INFLUENCE OF H1 RECEPTOR BLOCKERS ON EXPLORATORY BEHAVIOR AND LOCOMOTOR ACTIVITY OF RATS

Verginia Georgieva *, Roman Tashev*, Stiliana Belcheva**

**Department of Behavior Neurobiology, Institute of Neurobiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

***Faculty of Pre-School and Primary School Education, SU "Sv. Kl. Ohridsky", 69A Shipchenski prohod St., 1574 Sofia, Bulgaria*

ABSTRACT

In recent years, due to the greater frequency and severity of allergic diseases, the use of antiallergic drugs from the group of histamin1 (H1) receptor antagonists is increased. H1 receptor blockers, also called antihistamines act as a shift of the histamine from its receptors and terminate the effect of the released histamine. They are divided into three generations, according on the time of their synthesis, properties and side effects for therapeutic purposes. There are data that histamine receptors are involved in the regulation of several behaviors, such as sleep cycle - waking, drinking, locomotor activity and nociception. All of these determine the importance of the involvement of histamine receptors in the regulation of behavioral responses. The aim of this study was to investigate the effects of antihistamines (first, second and third generation), introduced repeatedly (15 days) at doses used in medical practice, on the exploratory behavior and locomotor activity.

The experiments were carried out on 3 months old male Wistar rats (180-200g). The influence of the first (Diphenhydramine - DPH, Chlorpheniramine - CLPH and Dexchlorpheniramine – DCLPH), second (Ketotifene - KET, Terfenadine - TER and Claritine - CLAR) and third generation (Xyzal - KSYS and Aerins – AER) antihistamines on exploratory behavior and locomotor activity of rats were studied. The changes in number of horizontal and vertical movements were registered in Opto Varimex apparatus (Columbus Instruments, USA) according to the method of Kohler и Lorens for 5-minute period of observation. The animals were treated with the H1- blockers or saline i.p. for 15 days as follow: DPH - 5 μg/kg and 10 μg/kg; CLPH - 10 mg/kg and 20 mg/kg, DCLPH -10 mg/kg and 20 mg/kg; KET - 0.03 mg/kg; TER - 0.04 mg/kg; CLAR - 0.04 mg/kg; KSYS - 0.05 mg/kг and AER - 0.05 mg/kг 30 min before behavior test.

ANOVA analysis followed by post-hoc comparisons of the data about the number of horizontal and vertical movements for the period 1^{st} - 5^{th} minute showed that first generation antihistamines significantly decreased exploratory and locomotor activity, while second and third generation antihistamines did not affect the exploratory behavior and locomotor activity of rats as compared to the saline-treated controls. Analysis of the changes in the number of horizontal and vertical movements showed that the habituation was not disturbed.

The present study extended the understanding of the effects of antihistamines on the behavior in rats. The data showed that H1 receptors were involved in the exploratory and locomotor activity. Their inhibition by first generation antihistamines suppressed these activities. Our results support the suggestion that inhibition of locomotor activity by H1-blockers did not affect the impaired learning and memory processes (previously observed in our lab) suggesting a different mechanisms underlying in these behaviors. The data could be of interest to clinical practice where H1- blockers are widely used as antiallergic drugs.

Key words: antihistamines, H1- antagonists, exploratory behavior, locomotor activity, rat

Introduction

Histamine is one of the most extensively studied biological amines in medicine. It stimulates smooth muscle contraction and gastric acid secretion, increases vascular permeability, functions as a neurotransmitter, and plays various roles in immunomodulation, allergy, inflammation, haematopoiesis and cell proliferation[11]. Histamine exerts its actions through interacting with four distinct G-protein coupled receptors (GPCRs) (H1R (Histamine 1 receptor), H2R, H3R, and H4R)

in different patho-physiological processes such as allergy, anaphylaxis, and gastric acid secretion, neurotransmission in the peripheral nervous system and mainly in the central nervous system [5].

In recent years, due to the greater frequency and severity of allergic diseases, the use of antiallergic drugs from the group of Histamin1 (H1) receptor antagonists is increased. H1 receptor blockers, also called antihistamines act as a shift of the histamine from its receptors and terminate the effect released histamine. They are divided into three generations, according on the time of their synthesis, properties and side effects for therapeutic purposes [7]. H1 antihistamines have well-documented anti-allergic and anti-inflammatory effects and are well established in the treatment of a variety of allergic disorders. First generation antihistamines are used as sleep aid drugs [1] and for travel sickness relief [12]. It is well known that the first-generation antihistamines are hydrophilic molecules that can easily go across the blood brain barrier and affect the central nervous system [2,8]. There are data that histamine receptors are involved in the regulation of a number of behaviors, such as sleep cycle - waking, drinking, locomotor activity and nociception [4]. These antihistamines have been shown to impair cognitive function [10,13]. Some literatures have reported that the disturbance of central histaminergic receptors by the first-generation antihistamines may underlie their neuronal toxic effects on the neuronal system [6]. However, the detailed mechanism has not been fully revealed yet. All this determines the importance of the involvement of histamine receptors in the regulation of behavioral responses. The aim of this study was to investigate the effects of antihistamines (first, second and third generation), applied introduced repeatedly (15 days) at doses used in medical practice, on the exploratory behavior and locomotor activity.

MATERIALS AND METHODS

Animals. The experiments were carried out on 80, 3-months old male Wistar rats (180-200g). The experiments were performed according to the *"Rules for care and experiments on laboratory animals"* of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences.

Test for exploratory and locomotor activity. Locomotor activity was recorded in Opto Varimex apparatus (Columbus Instruments, USA), according to the method of Kohler and Lorens [9]. The experimental chamber was 50 cm x 50 cm x 25 cm. This apparatus records the number of photobeam interruptions during the movements of the animal. It provides selective counting of the number of horizontal and vertical movements in arbitrary units (AU). The information obtained was recorded automatically every minute in a 5-minute period of observation. The experiments were carried out at one and the same time (between 10:00 am and 1:00 pm). The influence of the first (Diphenhydramine - DPH, Chlorpheniramine - CLPH and Dexchlorpheniramine – DCLPH), second (Ketotifene - KET, Terfenadine - TER and Claritine - CLAR) and third generation (Xyzal - KSYS and Aerins – AER) antihistamines on exploratory behavior and locomotor activity of rats were studied. The animals were treated with the H1-blockers or saline i.p. for 15 days as follow: DPH - 5 μg/kg and 10 μg/kg; CLPH - 10 mg/kg and 20 mg/kg, DCLPH -10 mg/kg and 20 mg/kg; KET - 0.03 mg/kg; TER - 0.04 mg/kg; CLAR - 0.04 mg/kg; KSYS - 0.05 mg/kg and AER - 0.05 mg/kg 30 min before behavior test.

Statistical analysis. behavioral data were analyzed by analysis of variance (ANOVA). Separate two-way repeated-measure ANOVA was used to process the data obtained for horizontal and for vertical movements. For DPH factors were: dose of DPH with 3 levels (5 μg/kg, 10 μg/kg and saline) and time 5 levels (1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} min), for CLPH factors were dose CLPH with 3 levels (10 mg / kg, 20 mg / kg and saline) and time 5 levels (1^{st} , 2^{nd}, 3^{rd} , 4^{th} and 5^{th} min) and for DCLPH were dose of DCLPH with 3 levels (10 mg/kg, 20 mg/kg and saline) and at 5 levels (1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} min). Separate one-way ANOVA was used to process the data obtained for the total number of horizontal and vertical movements during the whole 5-minute observation period. ANOVA data were further analyzed by post hoc t-test, where appropriate.

RESULTS

Effects of first generation antihistamines on the exploratory behaviour and locomotor activity.

ANOVA for the number of horizontal movements after 15-day treatment with DPH in two doses (5 μg/kg and 10 μg/kg) showed significant effects for factors "dose" of DPH ($F_{2, 179} = 24.93$; $P \leq 0.01$) and for factor "time" ($F_{4, 179} = 156.23$; $P \leq 0.001$). Post-hoct test showed that DPH at doses of 5μg/kg and 10μg/kg significantly reduces the number of horizontal movements on the 1st ($P \leq 0.001$ and $P \leq 0.001$ resp.); 2nd ($P \leq 0.001$ and $P \leq 0.001$ resp.), 3rd ($P \leq 0.05$ and $P \leq 0.01$ resp.) and 4th min ($P \leq 0.05$ and $P \leq 0.05$ resp.) (Fig. 1).

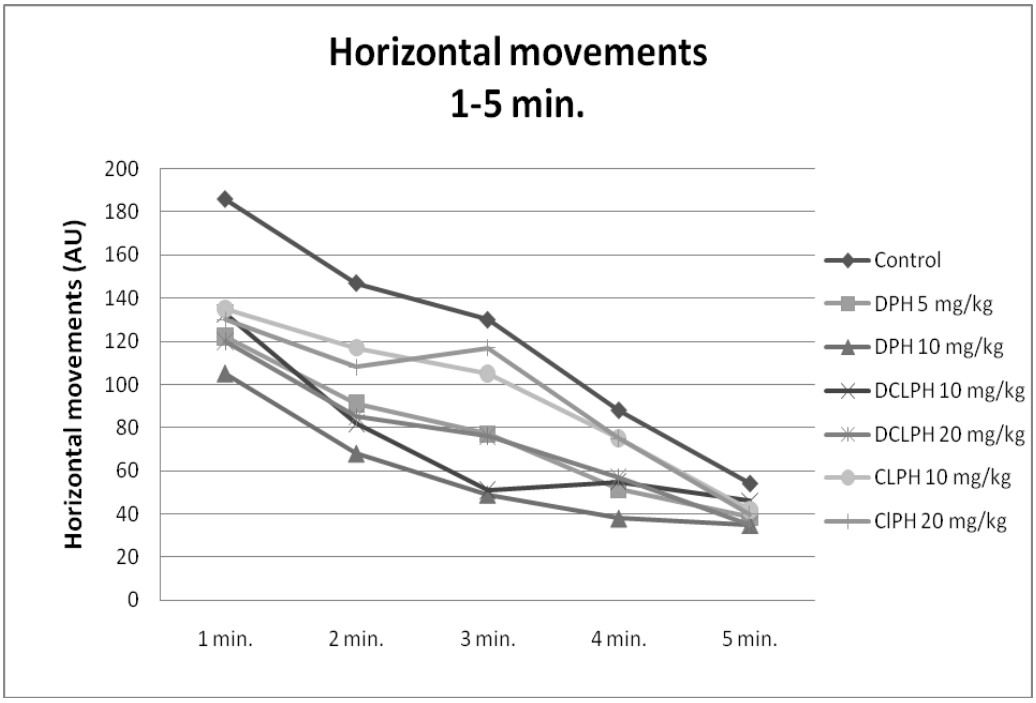

Fig.1. Effects of *first generation antihistamines*- DPH (5 μg/kg and 10 μg/kg), CLPH – (10 mg/kg and 20 mg/kg) and DCLPH (10 mg/kg and 20 mg/kg) on the number of horizontal movements, recorded every minute for a 5-minute period. AU - arbitrary units.

ANOVA for the number of horizontal movements after 15-day treatment with CLPH showed no significant effects for the factor "dose" of CLPH ($F_{2, 179} = 1.09341$, P = NS) and for factor "time" ($F_{2, 179} = 1.4232$, P = NS). Post-hoc test showed that CLPH at doses of 10 mg/kg and 20 mg/kg reduced significantly the number of horizontal movements only on the 1st min ($P \leq 0.05$ and $P \leq 0.01$ resp.) and 2nd min ($P \leq 0.001$ and $P \leq 0.001$ resp.) without changing significantly the number of horizontal movements on 3rd, 4th and 5th min compared with the controls (Fig. 1).

ANOVA for the number of horizontal movements after 15-day treatment of DCLPH showed significant effects for the factor "dose" of DCLPH (F2, 179 = 2.995, $P \leq 0.02$) and factor "time" ($F_{2, 179} = 77.266$, $P \leq 0.005$). Post-hoc test indicated that DCLPH in doses of 10 mg/kg and 20 mg/kg decreased the number of horizontal movements in the first 3 minutes: on the the1st ($P \leq 0.001$ and $P \leq 0.001$ resp.), 2nd ($P \leq 0.001$ and $P \leq 0.01$ resp.) and 3th min ($P \leq 0.05$ and $P \leq 0.05$ resp.) without changing significantly the 4th and 5th min compared with controls (Fig. 1).

Separate one-way ANOVA analysis of the total number of movements for the whole 5-minute period of observation showed a significant effect for "dose" of DPH ($F_{2, 29} = 16.688$, $P \leq 0.01$). Post-hoc t-test showed that the doses of 10 mg/kg ($P \leq 0.05$) and 20 mg/kg ($P \leq 0.01$) decreased significantly total number of horizontal movements in comparison with the controls. One-way

ANOVA showed no significant effects of DCLPH ($F_{2, 29}$ = 1.3402, P = NS) and CLPH ($F_{2, 29}$ = 1.1240, P = NS) on the total number of horizontal movements for the whole 5-minute period of observation (Fig. 2).

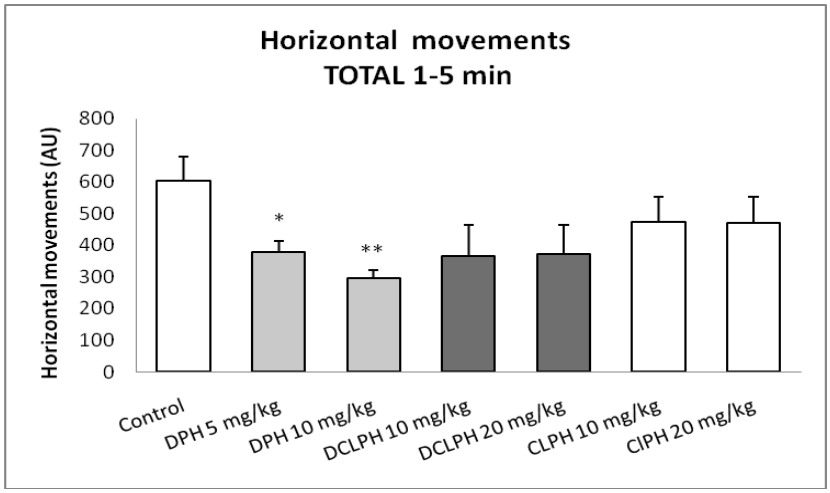

Fig. 2. Effects of *first generation antihistamines*- DPH (5 μg/kg and 10 μg/kg), CLPH – (10 mg/kg and 20 mg/kg) and DCLPH (10 mg/kg and 20 mg/kg) on the total number of horizontal movements for the whole 5-minute period of observation. AU - arbitrary units, *P ≤ 0.05; **P ≤ 0.001 - comparison to the respective saline-injected OBX controls. n = 10. Means (± S.E.M.) are presented.

The number of vertical movements were not changed after 15-day treatment with the first, generation antihistamines (data not shown).

Effects of second and third generation antihistamines on the exploratory behavior and locomotor activity.

ANOVA analysis followed by post-hoc comparisons of the data about the number of horizontal and vertical movements after 15-day treatment with second (KET - 0.03 mg/kg; TER - 0.04 mg/kg; CLAR - 0.04 mg/kg) and third (KSYS - 0.05 mg/kg and AER - 0.05 mg/kg) generation antihistamines did not affect the number of horizontal and vertical movements during the whole observation period as compared to the saline-treated controls (Fig. 3).

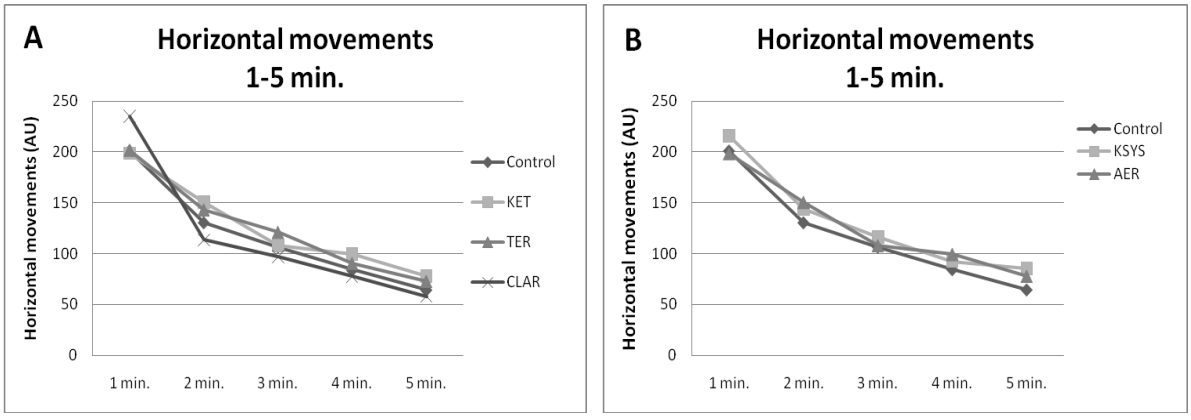

Fig.3. Effects of (**A)** *second* (KET - 0.03 mg/kg; TER - 0.04 mg/kg and CLAR - 0.04 mg/kg) *and* (**B)** *third (*KSYS - 0.05 mg/kg and AER - 0.05 mg/kg) *generation antihistamines* on the

number of horizontal movements, recorded every minute for a 5-minute period. AU - arbitrary units.

DISCUSSION

The present study extended the understanding of the effects of antihistamines on the behavior in rats. The data showed that H1 receptors were involved in the exploratory and locomotor activity. Their inhibition by first generation antihistamines suppressed these activities. The analysis of changes in the number of horizontal movements every minute after administration of first (Diphenhydramine, Chlorpheniramine and Dexchlorpheniramine), second (Ketotifene, Terfenadine and Claritine) and third generation (Xyzal and Aerins) antihistamines at all used doses showed that habituation was not disturbed. Habituation is considered as elementary form of learning. A form of habituation is the change in exploratory activity, seen in rodents, in response to continued exposure to a novel environment.

Our results support the suggestion that inhibition of locomotor activity by H1-blockers did not affect the impaired learning and memory processes [3] suggesting a different mechanisms underlying in these behaviors. The data could be of interest to clinical practice where H1- blockers are widely used as antiallergic drugs.

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