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Journal of Nanjing Medical University, 2009, 23(1):64-68
www.elsevier.com/locate/jnmu
Research paper

Neural histamine in the tuberomammillary nucleus regulates the onset of neurogenic pulmonary edema in rabbits

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Received 5 November 2008

Abstract

Objective: To explore the effect of neural histamine in the tuberomammillary nucleus(TM) on neurogenic pulmonary edema (NPE) onset in rabbits and the function of the rostral ventrolateral medulla(RVLM) in the neural histamine modulation of NPE. **Methods:** NPE was produced by the intracisternal injections of fibrinogen and thrombin. The contents of histamine in the TM and RVLM in rabbits were measured with high performance liquid chromatography(HPLC). Rabbits were placed on a stereotaxic frame and microinjection cannulae were inserted into the TM and RVLM using brain atlas coordinates. Animals were pretreated with R- α -methylhistamine(MeHA) in the TM and chlorphenamine Mmaleate/cimetidine in the RVLM prior to establishing the NPE model. Changes in the lung water ratio and mean arterial pressure(MAP) were recorded, and paraffin sections of lung tissue were observed by light microscope. **Results:**We found that the contents of histamine(HA) in the TM and RVLM increased significantly with the onset of NPE. Pretreatment with MeHA in the TM and chlorphenamine Mmaleate in the RVLM significantly decreased MAP, and the lung water ratio and histological characteristics of the NPE in the rabbit model. Pretreatment with cimetidine in the RVLM had no effect on NPE. **Conculsion:**The results suggest that neural histamine in the TM is involved in the onset of NPE, and this effect of neural histamine is mediated by H_1 receptor in the RVLM.

Key words: pulmonary edema; histamine; medulla oblongata; hypothalamus;posterior; rabbits

INTRODUCTION

Neurogenic pulmonary edema(NPE), is a severe complication of the respiratory system secondary to an acute lesion of the central nervous system, and is a common cause of death following head injuries or diseases resulting in increased intracranial pressure. The shifting of blood from systemic circulation to pulmonary circulation results in pulmonary capillary leakage and is caused by over-stimulation of the sympathetic nervous system[1]. We know little about how acute lesions of the central nervous system produce excessive excitation of the sympathetic nervous system. The hypothalamus is considered the major sub-cortical visceral activity integration centre, and the posterior portion, the posterior hypothalamus area (PHA), controls the peripheral sympathetic nervous system. Histaminergic neurons in the tuberomammillary nucleus(TM) of the PHA[2,3] release neural histamine to regulate sympathetic nerve activity in stress to maintain homeostasis of the internal environment. Traumatic lesions of the central nervous system are one type of stress. As the over-excitation of the sympathetic nervous system is a major feature of NPE, we hypothesized that NPE may result from the mass release of neural histamine when a central nervous system lesion occurs. The rostral ventrolateral medulla(RVLM)[4] is regarded as the sympathetic nerve centre. It is rich in histamine receptors and is innervated by histaminergic fibers originating in the TM. The present study was designed to determine if neural histamine released from TM histaminergic neurons resulted in RVLM-mediated sympathetic activity that caused NPE.

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MATERIALS AND METHODS

Material

R-α-methyl histamine and thioperomide were obtained from Sigma Chemical Co.(USA), chlorphenamine Mmaleate and cimetidine were obtained from Pengyao Chemical Co.(Jiangsu, China); thioperamide and thrombin was obtained from Hangkang Biological Pharmaceutical Co., Ltd., (Zhejiang, China); fibrinogen was obtained from Laishi Blood Products Co., Ltd.,(Shanghai, China) and histamine diphosphate monohydrate was obtained from Boao Biological Technology Co., Ltd., (Shanghai, China).

Experimental protocol

Rabbits were anesthetized with a marginal ear vein injection of urethane(1g/kg).A tracheal tube was inserted after performing a tracheotomy, and a catheter inserted in the common carotid artery and the transducer interfaced with a PowerLab,Chart v 3.6. 4,AD Instruments Pty Ltd Company(Australia), for the determination of mean arterial pressure(MAP). Rabbits were placed in a stereotaxic apparatus and catheters were located in the TM and RVLM using coordinates obtained from the Swayer atlas[5]. (The coordinates for TM were AP-3 mm,LR 1.5 mm, H_0-5 mm, and the coordinates for RVLM were AP 2.0 mm, LR 2.5 mm, H 4.0 mm[6].) A volume of 1 μl of saline(pH7.2-7.4) or 10 μg of thioperamide /R-α-methylhistamine(MeHA)[6] dissolved in 1 μl saline (pH 7.2-7.4) was microinjected in the TM of untreated and treated rabbits, respectively. A volume of 1 μl of saline(pH 7.2-7.4) or 10 μg of chlorphenamine Mmaleate/cimetidine[7] dissolved in 1 μl saline (pH7.2-7.4) was microinjected in the RVLM of untreated and treated rabbits, respectively. For induction of NPE, rabbits were consecutively treated with intracisternal microinjections of thrombin and fibrinogen at doses of 35 U/kg and 20 mg/kg respectively, five minutes apart[8]. The cisterna magna was accessed at the base of the dorsal side of the cranium using a needle.

The animal model was considered positive for NPE when edema fluid appeared in the tracheal tube within 15 min after intracisternal injection, or if, after a 15-min period was allowed to elapse, edema fluid could be obtained from the trachea or the bronchi after the chest was opened[9].

At the end of the experiment, methylthionine chloride was microinjected and the brain was frozen in a cryostat and coronal sections were cut(20 μm) and examined under the light microscope to determine whether the placements of the microinjection cannulae were correct.

Measurement of the contents of neural histamine in the TM and RVLM with high performance liquid chromatography(HPLC)

At the end of the experiment, animals were decapitated, the brains removed and placed on dry ice. The TM and RVLM were dissected[10,11], weighed and homogenized in 1%(w/v) trichloracetic acid in an ice bath. The homogenates were centrifuged for 10 mins at 3,000 rpm and the supernatants stored at −20℃ for subsequent analysis. The supernatant was then filtered through a membrane(0.45 μm). Histamine was measured by HPLC(LC-6A, Daojin, Japan) on a Lichrospher C_{18}, 4.6ID ± 25 cm,dp5 μm column (Hanbang, Jiangsu, China), and the mobile phase was 27.4% phosphoric acid: methanol(97:3) [12]. The peak area was recorded under the following condition; column temperature:25℃; rate of flow, 0.8 ml/min; sensitivity,0.02. A histamine standard curve was run with each sample, samples and standard curves were run in triplicate, and the mean concentration calculated from the maximum peak values.

Calculating the lung water ratio

The lungs were removes and weighed after the animals were euthanized at the end of and the experiment, and dried in an oven for 2 days at 70℃ until constant weigh. The lung water ratio was calculated according to the following formula.

Lung water ratio = (Lung wet weight-Lung dry weight)/Lung dry weight

Histology

Formalin fixed lung tissue was embedded in paraffin and sections stained with hematoxylin and eosin for examination by light microscopy.

Animals in each group finished with paraformaldehyde perfusion, get the right leave of the lung, put in the 4% paraformaldehyde 4℃ fixed with 24 hours. Put the tissue into the 70%、80%、95%、100% alcohol in the 45-minute to gradient dehydration, xylene to transparent the tissue, embedded in paraffin, slicing, stained with hematoxylin and eosin.

Statistics

Data are presented as mean ± SD. Differences between two means were evaluated using Student t-test, and differences between more than two means were evaluated using analysis of variance. $P < 0.05$ was considered statistically significant.

RESULTS

Animals that were not manipulated were assigned to the control group, animals in which saline was microinjected in the TM were assigned to the TM+NS

group, while animals in which saline was microinjected in the RVLM were assigned to the RVLM+NS group. NPE model rabbits in which saline was microinjected into the TM or RVLM were assigned to the NPE+NS_t group and NPE+NS_r group, respectively, while NPE model animals .that were microinjected with Thio and MeHA at 10 min and 5 min before inducing the NPE were assigned to the Thio+MeHA group. Animals in which the NPE model was induced after microinjection of chlorphenamine mmaleate or cimetidine into the RVLM were assigned to the H1 group and H2 group respectively, while those animals in which MeHA was microinjected into the TM prior to inducing the model were assigned to the H3 group.

The contents of neural histamine in the TM and RVLM

The recovery of histamine measured by HPLC was 85%, and the mean retention time of the histamine peak was 3.433s.(***Fig. 1***). The contents of histamine in the TM and RVLM of the **control** group were 1689.04 ± 446.37 nmol/L and 1420.87 ± 159.17 nmol/L, respectively, while the histamine contents in the TM and RVLM in the NPE model group(n = 6) were significantly increased to 2443.14 ± 407. 77 and 2392.76 ± 596.54, respectively($P < 0.01$, $P < 0.001$).

The histamine contents in the RVLM of NPE model animals microinjected in the TM with saline or MeHA(NPE+NS_t or H_3 group, respectively) were 2184.64 ± 483.42 and 1682.69 ± 394.28($P < 0.01$).

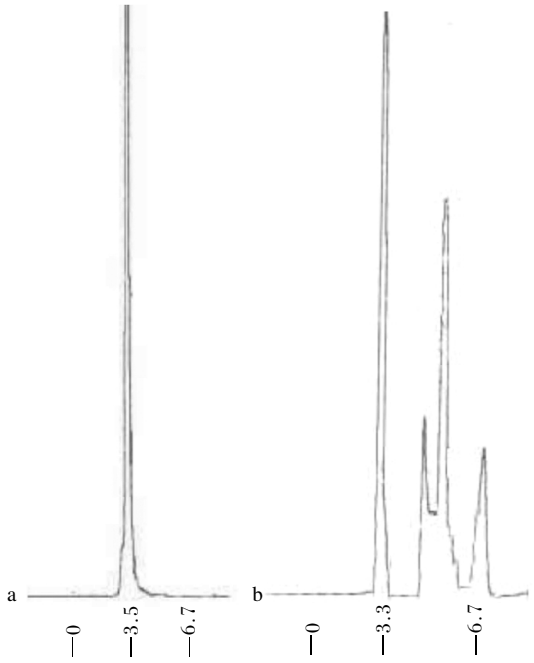


Fig. 1 a: Chromatogram of histamine standard; b: Chromatogram of sample

MAP

There was no significant difference in MAP between the control group and TM+NS or RVLM+NS groups (***Table 1***,***2***). Producing the NPE models in animals in which saline was microinjected in either the TM or RVLM resulted in a significant increase in MAP ($P < 0.001$). MAP of NPE+NS_t group increased dramatically compared to TM+NS group($P < 0.001$). After microinjection of MeHA into the TM, MAP decreased significantly($P < 0.001$) when compared to NPE+NS_t group(***Table 1***).

MAP of the NPE+NS_r group animals increased dramatically compared to RVLM+NS group($P <$ 0.001), but after microinjection of chlorphenamine Mmaleate into the RVLM (H_1 group), MAP decreased significantly($P < 0.001$).No significant difference in MAP was observed between the NPE+NS_r group and the H_2 group(***Table 2***).

Lung water ratio

No significant change in lung water ratio was obser-ved between the control group and the TM+NS group. The ratio of the NPE+NS_t group was significantly increased when compared to TM+NS group ($P < 0.01$). After the microinjection of MeHA into the TM, the lung water ratio decreased significantly ($P < 0.05$)(***Table 1***).

There was also no significant difference in lung water ratio between the control group and the RVLM+NS group, while the ratio in the NPE+NS_r group was increased significantly($P < 0.01$). When

Table 1 **Effects of microinjection of MeHA in TM (H_3) on MAP(mmHg) and lung water ratio in NPE rabbits** (mean ± *SD*)

Group	*n*	MAP	lung water ratio
Control group	6	100.85 ± 10.32	4.85 ± 0.40
TM+NS group	6	98.12 ± 12.74	4.90 ± 0.62
NPE+NS_t group	6	120.81 ± 28.74***	6.46 ± 1.47**
H_3 group	6	101.07 ± 9.12$^{\#\#\#}$	5.37 ± 1.08$^{\#}$
Thio+MeHA group	6	119.25 ± 33.01***	6.82 ± 1.82*

$^{**}P < 0.01$,$^{***}P < 0.001$ vs TM+NS group; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs NPE+NS_tgroup; $^{\star}P < 0.05$, $^{\star\star\star}P < 0.001$ vs H_3 group.

Table 2 **Effects of microinjection of chlorphenamine Mmaleate(H_1) and cimetidine(H_2) in the RVLM on MAP(mmHg) and lung water ratio in NPE rabbits** (mean ± *SD*)

Group	*n*	MAP	lung water ratio
Control group	6	100.85 ± 10.32	4.85 ± 0.40
TM+NS group	6	103.18 ± 12.82	4.91 ± 0.85
NPE+NS_t group	6	121.00 ± 28.39***	6.72 ± 1.36**
H_1 group	6	103.59 ± 12.97$^{\#\#\#}$	5.06 ± 0.59$^{\#\#}$
H_2 group	6	119.80 ± 25.56	6.12 ± 1.38

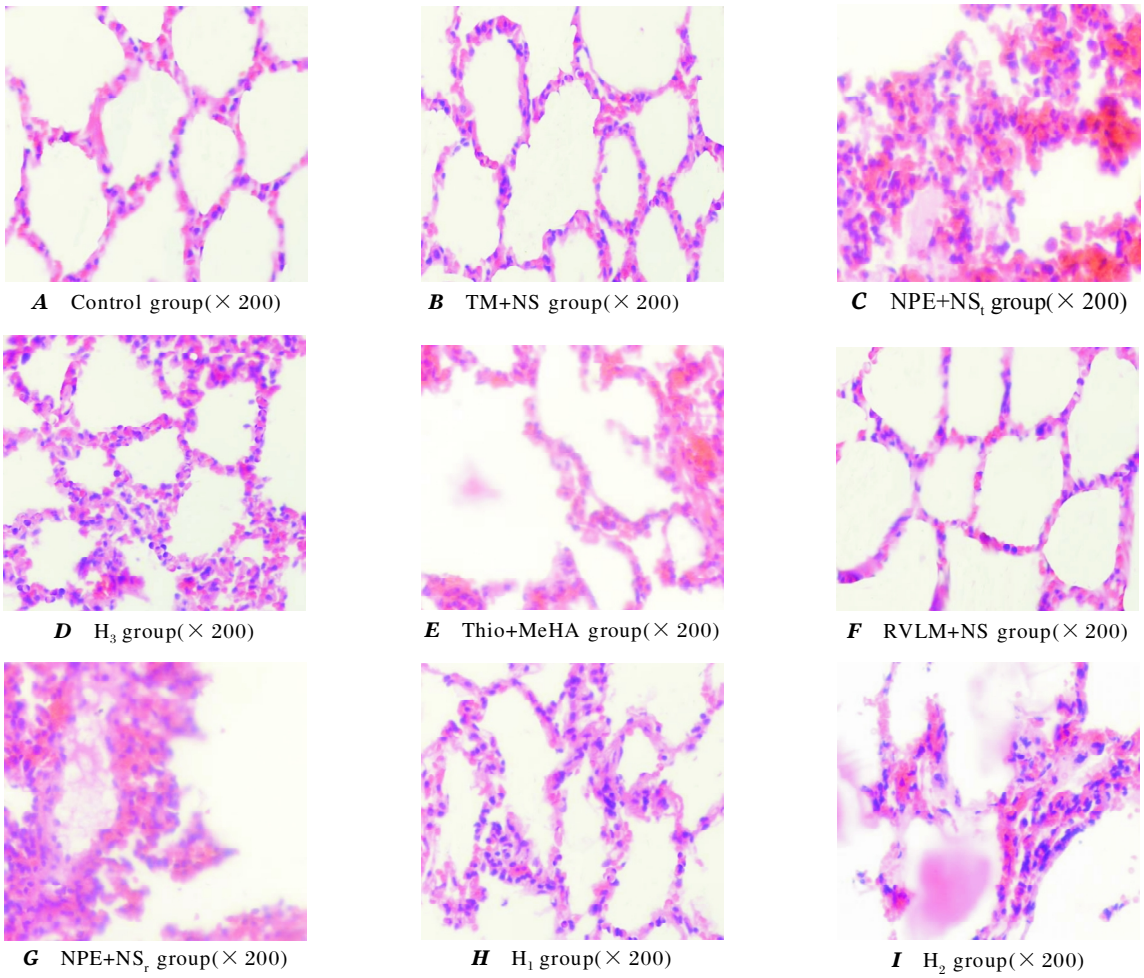
$^{**}P < 0.01$,$^{***}P < 0.001$ vs RVLM+NS group; $^{\#\#}P < 0.01$, $^{\#\#\#}P <$ 0.001 vs NPE+NS_r group.

the RVLM was pretreated with chlorphenamine Mmaleate the lung water ratio was significantly decreased when compared to the NPE+NS_r group($P < 0.01$), while microinjection of cimetidine into the RVLM produced no change(***Table 2***).

Histology

There were normal pulmonary alveoli and alveolar septa in the lung tissue of the control and TM+NS groups, there was no evidence of liquid exudation, and the alveolar epithelium was structurally intact (***Fig. 2-A,B***). In the NPE+NS_t group there was substantial fusion of the alveoli, evidence of exudates, thickening of the alveolar septa and erythrocyte infiltration(***Fig. 2-C***). In the H_3 group there was no evidence of exudate in the pulmonary alveoli, while the appearance of Thio+MeHA group sections was similar to that of the NPE+NS_t group(***Fig. 2-D,E***).

The lung tissue of the RVLM+NS group showed no evidence of pulmonary edema(***Fig. 2-F***), while in the NPE+NS_r group there was fusion of pulmonary alveoli and a significant amount of exudate(***Fig. 2-G***). In the H_1 group the lung tissue was free of evidence of exudate(***Fig. 2-H***). The lung tissue of the H_2 group had no normal alveolar structure, alveolar septum thickening was obvious, and there was significant exudate(***Fig. 2-I***).



A Control group(× 200) ***B*** TM+NS group(× 200) ***C*** NPE+NS_t group(× 200)

D H_3 group(× 200) ***E*** Thio+MeHA group(× 200) ***F*** RVLM+NS group(× 200)

G NPE+NS_r group(× 200) ***H*** H_1 group(× 200) ***I*** H_2 group(× 200)

Fig. 2 The role of neurogenic histamine on neurogenic pulmonary edema in rabbit

DISCUSSION

Over-excitation of the sympathetic nervous system is obvious in NPE, and neural histamine appears to play a major role. For this reason we first measured neural histamine. We found that histamine in the TM in NPE rabbits was significantly higher than in control rabbits. At the same time, the MAP in NPE rabbits increased significantly, which hinted indirectly at the up-regulation of the sympathetic nervous system. The results support our conjecture that neural histamine released by TM histaminergic neurons results in NPE that is mediated through the peripheral sympathetic nervous system.

The histamine H_3 receptor is an autoreceptor located on the histaminergic neuron presynaptic membrane to regulate the release and synthesis of histamine[13].

MeHA is an H_3 receptor agonist, and when it was microinjected into the TM it decreased the release of neural histamine. After microinjection of MeHA in the TM, the lung water ratio was also significantly decreased, and no exudate was present in the pulmonary alveoli. Alveolar septa thickened slightly, but epithelial cell structure was intact. The amelioration of the NPE was obvious. At the same time, MAP decreased dramatically, which suggested down-regulation of the sympathetic nervous system. This amelioration of NPE after down-regulation of the sympathetic nervous system by pretreating the TM with MeHA further confirms that neural histamine released by TM histaminergic neurons takes part in regulating NPE through the peripheral sympathetic nerve system.

Iwase and colleagues[14] discovered that enhancement of the electrical discharge of cervical sympathetic nerves produced by electrically stimulating the TM required a relatively long incubation period, from 150 ms to 200 ms. This indicated that neural histamine regulated the peripheral sympathetic nerve system through a multi-synaptic pathway. Histamine neurons in the TM send out histaminergic fibers innervating the RVLM. For this reason, when we measured the contents of histamine in the TM, we also determined the content in the RVLM, and found that in the NPE model the contents of histamine increased in both the TM and the RVLM. At the same time, microinjection of MeHA in the TM dramatically decreased the contents of histamine in the RVLM. The results indicate that neural histamine in the TM possibly influences the action of the sympathetic nervous system to regulate NPE through the RVLM. RVLM is rich in histaminergic receptors. To further study the role of the RVLM in NPE, we microinjected chlorphenamine Mmaleate in the RVLM. The lung water ratio and MAP decreased markedly, and NPE was obviously ameliorated These results were similar to those of experiments involving pretreatment with MeHA and indicate that H_1 receptors in the RVLM participate in the process of NPE. The lack of an effect of cimetidine microinjection in the RVLM on NPE indicate that 1) H_2 receptors may not be involved in the onset of NPE, or 2) there may be too few H_2 receptors present to manifest an effect. In summary, there is an excitatory pathway between the TM and sympathetic preganglionic spinal neurons. In this pathway, neural histamine released by TM histaminergic neurons regulates the sympathetic nervous system through the histamine H_1 receptors in the RVLM. NPE possibly results from abnormalities in this pathway.

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