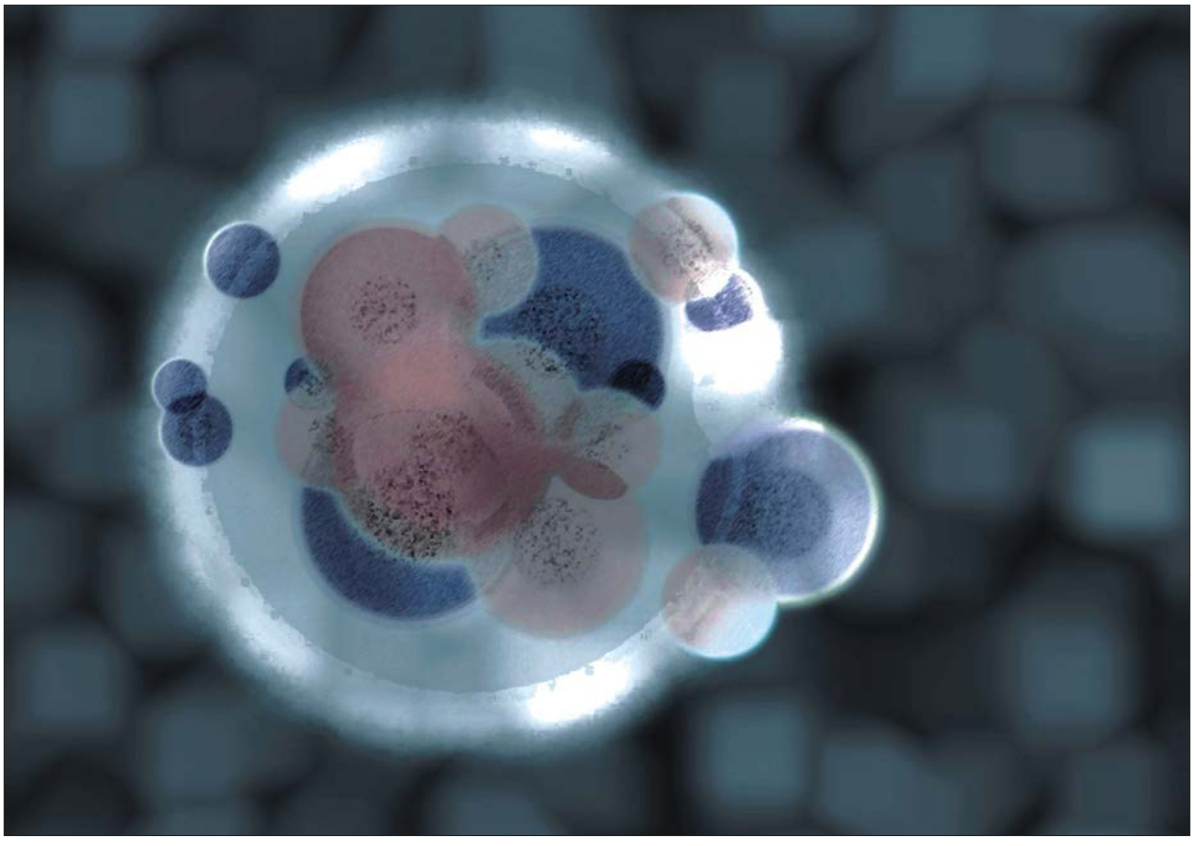

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The Effects of Thioperamide on the Histamine Level and Histidine Decarboxylase Activity of Mouse Brain

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Abstract: The aim of this study was to determine the effects of thioperamide on the total brain histamine content and histidine decarboxylase activity of mouse brain. Mice (n = 24) was used in this study. The animals were housed six per cage, water and food were allowed. All experimented manipulations were carried out with the animals under ether inhalation anesthesia. Mice were injected with 20 mg kg^{-1} of thioperamide 30 min and 5 h after IP injection of thioperamide and brains were removed. The brain homogenate was centrifuged and supernatant was obtained. Aliquots were removed for determination of histamine content and HD activity. The florescence was measured at 440 nm with excitation at 360 nm. The HD activity of brain was higher of treated mice (63.8±4.6 nmol mg/protein/min) than in control group (49.1±3.4 nmol mg/protein/min). Treated mice had a statistically significant increase in mean brain histamine level (243.7±7.9 nmol mg/protein) when compared with control group (197.3±8.2 nmol mg/protein). The increase in the histamine content and HD activity in the brain after injection of thioperamide in the brain, may contribute of the stimulate of histidine decarboxylase enzyme by thioperamide.

Key words: Thioperamide, histamine, histidine decarboxylase activity, brain

INTRODUCTION

Thioperamide, a histamine H3-receptor antagonist[1]. Thioperamide is a popular H3 receptor antagonist which has been used applied to many studies[2]. The effects of the antagonist thioperamide on stimulant-induced locomotor activity in the mouse were examined, they conclude that antagonism of the central histamine H3 receptor inhibits, to a varying degree, the effects of locomotor stimulants[3]. The effects of histamine, thioperamide at the noradrenaline release-modulating H3 receptor in the mouse brain were examined. The effects of histamine and thioperamide at the H3 receptor are readily reversible antagonist[4]. After bolus intravenous administration of thioperamide (10 mg kg^{-1}), the plasma concentration decreased monoexponentially with a half-life of 26.9 min[5]. Following peripheral administration, thioperamide penetrate the brain where they can subsequently interact with H3-receptors. It would appear that binding of thioperamide to H3 receptors is linked with a concomitant increase in histamine turnover in the brain[6]. Investigators assessed the functional role of the histamine H3-receptor in conscious intact rats during activation of the sympathoadrenal axis. They demonstrate an involvement of peripheral histamine H3 prejunctional receptors in the inhibitory modulation of peripheral noradrenergic responses during stress[7]. The effect of thioperamide, a histamine H3 receptor antagonist, on learning and memory was studied in the senescence-accelerated mice prone strain and normal-rate aging strain. Thioperamide administration significantly potentiated HDC activity in the forebrain of mice as well as improving learning and memory. These results suggests that central histaminergic neurons may be involved in learning and memory impairment of SAM-P/8 mice, although other possibilities are not ruled out[8]. The effects of the histamine H3 receptor ligands thioperamide and (R)-α-methylhistamine on the histidine decarboxylase (HDC) activity and histamine content of mouse brain were examined[9]. The effect of selective histamine H3-receptor antagonist thioperamide was studied on PTZ-induced seizures in mice. Thioperamide significantly protected clonic seizures induced by PTZ in a dose-dependent manner. The effect of thioperamide was completely countered by pretreatment with (R)-α-methylhistamine (RAMH), a selective H3-receptor agonist suggesting that the observed effect of thioperamide was elicited by

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histamine H3-receptors[10]. The histamine H3 antagonist, [3H]-thioperamide, can be used as a radioligand to study the histamine H3 receptor in rat brain, provided that subnanomolar concentrations are used in displacement studies[11]. Thioperamide at ICV doses of 40.8-408.5 μg/10μ microl had no effect on food intake in sated rats[12]. The effect on feeding of intracerebroventricular administration of a specific histamine H3-receptor antagonist prior to ICV administration of PYY in rats. These results suggest that the effect of PYY on appetite is different than that induced by fasting and may involve a histaminergic mechanism[13]. Histamine is synthesized from L-histidine by histidine decarboxylase and histamine released from neurons is predominantly methylated to tele-methylhistamine, which is further metabolized by mono amino oxidize[14-16]. Histidine decarboxylase (HD) is an enzyme involved in the formation of histamine, which is a bioactive substance in peripheral and central tissues[17]. HD, the enzyme which forms histamine in mammalian tissues, has not been studied at a molecular level as extensively as the enzymes that participate in the formation of other biogenic amines[18]. The objective of this study was to determine the effects of thioperamide on the brain histamine content and histidine decarboxylase activity of mouse brain.

MATERIALS AND METHODS

Thioperamide, percholoric acid, EDTA-Na_2, histidine decarboxylase, histamine, sodium phosphate, perchloric acid, o-phthaldehyde (OPA), NaOH methanol, H_3PO_4 were obtained from Sigma. All solvents were of analytical grade and were tested for purity by carrying out blank runs. Distilled and deionized water was used in all experiments.

Animals: This research has been done in 2 years from November 2002 to December 2004. Male mice (n = 24), 6-8 week age, were used in this study. All animals survived the study without signs of illness. The animals were six per cage, water and food were allowed. The animals were maintained in an air conditioned room at 19-25°C, with a 12 h light-dark cycle. All experimented manipulations were carried out with the animals under ether inhalation anesthesia. For each experiment the animals were killed between 7: 30 and 10: 30 a.m. Mice were injected with saline or 20 mg kg^{-1} of thioperamide. After 5 h and 30 min IP injection of thioperamide, the mice were killed by decapitation and brains were quickly removed and further divided into the cerebellum, cerebrum, thalamus and hypothalamus according to the method described as previously[2,15,17]. Less than 1 min were required to remove the brain from the skull and the brain was frozen within 3 min after decapitation. Brain tissues were weighed and homogenized in 3 mL of 0.5 M sodium phosphate buffer in a polytron homogenizer for 20 sec periods. The homogenate was centrifuged and supernatant was obtained. Aliquots were removed for determination of histamine content and HD activity.

Measurements of histamine: Measurement of histamine was carried out as previously described[17,18]. Briefly, the supernatant (2 mL) of crude extract was put into a microcentro filter tube (exclusion molecular weight 30000, Millipore) and centrifuged at 3000 g for 30 min. After concentration of crude extracts, 1 mL of 0.5 M sodium phosphate buffer (pH = 7.2) was added and centrifuged again. The part containing the filter and 1 mL of sodium phosphate buffer was added. The incubation was started by addition of 200 μL of 1.0 mM of histidine in sodium phosphate solution at 37°C. After 3 h the reaction was terminated by adding 0.5 mL of 80% perchloric acid. Histamine formed was measured fluorometrically using OPA[17]. The sample (1 mL) was mixed with 3 M NaOH (0.5 mL) then 0.5 mL of OPA reagent (0.2% in methanol) was added and mixed by shaking. After the reaction mixture was allowed to stand for 10 min at room temperature. Then 0.5 mL of 4 M H_3PO_4 was added to the mixture. The florescence was measured at 440 nm with excitation at 360 nm.

Enzyme assay: One unit HD enzyme is defined as 1 nmol of product formed per min at 37°C specific activity is in terms of units per mg of protein. The protein contents of various preparations were determined by a modified Lowery *et al.*[19] method. All assays were performed in duplicate.

Statistics: Data of brain histamine content and HD activity between thioperamide and saline-treated groups were analyzed using the students t-test. Values are expressed as Mean±SD at $p<0.05$.

RESULTS AND DISCUSSION

The HD activity in brain regions ranged from 57.3±2.5 nmol mg/protein/min to 74.5±3.2 nmol mg/protein/min. The histamine content in the brain of thioperamide treated mice was higher than controls (Table 1). Treated mice had a statistically significant increase in mean brain histamine level (243.7±7.9 nmol mg/protein) when compared with control group (197.3±8.2 nmol mg/protein), ($p<0.05$) (Table 2). The activity of this enzyme of brain of thioperamide treated mice was higher than controls. Saline

Table 1: Effects of thioperamide on the histamine content of mice were injected i.p. into mice and 5 h later the histamine content was measured

Histamine content (nmol mg/protein)	Control group	Treated group
Cerebellum	178.5±8.4	217.5±5.4
Cerebrum	194.6±7.5	235.2±7.6
Hypothalamus	197.3±8.2	243.7±7.3
Thalamus	186.4±8.3	229.3±9.3

Values are expressed as mean±SD. Each value is the mean of triplicate determinations with the standard deviation.

Table 2: Effects of thioperamide on the histidine decarboxylase activity of mice were injected i.p. into mice and 5 h later the histidine decarboxylase activity was measured

Histidine decarboxylase activity (nmol mg/protein/min)	Control group	Treated group
Cerebellum	38.5±2.8	42.5±3.4
Cerebrum	47.3±3.6	61.9±3.1
Hypothalamus	49.1±3.4	63.8±4.6
Thalamus	39.3±2.7	53.6±3.2

Values are expressed as mean±SD. Each value is the mean of triplicate determinations with the standard deviation.

did not show any effect on the histamine content and HD activity in comparison with untreated group. It is well established that histamine systems may be involved in the control of states of sleep and wakefulness. It is interesting that the antagonist which appears to be the first agent able to elicit a marked facilitation of histaminergic transmission in the central nervous system, should be useful, in behavioral and other studies, for elaborating the functions of histamine systems. The goals of the present study was to determine the effect of thioperamide on histamine system. We have shown that the histamine content and HD activity of the cerebellum was lowest, the highest content and activity was found in the hypothalamus, in good agreement with the previous findings[17,18]. It is particularly interesting that the HD activity and the brain histamine content were increased by 20 mg kg^{-1} of thioperamide. Treated mice had a statistically significant increase in their mean brain histamine level (243.7±7.9 nmol/mg protein) when compared with control group (197.3±8.2 nmol mg/protein) ($p<0.05$). The results obtained here clearly showed that thioperamide increased the formation of histamine from histidine in the cerebellum, cerebrum, thalamus and hypothalamus. The mean HD activity in the four brain regions of treated group was significantly higher than in the controls. The HD activity in brain was higher in treated mice (63.8±4.6 nmol mg/protein/min) than in control group (49.1±3.4 nmol mg/protein/min), ($p<0.05$). Present results were in good agreement with those reported previously[12,13]. The increase in the histamine content and HD activity in the cerebellum, cerebrum, thalamus and hypothalamus, where cell bodies of the histaminergic neurons are located, may contribute to the replenishment of histaminergic terminals after histamine release caused by thioperamide. Also, in the present study there was also an excellent relationship between HD activity and histamine levels in the treated group.

In conclusion, the increase in the histamine content and HD activity after injection of thioperamide in the brain, where cell bodies of the histaminergic neurons are located, may contribute to the stimulation of histidine decarboxylase enzyme by thioperamide.

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