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Original Article

Protective effect of histamine microinjected into the cerebellar fastigial nucleus on stress-induced gastric mucosal damage in rats

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Abstract: Aims: We investigated the effffects and the possible mechanism of microinjection of histamine into cerebellar fastigial nucleus (FN) on stress-induced gastric mucosal damage (SGMD) in rats. The effect of microinjection of histamine into FN on SGMD was observed. Methods: The model of SGMD was established by restraint and water (21 ± 1°C)-immersion (RWI) for 3 h in rats. The gastric mucosal damage index indicated the severity of SGMD. Western blotting was performed to assess gastric mucosal cell apoptosis and proliferation. Results: We observed that histamine microinjection into the FN markedly attenuated SGMD in a dose-dependent manner, and was prevented by pre-treatment with the ranitidine (a selective histamine H_2 receptor antagonist) into the FN. The effect of histamine was abolished by pre-treatment with 3-MPA (a glutamic acid decarboxylase antagonist) into the FN. There was a decrease in the discharge frequency of greater splanchnic nerve, and an increase in gastric mucosal blood flow after histamine injection into the FN. Additionally, anti-apoptotic and anti-oxidative factors of gastric mucosa might be involved in this process. Conclusion: The exogenous histamine in FN participates in the regulation of SGMD, and our results may help to provide new ideas on the treatment of gastroenterological diseases.

Keywords: Histamine, fastigial nucleus, lateral hypothalamic area, stress-induced gastric mucosal damage, decussation of superior cerebellar peduncle

Introduction

Histamine is well-known pro-inflammatory substances. Besides its functions in allergic reactions and inflammations, histamine has also been implicated with learning, memory, sleep/wakefulness, attention, anxiety, pain perception, homeostasis of fluid balance, appetite and body temperature [1-3]. However, it was not until the 1970's that the role of histamine was revealed as an important neurotransmitter/neuromodulator in learning and memory processes in the mammalian brain [4]. Central histaminergic nervous system originating from the tuberomammillary nucleus in the hypothalamus has been shown to innervate almost all areas of the cortices in the brain and three deep nuclei in the cerebellum, *i.e.*, the cerebellar fastigial nucleus (FN), interposed cerebellar nucleus (Int) and lateral dentate cerebellar nucleus (Lat) [5]. Autoradiographic mapping and *in situ* hybridization experiments have demonstrated the presence of H_1 and H_2 receptors in the rat cerebellar cortex and deep cerebellar nuclei, while H_3 receptors are scarce in the cerebellum [1, 6]. In the last decade, neuroanatomical studies revealed that the cerebellum and the hypothalamus are interconnected with direct hypothalamocerebellar and cerebellohypothalamic projections and via a multitude of indirect pathways [7]. The lateral hypothalamic area (LHA) is an important site in the brain

which protects from gastric damage. Our previous studies have already confirmed that cerebellum is involved in the regulation of stress-induced gastric mucosal damage (SGMD) [8]. These intriguing findings have greatly enriched the notion that the cerebellum also participates in the regulatory functions of autonomic viscera [9]. Besides its role as an important subcortical locomotor centre, the cerebellum is also an essential component in the central integration of visceral activities.

Immunocytochemical experiments have indicated that some of the hypothalamo cerebellar fibres are histaminergic [10]. The histamine-containing fibres project from the tuberomammillary nucleus of the hypothalamus to the cerebellar cortex and deep cerebellar nuclei, which suggests that the hypothalamo cerebellar histaminergic fibres may play a pivotal role in cerebellar functions [11]. FN, the phylogenetically oldest nucleus in the cerebellum and a key pivot in the ultimate outputs of the spinocerebellum, has been considered to participate in the protection of SGMD according to our previous studies. There are also studies that demonstrated that histamine excites FN mainly by modulating the electrophysiological properties of cerebellar nuclear neurons [8]. In this study, we investigated whether histamine is involved in the regulation of visceral activities by microinjection of histamine into cerebellar FN and observe its effect on stress-induced gastric mucosal damage in rats.

Material and methods

Animals

Adult Sprague-Dawley (SD) rats, weighing 210 ± 10 g, were provided by the Experimental Animal Centre of Xuzhou Medical College, Jiangsu, China (usage certificate No: SYXK (Su) 2002-0038). The rats were randomly divided the different groups (n = 6 each). The room temperature was maintained at 23 ± 1°C under a 12/12 h day/night period circle. Prior to the experiments, all rats were fasted for 24 h, except for access to tap water.

Ethical considerations: All animal experiments were approved by the Committee on Research Animal Care and Use of the Xuzhou Medical College.

Reagents

Histamine, ranitidine, triprolidine, 3-mercaptopropionic acid (3-MPA), gamma-aminobutyric acid (GABA), and kainic acid (KA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Rabbit anti-Caspase-3, rabbit anti-Bcl-2, rabbit anti-Bax, rabbit anti-β-actin and alkaline phosphorylase-tagged goat anti-rabbit IgG anti-body were obtained from Zhongshan Golden Bridge Biotech Co (Beijing, China). The SABC rabbit IgG POD kit and BCIP/NBT assay kit were purchased from Boster Bio-engineering (Wuhan, China). The malondialdehyde (MDA) and superoxide dismutase (SOD) assay kits were obtained from Jiancheng Bioengineering (Nanjing, China).

Orientations of brain nuclei

Prior to the experiments, the rats were anesthetised with sodium pentobarbital (40 mg/kg, i.p.), and then mounted onto a stereotaxic apparatus. The rats underwent an incision in the scalp, following by a drilling, with a hole of 0.5 mm in diameter, in the cranium dorsal of the target site. The coordinates for the placement parameters of FN, decussation of superior cerebellar peduncle (DSCP), LHA were determined in accordance with the rat brain atlas in stereotaxic coordinates [12].

Microinjection of brain nuclei and electrical destruction of DSCP

In all experiments, FN, and LHA underwent drug administration. The different doses of histamine (0.05, 0.5, and 5 μg in a volume of 0.3 μl normal saline) was microinjected into FN with a microsyringe in histamine groups, and vehicle (saline) in the vehicle control group. 15 min before microinjection of histamine (H, 5 μg), ranitidine (R, 0.1 μg), triprolidine (T, 0.1 μg) and glutamic acid decarboxylase antagonist, 3-MPA (5 μg) were microinjected into FN in the rats, hence the designation of R + H group, T + H group, and 3-MPA + H group, respectively [8, 13]. GABA (50 μg in 0.3 μl normal saline) was bilaterally microinjected into the LHA in GABA groups [8, 14]. All microinjection was slowly performed in 2 min for dispersion and the injection cannula was left in the position for an additional 3 min to prevent backflow. Electrical ablation of DSCP was conducted by the passage of a positive DC current of 1 mA for 10 s [8]. The same procedures were performed in Sham DSCP ablation group, except for the passage of DC current. The chemical ablation of FN, or LHA was made by bilateral microinjection of KA (0.3 μg in 0.3 μl normal saline) into the FN

and LHA, respectively [15, 16]. The vehicle (normal saline) was bilaterally microinjected into the FN and LHA in vehicle groups and sham chemical ablation groups, respectively.

Finally, the brains were sliced to certify the sites of lesion and microinjection. The data from the rats whose target sites failed to comply with the histological criteria were excluded from the analysis.

Model preparation of SGMD and assessment of gastric mucosal injury index

According to the previous method [17, 18], the model of SGMD and gastric mucosal damage index (GMDI) were established by restraint and water-immersion (RWI) (21 ± 1°C) for 3 h. The rats were sacrificed for the removal of the stomach, which was sliced along the greater curvature and washed with ice-cold phosphate-buffered saline (PBS; 0.1 mol/L). The GMDI was based on a cumulative-length scale on which an individual lesion limited to the mucosal epithelium was scored according to its length as follows: 1, ≤ 1 mm; 2, > 1 mm and ≤ 2 mm; 3, > 2 mm and ≤ 3 mm. If the lesions were > 1 mm in width, the lesion score was doubled. To avoid researcher bias, the gastric mucosal injury index was determined by a researcher who was blind to treatments. Thereafter, the scraped stomach mucosa was kept in the refrigerator at -80°C.

Measurement of the discharge frequency of greater splanchnic nerve (GSN)

The discharge frequency of greater splanchnic (GSN) was measured as reported before [19]. The rat was anesthetised with sodium pentobarbital (40 mg/Kg, i.p.) and mounted onto a stereotaxic apparatus. The coordinates of FN was the same as the above, and the histamine (5 μg in a volume of 0.3 μl 0.9% NaCl) was microinjected into FN). The discharge frequency of GSN was recorded for 2~3 min by single barrel silver wire electrode before and after microinjection of histamine into the FN. The discharge signals were amplified and filtered via a biological signal acquisition system, MedLab-U/4C501 (Nanjing MedLab Science and Technology Co., Ltd.), which automatically recorded the discharge frequency of GSN; with only GSN activities in the stable discharge recorded.

Measurement of the gastric mucosal blood flow (GMBF)

The measurement of GMBF was performed as described above [8] with Laser-Doppler flowmeter (LDF-2, Nankai University, China). Briefly, the rats were anesthetized with sodium pentobarbital (40 mg/kg). Then, the abdomen was opened, followed by the exposure and incision of the stomach at the minor curvature, with the gastric content slightly evacuated to the exterior through the cut (5 mm). The ultrasound probe was placed 0.5 mm above and perpendicular to the mucosal surface to observe GMBF on the digital panel of the flowmeter. When GMBF was stable, four sites were selected to record (1 min for each point) and then the average value was calculated.

Western blotting

The scraped stomach mucosa stored at -80°C was for Western blotting performed as reported before [20]. Protein concentration was determined with a BCA protein assay kit. The sample protein was separated by 10% SDS-polyacrylamide gel electrophoresis and subsequently transferred to a nitrocellulose membrane. The membranes were immunoblotted with primary antibodies that recognize MAPKs (anti-Bax antibody 1:500, anti-bcl-2 antibody 1:500, anti-Caspase-3 antibody 1:750 and anti-β-actin antibody 1:2000). Afterwards, the detection was performed with the addition of alkaline phosphatase goat anti-rabbit IgG (1:1000) and then developed with BCIP/NBT assay kit. In the end, the bands were scanned and analyzed by Image-Pro Plus 6.0.

Measurement of malondialdehyde (MDA) content and superoxide dismutase (SOD) activity

The measurement of MDA and SOD was performed as demonstrated [21]. To detect the MDA content and the SOD activity, the gastric mucosa was stored at 4°C. The homogenate was centrifuged at 3000 g for 10 min, with the supernatant retained. The protein concentration was determined by Coomassie brilliant blue protein assay. The level of lipid peroxidation was detected by thiobarbituric acid-reactive substances and was revealed spectrophotometrically at 532 nm. SOD activity was revealed spectrophotometrically at 550 nm by

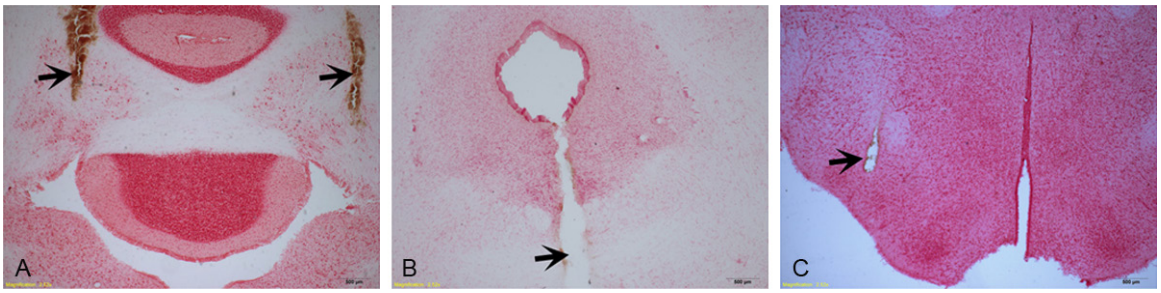

Figure 1. The histological verifications of target sites of the needle tip of microinjection into the FN, LHA, and the electrically damaged sites of DSCP in the rat brain. A-C: Photomicrographs of the target sites of microinjection into the FN, LHA , and the electrolytic damaged sites of DSCP, respectively in the rat brain. The coronal sections were stained with neutral red, showing the target sites of microinjection of FN and LHA, or damage of DSCP with the passage of DC of 1 mA for 10 s, which indicates that the placements within the FN, LHA, and DSCP, respectively. Scale bar = 500 μm.

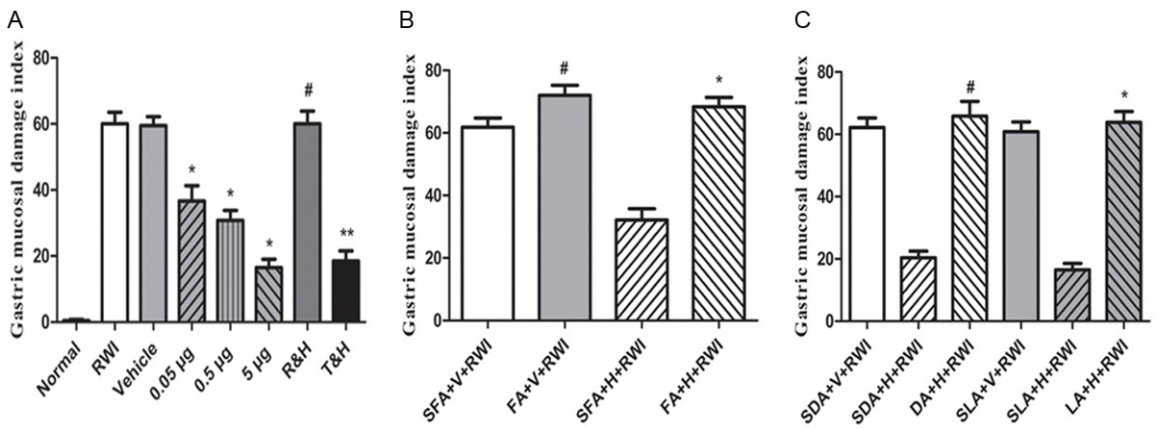

Figure 2. A: Effect of histamine microinjected into FN on SGMD and role of FN, DSCP and LHA. Mean ± SD; $n = 6$; Normal: normal; RWI: restraint and water-immersion (21 ± 1°C) alone; Vehicle: microinjection of vehicle + RWI; 0.05, 0.5, and 5 μg: histamine microinjection at three respective doses into FN + RWI. R + H: ranitidine microinjected into FN prior to histamine + RWI; T + H: triprolidine into FN prior to histamine + RWI. $^{*}P < 0.05$ *vs*. vehicle; $^{\#}P < 0.05$, $^{**}P > 0.05$ *vs*. histamine (5 μg). B: SFA + V + RWI: sham FN ablation + vehicle + RWI; FA + V + RWI: FN ablation + vehicle + RWI. SFA + H + RWI: sham FN ablation + histamine + RWI; FA + H + RWI group: FN ablation + histamine + RWI. $^{\#}P > 0.05$ *vs*. SFA + V + RWI; $^{*}P < 0.05$ *vs*. SFA + H + RWI. C: SDA + V + RWI: sham DSCP ablation + vehicle + RWI; SDA + H + RWI: sham DSCP ablation + histamine + RWI; DA + H + RWI: DSCP ablation + histamine + RWI. $^{\#}P < 0.05$ *vs*. SDA + H + RWI. SLA + V + RWI: sham LHA ablation + vehicle + RWI; SLA + H + RWI: sham LHA ablation + histamine + RWI; LA + H + RWI: LHA ablation + histamine + RWI. $^{*}P < 0.05$ *vs*. SLA + H + RWI.

the xanthine/xanthine oxidase reaction method. MDA content and SOD activity were expressed in nmol/mg and U/mg, respectively.

Statistical analysis

All results were expressed as mean ± SD. Comparisons between two groups were performed by Student's *t*-test, and multiple-group analyses were conducted by one-way analysis of variance (one-way ANOVA). Statistical analysis was performed by GraphPad Prism 5 and SPSS 16.0. The results were considered significantly different when $P < 0.05$.

Results

Histological verification

The histological verification was performed on all target sites of electrical and chemical lesions and of microinjections by referring to the stereotaxic atlas (**Figure 1A-C**).

Effects of histamine microinjected into the FN on SGMD in rats

As indicated in **Figure 2A**, the GMDI was 60.88 ± 8.69 in the RWI group, no significant differ-

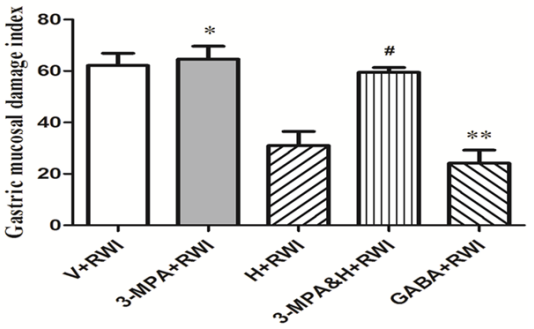

Figure 3. The role of GABA microinjected into the LHA, L-glutamic acid decarboxylase (GAD) antagonist, and the 3-MPA, in the histamine microinjected into the FN. V + RWI: vehicle microinjection into the FN + RWI; 3-MPA + RWI: 3-MPA microinjected into FN + RWI; H + RWI: histamine microinjected into the FN + RWI; 3-MPA + H + RWI: microinjection of 3-MPA and histamine into the FN + RWI; GABA + RWI: GABA microinjected into the LHA + RWI. Mean ± SD; n = 6; $^{*}P > 0.05$ *vs*. V + RWI group; $^{\#}P < 0.05$ compared with H + RWI group; $^{**}P > 0.05$ compared with H + RWI group.

ence was observed compared with vehicle group (59.50 ± 6.69, $P > 0.05$). Compared with the vehicle group, the GMDI was evidently decreased (36.67 ± 9.29, 30.83 ± 7.30, and 16.50 ± 6.12, respectively) after microinjections of different doses (0.05, 0.5, and 5 μg in a volume of 0.3 μl 0.9% NaCl) of histamine into FN plus RWI groups ($P < 0.05$) in a dose-dependent manner. The results suggested also that the 5 μg histamine was the optimal protective dose, which was used in the subsequent experiments.

In **Figure 2B**, the GMDI was 61.83 ± 7.25 in sham FN ablation + vehicle + RWI group, difference was observed compared with FN ablation + vehicle + RWI group (72.00 ± 7.95, $P < 0.05$). Compared with sham FN ablation + histamine + RWI group (32.17 ± 8.84), after chemical ablation of FN, a remarkable growth was observed in FN ablation + histamine + RWI group (68.30 ± 7.42, $P < 0.05$).

Role of histamine receptor in the histamine microinjection into FN on SGMD in rats

In addition, in **Figure 2A**, the GMDI was 60.00 ± 9.61 in ranitidine (a selective histamine H_2 receptor antagonist) + histamine group, and no statistical difference was found compared with the vehicle group ($P > 0.05$). However, the GMDI was remarkably decreased (18.50 ± 7.50, $P < 0.05$) in triprolidine (selective histamine H_1 receptor antagonist) + histamine group. The GMDI in triprolidine (selective histamine H_1 receptor antagonist) + histamine group was similar to the histamine microinjection into FN group ($P < 0.05$).

Role of DSCP and LHA in the protective effect of microinjection of histamine into FN on SGMD in rats

In **Figure 2C**, the GMDI was 62.17 ± 7.14 in sham DSCP ablation + vehicle + RWI group; 20.33 ± 5.28 in sham DSCP ablation + histamine group, and was subsequently increased to 65.55 ± 9.56 in the group after electrolytic ablation of DSCP + histamine + RWI group. Comparison indicated that the difference between the sham DSCP ablation + histamine group and the electrolytic ablation of DSCP + histamine + RWI group were significant ($P < 0.05$).

The GMDI was 60.83 ± 7.63 in the sham ablation of LHA + vehicle + RWI group; and 16.50 ± 5.01 in sham LHA ablation + histamine + RWI group, and was elevated to 63.83 ± 8.49 in the ablation of LHA + histamine + RWI. Difference between the latter two groups was significant ($P < 0.05$, **Figure 2C**).

Role of gamma-aminobutyric acid (GABA) in the protective effect of microinjection of histamine into FN on SGMD in rats

In **Figure 3**, the GMDI was 62.17 ± 4.15 in vehicle group, and was 64.17 ± 4.97 in 3-MPA (a glutamic acid decarboxylase antagonist) + RWI group, there was no statistical significance was found ($P > 0.05$) in the both between; Moreover, it was observed that the GMDI was 59.50 ± 1.87 in 3-MPA + histamine + RWI group, and the GMDI fell to 31.00 ± 5.11 in histamine + RWI group ($P < 0.05$). Microinjection of GABA alone into the LHA resulted in the GMDI of 24.17 ± 5.04, which indicated that the GMDI was similar to histamine + RWI group ($P > 0.05$), the protective effect of histamine may be associated with the GABA.

Effect of histamine microinjected into FN on the change in discharge of greater splanchnic nerve (GSN) and gastric mucosal blood flow (GMBF) on SGMD in rats

In **Figure 4A** showed that the compared with before histamine microinjection into FN, the

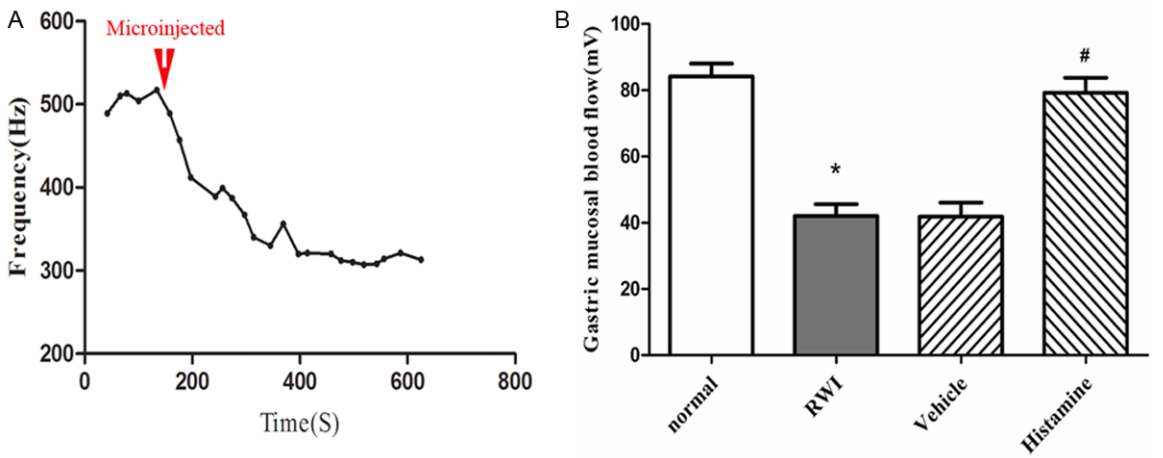

Figure 4. A: The variations of frequency of GSN after histamine injection into FN on SGMD. B: Effect of histamine microinjected into the FN on GMBF in the stomach. Normal: without treatment; RWI: restraint and water-immersion (21 ± 1°C) alone; Vehicle: vehicle microinjected into the FN; Histamine: histamine microinjected into the FN. Mean ± SD, $n = 6$; $^{*}P < 0.05$ *vs*. the normal group; $^{\#}P < 0.05$ *vs*. the vehicle group.

mean discharge frequency of GSN was 504.62 ± 17.45 Hz, decreased to 328.91 ± 7.12 Hz after histamine microinjection into FN ($P < 0.05$).

In **Figure 4B** showed that the GMBF in the histamine group revealed a notable increase (79.17 ± 4.56 mV) compared with the RWI group (42.00 ± 8.76 mV), and the vehicle group (41.32 ± 3.58 mV) have significant difference ($P < 0.05$); Moreover, there was also significant difference between RWI group (42.00 ± 8.76 mV) and the normal group (84.17 ± 3.85 mV) ($P < 0.05$).

Effects of histamine microinjection into FN on the expression of Bcl-2, bax and caspase-3 of gastric mucosal cells on SGMD in rats

In **Figure 5A-C**, the ratio of Bax/β-actin and caspase-3/β-actin in the normal group was 0.087 ± 0.219, and 0.113 ± 0.209, respectively; whereas the ratio was obviously increased (0.445 ± 0.048, and 0.490 ± 0.051) in RWI groups, and vehicle plus RWI groups (0.437 ± 0.055 and 0.512 ± 0.032), respectively. Comparisons revealed significant differences between the latter two groups and the normal group ($P < 0.05$). But the ratios were 0.210 ± 0.044, and 0.240 ± 0.043 in histamine groups, with statistical differences as compared to the plus RWI groups ($P < 0.05$).

The ratio of Bcl-2/β-actin was 0.571 ± 0.035 in the normal group. However, it was 0.300 ± 0.042 in RWI groups, and 0.310 ± 0.050 in the SGMD after vehicle group. Statistics revealed that the Bcl-2/β-actin of histamine group was significantly higher (0.477 ± 0.039) than that of the RWI group and the vehicle group ($P< 0.05$).

Effects of histamine microinjection into FN on the apoptosis and proliferation of gastric mucosa on SGMD in rats

Figure 6 shows that the average percentage of apoptotic cells was approximately 6.53% ± 2.90% in the normal group. In the vehicle group, the percentage of apoptotic cells was significantly higher (40.27% ± 2.42%) than that in the normal group ($P < 0.05$). In the histamine group compared with the vehicle group, we observed a decrease in the percentage of cells undergoing apoptosis (12.63% ± 3.01%, $P < 0.05$). Similarly, we observed an increase in the percentage of proliferation cells (38.63% ± 2.81%, $P < 0.05$) in the histamine group compared with the vehicle group.

Effects of microinjection of histamine into FN on MDA content and SOD activity on SGMD in rats

In **Figure 7**, MDA content was low (4.07 ± 0.23 nmol/mg) in normal group, was significantly increased (10.14 ± 0.24 nmol/mg, and 10.75 ± 0.26 nmol/mg) in the RWI group, and vehicle + RWI group, respectively. There was an obviously decrease in the histamine group (6.21 ± 0.48 nmol/mg) compared to the RWI group and vehicle + RWI group ($P < 0.05$).

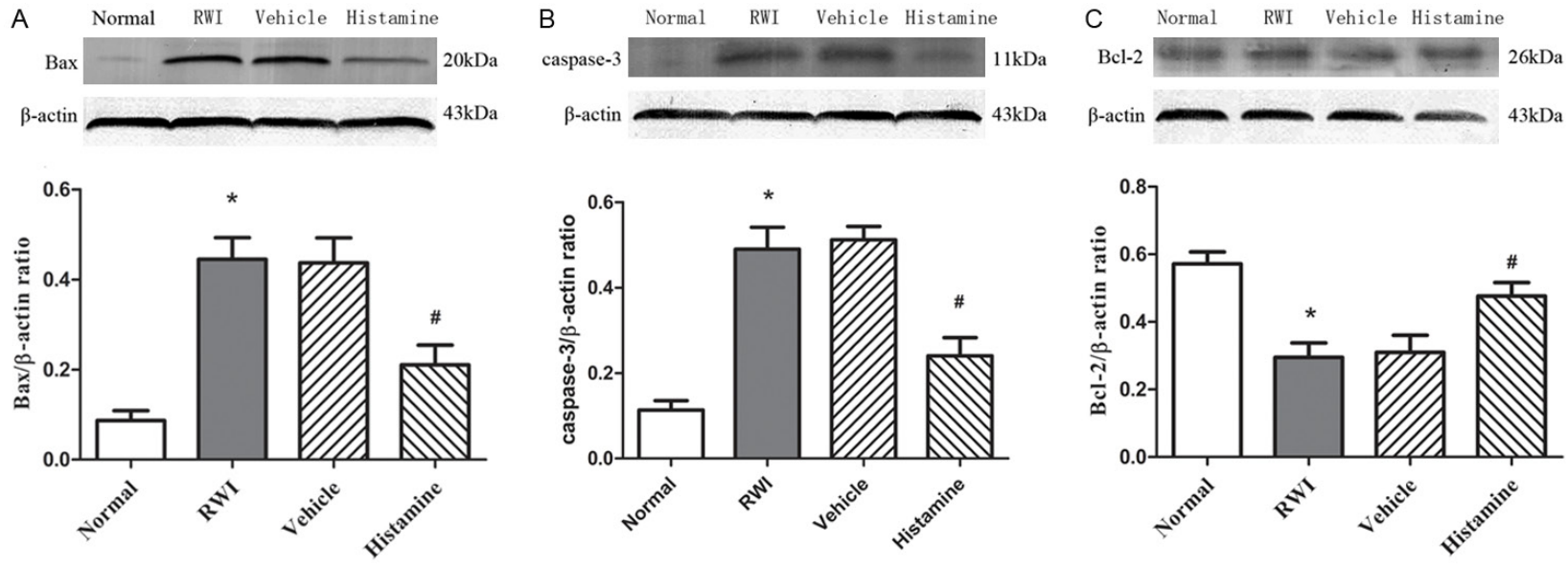

Figure 5. The role of histamine microinjected into the FN on proliferation, apoptosis and oxidation of gastric on SGMD. Normal: without treatment; RWI: restraint and water-immersion (21 ± 1°C) alone; Vehicle: vehicle microinjected into the FN + RWI; Histamine: histamine + RWI. A-C: The role of histamine microinjected into the FN on the expressions of Bax, caspase-3 and Bcl-2 in the gastric mucosal cells following RWI in rats respectively. Mean ± SD, $n = 6$; $^{*}P < 0.01$ *vs*. the normal group; $^{\#}P < 0.05$ *vs*. the vehicle group.

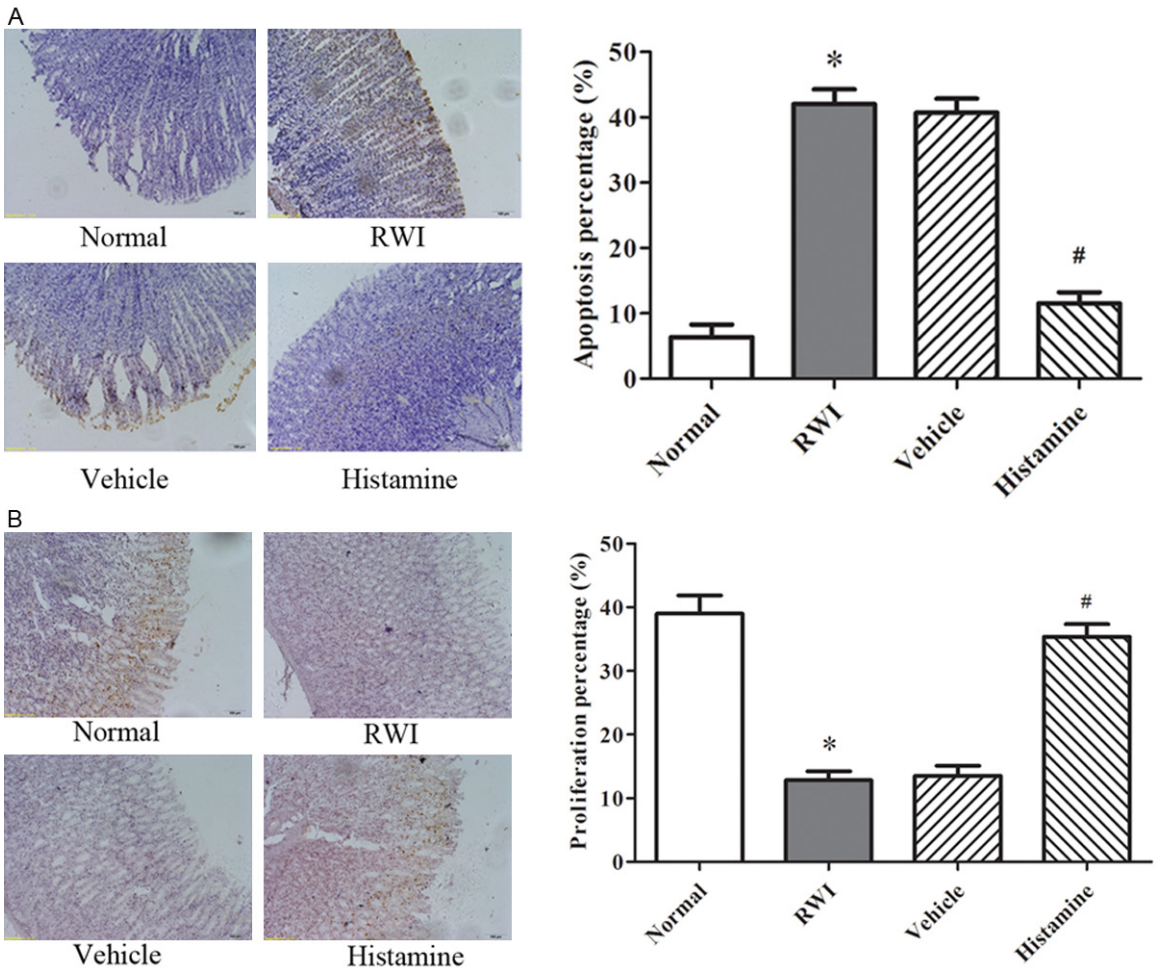

Figure 6. Effect of Histamine microinjection into FN on the proliferation and apoptosis of gastric mucosa on SGMD in rats. Normal: without treatment; RWI: restraint and water-immersion (21 ± 1°C) alone; Vehicle: vehicle microinjected into the FN + RWI; Histamine: histamine + RWI. (Scale Bar = 100 μm). Mean ± SD; n = 6; $^{\#}P < 0.05$ compared with vehicle group.

The SOD activity in the normal group reached a high level (124.20 ± 4.30 U/mg), and decreased in the RWI group (77.12 ± 2.12 U/mg), and vehicle plus RWI group (80.42 ± 1.57 U/mg), respectively. By comparison between RWI group and vehicle group, the SOD activity in histamine group was significantly increased (96.23 ± 3.51 U/mg, $P < 0.05$) (**Figure 7**).

Discussion

Recent neuro-anatomical, neuro-physiological, and behavioural researches have revealed that the cerebellum is the classical sub-cortical motor control center. However, accumulated experimental and clinical evidence has revealed that the cerebellum also plays an important role in cognition, for instance, in learning and memory [22, 23], as well as in emotional behaviour and in non-somatic activities (mainly the visceral activities) [24, 25]. The cerebellum participates in the regulations of cardiovascular activity, gastrointestinal function and micturition. Besides its role in the central nervous system as a potential neurotransmitter/neuromodulater which is used by the hypothalamocerebellar afferent fibres, histamine is confirmed to excite most of the FN neurons [5]. Immunohistochemical labelling of inhibitory amino acids, glycine and GABA in the cerebellum, spinal cord and brainstem revealed the coexistence of these two amino acids in the neuronal soma [26]. Thus, we might as well presume that the increased synthesis of GABA might be mediated by L-glutamic acid decarboxylase (GAD) after histamine microinjection into FN.

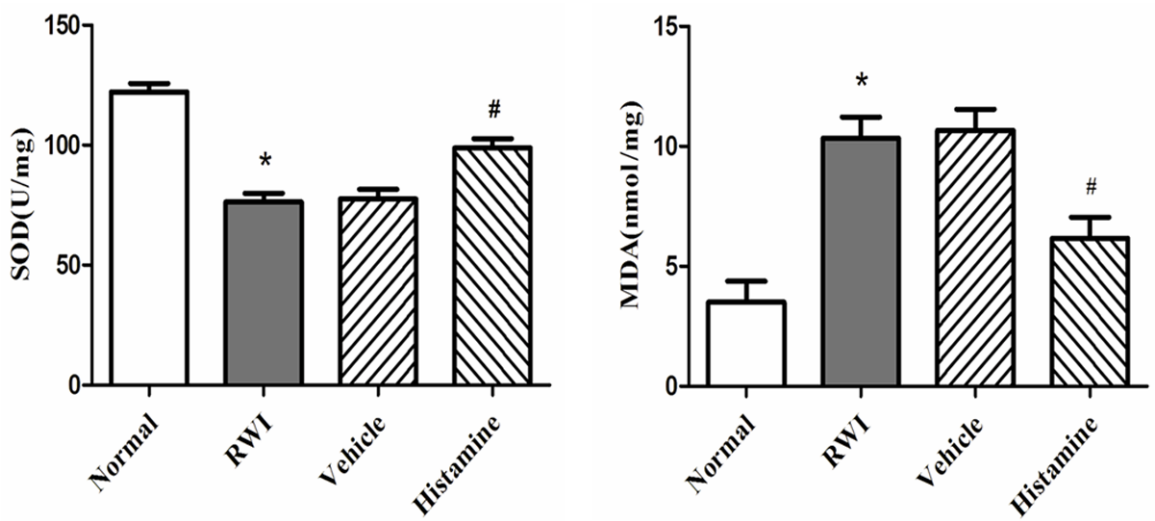

Figure 7. Effects of histamine on MDA content and SOD activity in the stress gastric mucosal cells. Normal: without treatment; RWI: restraint and water-immersion (21 ± 1°C) alone; Vehicle: vehicle microinjection into the FN + RWI; Histamine: histamine microinjected into the FN + RWI. Mean ± SD, $n = 6$, $^{*}P < 0.05$ *vs*. the normal group, $^{\#}P < 0.05$ *vs*. the vehicle group.

Recent neuroanatomical studies have revealed the direct bidirectional connections between the cerebellum and the hypothalamus [27]. Given that the hypothalamus is an important high autonomic centre for the regulation of visceral functions, it is suggested that the cerebellar-hypothalamic circuits might be potential neuroanatomical substrates underlying the cerebellar extensive modulation of non-somatic activities. Some reports indicated that the histamine induced excitation of FN neurons [5, 28]. The present study revealed that the unilateral microinjection of histamine at different doses into the FN obviously attenuated stress-induced gastric mucosal damage (SGMD) in a dose-dependent manner. We also showed that the effect of histamine microinjection into the FN was blocked by the pretreatment of ranitidine, a selective histamine H_2 receptor antagonist, rather than triprolidine, a selective histamine H_1 receptor antagonist. These results indicated that the histamine microinjection into the FN exerting protective effects on SGMD may be mediated through postsynaptic histamine H_2 receptor, then lead to the excitatory effect of FN neurons, meanwhile, which demonstrated that the FN neurons, not crossing fiber, participate in the regulation of SGMD, is an important locus in the central nervous system.

Recently, Immunohistochemical labelling of glycine and GABA in the cerebellum, spinal cord and brainstem revealed the coexistence of these two amino acids in neuronal soma [29]. Therefore, we inferred that the histamine microinjection into FN on LHA neurons response may be mediated by inhibitory neurotransmitter GABA in this study. However, the detailed mechanism is not clear at present. In our present study, we also observed that the microinjection of 3-MPA, an L-glutamic acid decarboxylase antagonist, into the FN blocked the protective effects of histamine microinjection into the FN on SGMD. The mechanism of protective effect may be achieved by the increase of synthesis and release of GABA after histamine microinjection into FN, with the glutamic acid decarboxylase exerting an important role in the synthesis of GABA.

Other studies revealed that the projections of cerebellar-hypothalamus arise from the three deep nuclei of the cerebellum (including dentate nucleus, fastigial nucleus, and interposed nucleus), pass through DSCP and project to the LHA, lateral mammillary nucleus (LMN), posterior hypothalamic area (PHA), paraventricular nucleus (PVN), and other areas of the hypothalamus [29, 30]. These hypothalamic nuclei/areas are just the origins of hypothalamocerebellar projections, suggesting that the connections between cerebellum and hypothalamus are reciprocal. Our present study also demonstrated that the protective effect of histamine

microinjected into FN on SGMD was reversed after electrolytic ablation of decussation of superior cerebellar peduncle (DSCP), or chemical damage of the LHA. These results suggested that the cerebellar-hypothalamic circuits play an important role in the protective effect of exogenous histamine in the FN, the DSCP and hypothalamic LHA, which are significant nodes in the cerebellar-hypothalamic circuits. The result indicated that the expression of inhibitory amino acid, GABA receptor of the LHA was significantly increased after histamine microinjection into the FN. We also observed that the GMDI was obviously decreased after GABA alone was microinjected into the LHA, with similar effect to that of histamine microinjection into FN. These results indicated that the effect of histamine microinjection into the FN on hypothalamic LHA neuronal response might be associated with the inhibitory neurotransmitter GABA. Accordingly, we believed that this possible process is that the histamine microinjection into the FN could elevate the neuronal excitability of the FN via histamine H_2 receptor, and could increase the synthesis and release of the inhibitory neurotransmitter, GABA, pass through DSCP and project to the LHA. Then, the GABA was integrated with GABA receptors in the neuronal membrane of LHA. Thus, the inhibitory effect of LHA is displayed.

Recently, a number of studies have revealed that the hypothalamus is an important high autonomic centre for the regulation of visceral functions. Neuroanatomy and our previous studies indicated that the hypothalamic PVN, and LHA are important brain area links greater splanchnic nerve (GSN) [30]. Meanwhile, we have also observed that the chemical or electrical stimulation of LHA can aggravate SGMD [8]. In present study, we also demonstrated that the discharge frequency of GSN was reduced, and the GMBF was increased after histamine microinjection into the FN. The attenuation of discharge frequency of GSN may induce visceral vasodilatation in mucosa and sub-mucosa, and the gastric mucosal blood flow was increased, which redound to ameliorate the stress-induced gastric mucosal damage after histamine microinjection into the FN.

In recent years, numerous data indicated that the mechanisms of SGMD might be related with the apoptosis, proliferation, and damage from oxidizing substances in the cells [31, 32]. In present study, we observed that the histamine microinjection into the FN could effectively inhibition of cellular apoptosis and the promotion of proliferation in the stress gastric mucosa, further, the protein expression of Bax, and caspase-3, the pro-apoptosis factor were down-regulated and the protein expression of Bcl-2, the anti-apoptosis factor was up-regulated. These results indicated that the protective effect of histamine microinjection into the FN on stress gastric mucosal cells might be mediated through the inhibition of cellular apoptosis and the promotion of proliferation.

MDA and SOD have previously been known as the oxidative/anti-oxidative index. In this study, we observed that MDA content was markedly decreased while SOD activity was elevated in stress gastric mucosal cells after microinjection of histamine into the FN. These results elucidated that the anti-oxidative effect might be involved in the regulatory mechanism of histamine function in the FN.

To best of our knowledge, we have, for the first time, reported that histamine microinjection into the FN might triggering FN neurons, facilitating the synthesis and release of GABA, and passage via the decussation of superior cerebellar peduncle (DSCP) and cerebellar-hypothalamic circuits to the hypothalamic LHA. Then, the GABA was conjugated with GABA receptors in the neuronal membrane of LHA, sequentially, inhibitory effect of LHA neurons were arose, the discharge frequency of GSN was suppressed, the blood vessels of the gut were relaxed, and the gastric mucosal blood flow was increased. Consequently, the protection against the SGMD is thus achieved via the inhibition apoptosis of and the promotion of proliferation in gastric mucosal cellular. Our new findings might help to understand the somatic-visceral integration mechanism of cerebellar nucleus, and further contribute to the therapeutics of gastrointestinal diseases.

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Disclosure of conflict of interest

None.

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