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NMDA Inhibits Oxotremorine-Induced Acid Secretion via the NO-Dependent Cyclic GMP System in Rat Stomach

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Abstract

The mechanism of N-methyl-D-aspartate (NMDA) inhibits oxotremorine-induced acid secretion was examined in rat stomach, in relation to the cyclic GMP system. NMDA (10^{-7} M) did not affect the spontaneous acid secretion from the everted preparations of isolated rat stomach, but inhibited the acid secretion stimulated by oxotremorine, and this effect of NMDA was antagonized by 2-amino-5-phosphonovaleric acid (AP-5), (±)3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) or N^G-nitro-L-arginine (L-NNA). NMDA also elevated the cyclic GMP content of mucosal slices from rat stomach, and this effect of NMDA was antagonized by L-NNA. These results indicate that NMDA receptors are present in the rat stomach and regulate the gastric acid secretion. The mechanism underlying the effect of NMDA inhibits oxotremorine-induced acid secretion may be mediated by the NO-dependent cyclic GMP system.

Key Words: N-methyl-D-aspartate (NMDA), cyclic GMP, nitric oxide, acid secretion

Introduction

Excitatory amino acids (EAAs), e.g. L-glutamate (L-Glu) and L-aspartate (L-Asp), exist in various parts of the vertebrate central nervous system (CNS) and serve as major excitatory neurotransmitters (9, 21). As generally accepted, EAAs exert their physiological functions through interaction with either ionotropic or metabotropic receptors. The ionotropic EAAs receptors can be further distinguished on the basis of different ligands, such as methyl-D-aspartate (NMDA), as NMDA receptors or by kainate/ a-amino-3-hydroxy-5-methyl-4-isoxalone propionate (AMPA) (non-NMDA receptors).

In addition to their function as a major excitatory neurotransmitter in the CNS, EAAs and their receptors are also found in the mammalian enteric nervous system and are believed playing important roles there (20, 22, 24). It has been demonstrated by immunohistochemical method (19, 28) and physiological method (25) that glutamatergic neurons are present in the gastrointestinal tract. EAAs are shown to act as neurotransmitters via NMDA receptor to control the motility of intestine perhaps through their interaction with cholinergic neurons (20, 22, 24). The mRNA coding for the glutamate N-methyl-D-aspartate (NMDA) receptor is expressed by rat enteric neurons (5). Myenteric ganglia and nerve bundles in the circular muscle and longitudinal muscle are found to contain glutamate- and glutaminase-positive nerve fibers (28). The glutamate receptors are shown to be present in the submucous and myenteric plexuses (13, 19, 22, 24, 25, 28).

Activation of non-NMDA ionotropic glutamate receptor was found to inhibit the stimulated acid secretion in the isolated rat stomach (28), but it is unclear whether the NMDA receptors participate in the acid secretion. Since the pathway of NMDA receptor-nitric oxide (NO) synthesis-cyclic GMP has been

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accepted in the central nervous system (8), the present study was attempted to examine whether the similar pathway is involved in regulation of oxotremorine-induced gastric acid secretion.

Materials and Methods

Experiments on Everted Whole Stomach

Experiments on everted whole stomach were performed as described elsewhere (27, 28). Briefly, male Sprague-Dawley rats (weight 180-250 g) were deprived of food overnight, but allowed free access to water to ensure that the stomach was free of solid contents. The rats were decapitated, and the stomach was removed immediately for use. The isolated everted whole stomach was prepared as described elsewhere, with only slight modifications. The entire everted organ was then placed in a 20-ml organ bath containing the mucosal saline solution (in mM, NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; glucose, 5.6; pH 5.2) at 30 ± 1 ℃ and continuously bubbled with 100% O_2. The serosal side was perfused with serosal saline solution (in mM, NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25; KH_2PO_4, 1.03; glucose, 5.6; pH 7.4) at the rate of 1 ml/min under the same conditions as mentioned above except 100% O_2 was replaced by a mixture of 95% O_2 and 5% CO_2. One hour after equilibration of the organ, the mucosal saline solution was replaced every 15 min during the experiment. Only the serosal side of the preparation was exposed to the test drugs.

Spontaneous acid secretion was followed for 60 min prior to adding the test drugs and then the acid secretion accumulated continuously for an additional hour. The acid solutions accumulated from the mucosal side, and were titrated initially to pH 5.2 and then to pH 7.0 with 0.1 mM NaOH. Responses of stomach to drug treatments were expressed as the secretory ratio (R), which is defined

R= (Secretion evoked by drugs) / (Average spontaneous secretion)

The average spontaneous secretion was calculated using samples from the four periods immediately before exposure to the test drugs. Finally, measuring the secretory ratio at the peak response was uses to assess the concentration-response curves.

Measurement of Cyclic GMP Content

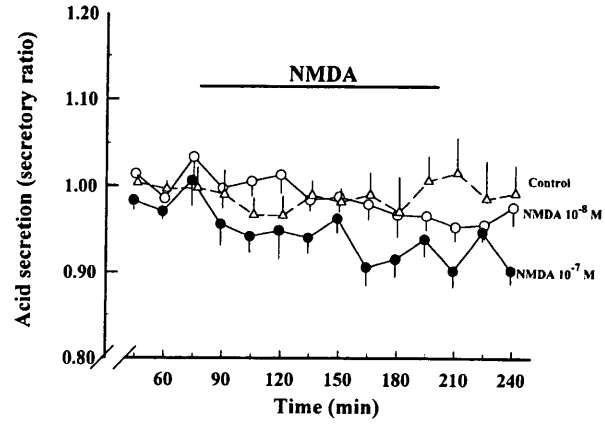

Fig. 1. Effect of N-methyl-D-aspartate (NMDA) on spontaneous acid secretion in the everted preparation of isolated rat stomach. Acid secretion was expressed as secretory ratio, in the absence (△) and presence of 10^{-8} M (○) and 10^{-7} M (●) of NMDA treatment. Each point represents the mean ± SEM from 6 animals.

Gastric mucosal slices were cut into 0.4 x 0.4-mm cubes with a Mellwain tissue chopper. After preincubation in serosal saline solution containing 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) maintained at 30 ±1 ℃ and continuously bubbled with 95% O_2 and 5% CO_2, preparations were incubated in the medium containing 0.5 mM IBMX and various concentrations of NMDA for various periods. At the end of incubation, tissues were homogenized in 6 % trichloroacetic acid, followed by centrifugation at 3000 g for 15 min at 4℃. The supernatant thus obtained was neutralized to pH 7.4 with 1 M Tris, followed by four-time ether extraction. The extracts were collected, air-dried, and the content of cyclic GMP was determined using a commercial RIA kit. Finally, the homogenized solution obtained was solubilized in 3 N NaOH and used for protein determination as described (3).

Chemicals

N-methyl-D-aspartic acid (NMDA), 2-amino-5-phosphonovaleric acid (AP-5), (±)3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), N^G-nitro-L-arginine (L-NNA), oxotremorine, and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma Chemical (St. Louis, MO, USA). [^{3}H] Cyclic GMP assay system was obtained from Amersham (Buckinghamshire, UK). Other chemicals used were of reagent grade and were obtained from various commercial sources.

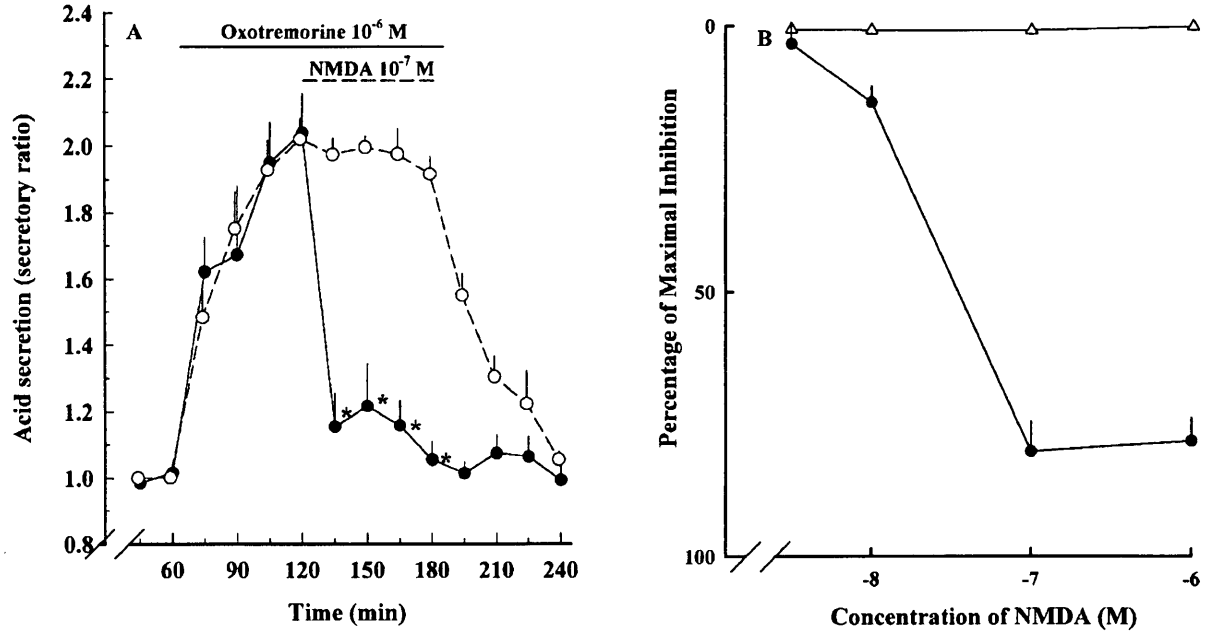

Fig. 2. A: Effect of N-methyl-D-aspartate (NMDA) on acid secretion stimulated by oxotremorine in the everted preparation of isolated rat stomach. Oxotremorine (10^{-6} M) was present in the medium for 120 min. NMDA (10^{-7} M) was added 60 min after administration of oxotremorine. B: Dose-dependent curve of the inhibitory effect of NMDA on oxotremorine-induced acid secretion. Inhibition of oxotremo-induced acid secretion was plotted according to various concentrations of NMDA. Acid secretion induced by oxotremorine 10^{-6} M alone was determined at each concentration of NMDA and served as control. Each point represents mean ± SEM. * $P<0.05$ compared to oxotremorine alone.

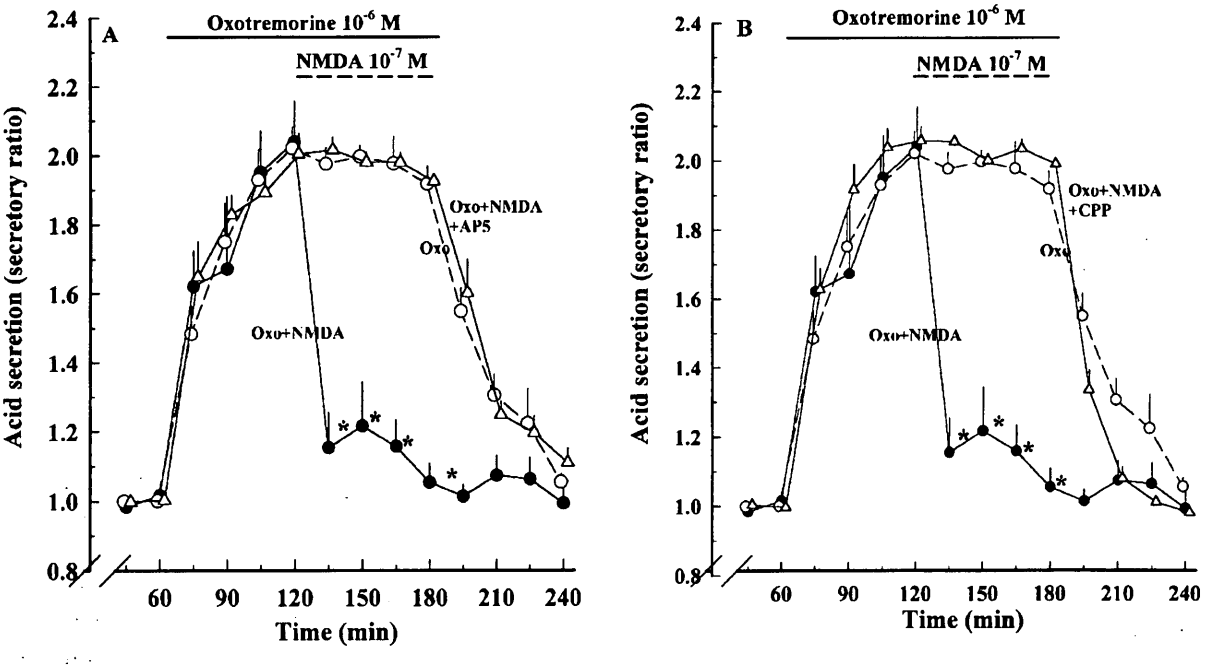

Fig. 3. Effects of N-methyl-D-aspartate (NMDA) and 2-amino-5-phosphovaleric acid (AP-5) or (±) 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) on oxotremorine-induced acid secretion in the isolated rat stomach. Oxotremorine (10^{-6} M) were present in the medium for 120 min. NMDA (10^{-7} M) and NMDA (10^{-7} M) plus AP-5 (10^{-6} M) or CCP (10^{-7} M) were added 60 min after administration of oxotremorine. Each point represents the mean ± SEM from 6 animals. * $P<0.05$ compared to oxotremorine alone.

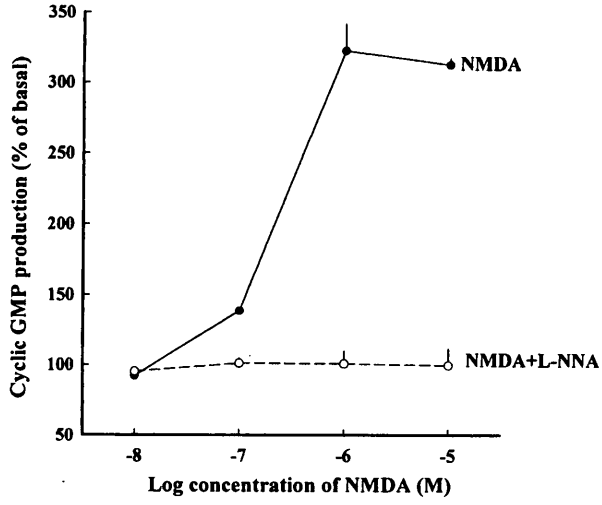

Fig. 4. Dose-dependent curve of NMDA with and without L-NNA (10^{-4} M) effects on cyclic GMP content in the mucosal slices of rat stomach. Each preparation was incubated in serosal saline solution containing various concentrations of NMDA for 3 min after 30 min of preincubation. Each point represents the mean ± SEM of percent of basal level from 15 samples.

Statistical Analysis

Results were expressed as the mean SEM for each study (n = sample number). Data were analyzed by Dunnett's test or Student's t-test; a P value of 0.05 or less was considered statistically significant.

Results

Effect of NMDA on Spontaneous and Secretagogus-Stimulated Acid Secretion in the Everted Stomach

Spontaneous acid secretion reached a steady state after equilibration for 2 h. The average spontaneous acid secretion after equilibration was 1.59 ± 0.15 μmol/15 min, which was taken as the control value. NMDA at 10^{-8} and 10^{-7} M did not affect the spontaneous acid secretion (Fig.1). Effect of NMDA was examined on the acid secretion stimulated by oxotremorine. Oxotremorine at 10^{-6} M stimulated acid secretion approximately 2 times of spontaneous secretion (Fig. 2A). The responses to oxotremorine reached maximum at about 60 min after application of secretagogus, maintained the level during the presence of secretagogus, and declined after removal of secretagogus (Fig. 2A). NMDA at 10^{-8} M and over inhibited the acid secretion stimulated by oxotremorine. The IC_{50} of NMDA on oxotremorine-induced acid secretion was calculated to be 2.69 x 10^{-8} M (Fig. 2B). NMDA (10^{-7} M)-induced inhibition of oxotremorine-induced acid secretion was antagonized by AP5 and CPP at 10^{-6} and 10^{-6} M, respectively, two selective NMDA antagonists (Fig. 3).

Effect of NMDA on Cyclic GMP Content in the Mucosal Slices from Rat Stomach

Basal contents of cyclic GMP in mucosal slices from rat stomach were gradually decreased by incubation in serosal saline solution and stabilized at 0.31 ± 0.03 pmol/mg protein, 30 min after incubation. Thus, preparations were incubated in the medium containing drugs after 30 min preincubation. NMDA-induced elevation of cyclic GMP contents reached maximum at 2 min and was followed by subsequent stabilization of contents between 2 and 4 min of incubation. NMDA at 10^{-8}-10^{-5} M elevated the content of cyclic GMP (Fig. 4).

Effect of L-NNA on NMDA-Induced Inhibition of Acid Secretion and Elevation of Cyclic GMP Content

L-NNA at 10^{-4} M, a NO synthase inhibitor blocked the NMDA (10^{-7} M)-induced inhibition of acid secretion stimulated by oxotremorine (Fig. 5A). L-NNA at 10^{-4} M also inhibited NMDA (10^{-7} - 10^{-6} M)-induced elevation of cyclic GMP content in the mucosal slices (Fig. 4), and significantly inhibited the NMDA (10^{-7} - 10^{-6} M)-induced elevation of cyclic GMP content in the presence of oxotremorine (Fig. 5B).

Discussion

NMDA inhibited the oxotremorine-stimulated acid secretion, which was antagonized by AP-5 and CCP, two specific antagonists to NMDA receptor, thereby indicating that NMDA receptors are present in the rat stomach. Since previous study showed that activation of the quisqualate/kainate receptors inhibited the oxotremorine-stimulated acid secretion (28), ion tropic glutamate receptors, NMDA and non-NMDA receptors were found to be present in the rat stomach and participate in the regulation of gastric acid secretion. In intestinal tissue, the ionotropic glutamate NMDA receptors (6, 15, 19, 25) and the metabotropic glutamate

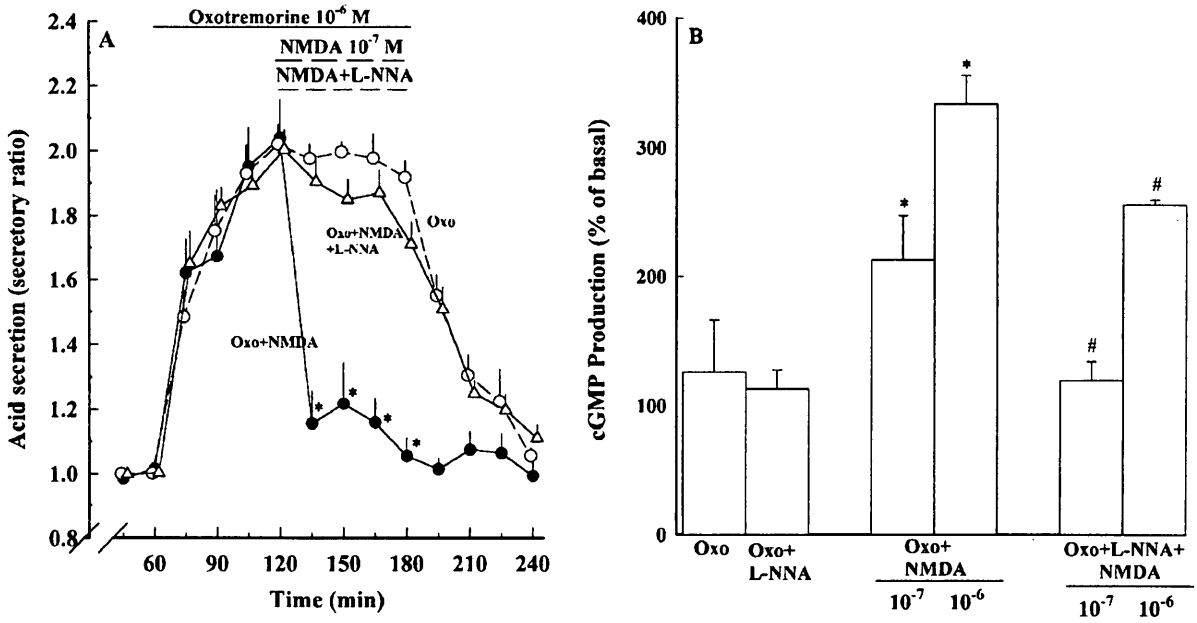

Fig. 5. Effect of N^G-nitro-L-arginine (L-NNA) on N-methyl-D-aspartate (NMDA)-induced inhibition of stimulated acid secretion (A) and elevation of cyclic GMP content (B). L-NNA (10^{-4} M) and NMDA (10^{-7}-10^{-6} M) were simultaneously added to the medium. Each point represents the mean ± SEM from 6 animals (A) and each column represents the mean ± SEM of percent of basal level from 15 samples (B). * $P<0.05$ compared to oxotremorine alone. # $P<0.05$ compared to oxotremorine and NMDA alone.

receptors (13) are shown to be present in the enteric nervous system. In contrast to neurons, in which glutamate receptors induce excitatory effects, the glutamate receptors appear to be inhibitory in the stomach in the gastrointestinal system.

In this communication, we have shown that NMDA-induced elevation of cyclic GMP content was also obtained in the presence of oxotremorine and the elevation of cyclic GMP content were antagonized by AP-5 and L-NNA, therefore NMDA increased cyclic GMP content via activation of NMDA receptor and NO system. Furthermore, NMDA inhibited the stimulated acid secretion in response to oxotremorine, the acting being an AP-5 sensitive manner and mediated by the cyclic GMP/NO system. These results are partly consistent with the previous observation by Barrachina et al. (1), Who showed that i.v. infusion of S-nitroso-glutathione inhibited vagally mediated gastric acid secretion by gastric distension and 2-deoxy-D-glucose. There have been several studies investigating the effect of NO synthase inhibitors on acid secreting, although the results controversial. For example, Pique et al. (23) found that the NO synthase inhibitors N (G)-nitro-L-arginine methyl ester (L-NAME) did not affect either basal or pentagastrin-stimulated acid secretion in rats. Kato et al. (16) showed that either exogenous or endogenous NO has an inhibitory action on gastric acid secretion through suppression of histamine release from enterochromaffin-like (ECL) cells. Furthermore, Hasebe et al. (10) and Horie et al. (12) reported that NO stimulated acid secretion in isolated stomachs of mice. They showed that both endogenous and exogenous NO secretion acid secretion mediated by histamine, through cyclic GMP. The present study demonstrated that NMDA reduced the acid secretion induced peripherally by oxotremorine but not histamine. L-NNA blocked the NMDA-induced elevation in cyclic GMP content in the presence of oxotremorine. Brown et al. (4) and Kim et al. (17) reported that S-nitroso-O-N-acetyl-penycillamine (SNP) inhibits acid secretory using rat parietal cells by a specific interaction that may involve cyclic GMP, suggesting a direct inhibitory action at the parietal cell. The site of action of NO in these reports is though to be on parietal cells. NO is known to stimulate soluble guanylate cyclase, resulting in increased levels of cyclic GMP due to the cyclic GMP system-dependent activation of phosphodiesterase 2 (2). In interaction between cyclic GMP system and Ca^{2+} mobilization system, activation of protein kinase G has been shown to regulate the intracellular Ca^{2+} levels; inhibition of phosphatidyl inositol turnover (14) the IP_3 receptor (18), Ca^{2+} release from the storage sites (11). NO-cyclic GMP-dependent system has shown to inhibit IP_3-induced Ca^{2+} release (26).

Thus, NMDA may inhibit the oxotremorine-induced stimulation of acid secretion by cyclic GMP system-mediated decrease in intracellular Ca^{2+} levels. Activation of NMDA receptor is known to increase the intracellular Ca^{2+} concentrations (7). In the parietal cells, elevation of the intracellular Ca^{2+} concentrations stimulates the acid secretion, as oxotremorine increased intracellular Ca^{2+} and stimulate acid secretion. The mucosal slices used in the present study contain some kinds of cells, therefore it is plausible that NMDA also acts on the cells other than parietal cells, NO formed in the cells penetrates the parietal cells, and then activation of the NO-guanylate cyclase-cyclic GMP-protein kinase G system in the parietal cells regulates the acid secretion. Further investigation NMDA-induced NO production on the parietal cells in the stomach remains studying.

Acknowledgements

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