receptor (Rock and Macdonald, 1995; Williams, 1997). In fact, polyamines, at low micromolar concentrations, enhance [^{3}H]MK-801 and [^{3}H]TCP binding to the NMDA receptor channel, whereas higher concentrations of polyamines do not alter the binding of these ligands, resulting in a biphasic concentration–response curve (Ransom and Stec, 1988; Saccan and Johnson, 1990; Williams, 1997). Electrophysiological studies have confirmed these findings since low concentrations of polyamines enhance NMDA-evoked currents, whereas higher concentrations of polyamines produce less enhancement of, or inhibit, NMDA receptor currents (McGurk et al., 1990; Sprosen and Woodruff, 1990; Williams et al., 1990; Rock and Macdonald, 1992). This dual modulation seems to exist at the behavioural level, also, since intrahippocampal administration of a polyamine site agonist at low, but not at high, concentrations improves inhibitory avoidance task performance (Rubin et al., 2000). These results support the view that polyamines improve memory and, to some extent, corroborate the findings of Kishi et al. (1998a), who reported that polyamines, by their interaction with the hippocampal NMDA glutamate receptors, attenuate deficits induced not only by NMDA receptor antagonists (Kishi et al., 1998a) but also by muscarinic and metabotropic glutamate receptor antagonists (Kishi et al., 1998b). Moreover, i.c.v. spermidine improves memory in the 14-unit T-maze task (Shimada et al.,1994), and systemic spermine attenuates NMDA receptor antagonist-induced impairment of acquisition in this task (Meyer et al., 1998).

The basolateral part of the amygdala is one of the brain regions containing high levels of NMDA type excitatory amino acid receptors and receives afferent glutamatergic projections from cortex and thalamus (Maren, 1999), a fact that makes putative polyamine modulation of this cerebral structure worth investigating. Indeed, it is well established that a variety of treatments, which affect the function of the amygdala, alter memory. The administration of ionotropic glutamate receptor antagonists (Jerusalinsky et al., 1992; Bianchin et al., 1993, 1999; Mesches et al., 1996), metabotropic glutamate receptor antagonists (Bianchin et al., 2000), scopolamine, Ca^{2+}/calmodulin-de-

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pendent protein kinase II or protein kinase C inhibitors (Bianchin et al., 1999) into the amygdala impairs memory, whereas picrotoxin and norepinephrine infusion enhances it (Bianchin et al., 1999). However, to our knowledge, there are no studies in the literature concerning the effects of polyamines on learning and memory involving amygdaloid receptors. Therefore, the present study was conducted to investigate the effect of post-training intra-amygdala administration of spermidine and arcaine on the inhibitory avoidance performance of adult rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (230–250 g) were housed five to a cage on a natural day/night cycle at a temperature of 21°C with free access to water and standard lab chow (Guabi, Santa Maria, RS, Brazil) ad libitum.

2.2. Behavioural evaluation

Rats were implanted under thionembutal anaesthesia (30 mg kg^{-1}, i.p.) with a 27-gauge guide cannula aimed 1 mm above the amygdala at the A −2.0, L 4.4, V 7.5 coordinates of the Atlas of Paxinos and Watson (1986). Four days after surgery, the animals were subjected to a single training session in a step-down inhibitory avoidance apparatus, returned to their home cage and tested for retention 24 h later. The apparatus consisted of a 25 × 25 × 35-cm box with a grid floor whose left portion was covered by a 7 × 25-cm platform, 2.5-cm high. The rat was gently placed on the platform facing the rear left corner, and when the rat stepped down with all four paws on the grid, a 3-s 0.4-mA shock was applied to the grid. Immediately after training, the animals were injected bilaterally into the amygdala with 0.5 μl of vehicle (200 mM phosphate buffer, pH 7.4), *N*-[3-Aminoprophyl]-1,4-butanediamine trihydrochloride (spermidine; Sigma, Saint Louis, MO) (0.02–20 nmol), 1,4-diguanidinobutane sulfate (arcaine; Sigma) (0.002–0.2 nmol), or spermidine (2.0 nmol) plus arcaine (0.002 nmol). The injection was performed using 30-gauge cannulas that were fitted into the guide cannula, with the tip of the infusion cannula protruding 1.0 mm beyond that of the guide cannula, and therefore aimed at the amygdala. Test step-down latency was taken as a measure of retention, and a cutoff time of 300 s was established. Immediately after the inhibitory avoidance testing session, the animals were transferred to an open-field measuring 56 × 40 × 30 cm with the floor divided into 12 squares measuring 12 × 12 cm each. The open-field session lasted 6 min and during this time, the number of crossing and rearing responses was recorded. Injection placements were histologically verified as described elsewhere (Rubin et al., 1997). Only data from the animals with correct cannula placement were analyzed.

2.3. Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) or by Kruskal–Wallis *H*-test analysis of variance followed by the individual Mann–Whitney *U*-test, depending on the case.

3. Results

Statistical analysis of test step-down latencies after post-training intra-amygdala administration of spermidine revealed a significant effect of spermidine administration ($H = 14.39$; $df = 4$; $P < 0.05$—Kruskal–Wallis *H*-test, Fig. 1). Post hoc analysis showed that 0.2 nmol ($U(5,7) = 3.00$; $P < 0.05$), 2.0 nmol ($U(5,5) = 0.00$; $P < 0.05$) and 20.0 nmol ($U(5,5) = 1.00$; $P < 0.05$) spermidine prolonged step-down latencies compared to those in the respective control group.

Fig. 2 shows the effect of intra-amygdala administration of arcaine (a competitive antagonist at the NMDA polyamine binding site) on test step-down latencies. Statistical analysis revealed a significant effect of arcaine administration ($H = 10.37$; $df = 3$; $P < 0.05$). Post hoc analysis showed that 0.02 nmol ($U(9,7) = 6.0$; $P < 0.05$) and 0.2 nmol ($U(9,8) = 12.5$; $P < 0.05$) arcaine decreased step-down latencies compared to those in the respective control group.

The effect of the co-administration of arcaine (0.002 nmol) on the spermidine-induced increase of test step-down latencies is shown in Fig. 3. Statistical analysis revealed a significant effect of groups ($H = 7.8$; $df = 3$; $P < 0.05$). Post hoc analysis showed that only the PO_4/spermidine

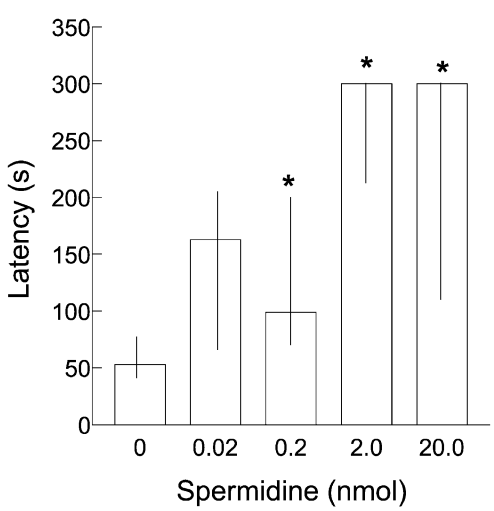

Fig. 1. Effect of immediate post-training intra-amygdala spermidine administration on the inhibitory avoidance task performance of adult rats measured as the test step-down latency. * $P < 0.05$ compared with vehicle using the Mann–Whitney *U*-tests. Data are median ± interquartile range for 5–7 animals in each group.

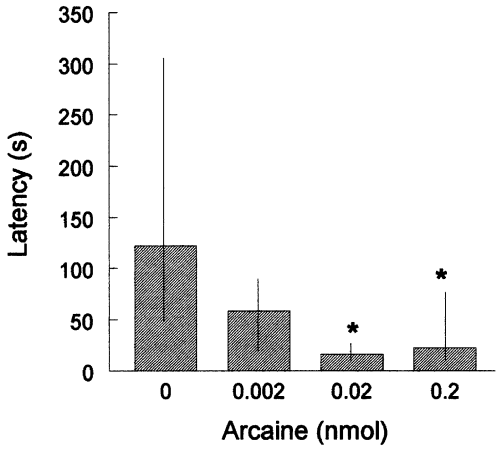

Fig. 2. Effect of immediate post-training intra-amygdala arcaine administration on the inhibitory avoidance task performance of adult rats measured as the test step-down latency. $^{*}P < 0.05$ compared with vehicle using the Mann–Whitney *U*-tests. Data are median ± interquartile range for 8–9 animals in each group.

group differed from the PO_4/PO_4 group ($U(9,9) = 14.0$; $P < 0.05$).

We have previously reported that prolonged testing step-down latencies may arise from decreased locomotor activity, and consequently be misinterpreted as a memory-enhancing effect (Rubin et al., 2000). Therefore, in the present study, we also assessed the locomotor behaviour of the animals immediately after the inhibitory avoidance testing session in order to identify any motor disability, which might influence inhibitory testing avoidance performance. Statistical analysis of open-field data (one-way ANOVA) revealed that intra-amygdala spermidine injection immediately after the inhibitory avoidance training session had no effect on the number of crossing ($F(4,22) = 0.40$; $P > 0.05$) or rearing responses ($F(4,22) = 0.69$; $P > 0.05$) in a subsequent open-field testing session. The same result was observed after intra-amygdala arcaine injection, which had no effect on crossing ($F(3,28) = 0.40$; $P > 0.05$) or rearing behaviour ($F(3,28) = 0.09$; $P > 0.05$, Table 1). These data suggest that the post-training effects of spermidine or arcaine on test step-down latencies were not due to an effect on locomotor activity.

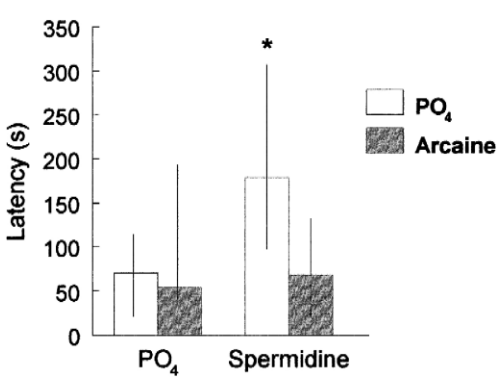

Fig. 3. Effect of intra-amygdala arcaine (0.002 nmol) administered immediately post-training on the improvement of the inhibitory avoidance task performance (test step-down latency) induced by 2.0 nmol spermidine. PO_4 (phosphate buffer) represents vehicle treatment. $^{*}P < 0.05$ compared with vehicle by the Mann–Whitney *U*-tests. Data are median ± interquartile range for 8–9 animals in each group.

Table 1
Effect of intra-amygdala spermidine or arcaine administration on the animals' crossing and rearing responses in the open-field immediately after the inhibitory avoidance testing session
Data are means ± S.E.M. for 5–9 animals in each group.

Group (nmol)	Crossing	Rearing
Control	46.40 ± 5.26	14.80 ± 1.31
Spermidine (0.02)	38.80 ± 5.22	15.40 ± 4.08
Spermidine (0.2)	39.42 ± 7.67	12.14 ± 1.42
Spermidine (2.0)	35.00 ± 5.54	10.20 ± 2.65
Spermidine (20.0)	38.80 ± 5.07	13.40 ± 2.42
Control	38.66 ± 6.34	13.78 ± 2.11
Arcaine (0.002)	38.75 ± 7.95	14.37 ± 2.96
Arcaine (0.02)	43.42 ± 8.49	15.71 ± 4.24
Arcaine (0.2)	31.87 ± 6.72	13.75 ± 2.59

4. Discussion

In the present study, we investigated for the first time the modulatory role of endogenous and exogenous polyamines in the memory of the step-down inhibitory avoidance task in the amygdala. The basolateral part of the amygdala contains a high density of NMDA receptors and many glutamate-immunoreactive terminals and is also a site of long-term potentiation (Maren, 1999). It is well established that the amygdala plays an important role in modulating the storage of affectively influenced memory (Jerusalinsky et al., 1992; Bianchin et al., 1993, 1999, 2000; Mesches et al., 1996; Maren, 1999; Spanis et al., 1999). In particular, the basolateral nucleus of the amygdala seems to be implicated in this function (Spanis et al, 1999; Bianchin et al., 2000).

It has been frequently argued that the NMDA receptor plays a pivotal role in the generation of various forms of synaptic plasticity, including processes thought to underlie learning and memory (Izquierdo and Medina, 1995, 1997; Pláteník et al., 2000). The involvement of NMDA receptors in the modulation of step-down inhibitory avoidance memory in the amygdala during the consolidation phase was proposed by Izquierdo et al. (1992) and Jerusalinsky et al. (1992), who have demonstrated that post-training intra-amygdala administration of aminophosphonopentanoic acid (AP5) impairs retention of this task. Indeed, in the present study, we obtained additional evidence for the participation of these receptors in the memory modulation of the amygdala since the NMDA polyamine binding site agonist improved, whereas the antagonist impaired consolidation of the memory of the task. Moreover, arcaine, at a

dose that had no effect per se, antagonized the effect of spermidine, suggesting some degree of specificity in such reversal.

It is well known that polyamines are released in the central nervous system in a Ca^{2+}-dependent manner in response to a variety of depolarizing stimuli (Shaw, 1994). This interesting finding supported the hypothesis that polyamines could act as physiological modulators of the NMDA receptor and its functions, including memory, but specific studies on this issue are lacking. The findings that Down's syndrome and Alzheimer's disease patients have lower levels of polyamines and memory deficits gave some support to this view (Seidl et al., 1996), inasmuch as these patients present some other neurochemical abnormalities, which certainly contribute to their cognitive deficit (Miranda et al., 2000; Siegel and Chauhan, 2000; Holland, 2000). Nonetheless, the present finding that intra-amygdala administration of arcaine impairs retention of the inhibitory avoidance task may represent evidence that endogenous polyamines exert a physiological modulation of NMDA receptors in this structure during the consolidation phase of memory. In this respect, it is interesting to point out that such an endogenous modulation may be site-specific since we have previously shown that intrahippocampal arcaine (in a wide range of doses) has no effect per se on step-down inhibitory avoidance performance. Moreover, the fact that higher doses of spermidine were required to induce memory enhancement in the amygdala, compared to our previous results for the hippocampus, further supports the idea that higher polyamine levels might be present in this structure, conferring a tonic stimulation of NMDA receptor functions. Indeed, polyamine concentrations vary throughout the various cerebral structures (Shaw, 1994), and this fact may explain the differential effect of arcaine and spermidine on the amygdala. Unfortunately, polyamine levels in the amygdala are not known, a fact that causes this interesting discussion to remain speculative, at least for the moment.

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