Experimental system: detection of hydrogen ion secretion rate of mouse gastric mucosa

Lifei Zheng*, Jinxia Zhu

Department of Physiology and Pathophysiology, School of Basic Medicine, Capital Medical University, 100069

*Corresponding author E-mail: 13581582937@163.com
Tel: 13581582937
Zheng Lifei: Ph.D., associate professor, master's supervisor, writer; Zhu Jinxia: Doctor, professor, doctoral supervisor, revised.
Mailing address: No. 10, West Toutiao, Youanmenwai, Fengtai District, Beijing

I. Purpose of the experiment

To understand the detection method of hydrogen ion in mouse gastric acid secreting glands.

II. Materials, appliances, instruments and drugs

Test sample: mouse gastric mucosa

Detection instrument: non-damage micro-measurement system (scientific research platform) or non-damage micro-measurement system (research and development platform)

Consumables: NMT dedicated solid H+flow sensor

Required drugs: 141.8 mmol · L^{-1} NaCl, 5.9 mmol · L^{-1} KCl, 1.2 mmol · L^{-1} $MgCl_2$, 10 mmol · L^{-1} HEPES acid, 5.6 mmol · L^{-1} Tris, 2.56 mmol · L-1 $CaCl_2$, 2 g glucose, pH 7.4 100 mmol · L^{-1} histamine, 300 mmol · L^{-1} cimetidine.

Tools: stereomicroscope, silica gel plate, culture dish, CO_2, pipette gun, several gun heads.

III. Principle

The gastric mucosa is mainly composed of parietal cells that secrete gastric acid, main cells that secrete pepsinogen and mucous cells that secrete mucus. These cells coordinate with each other to participate in the digestive process in the stomach. Gastric acid (HCl) is derived from parietal cells and is produced through the synergy of multiple transporters and ion channels in the apical and basal membranes of parietal cells. Under stimulation, H^+/K^+- ATPase, Cl^- secretion pathway, K^+circulation pathway and Cl - absorption pathway of basal membrane of parietal cells participate in the secretion of gastric acid. Histamine can stimulate the secretion of gastric acid. By binding with specific receptors on the parietal cell membrane, it can increase the level of cAMP in the cell, activate the ion channels and transporters on the cell membrane, and increase the secretion of HCl.

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IV. Method steps

1. Preparation of isolated gastric mucosa: mice were euthanized by inhaling carbon dioxide. Take out the stomach, keep the stomach body, cut the stomach body along the great curvature of the stomach, fix it on the silica gel plate after cleaning, and separate the mucosa layer under the posture microscope.
2. Put the gastric mucosa into a culture dish containing 5mL of test solution and fix it for 30min.
3. After replacing the test solution, put it into the NMT system to detect the flow rate of H^+in the facial mask of the gastric mucosa.
4. Instantly add the final concentration of 100mM histamine, and continue to detect the H^+flow rate for 10min.
5. Then add cimetidine with a final concentration of 300mM and continue to carry out H^+flow rate detection.

V. Experimental report

According to the experimental results, explain the physiological phenomenon and principle of H+change after adding histamine and cimetidine.

VI. Expected results

After histamine treatment, the secretion of H^+increased; After further addition of cimetidine, H^+secretion gradually decreased.

VII. Thinking questions

1. Why can NMT quickly detect the gastric mucosal H^+flow rate?

Answer: NMT is a technology with ultra-high sensitivity, non-contact method, based on the flow rate, to detect the molecular concentration and gradient of external ions of materials, which can quickly detect the changes of sample molecular ions.

2. Can NMT detect the hydrogen ion flow rate of cultured parietal cells, and is there any difference between NMT and the detection of acutely isolated gastric mucosa tissue?

Answer: It can detect cultured parietal cells. The normal growth of gastric mucosal parietal cells and the cultured parietal cells will differ in activity. The data reflected by the normal growth of gastric mucosal parietal cells will be closer to the real live data.

VIII. References

[1]Zheng LF, Ji T, Guo ZH, Wang T, Xiu XL, Liu XY, Li SC, Sun L, Xue H, Zhang Y, Zhu JX(2020). Na^+-K^+-$2Cl^-$ cotransporter 2 located in the human and murine gastric mucosa is involved in secretagogue-induced gastric acid secretion and is downregulated in lipopolysaccharide-treated mice. *Eur J Pharmacol* 880,173162. doi: 10.1016/j.ejphar.2020.173162.

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