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Research report

Activation of adenosine A_1 receptors alters behavioral and biochemical parameters in hyperthyroid rats

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Abstract

Adenosine acting on A_1 receptors has been related with neuroprotective and neuromodulatory actions, protection against oxidative stress and decrease of anxiety and nociceptive signaling. Previous studies demonstrated an inhibition of the enzymes that hydrolyze ATP to adenosine in the rat central nervous system after hyperthyroidism induction. Manifestations of hyperthyroidism include increased anxiety, nervousness, high O_2 consumption and physical hyperactivity. Here, we investigated the effects of administration of a specific agonist of adenosine A_1 receptor (N^6-cyclopentyladenosine; CPA) on nociception, anxiety, exploratory response, locomotion and brain oxidative stress of hyperthyroid rats. Hyperthyroidism was induced by daily intraperitoneal injections of L-thyroxine (T4) for 14 days. Nociception was assessed with a tail-flick apparatus and exploratory behavior, locomotion and anxiety were analyzed by open-field and plus-maze tests. We verified the total antioxidant reactivity (TAR), lipid peroxide levels by the thiobarbituric acid reactive species (TBARS) reaction and the free radicals content by the DCF test. Our results demonstrated that CPA reverted the hyperalgesia induced by hyperthyroidism and decreased the exploratory behavior, locomotion and anxiety in hyperthyroid rats. Furthermore, CPA decreased lipid peroxidation in hippocampus and cerebral cortex of control rats and in cerebral cortex of hyperthyroid rats. CPA also increased the total antioxidant reactivity in hippocampus and cerebral cortex of control and hyperthyroid rats, but the production of free radicals verified by the DCF test was changed only in cerebral cortex. These results suggest that some of the hyperthyroidism effects are subjected to regulation by adenosine A_1 receptor, demonstrating the involvement of the adenosinergic system in this pathology.

Keywords: Adenosine; Hyperthyroidism; Nociception; Anxiety; Free radicals; CPA

1. Introduction

Adenosine is a neuromodulator involved in an array of functions in a variety of physiological systems [5,26]. The levels of extracellular adenosine enhance during periods of increased metabolic demand, such as seizures, ischemia, hypoxia and stressful challenges [3,26]. Adenosine acts on four cloned and pharmacologically characterized receptors, A_1, A_{2A}, A_{2B} and A_3 [5]. However, the A_1 adenosine receptor is the most prevalent adenosine receptor subtype, having both a high level of expression and a widespread distribution [11]. The inhibitory actions of adenosine on the neurotransmitter release and reduction of cellular excitability are mediated by specific plasma membrane receptors of the A_1 subtype [5]. Actions due to adenosine A_1 receptors activation have been proposed to result from coupling to inhibitory protein G_i, inhibition of adenylate cyclase, inhibition of presynaptic voltage-sensitive Ca^{2+} channels and activation of postsynaptic K^+ channels [17,22,38].

In addition, adenosine-mediated inhibitory influences on nociceptive reflex responses have been demonstrated in experimental and clinical situations [25,39,24]. It is known

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that the antinociceptive properties of adenosine are associated with adenosine A_1 receptor activation at spinal sites [39].

Studies performed in mice lacking the adenosine A_1 receptor confirm the involvement of adenosine in motor activity, exploratory behavior, anxiety and aggressiveness [18]. Furthermore, adenosine antagonists like caffeine promote wakefulness, aggressive behavior in rats and nervousness and irritability in man [16], while adenosine analogues counteract these effects [33].

Adenosine is also able to attenuate the deleterious consequences of reactive oxygen species (ROS) through A_1 receptors activation in rat hippocampal slices [2]. In addition, oxidative stress induces expression of the A_1 receptors [32], which provide cytoprotection in the central nervous system [36].

Adenosine can be released as such through bi-directional transporters or can be produced from the ATP hydrolysis by the action of the ecto-nucleoside triphosphate diphosphohydrolase family (NTPDases) and a 5′-nucleotidase (CD73, EC 3.1.3.5) [42]. We have demonstrated that hyperthyroidism inhibits the sequential hydrolysis of ATP to adenosine in hippocampus and cerebral cortex of rats during different phases of development [6]. Furthermore, adenosine A_1 receptors and adenosine transport are reduced after the hypothyroidism induction [13].

Thyroid disease in adults is accomplished by both neurological symptoms and behavioral abnormalities [40]. Hyperthyroidism is characterized by anxiety, nervousness, irritability, tachycardia, emotional lability, weight loss, increased perspiration, sleep disturbances, weak muscles and physical hyperactivity, while extreme cases may include seizures, delirium and coma [40]. These excitatory effects can be associated with the increased synthesis of catecholamine [12] and ability of thyroid hormones to block $GABA_A$-induced Cl^- currents in adult brain [30].

Considering the protective and modulatory effects of adenosine via A_1 receptor activation, the present study investigates the consequences of the administration of a specific agonist of adenosine A_1 receptor on behavioral and biochemical parameters in adult hyperthyroid rats, verifying a possible relation between the adenosinergic system and this thyroid disorder.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 160–240 g were used throughout this study. Animals were housed in cages with food and water available ad libitum and were maintained under a 12-h light:12-h dark cycle (light on at 07:00 h) at a room temperature of 25 °C.

Initially, rats were randomized into four different groups: control, hyperthyroid (hyper), control injected with CPA (control + CPA) and hyperthyroid injected with CPA (hyper + CPA).

Procedures for the care and use of animals were adopted according to the regulations of Colégio Brasileiro de Experimentação Animal (COBEA), based on the *Guide for the Care and Use of Laboratory Animals* (National Research Council).

2.2. Induction of hyperthyroidism

Hyperthyroidism was induced by daily intraperitoneal injections of L-thyroxine (T4), 25 μg/100 g body weight, for 14 days [34]. T4 was dissolved using 0.04 M NaOH and the final solution was prepared with saline solution. Control animals received intraperitoneal injections of saline solution. This treatment results in a long-term moderate hyperthyroidism [34]. Animals were used 24 h after the last injection. After the behavioral tests, the animals were killed by decapitation.

2.3. Drug administration

The agonist of adenosine A_1 receptor, N^6-cyclopentyladenosine (CPA), was used because it readily reaches the brain after peripheral injection and has high specificity for adenosine A_1 receptor [23]. Hyperthyroid (hyper + CPA) and control (control + CPA) rats received an intraperitoneal injection of CPA (0.5 mg/kg) dissolved in saline solution 0.9%. Rats that not were injected with CPA (groups hyper and control) received an intraperitoneal injection of saline solution. CPA or saline were administrated 24 h after the last injection with T4 or saline.

2.4. Body temperature

The body temperature was determined in four groups of animals mentioned above, using a rectal thermistor probe (0.4 mm outer diameter microprobe). The temperature probe was inserted rectally 2 cm in rats. Rectal temperature was measured in rats injected with saline (control rats) and rats treated with T4, before (considered the basal measurement) and 30 min after the CPA injection. All testing were done in a room maintained at 23–25 °C.

2.5. Tail-flick measurement

In order to investigate the effects of the adenosine A_1 receptor agonist (CPA) on nociception in hyperthyroidism, rats injected with saline (controls) and rats treated with T4 were submitted to tail-flick measurement before (considered the basal measurement), 30, 60 and 120 min after the CPA injection.

Nociception was assessed with the tail-flick apparatus [8]. Rats were wrapped in a towel and placed on the apparatus. The light source positioned below the tail was focused on a point 2–3 cm rostral to the tip of the tail. Deflection of the tail activated a photocell and automatically terminated the trial. The tail-flick latency (TFL) represented the period of time (seconds) from the beginning of the trial to the tail deflection. A cut-off time of 10 s was used to avoid tissue damage. At the end of T4 treatment and 24 h before the first measurement, the animals were exposed to the tail-flick apparatus to familiarize them with the procedure, since the novelty of the apparatus can itself induce antinociception [31].

2.6. Open-field test

The open-field test was performed 30 min after the administration of CPA. The apparatus consisted of a 50-cm-high, 50-cm-wide, 39-cm-deep open field with black plywood wall and a brown floor divided into 12 equal squares by black lines. Animals were gently placed on the left rear square. The number of line crossings, rearings and the time on the central squares [21] were measured for a 5 min period. The number of crossings measured the locomotor activity and the number of rearings was used to determine the exploratory behavior. The time on the central quadrants was used as an anxiety measurement.

2.7. Plus-maze test

The elevated plus-maze test was conducted using a standard plus-maze apparatus kept 80 cm above the floor, consisting of two enclosed arms (50 × 10 cm) and two open arms (50 cm × 10 cm) [35]. The four arms were joined at the center by a 10 cm square platform. This test was realized 30 min after the administration of CPA. The animals were placed in center of the plus-maze facing one of the open arms. The time in the open and enclosed arms was measured for 5 min, in order to evaluate the anxiety of the animals. The number of entries in the

open and enclosed arms measured the locomotor activity. The ratio "time spent in the open arms/time spent in all arms (open and enclosed)" was calculated and multiplied by 100, to observe the percentage of time spent in open arms (%OA). This parameter is related to the inhibition of the anxiety level experienced by the animals.

2.8. Preparation of the brain samples

Animals were killed by decapitation 35 min after the injection of CPA or saline and their hippocampi and cerebral cortex were quickly dissected out and frozen in liquid N_2. Samples were stored at −70 °C until analysis, when they were homogenized in 10 vol (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4), containing 140 mM KC1 and 1.0 mM EDTA. The homogenate was centrifuged at 960 × *g* for 10 min and the supernatant was used for the experiments.

2.9. Free radical content (DCF)

To assess the free radicals content, we used 2′-7′-dichlorofluorescein diacetate (DCFH-DA) as a probe. An aliquot of the sample was incubated with DCFH-DA (100 μM) at 37 °C for 30 min. The reaction was terminated chilling the reaction mixture in ice. The formation of the oxidized fluorescent derivative (DCF) was monitored at excitation and emission wavelengths of 488 and 525 nm, respectively, using a fluorescence spectrophotometer (Hitachi F-2000). The free radicals content was quantified using a DCF standard curve and results were expressed as pmol DCF produced/mg protein. All procedures were performed in triplicate, in the dark, and blanks containing DCFH-DA were processed for measurement of autofluorescence [10].

2.10. Determination of TBA reactive substances (TBARS)

The formation of thiobarbituric acid reactive substances (TBARS) was used as an indicator of lipoperoxidation. Malondialdehyde (MDA), a product of lipoperoxidation, reacts with thiobarbituric acid (TBA) at low pH and high temperature. This test was based on the methods described by Buege and Aust [7]. Aliquots of samples were incubated with 10% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid. The mixture was heated (30 min) on a boiling water bath. Afterwards, *n*-butanol was added and the mixture was centrifuged. The organic phase was collected to measure fluorescence at excitation and emission wavelengths of 515 and 553 nm, respectively. 1,1,3,3-Tetramethoxypropane, which is converted to malondialdehyde, was used as standard. The formation of TBARS was expressed as MDA equivalents/mg of protein.

2.11. Total antioxidant reactivity (TAR) assay

The reaction mixture contained 2.0 mM 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) and 6.0 mM luminol in glycine buffer, pH 8.6. The addition of Trolox (antioxidant standard, 20 nM) or samples (1.0 μl) decreases chemiluminescence proportionally to the concentration of antioxidants. TAR values were determined by assessing the initial decrease of luminescence calculated as the ratio "I_o/I", where "I_o" is the chemiluminescence in the absence of additives and "I" is the chemiluminescence after addition of Trolox, or of samples [27]. TAR values were expressed as equivalents of Trolox concentration/mg protein.

2.12. Protein assay

The total protein concentrations were determined using the method described by Lowry et al. [28] with bovine serum albumin as the standard.

2.13. Statistical analysis

The data were analyzed using a commercial statistical package (SPSS/PC+). The results for the tail-flick were expressed as median (interquartile range) of at least eight animals and were analyzed by Mann–Whitney *U*-test (two-tailed) or Wilcoxon test. The analgesic response was calculated as percent of the maximum possible effect (%MPE) using the following equation: MPE = ((test latency − control latency)/(10 − control latency)) × 100. The results for %MPE and the body temperature measurement were analyzed by Student's *t*-test, whereas the results obtained in the open-field and plus-maze tests were expressed as means ± S.D. values of at least 10 animals and were evaluated by Two-Way ANOVA followed by Duncan's test. The data obtained to the DCF, TBARS and TAR tests were expressed as means ± S.D. values of at least four animals and were analyzed by Two-Way ANOVA followed by Duncan's test. Values of $P < 0.05$ were considered significant.

3. Results

The body temperature of the hyperthyroid rats (37.41 ± 0.54 °C, $n = 8$) was not significantly different from control rats (37.35 ± 0.63 °C, $n = 8$). CPA administration significantly decreased the temperature in control rats (35.73 ± 1.2 °C, Student's *t*-test for paired samples, $P < 0.05$), but did not affect the temperature in treated rats (37.67 ± 0.70 °C, $n = 8$) 30 min after the CPA injection.

Nociception was assessed in hyperthyroid and control rats with a tail-flick apparatus, before (basal measurement), 30, 60 and 120 min after the CPA injection (Fig. 1). In the basal measurement, the tail-flick latency was significantly decreased in hyperthyroid (Mann–Whitney *U*-test, $P = 0.02$) in comparison to control animals, demonstrating that the hyperthyroidism increases the pain threshold. CPA elicited a significant increase in the tail-flick latency at 30 and 60 min after the drug administration in control rats (Wilcoxon test, $P = 0.001$). However, at 120 min after the CPA administration, the tail-flick latency returned to basal levels in the control rats. Hyperthyroid rats also showed an increase in the tail-flick latency in response to CPA administration, characterizing antinociception (Wilcoxon test, $P = 0.02$). This antinociceptive effect was observed at 30, 60 and 120 min after the injection of CPA in rats treated with T4. These results indicate that the administration of CPA elicited an analgesic response in both control and animals treated with T4. However, control and hyperthyroid rats demonstrated distinct effects: at 30 min after the CPA injection, the tail-flick latency increased by 95% in control rats, but in hyperthyroid rats this increase was of 289% (Fig. 1). Furthermore, the %MPE after

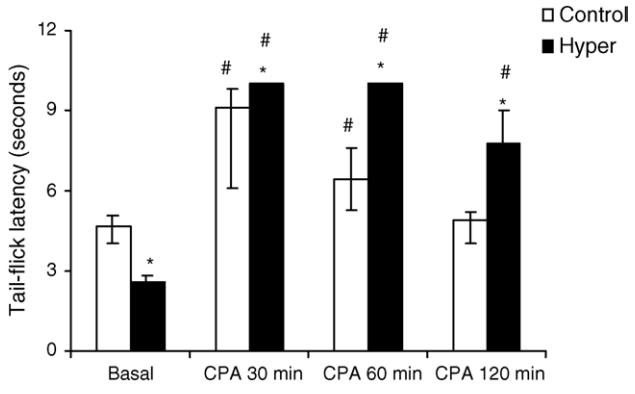

Fig. 1. Nociceptive response to the tail-flick test in control and hyperthyroid rats before (considered the basal measurement), 30, 60 and 120 min after the CPA administration. Results were expressed as median (interquartile range) of at least eight animals. $^{*}P < 0.05$ were considered significantly different from control (Mann–Whitney *U*-test) and $^{\#}P < 0.05$ were considered significantly different from basal (Wilcoxon test).

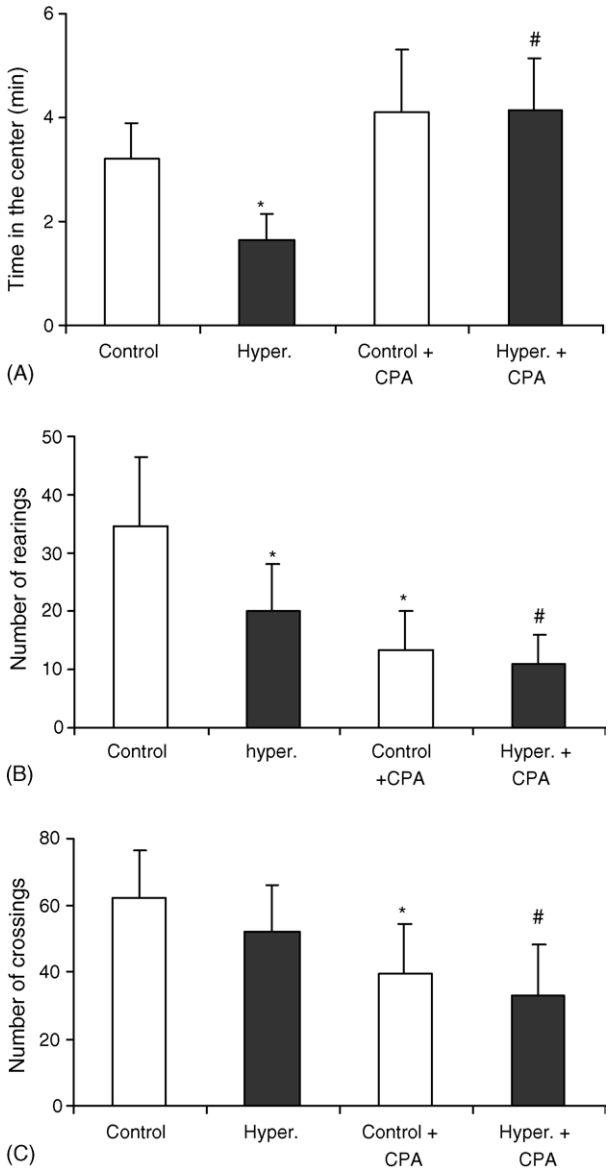

Fig. 2. Effect of administration of CPA in control and hyperthyroid rats in the open-field test on: (A) the time spent (min) on the central squares, (B) the number of rearings and (C) the number of crossings. Results were expressed as means ± S.D. values of at least 10 animals per group. $^{*}P < 0.05$ were considered significantly different from control and $^{\#}P < 0.05$ were considered significantly different from hyperthyroid rats (Two-Way ANOVA followed by Duncan's test).

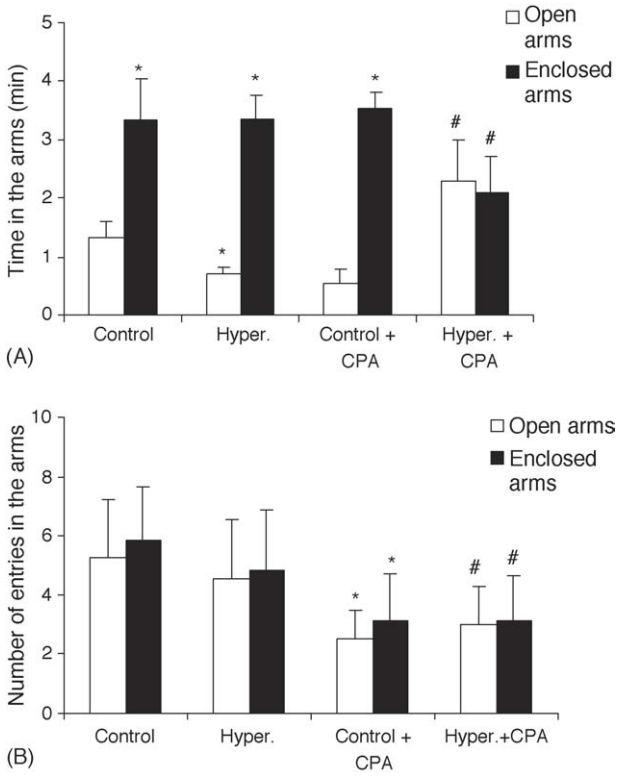

Fig. 3. Effect of administration of CPA in control and hyperthyroid rats in the plus-maze test on: (A) the time spent (min) in the open and enclosed arms ($^{*}P < 0.05$ were considered significantly different from open arms and $^{\#}P < 0.05$ were considered significantly different from hyperthyroid rats (Two-Way ANOVA followed by Duncan's test) and (B) the number of entries in the open and enclosed arms. Results were expressed as means ± S.D. values of at least 10 animals per group. $^{*}P < 0.05$ were considered significantly different from control and $^{\#}P < 0.05$ were considered significantly different from hyperthyroid rats (Two-Way ANOVA followed by Duncan's test).

the CPA injection was significantly higher in hyperthyroid rats at 60 and 120 min, respectively (92 and 57%, Student's *t*-test, $P = 0.001$), in relation to control rats (30 and −5%).

Fig. 2 shows the effect of CPA administration on parameters related to anxiety, exploratory response and locomotion, obtained by exposure of hyperthyroid and control rats to the open-field test. The time spent in the central squares of the open-field by the hyperthyroid rats was significantly lower than the time spent by the control animals (Two-Way ANOVA, $P = 0.01$, followed by Duncan's test; Fig. 2A), confirming data from the literature [4]. However, there was a significant interaction between hyperthyroidism and CPA administration (Two-Way ANOVA, $P = 0.01$), since CPA administration in hyperthyroid, but not in control rats, significantly increased the time in the central squares of the open-field (Duncan's test, $P = 0.02$), indicating an anxiolytic effect of CPA in hyperthyroidism (Fig. 2A). These results suggest an involvement of this agonist of adenosine A_1 receptor with the anxiety in hyperthyroid rats.

The number of rearings in the open field in hyperthyroid rats was significantly decreased in relation to control rats (Two-Way ANOVA followed by Duncan's test, $P = 0.04$), indicating that the treatment with T4 decreased exploratory behavior (Fig. 2B). CPA administration decreased the number of rearings in both hyperthyroid and control rats (Two-Way ANOVA, $P = 0.001$, followed by Duncan's test, $P = 0.01$ and 0.03, respectively).

The number of crossings in the open-field task was not significantly different in hyperthyroid rats when compared to control rats, but after CPA administration, the number of crossings decreased in control and hyperthyroid animals. This result indicates an effect of this agonist on rat locomotion activity (Two-Way ANOVA, $P = 0.02$, followed by Duncan's test, $P = 0.04$ and 0.03, respectively; Fig. 2C).

In the plus-maze test, we evaluated the effects of the administration of CPA on the anxiety and locomotion of the hyperthyroid rats (Fig. 3). Both hyperthyroid and control animals showed preference for the closed arms in relation to open arms (Two-

Way ANOVA followed by Duncan's test, $P = 0.0002$ and 0.0005; Fig. 3A). However, the time spent in the open arms by hyperthyroid rats was significantly lower than the control group, indicating an increased anxiety in hyperthyroidism (Two-Way ANOVA, followed by Duncan's test, $P = 0.04$). In control rats, CPA administration did not modify time spent in closed or open arms (Fig. 3A). In hyperthyroid rats, however, CPA administration reduced the time spent in closed arms, and increased the preference for the open arms (Duncan's test, $P = 0.04$ for enclosed arms and 0.03 for open arms). In addition, there was a significant interaction between T4 treatment and CPA administration on the time spent in the open arms (Two-Way ANOVA, $P = 0.006$). Similarly, comparing the percent of time spent in the open arms in relation to the total time spent in all arms (%OA), we also observed a significant interaction between T4 treatment and CPA administration (control group: $27 \pm 15\%$; hyper: $19.9 \pm 8.6\%$; control group + CPA: $18 \pm 10\%$; hyper + CPA: $52.2 \pm 23.3\%$, Two-Way ANOVA, $P = 0.01$), since this drug presented an anxiolytic effect only in hyperthyroid rats. These results indicate that the administration of the selective adenosine A_1 receptor agonist (CPA) decreased the anxiety in hyperthyroid rats, reinforcing the participation of the adenosine A_1 receptor in the anxiety induced by hyperthyroidism.

The locomotion activity was also evaluated by the plus-maze test (Fig. 3B). There was no significant difference between hyperthyroid and control rats in relation to number of entries in the enclosed or open arms. However, CPA administration reduced the number of entries in closed and open arms, in control (Two-Way ANOVA followed by Duncan's test, $P = 0.04$ for closed arms and 0.03 for open arms, respectively) and hyperthyroid rats (Two-Way ANOVA followed by Duncan's test, $P = 0.03$ for closed arms and 0.02 for open arms), indicating that this agonist decreases the locomotor activity (Fig. 3B).

In the present study, we also investigated production of free radicals, the lipoperoxidation, as well as the total antioxidant reactivity levels in rats treated with T4 after CPA administration (Fig. 4). To verify the effect of adenosine A_1 receptor on free radicals production in hippocampus and cerebral cortex of hyperthyroid rats, we used the DCF test. This measurement was increased by 78% in cerebral cortex of hyperthyroid rats when compared to control rats (Two-Way ANOVA followed by Duncan's test, $P = 0.001$; Fig. 4A). CPA administration decreased significantly the DCF measurement in cerebral cortex of control (81%) and hyperthyroid (84%) rats (Two-Way ANOVA followed by Duncan's test, $P = 0.002$ for control and 0.001 for hyper), but in hippocampus no significant effect was observed in the groups studied (Fig. 4A).

The effects of CPA administration on lipid peroxidation (TBARS) were studied in hippocampus and cerebral cortex of hyperthyroid and control rats (Fig. 4B). The results demonstrated a significant increase of TBARS (19%) in hippocampus of rats treated with T4 when compared to controls (Two-Way ANOVA followed by Duncan's test, $P = 0.04$). This effect, however, was not observed in cerebral cortex. Moreover, CPA administration decreased TBARS, demonstrating an effect on the lipid peroxidation in both hippocampus (17%) and cerebral cortex (34%) of control rats (Two-Way ANOVA followed by Duncan's test, $P = 0.04$ for hippocampus and 0.004 for cerebral cortex). When hyperthyroid rats received CPA, the lipid peroxidation was decreased by 26% in cerebral cortex (Two-Way ANOVA followed by Duncan's test, $P = 0.03$) but not in hippocampus.

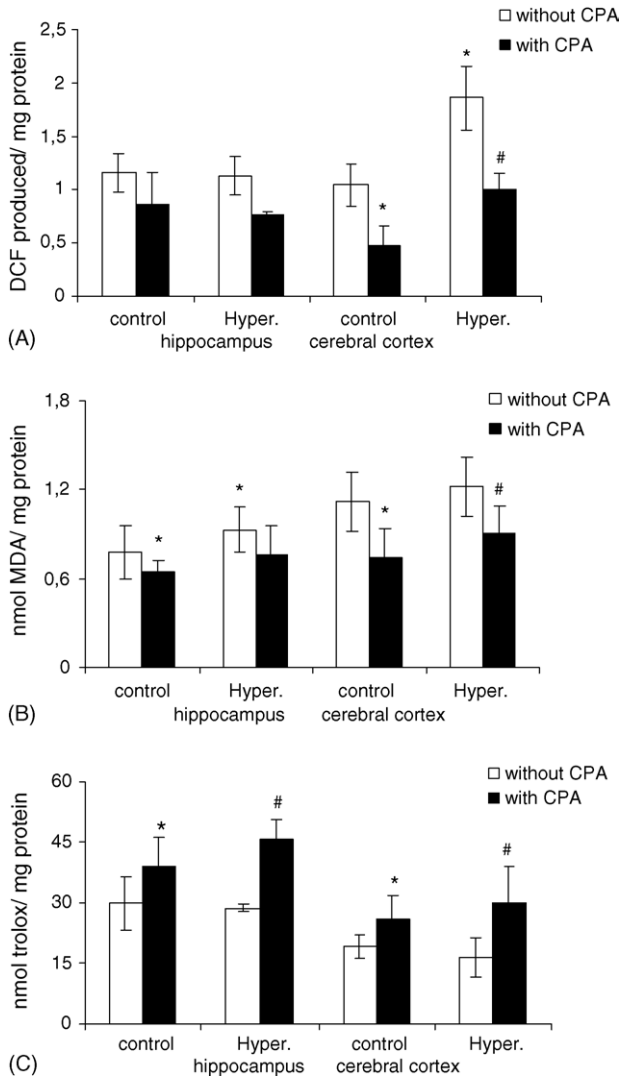

Fig. 4. Effect of administration of CPA in hippocampus and cerebral cortex of control and hyperthyroid rats on: (A) free radicals content by the formation of the oxidized fluorescent derivative (DCF); (B) thiobarbituric acid reactive substances (TBARS); (C) total antioxidant reactivity (TAR). Results were expressed as means ± S.D. values of at least four animals per group. $^{*}P < 0.05$ were considered significantly different from control and $^{\#}P < 0.05$ were considered significantly different from hyperthyroid rats (Two-Way ANOVA followed by Duncan's test).

Finally, TAR level, which is a measurement of the tissue capacity to protect itself from free radicals, was increased by 30% in hippocampus and by 37% in cerebral cortex of control rats after CPA administration (Two-Way ANOVA followed by Duncan's test, $P = 0.02$ for hippocampus and 0.01 for cerebral cortex; Fig. 4C). Furthermore, the administration of CPA in hyperthyroid rats significantly increased the TAR measurement in hippocampus (60%) and cerebral cortex (82%) (Two-Way ANOVA followed by Duncan's test, $P = 0.004$ for hippocampus and 0.002 for cerebral cortex). These results suggest that

the antioxidant defenses of the hippocampus and cerebral cortex were significantly altered by the use of CPA, indicating an increased capacity to regulate the damage associated with reactive species.

4. Discussion

Given the strong evidence that adenosine A_1 receptor agonists have protective effects in mature animals [15], this study investigated the effect of the agonists of adenosine A_1 receptor on some behavioral and biochemical parameters after the hyperthyroidism induction.

The present results confirm previous studies describing the influence of the adenosine A_1 receptors on the nociceptive response [39]: the administration of a selective adenosine A_1 receptor agonist elicited an antinociceptive response in control animals, as previously demonstrated by Reeve and Dickenson [37]. However, our results demonstrated that hyperthyroidism induces a hypernociceptive response in adult rats (Fig. 1). In addition, this hyperalgesic response was inhibited by the CPA administration, indicating the influence of the adenosine A_1 receptors on the nociception of hyperthyroid rats. Furthermore, the magnitude of analgesia induced by CPA was higher in hyperthyroid (289%) than in control rats (95%) (Fig. 1). Secondly, at 120 min after the CPA injection, the nociceptive threshold returned to basal levels in control rats, while the hyperthyroid animals remained analgesic (Fig. 1). These results indicate a distinct effect of the adenosine A_1 receptor in relation to hyperthyroid and control animals. Furthermore, the %MPE was significantly increased in hyperthyroid rats at 60 and 120 min after the CPA injection, demonstrating a higher analgesic effect in hyperthyroid rats. The permanence of the analgesic effect of CPA in hyperthyroid rats could reflect an up-regulation of the adenosine A_1 receptor and, consequently, an increase of the analgesic effects mediated by this receptor.

It is widely known the correlation between hyperthyroidism and some disorders, such as anxiety, hyperactivity and motor problems [4]. In agreement with the literature, our results demonstrated an increased anxiety in hyperthyroid rats [4] (Fig. 2A). In fact, endocrinological symptoms related to high level of thyroid hormones are correlated with the severity of anxiogenic state [41]. However, CPA administration in hyperthyroid rats induced an anxiolytic behavioral pattern in the open-field (Fig. 2A) and plus-maze tests (Fig. 3A). These results indicate that the anxiety observed in hyperthyroidism could be related to modulation of the adenosine A_1 receptor and possibly may be regulated by specific adenosinergic agents. Although the role of agonists of adenosine A_1 receptors in the modulation of anxiety has been previously shown [14,18], the anxiolytic effect of these receptors still not had been described to hyperthyroidism.

Our results also showed a significant effect of CPA administration on the exploratory behavior in hyperthyroid and control rats (Fig. 2B), suggesting that the exploratory behavior is modulated by the activation of adenosine A_1 receptors in a similar way in the control and hyperthyroid rats. Accordingly, locomotor activity was not changed by hyperthyroidism, but CPA administration decreased locomotion in both control and hyperthyroid animals. These results were obtained in the open-field (Fig. 2C) and in the plus-maze tests (Fig. 3B). Therefore, the reduction in locomotor activity elicited by CPA seems to be related to drug administration, and not to specific effects of this drug on the pathological condition studied.

Moreover, the body temperature was affected by the CPA administration only in control rats, as previously described in the literature [9], but not in hyperthyroid rats. These results are important considering the possible therapeutic application of drugs based on adenosine agonists in hyperthyroidism.

Furthermore, adenosine A_1 receptors provide protection against the effects of oxidative stress in the central nervous system [36,2]. In this context, it is well known that the brain is especially susceptible to oxidative damage because of its highly polyunsaturated fatty acids content and high O_2 consumption [20], especially in hyperthyroidism [1]. Our results demonstrated that the free radicals production verified by the DCF analysis was increased in cerebral cortex of hyperthyroid rats when compared to control rats (Fig. 4A). Additionally, CPA administration decreased significantly the free radicals levels in cortex cerebral of control and hyperthyroid rats, but in hippocampus no significant effect was observed in the groups studied (Fig. 4A).

Lipid peroxidation was significantly increased in hippocampus, but not in cerebral cortex from hyperthyroid rats in comparison with the control rats (Fig. 4B). This result indicates higher vulnerability of hippocampus to lipid peroxidation induced by T4 treatment. In agreement with the literature [2], our results have shown that CPA administration in control rats decreases the lipid peroxidation in both hippocampus and cerebral cortex. Lipid peroxidation is mediated by free radical reactions that produce peroxyl radical formation and fatty acid conformational alterations, leading to severe loss of membrane integrity [19]. In hyperthyroid rats that received CPA, the lipid peroxidation was decreased in cerebral cortex but not in hippocampus. These results concord with previous studies showing the neuroprotective effects of agonists of adenosine A_1 receptors and confer a data additional concerning to protective effect of A_1 receptor against the damages elicited by lipid peroxidation in hyperthyroidism. This result is relevant, because lipid peroxidation contributes significantly to the development of permanent neurological dysfunction and seems to be a major cause of death and posttraumatic cell damage [19].

Accordingly, the TAR measurement was increased in hippocampus and cerebral cortex of control and hyperthyroid rats after CPA administration (Fig. 4C). These results might be a consequence of action of adenosine via A_1 receptors on the antioxidant defense system [29,36]. Furthermore, the decrease in the free radicals levels (verified by the DCF test) in cerebral cortex of control and hyperthyroid rats after CPA administration is pertinent with the results obtained by TAR measurement in cerebral cortex. In contrast, the absence of significant effects in free radicals production in hippocampus after CPA administration (Fig. 4A) might be attributed to a higher vulnerability of hippocampus to oxidative stress, since CPA increased the total antioxidant reactivity in hippocampus but did not reduce the free radicals production content. In addition, the effects of

CPA on the total antioxidant reactivity were more pronounced in hyperthyroid rats, demonstrating an increase of brain capacity to modulate the damage associated with the production of free radicals in hyperthyroidism after the administration of this agonist.

We recently demonstrated that hyperthyroidism decreases the activity of ectonucleotidases responsible by the hydrolysis of ATP to adenosine in hippocampus and cerebral cortex of rats [6]. Therefore, a reduced availability of extracellular adenosine in the central nervous system might be contributing to genesis of some of the excitatory effects described in hyperthyroidism. Moreover, in the same way that hypothyroidism induces a down-regulation of the A_1 receptors in the rat brain [13], a decrease in adenosine levels after hyperthyroidism may up-regulate these receptors. Thus, the administration of a specific agonist of A_1 receptor in a central nervous system chronically altered could explain the differences observed between control and hyperthyroid rats on the behavioral and biochemical parameters studied. Furthermore, the results presented in this study suggest an important association between adenosinergic system and some of the symptoms of hyperthyroidism, and reinforce the importance of the protective role proposed for adenosine via A_1 receptor in pathological conditions.

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