Effects of sympathetic histamine on vasomotor responses of blood vessels in rabbit ear to electrical stimulation

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Abstract: Objective To investigate the effects of histamine receptor antagonists on vasoconstriction induced by electrical stimulation (ES) on posterior auricular nerve, and to explore the pre- and post-synaptic effects of sympathetic histamine on the vasomotor responses of vascular smooth muscle in rabbit ear. **Methods** ES was applied to posterior auricular nerves of the whole rabbit ear at 10 Hz, 20 Hz and 40 Hz, respectively. Besides, the whole ear was perfused with different histamine receptor antagonists under constant perfusion pressure, and the changes in the flow rate of perfusate were observed. **Results** The flow rate of venous outflow was decreased by ES at all the 3 frequencies. The ES-induced vasoconstriction at 20 Hz and 40 Hz could be partly inhibited by H₁ receptor antagonist chlorpheniramine ($P < 0.05$). After exhaustion of histamine in mast cells by pretreatment with specific mast cell degranulator compound 48/80, chlorpheniramine could still inhibit the ES-induced flow rate reduction. In contrast, H₂ receptor antagonist cimetidine could enhance the 40-Hz ES-induced flow rate reduction ($P < 0.05$). Moreover, ES-induced vasoconstriction at the 3 frequencies could all be enhanced by H₃ receptor antagonist thioperamide ($P < 0.05$). **Conclusion** Stimulation on the auricular nerve may evoke histamine release from sympathetic nerves rather than from mast cells. Moreover, the functions of sympathetic histamine vary from pre-synaptic modulation to post-synaptic vasoconstriction or vasodilatation, via activation of different histamine receptors.

Keywords: sympathetic nervous system; histamine; histamine receptors; blood vessels

1 Introduction

Receptors for histamine H₁, H₂ and H₃ are all present in the cardiovascular system in rabbit and perform different functions in vasculature. H₁ and H₂ receptors are reported to be present in the smooth muscle of blood vessels, and stimulation of them can cause vasoconstriction and vasodilation, respectively^[1]. H₃ receptors are mainly located presynaptically on sympathetic nerve terminals, and are responsible for the inhibition of norepinephrine release^[2]. However, up to

now, most research has been focused on the functions of these receptors, while little attention has been paid on the main sources of their natural ligands. Previous reports have suggested the possible presence of histamine in sympathetic nerves^[3,4]. Although it is widely accepted that central histamine plays a role in regulating the cardiovascular responses^[5], whether sympathetic histamine can also mediate similar functions seems obscure. Most researchers believe that in the peripheral nervous system, histamine that modulates or mediates the functions of autonomic nervous system or somatosensory system, is non-neurogenic^[6-9]. Previous investigations have revealed a wide distribution range of histamine in sympathetic nervous systems in different species^[10,11], and that sympathetic histamine can evoke direct contractile

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responses via post-synaptic H_1 receptors in guinea-pig vas deferens^[12]. In the present study, the effect of sympathetic histamine on vasomotion was examined in an isolated and perfused rabbit whole ear model.

2 Materials and methods

2.1 Animals All the experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by local Animal Care and Use Committee. A total number of 40 New Zealand white rabbits (weighing 2.2-3.0 kg) were supplied by the Experimental Animal Center of the Fourth Military Medical University. Among these animals, 24 rabbits were randomly selected and divided into 3 groups: group A receiving nerve stimulation at 10 Hz, group B receiving nerve stimulation at 20 Hz, and group C receiving nerve stimulation at 40 Hz. The other 16 rabbits were employed to observe the influence of compound 48/80 on chlorpheniramine maleate (CPM)-induced vasomotion.

2.2 Isolation and perfusion of rabbit ear The experiment was performed according to the previous report^[13]. Briefly, the animals were anesthetized with pentobarbital sodium (30 mg/kg, i.p.) and fixed in prone position. A 1.5-cm-long incision was made on the dorsal of the rabbit ear. Then the central artery and the posterior auricular nerve were carefully dissected from the central vein and the surrounding tissues. After cannulation in the central artery and transaction of the central vein, blood was washed out using heparin-containing perfusate. The whole ear was then excised proximal to the cannula and perfused with oxygenated (95% O_2 , 5% CO_2) Tyrode's solution (37 °C, pH 7.3-7.4) containing atropine (1×10^{-5} mol/L) under constant perfusion pressure (40 cmH₂O). The Tyrode's solution consisted of 137 mmol/L NaCl, 2.68 mmol/L KCl, 1.0 mmol/L $MgCl_2$, 0.32 mmol/L NaH_2PO_4 , 11.9 mmol/L $NaHCO_3$, 1.35 mmol/L $CaCl_2$ and 5.55 mmol/L glucose. Before drug application, the isolated and perfused rabbit ear was first equilibrated for at least 15 min to reach a stable flow rate. Then the drugs were applied to the ear vessels via the perfusate by the constant-pressure perfusion apparatus. The duration of perfusion and washout for each drug was 20 min, respectively.

2.3 Stimulation of the auricular nerve The auricular nerve was stimulated through bipolar platinum electrodes. This mixed nerve was segregated into different, usually 3 nerve bundles. Each fiber bundle was stimulated once at the specific parameter, and the one whose flow rate reduced was considered as sympathetic nerve bundle. The stimulation was rectangular pulses at the frequency of 10 Hz, 20 Hz or 40 Hz with the duration of 1 ms and an intensity of 10 V. Each rabbit ear was stimulated for 30 s, at one of these frequencies. The interval of the consecutive stimulation was no less than 5 min in favor of function recovery of the nerves and the blood vessels. Volumes (mL) of the outflow within 1 min before (F1) and after (F2) each stimulation were measured by transferpettor, and the change of flow rate was calculated as the ratio of F2 to F1 (F2/F1).

2.4 Reagents Prazosin hydrochloride, thioperamide (Thio), CPM, cimetidine and compound 48/80 were all purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemicals were all of high grade and commercially available in China. The concentrations of drugs indicated the final molar concentration in the perfusate.

2.5 Statistical analysis Data were expressed as mean $\pm$ SD, and analyzed by Student's *t*-test. The differences among the 3 frequencies were analyzed by one-way analysis of variance (ANOVA) using SPSS 12.0 (SPSS Inc., Chicago, IL). $P < 0.05$ was considered as significantly different.

3 Results

3.1 Responses of blood vessels to different frequencies of auricular nerve stimulation As shown in Table 1, flow rate of perfusate was reduced by auricular nerve stimulation, which was, to some extent, dependent on the frequency of stimulation ($P < 0.05$, 10 Hz vs 20 Hz, 20 Hz vs 40 Hz; $P < 0.01$, 10 Hz vs 40 Hz). However, in the presence of prazosin (1×10^{-5} mol/L), electrical stimulation (ES) failed to induce a significant decrease in the outflow rate, compared with that in the absence of prazosin ($P < 0.01$), at any of the 3 frequencies (Table 1).

3.2 Effects of CPM and cimetidine on ES-induced vasoconstriction in perfused rabbit ear The decrease in flow rate caused by ES at high frequencies (20 Hz and 40 Hz) could be

Table 1. ES-induced changes in the flow rate (mL/min)

Frequency	<i>n</i>	Control			Prazosin(1×10^{-5} mol/L)		
		Before	After	Decrease	Before	After	Decrease
10Hz	8	1.45 $\pm$ 0.09	1.14 $\pm$ 0.17	0.31 $\pm$ 0.12	1.45 $\pm$ 0.09	1.35 $\pm$ 0.15	0.10 $\pm$ 0.11**
20Hz	8	1.47 $\pm$ 0.08	1.02 $\pm$ 0.14	0.45 $\pm$ 0.10	1.47 $\pm$ 0.08	1.31 $\pm$ 0.10	0.16 $\pm$ 0.09**
40Hz	8	1.50 $\pm$ 0.09	0.90 $\pm$ 0.10	0.60 $\pm$ 0.16	1.50 $\pm$ 0.09	1.27 $\pm$ 0.07	0.23 $\pm$ 0.12**

** $P < 0.01$ vs Control.

reversed by histamine H_1 receptor antagonist CPM ($P < 0.05$, Fig. 1A), from (69.3 $\pm$ 7.1)% to (79.0 $\pm$ 8.3)% at 20 Hz, and from (60.4 $\pm$ 9.0)% to (70.3 $\pm$ 9.0)% at 40 Hz. In contrast, histamine H_2 receptor antagonist cimetidine preferentially enhanced the

ES-induced decrease in flow rate at 40 Hz ($P < 0.05$, Fig. 1B), from (60.4 $\pm$ 9.0)% to (51.3 $\pm$ 7.4)%.

3.3 Effects of prazosin and CPM on vasoconstriction induced by ES after pretreatment with compound 48/80 As shown in Fig. 2, after pretreatment with compound 48/80 for 20 min, CPM could still reverse the ES-induced flow rate reduction at 20 Hz ($P < 0.05$), from (69.9 $\pm$ 7.7)% to (78.7 $\pm$ 7.2)%. Moreover, the ES-evoked flow rate reduction was inhibited more significantly by co-treatment with prazosin and CPM, from (69.9 $\pm$ 7.7)% to (96.3 $\pm$ 4.4)%, than by prazosin alone, from (69.9 $\pm$ 7.7)% to (88.9 $\pm$ 7.4)%, at 20 Hz ($P < 0.05$).

Fig. 1 Effects of CPM (A) and cimetidine (B) on ES-evoked decrease in the outflow of perfusate. CPM could reverse the decrease at 20 Hz and 40 Hz, while cimetidine enhanced the decrease at 40 Hz. Each point represented the ratio of flow rate after ES to that before ES (F2/F1). * $P < 0.05$ vs control group at the corresponding frequency. $n = 8$ in each group.

Fig. 2 Effects of prazosin or CPM, or their co-effects on the ES-induced changes in the outflow rate at 20 Hz after pretreatment with compound 48/80. Data were analyzed by ANOVA analysis followed by Student-Newman-Keuls *t*-test. * $P < 0.05$, $n = 8$ in each group.

3.4 Effects of Thio on ES-induced vasoconstriction in perfused rabbit ear The histamine H_3 receptor antagonist Thio significantly strengthened the reduction in the flow rate of perfusate at 10 Hz, 20 Hz and 40 Hz ($P < 0.05$, Fig. 3), from (78.5 $\pm$ 8.8)% to (70.1 $\pm$ 6.2)% at 10 Hz, from (69.3 $\pm$ 7.1)% to (60.8 $\pm$ 8.2)% at 20 Hz, and from (60.4 $\pm$ 9.0)% to (51.2 $\pm$ 6.8)% at 40 Hz.

Fig. 3 Effects of Thio on ES-evoked changes in the outflow rate of perfusate. Each point represented the ratio of flow rate after ES to that before ES (F2/F1). Data were analyzed by Student's *t*-test. **P* < 0.05 vs control group at the corresponding frequency. *n* = 8 in each group.

4 Discussion

The isolated and perfused rabbit ear is a simple tool to study the peripheral vascular behavior. When perfused with physiological salt solution, blood vessels of this model retain their capacity of constriction or dilation for several hours. The isolated whole ear model is of interest just because the vascular resistance for the outflow is determined by the peripheral vessels, from arteries to veins. Although the perfused preparation of the great auricular nerve attached to the body has been used in some studies^[14], this method is time-consuming and hard to be performed. It is well-known that the sympathetic nervous system innervates the function of vasomotion. Although the auricular nerve is a peripheral mixed nerve, ES on the dissected fasciculus could still slow the outflow from the perfused rabbit ear, an effect that can be significantly blocked by 1×10^{-5} mol/L prazosin (Table 1). This means that ES triggers the release of neurotransmitters, mainly norepinephrine, from sympathetic nerve terminals, and that this model allows the examination of the function of endogenous sympathetic histamine. Based on the hypothesis that sympathetic histamine mediates differential effects under different strength of stimuli, here 3 different frequencies were selected according to the results of preliminary experiments.

In the present experiments, no significant changes in flow rate were observed after each perfusion or washout of

the antagonists used. The influence of CPM or cimetidine on the neurogenic vasoconstriction was studied, to explore whether histamine receptors located in blood vessels are activated by endogenous sympathetic histamine. We found that neither H_1 receptor antagonist nor H_2 receptor antagonist exerted any effect on the ES-induced flow rate reduction at 10 Hz. Besides, the vasoconstriction induced by ES at 20 Hz was suppressed by blockade of H_1 receptors, but not by blockade of H_2 receptors. Furthermore, the vasoconstriction induced by ES at 40 Hz could be suppressed by CPM and promoted by cimetidine.

Factors responsible for the differences in the effects of CPM and cimetidine are the density, the affinity and the location of histamine receptors. Since it has been reported that vasodilation could be mediated by NO and prostanoid signaling pathways through activation of endothelial histamine H_1 receptors^[15,16], it appears that the neurogenic histamine-induced vasoconstriction is associated with activation of smooth muscle H_1 receptors, whereas the vasodilatation is mediated by smooth muscle H_2 receptors. However, further investigations are needed to clarify the interactions between neurogenic or non-neurogenic histamine and histamine receptors at different sites.

To exclude the possible involvement of mast cell histamine in the flow rate changes, the specific mast cell degranulator compound 48/80 was used. Compound 48/80 can cause exocytosis of mast cell granular content, but exerts no effect on histamine release from axonal varicosities^[17]. Therefore, this compound can differentiate non-neuronal and neuronal resources of histamine^[18]. Preliminary experiments show that after pretreatment with compound 48/80 for 20 min, histamine in perfusate is undetectable by high performance liquid chromatography-electrochemical detection (HPLC-ECD) we set up. Even under this condition, the ES-evoked flow rate reduction at 20 Hz could still be inhibited by 4×10^{-6} mol/L CPM. Meanwhile, the attenuation of ES-induced flow rate reduction by co-treatment with α_1 receptor antagonist prazosin and H_1 receptor antagonist CPM was stronger than that by administration of prazosin alone (Fig. 2). These findings suggest that the neurogenic histamine would preferentially cause vasoconstriction via H_1 R activation, although

it has also been reported that vasodilation induced by mast cell-releasing histamine is mediated at least partially by H_1R ^[19]. Therefore, vascular receptors activated by sympathetic histamine may be distinctive from those activated by histamine from mast cells, or the effects of histamine on vasomotion may be correlated with the accumulation of histamine production.

We have also observed that the H_3 receptor antagonist Thio could enhance the ES-induced outflow reduction (Fig. 3), which means that the presynaptic H_3 receptors could be activated by sympathetic histamine and that Thio increases norepinephrine (NE) release probably by interrupting the inhibition of H_3 receptors. Since histamine H_4 receptors have a high homology with H_3 receptors, it is reported that Thio also has a weak affinity for H_4 receptors. However, H_4 receptors are expressed mainly in basophilic granulocytes, neutrocytes and mast cells. Thus, they are thought to be involved in immune reactions and inflammatory responses^[20], and the possibility of their involvement in the effects here is excluded. Moreover, the assumption that the enhancement on vasoconstriction by Thio has no correlation with the blockade of H_3 receptors is not convincing. Actually, the fact that peripheral endogenous histamine plays a role in regulating the effect of NE has been well demonstrated^[21,22]. However, since H_3R stimulation has been reported to reduce both exocytotic and carrier-mediated NE release^[23], this modulation can be related either to NE release inhibition or to the increase in its uptake. Thus, further studies are required to clarify the underlying mechanisms.

In conclusion, these results suggest that neurogenic histamine modulates sympathetic flow rate changes via activation of different histamine receptors under different conditions. This indicates that histamine may act as a neurotransmitter in sympathetic nerves innervating the vascular system, and the relative distribution of histamine receptors may mediate different functional responses.

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交感神经源性组胺释放对电刺激条件下兔耳血管舒缩功能的影响

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摘要 目的 研究不同的组胺受体拮抗剂对电刺激耳后神经引起的血管收缩的影响,并探讨交感组胺对兔耳血管平滑肌舒缩功能的突触前及突触后效应。方法 使用离体灌流的完整兔耳,研究不同频率的交感神经刺激引起的组胺释放对血管舒缩功能的影响。对耳后神经分别给予 10 Hz、20 Hz 和 40 Hz 的电刺激,并在恒压灌流条件下,观察不同的组胺受体拮抗剂对灌流液流速的影响。结果 三种频率的电刺激均能引起恒压灌流兔耳的静脉流出液流速降低。H₁受体的拮抗剂氯苯那敏能部分拮抗20 Hz及40 Hz电刺激引起的流速降低效应($P < 0.05$)。应用肥大细胞脱颗粒剂化合物 48/80 耗竭组织肥大细胞中的组胺后, H₁受体拮抗剂仍能抑制电刺激引起的流速降低。相反, H₂受体拮抗剂西咪替丁能增强电刺激引起的流速降低效应,且只针对更高的刺激频率(40 Hz, $P < 0.05$)。此外,在 H₃受体拮抗剂噻普酰胺的作用下,三种频率的电刺激所引起的血管收缩都更为显著($P < 0.05$)。结论 刺激耳后神经有可能引起组胺释放,且这种释放来自交感神经而非肥大细胞。通过刺激不同的组胺受体,交感组胺分别介导突触前调节和突触后血管收缩或舒张效应。

关键词: 交感神经系统; 组胺; 组胺受体; 血管