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Effects of chronic treatment with chlorpheniramine on anxiety and emotional memory in mice

Efeitos do tratamento crônico com clorfeniramina na ansiedade e memória emocional de camundongos

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Abstract

Introduction: H1 receptors mediate actions in brain activity, and antihistamines such as chlorpheniramine (CPA) act as H1 antagonists. **Objective:** This study investigated the effects of chronic treatment with CPA on anxiety and emotional memory in mice in the elevated plus-maze (EPM). **Method:** Male Swiss albino mice received chronic treatment with saline or CPA for 15 days. After this, the test was performed on two consecutive days. In Trial 1, mice received an injection of saline or CPA, and 40 minutes later they were exposed to the EPM. Twenty-four hours later, the mice received injections again and were retested. **Results:** Results showed no effects on anxiety or locomotor activity. During trial 2, open-arm exploration diminished in mice treated only with CPA with a dosage of 16 mg/kg (ANOVA, SNK<0.05). **Conclusion:** Results suggest that the aversive information obtained in the open arms in Trial 1 was remembered by the animals which were chronically treated with CPA (16mg/kg).

Key words: Anxiety; Chlorpheniramine; Maze learning; Memory.

Resumo

Introdução: A clorfeniramina (CPA) é um antagonista dos receptores H1, que medeiam ações na atividade cerebral. **Objetivo:** Investigar os efeitos de um tratamento crônico com CPA sobre a ansiedade e a memória emocional em camundongos. **Método:** Os camundongos receberam injeções de salina ou CPA, durante 15 dias. Após esse período, o teste no labirinto em cruz elevado (LCE) foi realizado. No Teste 1, os animais receberam uma injeção de salina ou de CPA, e após 40 minutos, foram expostos ao labirinto. Após 24 horas, no Teste 2, o mesmo procedimento foi realizado. **Resultados:** Não foram observados efeitos na ansiedade e na atividade locomotora dos animais. A exploração dos braços abertos do labirinto diminuiu nos camundongos tratados apenas com CPA, na dose de 16 mg/kg. **Conclusão:** A informação aversiva dos braços abertos obtida em T1 foi recordada apenas pelos animais que foram cronicamente tratados com CPA 16 mg/kg.

Descritores: Ansiedade; Clorfeniramina; Aprendizagem em labirinto; Memória.

Introduction

Histamine is a central neurotransmitter contained within and released from neurons whose cell bodies are clustered in the tuberomammillary nucleus of the posterior hypothalamus. These neurons project fibers to practically all brain areas[1]. Four types of receptors, H1, H2, H3 and H4, which differ in pharmacology, in localization, and in the intracellular response they evoke, mediate histamine action in the central nervous system (CNS)[2,3].

A number of studies have implicated the histaminergic neuron system in various brain functions, including control of the waking state; motivated behaviors and behavioral disorders[4]; and neuroplastic changes associated with functional recovery from brain damage[5]. Experiments performed with different research approaches have provided evidence that the histaminergic system is involved in modulating anxiety-like behaviors[6,7] and learning and memory processes[8,9]. The involvement of the histaminergic system in anxiety-like states was investigated by Malmberg-Aiello et al.[7], who demonstrated that substances able to enhance histaminergic transmission reduced animals' time spent in the light compartment of a light/dark transition model, indicating an anxiogenic effect. Moreover, the destruction of the rat tuberomammillary rostroventral E2 sub-region, from which histaminergic neuron fibers arise, induced anxiolytic-like effects in the plus-maze test[10]. Both facilitatory and inhibitory effects of cerebral histamine on the learning and memory process have been described in animal behavioral studies[8,11].

H1 receptors mediate actions in brain activity, and classic antihistamines such as chlorpheniramine (CPA) act as H1 antagonists[12]. Several studies have demonstrated anxiolytics effects of CPA in behavioral tests[6,13]. Furthermore, it has been proposed that CPA is involved in spatial learning and emotional memory processes; however, results in this area are controversial. While pharmacological blockade of the H1 receptor with CPA improves spatial learning in the Morris water maze[14], it conversely impairs spatial learning in the 8-arm radial maze[15]. Additionally, our previous study demonstrated that acute pre-trial CPA injections had no effect on anxiety or emotional memory in mice using the Trial 1/2 protocol in the elevated plus-maze (EPM)[11].

To date, little is known concerning the effects of chronic CPA treatment and its role in these processes. The chronic application of some types of antagonists can lead to receptor up-regulation (for example in the dopaminergic system), but there are no studies about this effect in the neural histaminergic system specifically. Therefore, the aim of this study was to evaluate the effects of chronic treatment with CPA on anxiety and emotional memory in mice using the EPM.

Material and methods

Animals

Adult male Swiss albino mice (30-40g) were housed in groups of ten per cage and maintained on a 12-hour light/dark cycle (lights on at 07:00) in a temperature- and humidity-controlled environment (23±1ºC/55±5%). Food and water were freely available. All mice were experimentally naïve, and the experimental sessions were conducted during the light period of the cycle (9:00–13:00 h).

Drugs

The H1 receptor antagonist chlorpheniramine maleate salt (CPA; 8 mg/kg and 16 mg/kg; Sigma, MO, USA) was dissolved in a 0.9% saline solution (SAL). SAL was used as an experimental control. SAL and CPA were administrated intraperitoneally (i.p.) at a volume of 2 ml/kg body weight. The doses were selected on the basis of previous research[11]. The substances were labeled with codes, which were unknown to the experimenter during the tests and behavioral analysis.

Apparatus

The apparatus used was similar to that originally described by Lister[16]. The EPM consisted of two open arms (30 × 5 × 0.25 cm) and two enclosed arms (30 × 5 × 15 cm) connected to a common central platform (5 × 5 cm). The apparatus was constructed from wood (floor) and transparent glass (clear walls) and raised to a height of 38.5 cm above floor level. All tests were conducted under moderate illumination (77 lx, measured on the central EPM platform).

Procedure

For 15 consecutive days, the animals received daily i.p. injections of CPA or SAL. After this period, the animals were submitted to the EPM on two consecutive days: Trial 1 (T1) and Trial 2 (T2). In T1, each mouse received i.p. injection of SAL or CPA and, 40 minutes later, was placed on the central platform of the maze facing an open arm. Each test sessions lasted five minutes and was videotaped. Between successive test runs, the maze was thoroughly cleaned with 20% ethanol and a dry cloth. In T2, 24 hours later, the mice were injected again with SAL or CPA and re-exposed to the EPM as described for T1 (Figure 1).

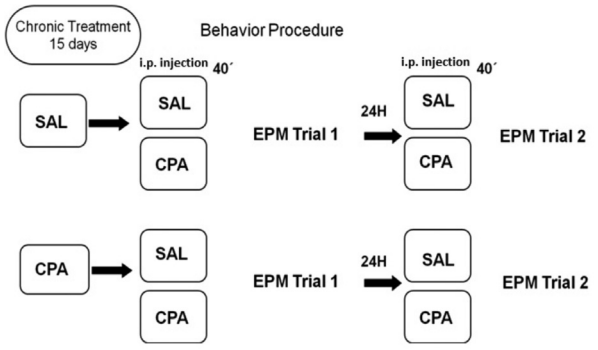

Figure 1: Experimental procedure

For each CPA dose administered (CPA 8 mg/kg and CPA 16 mg/kg), one control group was assigned. Therefore eight groups were formed based on chronic treatment: CPA 8 mg/kg (SAL+SAL, SAL+CPA, CPA+CPA, CPA+SAL); CPA 16 mg/kg (SAL+SAL, SAL+CPA, CPA+CPA, CPA+SAL) (see Table 1).

Table 1: Experimental groups

Chronic treatment	Injection pre-Trial 1	Injection pre-Trial 2	Group (n)
SAL	SAL	SAL	SAL+SAL (12)
CPA	CPA 8 mg/kg	CPA 8 mg/kg	SAL+CPA (14)
8 mg/kg	CPA 8 mg/kg	CPA 8 mg/kg	CPA+CPA (15)
	SAL	SAL	CPA+SAL (15)
SAL	SAL	SAL	SAL+SAL (14)
CPA	CPA 16 mg/kg	CPA 16 mg/kg	SAL+CPA (11)
16 mg/kg	CPA 16 mg/kg	CPA 16 mg/kg	CPA+CPA (12)
	SAL	SAL	CPA+SAL (11)

Behavioral analysis

Images were analyzed by a highly trained observer using X-PLO-RAT, an ethological analysis pack developed at the Laboratory of Exploratory Behavior, Universidade de São Paulo – USP/Ribeirão Preto[17]. Behavioral parameters were defined according to previous studies[16,18,19]: frequency of open- and enclosed-arm entries (OAE and EAE), defined as all four paws placed inside an arm; and total time spent in the open arms (OAT), in the enclosed arms (EAT), and in the central area (CT). These data were used to calculate the percentage of open arm entries [%OAE = (open entries/open + enclosed entries) x 100]; the percentage of time spent in the open arms [%OAT = (open time/300) x 100]; and the percentage of time spent in the enclosed arms [%EAT = (enclosed time/300) x 100]. The number of stretched-attend postures (SAP; exploratory posture in which the body stretches forward and then retracts to its original position without any forward locomotion) and the frequency of head dipping (exploratory movement of head/shoulders over the sides of the maze) were also scored. Total SAP was considered a primary index of risk assessment, and head dipping evaluated as exploratory behavior.

The conventional measures of anxiety consisted of %OAE and %OAT[18]. In the EPM, emotional memory was evaluated by the Trial 1/Trial 2 protocol. Decreased open-arm activity (%OAE and %OAT) in T2 was defined as the learning and memory index. The inclusion of a retest session has been made in recent years, which is consistent with the assumption that there is a learned component underlying the exploratory behavior during EPM re-exposure [9,11,20,21,22]. Total enclosed arm entries were measured as an index of locomotor activity[18].

The experiments carried out in this study were approved by the Animal Ethics Commission of the Federal University of São Carlos (CEEA 10/08), and they are in compliance with the norms of the Brazilian Neuroscience and Behavior Society (SBNeC), based on the US National Institutes of Health Guide for Care and use of Laboratory Animals.

Statistic analysis

All results were initially submitted to Levene´s test for homogeneity of variance. When appropriate, the data were transformed by taking their square root and then analyzed by two-way analysis of variance (ANOVA; factor 1: treatment, factor 2: day). When differences were indicated by significant *F* values, they were identified by the Student-Newman-Keuls (SNK) multiple comparisons test. A P value less than 0.05 was required for significance.

Results

The results of chronic treatment with CPA 8 mg/kg are summarized in Table 2. Two-factor ANOVA (Factor A: group; Factor B: day) did not reveal significant effects of CPA 8 mg/kg treatment on T1 among the groups SAL+SAL, SAL+CPA, CPA+CPA, and CPA+SAL for the conventional measure for the evaluated anxiety – %OAE and %OAT –, indicating that CPA 8 mg/kg shows no effect on anxiety. For these variables, no significant differences were shown between T1 and T2 (day factor) and there was no interaction between factors A and B. For the EAE [$F_{(3.220)}$=2.65; p>0.05], no significant statistical difference was detected between the groups, which means there were no significant changes in the locomotor activity. ANOVA did not show significant differences for the ethological measures – head dipping and SAP between groups and days in animals treated with CPA 8 mg/kg.

The results for treatment with CPA 16 mg/kg are summarized in Table 3.

Table 2: Effects of chronic administration of CPA (8 mg/kg) for conventional and ethological measures of mice

	Chronic saline								Chronic CPA							
	SAL+SAL				SAL+CPA				CPA+CPA				CPA+SAL			
	T1		T2		T1		T2		T1		T2		T1		T2	
%OAE	36.8 ±	6.5	28.2 ±	5.5	23.4 ±	3.8	20.7 ±	5.4	28.8 ±	5.4	21.3 ±	5.4	35.0 ±	4.3	32.1 ±	4.8
%OAT	21.9 ±	6.6	11.2 ±	2.0	10.7 ±	2.1	8.6 ±	2.8	17.7 ±	4.2	9.6 ±	3.3	19.2 ±	5.1	11.5 ±	3.0
EAE	7.2 ±	0.9	8.5 ±	1.2	8.4 ±	0.6	9.6 ±	1.2	8.0 ±	0.9	5.4 ±	0.6	6.9 ±	0.9	5.7 ±	0.9
%EAT	42.3 ±	5.7	62.9 ±	6.5	38.3 ±	4.1	53.6 ±	5.5	42.7 ±	5.1	68.0 ±	6.1	36.7 ±	4.1	58.1 ±	6.7
CT	107.5 ±	10.3	77.1 ±	19.0	152.0 ±	12.8	113.4 ±	16.5	118.4 ±	14.6	67.2 ±	12.2	132.7 ±	15.7	91.0 ±	20.0
SAP	2.7 ±	0.5	1.4 ±	0.3	1.4 ±	0.3	2.0 ±	0.7	2.4 ±	0.6	1.2 ±	0.6	1.5 ±	0.3	0.9±	0.3
HD	3.3 ±	0.8	1.2 ±	0.6	2.9 ±	0.4	1.4 ±	0.6	2.4 ±	0.6	2.0 ±	0.6	2.0 ±	0.4	1.0 ±	0.3

Values presented as mean ± SEM. %OAE= percentage open arms entries; % OAT= percentage of time spent in open arms; EAE= enclosed arms entries; %EAT= percentage time spent in enclosed arms; CT= central time; SAP= stretched-attend postures on open arms; HD= head dipping on open arms.

Table 3: Effects of chronic administration of CPA (16 mg/kg) for conventional and ethological measures of mice

	Chronic saline								Chronic CPA							
	SAL+SAL				SAL+CPA				CPA+CPA				CPA+SAL			
	T1		T2		T1		T2		T1		T2		T1		T2	
EAE	6.7 ±	1.1	7.9 ±	1.1	8.1 ±	1.6	11.5 ±	1.6	7.1 ±	1.0	10.0 ±	1.1	7.6 ±	1.0	9.8 ±	2.2
%EAT	38.0 ±	4.5	55.3 ±	5.9	57.7 ±	7.4	72.1 ±	5.2	40.9 ±	5.8	66.4 ±	6.7	54.8 ±	5.8	75.1 ±	4.7
CT	118.6 ±	16.3	91.8 ±	15.2	98.4 ±	17.0	75.4 ±	14.5	121.7 ±	19.2	81.9 ±	21.3	89.5 ±	15.5	51.8 ±	8.6
SAP	0.5 ±	0.3	0.0 ±	0.0	0.3 ±	0.1	0.3 ±	0.1	0.8 ±	0.3	0,6 ±	0.2	0.5 ±	0.2	0.4 ±	0.2
HD	0.3 ±	0.2	0.1 ±	0.1	0.3 ±	0.2	0.0 ±	0.0	0.3 ±	0.2	0.1 ±	0.1	0.5 ±	0.4	0.2 ±	0.2

Values presented as mean ± SEM. EAE= enclosed arms entries; %EAT= percentage time spent in enclosed arms; CT= central time; SAP= stretched-attend postures on open arms; HD= head dipping on open arms.* Significant difference between T1 and T2 (SNK, <0.05).

ANOVA showed significant differences between days in %OAE [$F_{(1.94)}$=22.78; p<0.0001] and %OAT [$F_{(1.94)}$=24.26; p<0.0001]. However, the *post hoc* test revealed these changes only in the CPA+CPA group. These animals showed a decrease in the exploration of open arms of the maze on T2 compared with T1 (p<0.05) (Figure 2A-B). There were no significant changes in the locomotor activity at a CPA dose of 16 mg/kg represented by EAE [$F_{(3.188)}$= 25.59; p>0.05]. ANOVA test did not reveal statistical significant differences in any of the factors for the ethological behaviors (head dipping and SAP) of the animals treated with CPA at this dose.

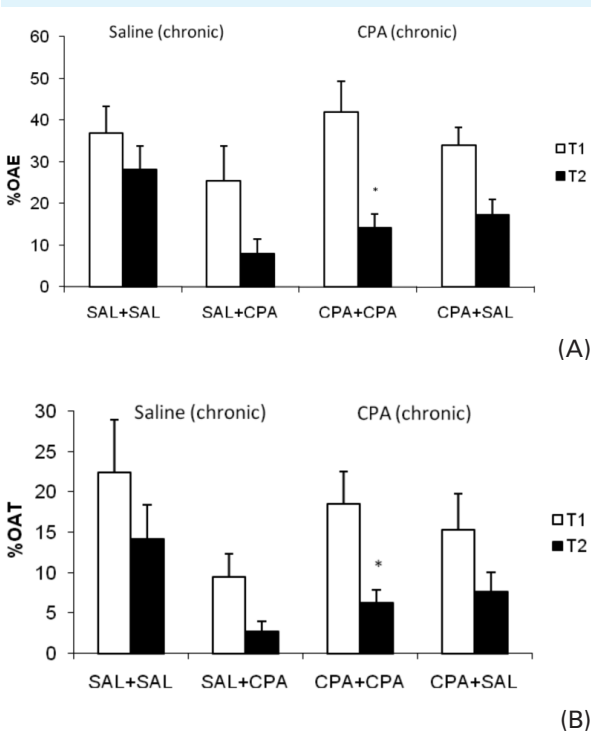

Figure 2: Chronic treatment with CPA 16 mg/kg. Means and standard error of means of: (A) percentage of entries into open arms in the EPM in T1 and T2; (B) percentage of time spent in open arms in the EPM in T1 and T2. SAL+SAL (chronic treatment with SAL, injection pre trial with SAL in T1 and T2). SAL+CPA (chronic treatment with SAL, injection pre trial with CPA in T1 and T2); CPA+CPA (chronic treatment with CPA, injection pre trial with CPA in T1 and T2); CPA+SAL (chronic treatment with CPA, injection pre trial with SAL in T1 and T2). * Significant difference between T1 and T2 (SNK, <0.05).

Discussion

The main experimental finding of this study is that chronic treatment with chlorpheniramine (8 mg/kg and 16 mg/kg) did not show any effect on anxiety-like behaviors, since there was no difference between treatments on open arm exploration in Trial 1. The results revealed that the control group treated with saline showed no reduction in the exploration of the open arms, which indicates that these animals did not acquire the avoidance behavior of the open arms of the EPM. The chronic treatment with chlorpheniramine (16 mg/kg) was able to revert this effect and facilitate emotional memory in mice on the elevated plus maze. The same

effect was not found in animals treated with chlorpheniramine (8 mg/kg), which may suggest a dose-dependent response.

The EPM is a test that assesses the behavior of animals that is associated with an emotional component, as the behavior expressed during the test is due to a conflict between the motivation to explore the maze and the natural tendency to avoid open spaces[16,20]. A general aspect of the exploration of animals in the EPM shows that they have a clear preference for enclosed arms[22]. Studies indicate that drugs that increase activity in the open arms are anxiolytic compounds, while those that decrease this activity have an anxiogenic characteristic.

Ethological analysis has been an important tool in the analysis of behavioral responses to innate and acquired anxiety-inducing stimuli. Behavior acts representing risk assessment, such as the stretched attend posture, have been taken as measure of anxiety[18,19]. Ethological measures often are more sensitive to anxiolytic drugs, and their use in association with the conventional measures is very important to confirm some results about anxiety[19].

In this study, the CPA doses of 8 mg/kg and 16 mg/kg did not affect ethological behavior, and the conventional measures showed no significant differences in activity in the open arms in T1 (%OAE and %OAT) between the groups, indicating no effects on anxiety. These results agree with previous reports from our group showing no effect of CPA on anxiety[11,23]. However, Privou et al.[13] showed that CPA induced an anxiolytic-like effect following its unilateral injection (0.1 and 20 µg) into the vicinity of the *nucleus basalis magnocellularis* of rats subjected to the EPM.

After the initial exploration of the EPM, the animal acquires, consolidates, and evokes some kind of memory related with potentially aversive areas in the maze[11,21]. Increased avoidance of open arms in a second exposure is observed in various studies, suggesting that re-exposure is associated with behavioral changes indicative of aversive learning. This hypothesis was further strengthened when tests separated by intervals of one or two weeks showed a significant reduction in the exploration of open arms. Furthermore, when a high dose (75 mg/kg) of chlordiazepoxide, considered amnesic, was given in trial 1, this behavior was not expressed[24,25].

The results of this study revealed that the control group showed no reduction in the exploration of the open arms, which indicate that these animals did not acquire avoidance behavior to them. However, the animals that received chronic injections of CPA (16 mg/kg) and pretest injections of the same drug in T1 and T2 showed a significant reduction in the exploration of open arms (%OAE and %OAT) in T2 compared to T1. Those animals treated exclusively with CPA (16 mg/kg) acquired and evoked aversive information of open arms from the first exposure. The groups treated with CPA (8 mg/kg) showed no significant reduction in the exploration of open arms in T2 compared to T1. In all groups tested, there was no significant difference in the total of entries into enclosed arms, indicating that the treatments did not alter the locomotor activity of animals.

In this study, the impairment of the acquisition of avoidance to the open arms of the EPM can be explained by positing that the chronic injection procedure and the daily handling of animals would have represented a form of stress, impairing the emotional memory of these animals. Studies have shown that different kinds of stress are capable of delaying or impairing learning[26,27], and it seems that cognitive impairment caused by stress is related to specific alterations in brain homeostasis, involving the neuro-immune and neuroendocrine systems as well as neurogenesis[27].

In a study of Lapin[28], animals subjected to procedures of handling, intraperitoneal injection of saline and *sham* injection (needle insertion without infusion of the liquid) had increased latency in the second exposure to the EPM when compared to naïve animals. The latency was the parameter used as a test of memory and learning in the EPM, and meant the time

spent after the first exposure to the animal to move from the end of the open arm to the enclosed arm. According to the author, these procedures induce an amnesic effect in this protocol, and groups treated with saline used as controls in pharmacological experiments have a behavioral profile of stress in EPM. Animals submitted to stress presented higher latencies to enter the feeding area than non-stressed animals. Furthermore, a study by Kompagne et al.[29] reported that exposure to chronic stressors affects the ability of rats to respond normally to situations of acute stress, which may have occurred with the animals in our study, as demonstrated by their aversion to the exploration of the EPM.

The results of this study showed that the chronic treatment of CPA (16 mg/kg) was able to reverse this effect, suggesting that these animals acquired and evoked the aversive information of the open arm of the EPM. These effects could be related to state-dependent memory, since mice were able to evoke emotional memory of the previous experience after 24 hours when put in the same conditions. The state-dependent memory is a phenomenon in which the retrieval of an event from memory may require that the organism be in a state similar to that in which the event was initially acquired.

Studies have shown involvement of histaminergic drugs on state-dependent memory[9,11,30]. In a study by Zarrindast et al.[30], the results indicated a significant increase in retrieval when histamine was taken at both pre-training and pre-test times. However, Serafim et al.[9] showed that treatment with L-histidine (LH), histamine precursor, pre-T1 and pre-T2, impaired the ability to evoke memory during the second trial in this state, indicating a state-dependent memory retrieval deficit in mice exposed and re-exposed to the EPM.

Our results suggest that CPA improves retrieval of state-dependent memory, since animals treated with CPA (16 mg/kg) were able to evoke emotional memory after 24 hours, when they were exposed to the same sensory context and physiological state as during the encoding phase. These results corroborate our previous study which showed that the LH provoked state-dependent memory retrieval deficit in mice re-exposed on the elevated plus maze[9].

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