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ARTICLE

Caffeine improves spatial learning deficits in an animal model of attention deficit hyperactivity disorder (ADHD) – the spontaneously hypertensive rat (SHR)

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Abstract

The spontaneously hypertensive rat (SHR) is generally considered to be a suitable genetic model for the study of attention deficit hyperactivity disorder (ADHD), since it displays hyperactivity, impulsivity, poorly sustained attention, and deficits in learning and memory processes. Converging evidence suggests a primary role of disturbance in the dopaminergic neurotransmission in ADHD patients and in SHR, and in addition, some studies have also demonstrated alterations in adenosinergic neurotransmission in SHR. In the present study, adult female Wistar (WIS) and SHR rats received caffeine (1–10 mg/kg i.p.) 30 min before training, immediately after training, or 30 min before a test session in the spatial version of the Morris water maze. The effect of caffeine administration on WIS and SHR blood pressure was also measured. SHR needed significantly more trials in the training session to acquire the spatial information, but they displayed a similar profile to that of WIS rats in the test session (48 h later), demonstrating a selective deficit in spatial learning. Pre-training administration of caffeine (1–10 mg/kg i.p.) improved this spatial learning deficit in SHR, but did not alter the WIS performance. In contrast, post-training administration of caffeine (3 mg/kg i.p.) did not alter the SHR test performance, but increased memory retention in WIS rats. No dose of caffeine tested altered the mean blood pressure of WIS or SHR. These results demonstrate a selective spatial learning deficit in SHR which can be attenuated by pre-training administration of caffeine. In addition, the present findings indicate that the spatial learning deficit in SHR is not directly related to hypertension.

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Key words: Attention deficit hyperactivity disorder (ADHD), caffeine, Morris water maze, spatial learning, spontaneously hypertensive rats (SHR).

Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most common childhood psychiatric disorders affecting between 1.3 and 5% of primary-school children (Swanson et al., 1998; Taylor, 1998). The disorder usually manifests itself before the child is 7 yr old. In childhood, the disorder is more common in boys than in girls and at least 75% will continue to suffer from the disorder after they have grown up. In adulthood, more females suffer from the disorder than males (Biederman et al., 1994). ADHD is characterized by the presence of three primary symptoms: hyperactivity, inattention and impulsivity (Himelstein et al., 2000; Taylor, 1998). Additionally, ADHD children have problems with cognitive impulsiveness that may be defined as planning deficits, forgetfulness, poor use of time and impetuous behaviour (Sagvolden, 2000).

Spontaneously hypertensive rats (SHR) have often been used as an animal model of ADHD, since they display hyperactivity, impulsivity, impaired ability to withhold responses and poorly sustained attention in comparison with normotensive Wistar–Kyoto (WKY) control rats (Russel, 2002; Sagvolden, 2000; Sagvolden and Sergeant, 1998). Furthermore, reduced performance by SHR has been observed in different

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paradigms used to investigate learning and memory processes: the conditional avoidance task (Hecht et al., 1978), the two-way shuttle box avoidance task (Sutterer et al., 1980), the radial-arm maze (Mori et al., 1995; Nakamura-Palacios et al., 1996; Wyss et al., 1992), and also the Morris water maze (De Bruin et al., 2003; Gattu et al., 1997; Terry et al., 2000; Wyss et al., 2000). For this reason, it has been suggested that these rats could provide an interesting model in the evaluation of substances with therapeutic potential for the treatment of disorders in memory and attention (De Bruin et al., 2003; Meneses and Hong, 1998).

To date, the 'dopaminergic hypothesis', based on a dysregulation in dopaminergic neurotransmission, has been the most widely accepted hypothesis regarding the behavioural alterations both in ADHD patients and SHR. There is considerable evidence suggesting that ADHD patients have disturbances in dopamine uptake, storage and/or metabolism (Castellanos and Tannock, 2002; Ernst et al., 1998; Swanson et al., 2000). Furthermore, the most effective and frequently prescribed drugs for ADHD, methylphenidate (Ritalin®) and d-amphetamine, are psychostimulants that inhibit re-uptake and stimulate release of dopamine in the central nervous system (CNS), thereby increasing the temporal and spatial presence of dopamine at post-synaptic receptors (Krause et al., 2000; Safer and Krager, 1988). Alterations in dopamine neurotransmission have been also extensively described in SHR, including reduced release of dopamine in the prefrontal cortex, nucleus accumbens and striatum, and decreased dopamine turnover in the substantia nigra, ventral tegmental area and frontal cortex (Russel, 2002), as well as reduced dopamine vesicular storage (Russel, 2002; Russel et al., 1998), and increased density of binding sites for the dopamine D_1/D_5 receptor family in the anterior forebrain (Carey et al., 1998; Papa et al., 2002).

In addition to this well-documented role of dopamine neurotransmission imbalance in the pathophysiology of ADHD and in the behavioural alterations of SHR, some reports have shown functional alterations in the adenosinergic neurotransmission in SHR (Davies et al., 1987; Illes et al., 1989; Kamikawa et al., 1980; Matias et al., 1993). Davies et al. (1987) have demonstrated a reduced adenosine deaminase activity (ADA, the enzyme which catabolizes adenosine to inosine) in the CNS of SHR. Moreover, the affinity of agonists to brain adenosine receptors is altered in SHR (Matias et al., 1993). Adenosine functions as a neuromodulator in the CNS, acting through cell-surface receptors (see Cunha, 2001) which were initially recognized on the basis of the ability of caffeine to act as an antagonist at A_1 and A_2 receptors (Snyder et al., 1981). At the moment, four adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) have been cloned and characterized from several mammalian species, including humans and rats, and they all belong to the G-protein-coupled receptor (GPCR) family (see Fredholm et al., 2001). There is considerable evidence supporting a modulatory role of adenosine in learning and memory, including processes such as hippocampal long-term potentiation (de Mendonça and Ribeiro, 1994) and long-term depression (de Mendonça et al., 1997) which may represent cellular mechanisms underlying memory. Moreover, previous studies have demonstrated that adenosine receptor agonists (mainly adenosine A_1 agonists) disrupt learning and memory in rodents (Homayoun et al., 2001; Normile and Barraco, 1991; Ohno and Watanabe, 1996; Zarrindast and Shafaghi, 1994), while the non-selective blockade of adenosine receptors by theophylline or caffeine, as well as the selective blockade of adenosine A_1 and A_{2A} receptors, facilitates rodent learning and memory in the inhibitory avoidance task (Kopf et al., 1999; Nehlig et al., 1992; Pereira et al., 2002; Suzuki et al., 1993) and in the water maze task (Angelucci et al., 2002; Dudley et al., 1994; Hauber and Bareiß, 2001).

The understanding of the modulatory influence of adenosine receptors on dopamine neurotransmission has increased in recent years, providing evidence of an antagonistic interaction between adenosine A_1/dopamine D_1 and adenosine A_{2A}/dopamine D_2 subtype receptors in different brain areas, such as the striatum (Franco et al., 2000; Fuxe et al., 1998). Thus, there is sufficient evidence that the adenosine receptors represent a target for the development of drugs for the treatment of diverse disorders associated with the dysregulation in dopamine neurotransmission that occurs in Parkinson's disease, schizophrenia and ADHD (see Ribeiro et al., 2003).

The purpose of the present study was to evaluate whether SHR exhibited altered spatial learning and memory compared to normotensive Wistar (WIS) rats using the spatial version of the Morris water maze. Although WKY rats have often been used as non-hypertensive controls for SHR, we did not use this strain since some previous studies have demonstrated learning impairment in young adult WKY rats (Bull et al., 2000; Diana, 2002; Grauer and Kapon, 1993). In addition, we systematically investigated the effect of the non-selective adenosine receptor antagonist caffeine on acquisition, consolidation and retrieval of WIS and SHR in the Morris water maze.

Materials and methods

Subjects

Adult female WIS and SHR rats (3 months old), weighing 220–350 g, from our own colony were used. They were kept in groups of five animals per cage and were maintained in a room under controlled temperature (23 ± 1 °C). They were subjected to a 12 h light/dark cycle (lights on 07:00 hours) with free access to food and water. We decided to use adult female rats to reproduce the fact that in human adulthood more females suffer from this disorder than males (Biederman et al., 1994). Although a previous study has shown fluctuations of the performance in the Morris water maze across the oestrous cycle of the female rat (Warren and Juraska, 1997), we did not assess the stage of the oestrous cycle of the WIS and SHR females utilized in the present experiments in order to represent an heterogeneous population. Thus, females were tested randomly throughout their cycle and all experiments were carried out between 09:00 and 12:00 hours. The procedures used in the present study complied with the guidelines on animal care of the UFSC Ethics Committee on the Use of Animals which follows the 'Principles of Laboratory Animal Care' from NIH publication no. 85-23, revised 1985.

Drugs and treatment

Caffeine (Sigma, St Louis, MO, USA) was dissolved in saline (0.9% NaCl) and was administered by intraperitoneal (i.p.) route in a volume of 0.1 ml/100 g of body weight. In the first experiment, groups of WIS and SHR received saline or caffeine (1, 3 or 10 mg/kg) 30 min before the water maze training session (see below). In the second experiment, groups of WIS and SHR received saline or a selected dose of caffeine (3 mg/kg) immediately after the training session. In the third set of experiments, groups of WIS and SHR received saline or a selected dose of caffeine (3 mg/kg) 30 min before the test session.

Apparatus

The water maze tasks were performed in a circular swimming pool similar to that described by Morris et al. (1982). The pool was made of black painted fibreglass, 1.70 m inside diameter, 0.8 m high, and filled to a depth of 0.6 m with water maintained at 25 °C. The target platform (10 × 10 cm) was made of transparent Plexiglas and submerged 1–1.5 cm beneath the surface of the water. Starting points for animals were marked on the outside of the pool as north (N), south (S), east (E) and west (W). The platform was located in the centre of the southwest (SW) quadrant at a point 35 cm from the wall of the pool. Four distant visual cues (55 × 55 cm) were placed on the walls of the water maze room. They were all positioned with the lower edge 30 cm above the upper edge of the water tank and in the standard setting the position of each symbol marked the midpoint of the perimeter of a quadrant (circle = NE quadrant, square = SE quadrant, cross = SW quadrant, and diamond = NW quadrant). The apparatus was located in a room with indirect incandescent illumination. A monitor and a video-recording system were installed in an adjacent room.

Behavioural procedures

The experiments were video-taped and the scores for latency of escape to the platform, distance travelled from the starting point to the platform, and swimming speed were later measured through an image analyser (CEFET, Curitiba, PR, Brazil). The training session consisted of 10 consecutive trials during which the animals were left in the tank facing the wall and allowed to swim freely to the escape platform. If the animal did not find the platform during a period of 120 s, it was gently guided to it. The animal was allowed to remain on the platform for 10 s after escaping to it and was then removed from the tank for 20 s before being placed at the next starting point in the tank. This procedure was repeated 10 times, with the starting points (the axis of one imaginary quadrant) varying in a pseudo-randomized manner. The test session was performed 48 h later and was similar to the training session, except that the number of trials was reduced to three.

Blood pressure (BP)

The arterial BP (mmHg) of the adult female WIS and SHR was measured 30 min after the i.p. injection of saline or caffeine (1, 3 or 10 mg/kg), using a protocol identical to that described by Ramos et al. (2002). Under anaesthesia with ketamine and xylazine (90 and 15 mg/kg respectively), a heparinized PE 20 polyethylene catheter was inserted into the right carotid artery for the recording of systolic and diastolic arterial pressure. To prevent clotting, an i.p. dose of heparin (300 IU) was injected 10 min before the ketamine/xylazine injection. Animals were allowed to breathe spontaneously via a cannula and body temperature (maintained at 36 ± 1 °C) was monitored by a rectal thermometer. After the surgical procedure, a period of 30 min was allowed for stabilization and 30 min after the i.p. administration of saline or caffeine

(1, 3 or 10 mg/kg) the systolic and diastolic arterial BP from female adult WIS and SHR were recorded for 60 min. BP data were recorded (at a 10-s sampling rate) with a Digit-Med BP Analyser system (Model 190) connected to a Digit-Med System Integrator (Model 200; Louisville, KY, USA). At the end of the experiment, the animals were sacrificed by pentobarbital overdose.

Statistical analysis

All values are expressed as means ± S.E.M. (n equals the number of rats included in each analysis). The statistical comparison of results was carried out using two-way ANOVA with strain, treatment or the number of trials (repeated measure) as independent variables. Following significant ANOVAs, differences between groups were evaluated by the post-hoc Newman–Keuls test. The accepted level of significance for the tests was $p \leqslant 0.05$. All tests were performed using the STATISTICA® 5.0 software package (StatSoft Inc., Tulsa, OK, USA).

Results

Strain differences between WIS and SHR in the Morris water maze

The results of the escape latency and the distance travelled by adult female WIS and SHR in the Morris water maze are summarized in Figure 1a, b respectively. Two-way ANOVA (strain vs. repeated measures) revealed inter-strain differences in both escape latency [$F(1,15)=3.83$, $p=0.04$] and distance travelled [$F(1,15)=10.56$, $p=0.006$] to find the platform during the training session. Subsequent Newman–Keuls tests indicated that WIS control rats learned more quickly than SHR, since although WIS and SHR demonstrated similar final escape latencies and distances travelled to find the platform, the SHR learning curve was clearly offset to the right, i.e. they needed a greater number of trials to satisfactorily acquire the spatial information (Figure 1a, b). However, statistical analysis of the test session revealed no significant effect for the strain factor in the escape latency [$F(1,15)=1.94$, $p=0.18$] and distance travelled [$F(1,15)=4.45$, $p=0.61$]. These results indicated a selective deficit in SHR spatial learning, but not in spatial memory.

The swimming speeds of WIS and SHR are illustrated in Figure 1c. ANOVA revealed a significant effect for the strain factor [$F(1,15)=9.04$, $p=0.01$] in the training session. Post-hoc comparisons indicated that SHR were significantly faster than WIS rats in the training session ($p \leqslant 0.05$, Newman–Keuls test), but no significant difference was found during the test session [$F(1,15)=0.29$, $p=0.59$] (Figure 1c).

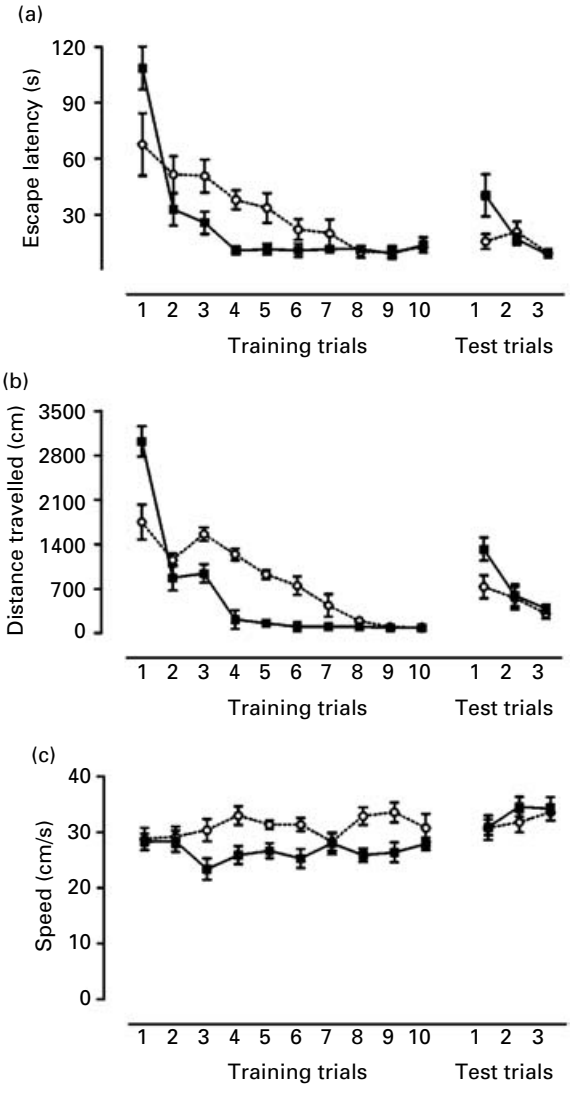

Figure 1. Comparison of spatial learning between adult female normotensive Wistar rats (WIS, –■–) and spontaneously hypertensive rats (SHR, --○--) in the Morris water maze ($n=8$ animals in each group). Date are presented as mean ± S.E.M. of (a) escape latency (s); (b) distance travelled (cm) to find a submersed platform and (c) swimming speed (cm/s). The test trials were performed 48 h after the training trials. ANOVA showed that SHR had a longer escape latency ($p<0.05$), greater distance travelled to platform ($p<0.01$) and higher velocity ($p=0.01$) compared to WIS control group in the training session. No significant effects were found in the test session.

Another marked difference between WIS and SHR performance in the Morris water maze was that

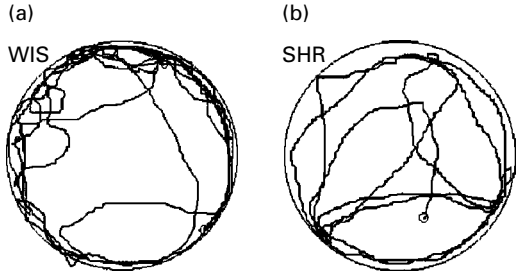

Figure 2. Tracings of the typical swimming patterns in the first trial of the training session of adult female (a) normotensive Wistar rats (WIS) and (b) spontaneously hypertensive rats (SHR) in the Morris water maze.

SHR were significantly faster in finding the platform in the first trial of the training session (Figure 1a). The analysis of the swimming patterns indicates that this result can reflect a different strategy of WIS and SHR to find the platform. WIS rats presented a significant thygmotaxis, i.e. the tendency to swim close to the maze wall (Figure 2a), while SHR displayed markedly wider loops in their search strategy (Figure 2b).

Effects of pre-training administration of caffeine on spatial learning of WIS and SHR in the Morris water maze

Figure 3 shows the results obtained when caffeine (1, 3 or 10 mg/kg i.p.) was administered to WIS rats 30 min before the training session. ANOVA indicated that the caffeine treatment did not affect the training scores of WIS rats: escape latency [$F(3,28)=1.04$, $p=0.39$], distance travelled [$F(3,28)=0.83$, $p=0.49$], and swimming speed [$F(3,28)=0.73$, $p=0.54$]. Moreover, pre-training administration of caffeine did not affect the retention scores (test session) of WIS rats [$F(3,28)=2.80$, $p=0.16$].

The effects of pre-training caffeine administration (1, 3 or 10 mg/kg i.p.) in the water maze performance of SHR are summarized in Figure 4. ANOVA revealed a significant effect for the treatment factor in the escape latency [$F(3,29)=11.42$, $p<0.0001$] and distance travelled [$F(3,29)=12.99$, $p<0.0001$] to find the platform during the training session. Subsequent Newman–Keuls tests indicated that the pre-training administration of all test doses of caffeine improved the spatial learning deficits of SHR, i.e. promoted a significant reduction in the escape latency and distance travelled to find the platform in the training session (Figure 4a, b).

Figure 4c shows the effect of caffeine treatment on the swimming speed of SHR. Two-way ANOVA (treatment vs. repeated measures) indicated that the swimming speed increased as a function of the number of trials [$F(9, 29)=3.57$, $p<0.001$], but that it was not affected by caffeine administration [$F(3,29)=0.15$, $p=0.93$].

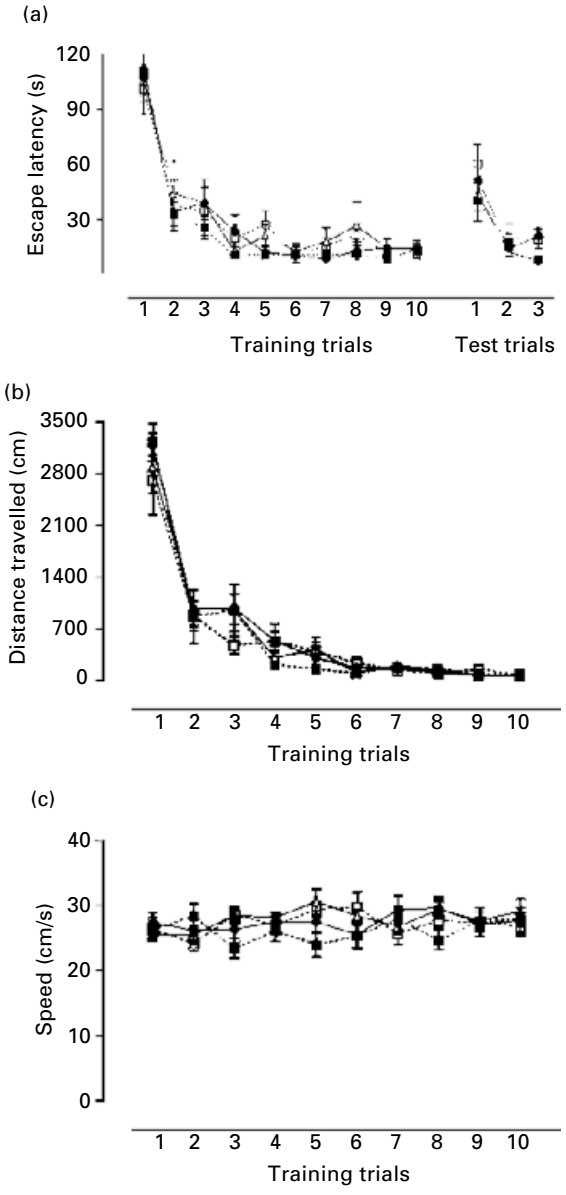

Figure 3. Effects of the administration of caffeine (1, 3 or 10 mg/kg i.p.) 30 min before the training session in the (a) escape latency (s), (b) distance travelled (cm) and (c) swimming speed (cm/s) of adult female WIS rats in the Morris water maze ($n=7$–8 animals in each group). ANOVA indicated that the pre-training administration of caffeine did not affect either the training or the test scores of WIS rats in the water maze. --■--, Saline; –●–, caffeine (1 mg/kg); –△–, caffeine (3 mg/kg); --□--, caffeine (10 mg/kg).

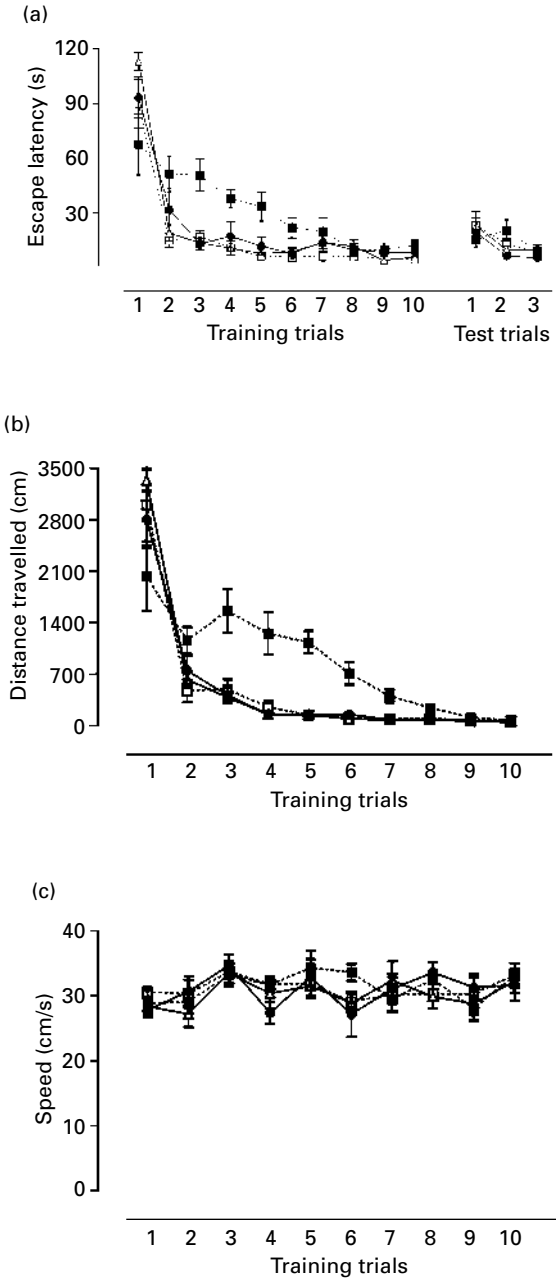

Figure 4. Effects of the administration of caffeine (1, 3 or 10 mg/kg i.p.) 30 min before the training session in the (a) escape latency (s), (b) distance travelled (cm) and (c) swimming speed (cm/s) of adult female SHR in the Morris water maze ($n = 7$–8 animals in each group). ANOVA showed that pre-training administration of caffeine promoted a significant reduction in the escape latency ($p < 0.0001$) and distance travelled ($p < 0.0001$) by SHR to find the platform in the training session, without effect on their swimming speed ($p = 0.93$). No significant effects were found in the test session. --■--, Saline; –●–, caffeine (1 mg/kg); –△–, caffeine (3 mg/kg); –□–, caffeine (10 mg/kg).

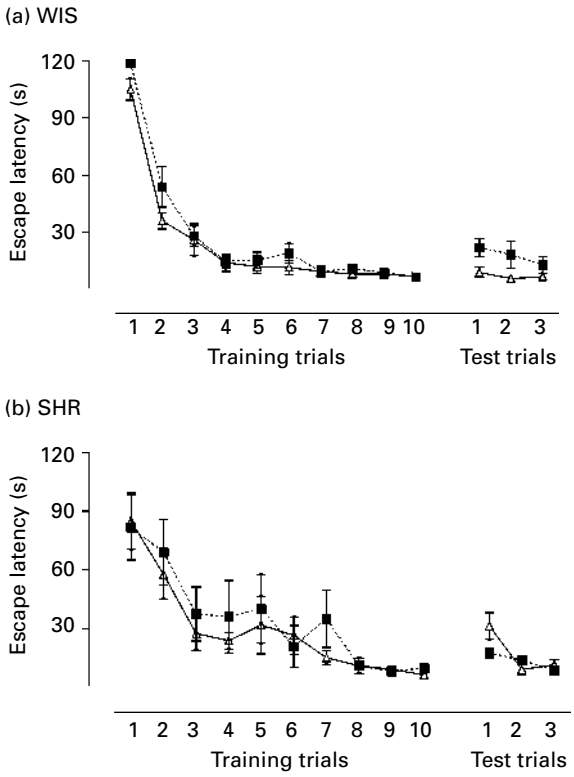

Figure 5. Effects of the administration of a selected dose of caffeine (3 mg/kg i.p.) immediately after the training session on the escape latency (s) of adult female (a) WIS rats and (b) SHR to find the platform ($n = 7$–8 animals in each group). ANOVA indicated that the post-training administration of caffeine promoted a significant reduction in the escape latency ($p = 0.03$) of WIS rats to find the platform in the test session, but that it did not alter the scores of SHR in the water maze ($p = 0.16$). --■--, Saline; –△–, caffeine (3 mg/kg).

Effects of post-training administration of caffeine on spatial memory retention of WIS and SHR in the Morris water maze

The effect of post-training administration of a selected dose of caffeine (3 mg/kg i.p.), injected immediately after the training session to WIS and SHR, are presented in Figure 5(a, b) respectively. In the training session, caffeine-treated groups did not differ from their respective controls in the escape latency [WIS: $F(1, 14) = 1.20$, $p = 0.29$; SHR: $F(1, 12) = 1.15$, $p = 0.30$]. Analysis of the test session scores showed that post-training administration of caffeine (3 mg/kg i.p.) improved the spatial retention of WIS rats [$F(1, 14) = 4.07$, $p = 0.03$] (Figure 5a), but not of the SHR group [$F(1, 12) = 2.25$, $p = 0.16$] (Figure 5b).

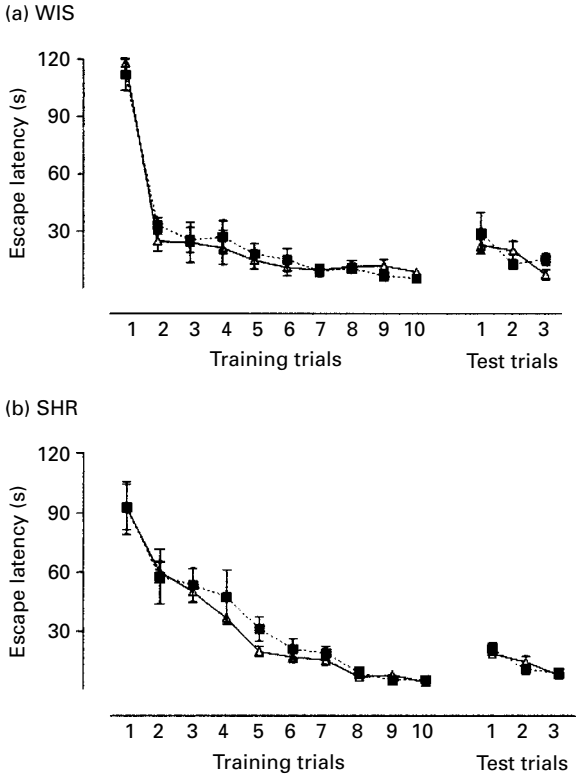

Figure 6. Effects of the administration of a selected dose of caffeine (3 mg/kg i.p.) 30 min before the test session on the escape latency (s) of adult female (a) WIS rats and (b) SHR to find the platform ($n = 7$–8 animals in each group). ANOVA indicated no significant effects for the pre-test administration of caffeine in the test scores of either WIS or SHR in the water maze. --■--, Saline; –△–, caffeine (3 mg/kg).

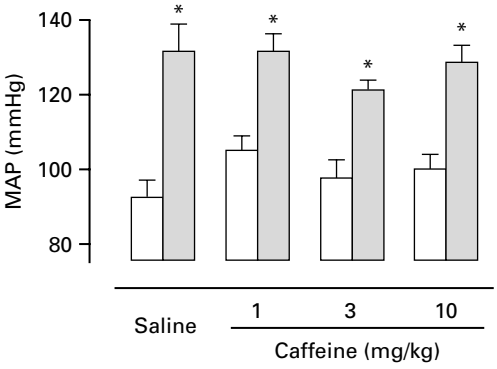

Figure 7. Effects of the administration of caffeine (1, 3 or 10 mg/kg i.p.) on mean arterial pressure (mean ± S.E.M., in mmHg) of adult female normotensive Wistar rats (WIS, □) and spontaneously hypertensive rats (SHR, ▣) ($n = 4$–5 animals in each group). ANOVA revealed that SHR presented a significantly higher mean arterial pressure ($p < 0.0001$) compared to WIS controls, but it indicated that the caffeine treatment did not significantly alter the mean arterial pressure of either WIS or SHR groups ($p = 0.33$). * $p \leqslant 0.05$ compared to the WIS of the same treated group (Newman–Keuls test).

Effects of pre-test administration of caffeine on spatial memory retrieval of WIS and SHR in the Morris water maze

The results obtained with the administration of a selected dose of caffeine (3 mg/kg i.p.) 30 min before the test session to WIS and SHR are summarized in Figure 6(a, b) respectively. In the training session, caffeine-treated groups did not differ from their respective controls in the escape latency [WIS: $F(1, 12) = 0.08$, $p = 0.78$; SHR: $F(1, 12) = 1.83$, $p = 0.20$]. Analysis of the test session scores showed that the pre-test administration of caffeine (3 mg/kg i.p.) did not alter the spatial retrieval of either the WIS [$F(1, 12) = 0.11$, $p = 0.74$] (Figure 6a), or the SHR group [$F(1, 12) = 0.31$, $p = 0.59$] (Figure 6b).

Effects of caffeine administration on arterial BP of WIS and SHR

Figure 7 shows the results of the caffeine administration (1, 3 or 10 mg/kg i.p.) on the arterial BP (mmHg) of WIS and SHR adult females. Two-way ANOVA (strain vs. treatment) revealed a significant effect for the strain factor [$F(1, 35) = 76.70$, $p < 0.0001$], but indicated no significant effect for the treatment factor [$F(3, 35) = 1.17$, $p = 0.33$] or for the interaction factor between strain and treatment [$F(3, 35) = 1.02$, $p = 0.40$]. Post-hoc comparisons indicated that, as expected, vehicle-treated SHR were hypertensive in relation to their WIS controls. However, the administration of the same doses of caffeine (1, 3 or 10 mg/kg i.p.) that were able to improve the spatial learning deficits in SHR did not significantly alter the mean arterial pressure of either WIS or SHR (Figure 7).

Discussion

The present findings demonstrate selective deficits in spatial learning, but not in spatial memory, of adult female SHR compared to normotensive female WIS rats. More importantly, our results demonstrate for the first time that single pre-training administration of caffeine (1–10 mg/kg i.p.) improves the spatial learning deficits of SHR in the Morris water maze, without altering their hypertensive state.

In accordance with previous studies reporting a reduced performance of SHR in different paradigms used to investigate learning and memory processes, our results demonstrate a poor performance of SHR compared to WIS rats in the spatial version of the

Morris water maze. However, in contrast to reports that have demonstrated impairment of SHR in both spatial learning and memory in the water maze (Gattu et al., 1997; Wyss et al., 2000), the present results indicate a selective deficit in spatial learning (but not memory) of SHR, since they performed in a similar way to control WIS rats in the test session. This discrepancy with early data may be explained by differences in the age and/or gender of the subjects utilized, but we believe that it mainly reflects the difference between the protocols utilized to evaluate the spatial learning and memory in the water maze. In these previous studies, each rat was given 2–4 trials per day for 4–5 consecutive days to find the platform (Gattu et al., 1997; Wyss et al., 2000), while in the current study each rat was given 10 consecutive trials during the training session (only 1 day) and the test session occurred 48 h later. Thus, it is possible that a training schedule with a higher number of consecutive trials instead of repeatedly training over a number of days promotes an equivalence in the learning performance for both strains, which can be observed in the similar pattern of the escape latencies of the latter's training trials (see Figure 1). This equalization in the latency to find the platform may reflect the fact that learning deficits presented by SHR can be softened by the repetitions of the task, given the evidence that if they were utilized in a properly arranged protocol, they would be able to acquire the information just as well as the WIS rats. This affirmation is reinforced by the present lack of differences between SHR and WIS rats in the test session after 10 trials of the training session.

The exact mechanism responsible for this cognitive deficit in SHR is still unknown. Converging evidence suggests a primary role of disturbance in dopamine neurotransmission in SHR (Carey et al., 1998; Papa et al., 2002; Russel, 2002; Russel et al., 1998). Additionally, some reports have also shown functional alterations in the adenosinergic neurotransmission in SHR (Davies et al., 1987; Illes et al., 1989; Kamikawa et al., 1980; Matias et al., 1993). The fact that SHR present a reduced activity of adenosine deaminase (Davies et al., 1987), the enzyme which catabolizes adenosine to inosine, and consequently an increase in the activity of the adenosinergic system, may be partially responsible for the disruption in cognitive processes, since there is considerable evidence supporting a negative effect of adenosine receptor agonists on learning and memory in rodents (Homayoun et al., 2001; Normile and Barraco, 1991; Ohno and Watanabe, 1996; Zarrindast and Shafaghi, 1994). In the present work, the pre-training administration of acute doses of caffeine (1–10 mg/kg i.p.), a non-selective adenosine receptor antagonist, improved the spatial learning deficits in SHR, promoting a significant reduction in the escape latency and distance travelled by SHR to find the platform in the training session. These results cannot be explained by a direct increase in locomotor performance, since no alteration was observed in the swimming speed of caffeine-treated groups. In contrast, post-training administration of caffeine (3 mg/kg i.p.) increased the spatial memory retention of WIS, but not SHR, indicated by a significant reduction in the escape latency in the test session. This same dose of caffeine was ineffective when administered before testing in both WIS and SHR.

Our results suggest that caffeine presents different effects depending on the stages of memory processing (acquisition, consolidation and retrieval) and that these effects depend on the strain studied. The present selective effect of caffeine on memory consolidation of the WIS group (control) agrees with most of the previous studies which have reported improvement of memory retention in rodents after a post-training administration of caffeine (Angelucci et al., 1999, 2002; Cestari and Castellano, 1996; Yonkov and Roussinov, 1983). Moreover, reinforcing early studies in which pre-training administration of caffeine was ineffective or even impaired memory retention in rodents (Angelucci et al., 1999, 2002; Izquierdo et al., 1979), the present pre-training administration of caffeine failed to improve the spatial learning in WIS rats. Nevertheless, the pre-training administration of the same range of doses of caffeine (1–10 mg/kg i.p.) was able to improve the spatial learning deficits of SHR in the Morris water maze, significantly reducing the escape latency and the distance travelled to find the platform in the training session. A conceivable explanation for the present results is that the difficulty experienced by SHR in finding the platform during the training session is a consequence of their impulsivity and poorly sustained attention. Thus, the administration of caffeine, a drug traditionally known to improve attention (Nehlig et al., 1992), prior to the training session can reverse the attention deficit in SHR and facilitate their spatial learning (De Bruin et al., 2003).

Another important discussion concerning the cognitive defficits in SHR is whether these symptoms are directly associated with hypertension or whether these two phenomena present distinct mechanisms. The activation of adenosine receptors has been shown to lower BP, whereas their blockade causes hypertension (Abdel-Rahman and Tao, 1996; Azevedo and

Osswald, 1992; Biaggioni, 1992). However, our results indicate that the doses of caffeine that were able to improve the spatial learning deficits in SHR did not alter the hypertensive state, demonstrating that this cognitive impairment in SHR might not be entirely explained by hypertension. These results are in accordance with previous studies that demonstrate a decline in the cognitive functions of the normotensive WKY control strain (Bull et al., 2000; Diana, 2002; Diana et al., 1994; Grauer and Kapon, 1993) and the improvement of SHR learning with the administration of drugs that do not interfere with BP, such as the combination of uridine and choline (De Bruin et al., 2003). In brief, these studies suggest that some other factors different from hypertension could contribute to the learning deficits of SHR.

To date, the most effective and frequently prescribed drugs for ADHD, methylphenidate (Ritalin®) and d-amphetamine, are psychostimulants that inhibit re-uptake and stimulate the release of dopamine, thereby increasing the temporal and spatial presence of dopamine at post-synaptic receptors (Krause et al., 2000; Safer and Krager, 1988). However, the increasing amount of evidence indicating an antagonistic interaction between adenosine and dopamine receptors in different brain areas (Fuxe et al., 1998; Franco et al., 2000), associated with the present improvement of spatial learning deficits in a model of ADHD by caffeine, suggest that adenosine receptor antagonists might represent an important therapeutic tool for the treatment of ADHD. Results from previous clinical studies on the efficacy of caffeine in ADHD have been inconsistent, with some authors demonstrating an improvement of general condition in ADHD children (Dalby, 1985; Garfinkel et al., 1981; Reichard and Elder, 1977; Schechter and Timmons, 1985), while others have not found convincing positive caffeine effects at all (Arnold et al., 1978; Firestone et al., 1978; Gross, 1975; Huestis et al., 1975; Kupietz and Winsberg, 1977). These studies, performed with a small number of subjects, have had no substantial impact on the alteration of clinical treatment strategies, despite the fact that they have not been systematically refuted. For this reason, the consensus in the field is that adjunctive caffeine is not contra-indicated for the treatment of ADHD, but it is also not a viable replacement for the currently used drugs (see Castellanos and Rapoport, 2002). Thus, the performance of additional longitudinal studies to verify the effects of caffeine in ADHD therapy appears to be important.

In conclusion, the present findings confirm and extend the presence of cognitive impairments in SHR, demonstrating a selective deficit in the spatial learning, but not the spatial memory, of adult SHR compared to normotensive WIS rats. Furthermore, our results demonstrate for the first time that a single pre-training administration of caffeine (1–10 mg/kg i.p.) improves the spatial learning deficits of SHR in the Morris water maze, and that this effect is not directly related to hypertension. Additional research is necessary to evaluate whether there exists any interaction between the adenosinergic and other neurotransmitter systems in this effect of caffeine. Finally, a better evaluation of the potential of adenosine receptor antagonists in ADHD therapy is also indicated.

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Statement of Interest

None.

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