

Answering to Reviewers for WJG-53524

Dear editor,

Thanks for giving us the chance to modify my manuscript entitled "Nervous Mechanisms of Restraint Water-Immersion Stress induced Gastric Mucosal Lesion".

We would like to answer the reviewers' comments firstly.

Reviewer #1: The manuscript is well-structured and clear. It represents an exhaustive review of the current available evidence regarding the possible nervous mechanisms of RWIS-induced gastric mucosal lesions. The title clearly outlines the content of the manuscript and the Authors' aims, but the last two sentences of the Abstract, in particular, could be rephrased in order to better describe the conclusions. A clearer focus on the complexity and multifactorial pathways leading to GML, the different nuclei involvement with the time of stress, the different levels of involvement of the sub-regions of the same nucleus, and the diverse signalling molecules.

Reply: Thanks for this forward-looking suggestion. Modifying our manuscript according to the suggestions would point out the direction for the subsequent research.

The last two sentences of Abstract were "However, inconsistent and even contradictory results have been obtained regarding the actual roles of each nucleus, how they functioned and the direct pathway of their action. Therefore, the nervous mechanism of RWIS-induced GML in rats remains to be further elucidated." We revised these sentences into "However, inconsistent and even contradictory results have been obtained regarding the actual roles of each nucleus, the nervous mechanism of RWIS-induced GML in rats, such as the different nuclei involvement with the time of stress, the different levels of involvement of the sub-regions of the same nucleus, the diverse signalling molecules, remains to be further elucidated."

Secondly, to meet the requirements of WJG, we have revised our manuscript Step by step.

1. Running title: Zhao DQ et al. Nervous mechanisms of RWIS-induced GML

2. References

Some references in Chinese and master's or doctoral theses have been deleted, and the first page of references that are not indexed by PubMed are provided.

Reference 106:

DOI: 10.1681/j.cnki.2008-4646.2007.03.011

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大鼠孤束核微量注射乙酰胆碱对电针抗大鼠 应激性胃黏膜损伤的影响

余宙¹, 王盼¹, 谈小琴¹, 徐秀良², 李彩伟², 黄碧兰^{3*}

(咸宁学院医学院 1.2003 级临床医学学生; 2.2004 级临床医学学生;
3.生理学教研室, 湖北咸宁 437100)

摘要:目的 观察孤束核内乙酰胆碱(Ach)对电针(EA)抗大鼠应激性胃黏膜损伤的影响。方法 84 只雄性 SD 大鼠, 随机分为正常组、应激组、EA+应激组、NS+EA+应激组、Ach+EA+应激组、阿托品+EA+应激组。采用束缚-浸水制备大鼠应激性胃溃疡模型, 观察孤束核微量注射 Ach、M 受体阻断剂阿托品, 大鼠胃黏膜溃疡指数(UI)、超氧化物歧化酶(SOD)活性、丙二醛(MDA)含量的变化。结果 与单纯应激组比较, EA+应激组胃黏膜 UI、MDA 含量降低, SOD 活性增高, Ach+EA+应激组上述作用更明显。孤束核给予阿托品处理, 可减弱电针抗胃黏膜损伤的作用。结论 孤束核内 Ach 参与了电针对大鼠应激性胃黏膜损伤保护作用过程。

关键词: 乙酰胆碱; 孤束核; 电针; 应激性溃疡; 超氧化物歧化酶; 丙二醛

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The Effects of Microinjection of Acetylcholine into Nucleus Tractus Solitarius on Electroacupuncture Against Stress Mucosa Injury in Rats

YU Zhou, WANG Pan, TAN Xiao-qin, et al

(2003 Grade Clinic Medical Student, Xianning College, Xianning Hubei 437100, China)

ABSTRACT: **Objective** To observe the effects of acetylcholine (Ach) in nucleus tractus solitarius on electroacupuncture (EA) against gastric stress ulcer in rats. **Methods** A total of 84 male SD rats were randomly divided into normal control group, stress group, EA+stress group, NS+EA+stress group, Ach+EA+stress group and atropine+EA+stress group. The gastric stress ulcers models were established by restrain and water immersion. The nucleus tractus solitarius was injected by the Brain Stereotaxic Frame. The variation of the gastric ulcer index (UI) and superoxidase (SOD) activity and malondialdehyde (MDA) level were observed in rats that were separately microinjected Ach and M receptor antagonist-atropine in the nucleus of tractus solitarius. **Results** Compared with stress group, EA+stress group showed that the UI and MDA level were decreased but SOD activity was increased. Pretreatment with Ach into nucleus tractus solitarius enhanced the effects of electroacupuncture against stress mucosa injury, by contrast, pretreatment with atropine significantly attenuated the same effects. **Conclusion** The Ach in the nucleus of tractus solitarius involve in the protective effects process of the stressing gastric mucosa lesion by EA in rats.

KEY WORDS: Acetylcholine; Nucleus tractus solitarius; Electroacupuncture; Stress gastric ulcer; SOD; MDA

* 通讯作者: E-mail: huangbilan@sina.com

Reference 127:

Neurons in the Medulla Oblongata Related to Gastric Mucosal Lesion of Rats Subjected to Restraint Water-Immersion Stress

Dong-qin Zhao, Ph.D., Yan-jiao Bi, M.D., Sheng-nan Gong, M.D., and Peng-fei Li, M.D.

OBJECTIVE: Restraint water-immersion stress (RWIS) can induce a gastric mucosal lesion. Our objective was to detect the phenotype of activated neurons in the medulla oblongata of rats subjected to RWIS.

STUDY DESIGN: Male Wistar rats were divided into 2 groups in accordance with the duration of RWIS: a control group and the RWIS group. The brain sections were treated with a dual immunohistochemistry of Fos and choline acetyltransferase (ChAT) or tyrosine hydroxylase (TH) or oxytocin receptor (OTR) or vasopressin 1b receptor (V1bR) or methionine-enkephalin (M-ENK).

RESULTS: Compared to the control group, the number of Fos+ChAT-immunoreactivity (IR) neurons in the dorsal motor nucleus of the vagus (DMV) and nucleus ambiguus (NA) and that of Fos+TH-IR neurons in the nucleus of the solitary tract (NTS) were evidently increased in the RWIS group; meanwhile, more OTR-IR and V1bR-IR neurons in the DMV and NTS were activated. A few M-ENK-IR perikarya were observed only in the NTS, with no difference between the RWIS and control groups.

CONCLUSION: Data suggest that the hyperactivity of cholinergic neurons in the DMV and NA and catechol-

amine neurons in the NTS is one of the main mechanisms of gastric mucosal lesions induced by RWIS; the OT-sensitive and AVP-sensitive neurons, excepting M-ENK ones, are involved in the modulation of the gastric mucosal lesion during RWIS. (Anal Quant Cytopathol Histopathol 2019;41:127–138)

Keywords: acute gastric mucosal lesion; disease models, animal; gastric mucosa/pathology; gastric ulcer; medulla oblongata; neurons; neurotransmitter; rats, Wistar; restraint water-immersion stress; stomach ulcer; stress; stress, psychological; stress gastric mucosal lesion.

Stress can cause behavior, immune, and neural responses, and intense and persistent stress responses can lead to a wide range of physiological and psychological dysfunctions in the body.¹⁻³ Restraint water-immersion stress (RWIS) can induce a gastric mucosal lesion, one of the most common visceral complications after wound. Exploring the pathomechanism of the occurrence and development of stress gastric mucosal lesions to find suitable treatment measures has become a focus in

From the Key Laboratory of Animal Resistance of Shandong Province, School of Life Sciences, Shandong Normal University, and Shandong Academy for Environmental Planning, Jinan, Shandong Province, P.R. China.

Dr. Zhao is Professor, Key Laboratory of Animal Resistance of Shandong Province, School of Life Sciences, Shandong Normal University. Drs. Bi and Gong are Researchers, Key Laboratory of Animal Resistance of Shandong Province, School of Life Sciences, Shandong Normal University.

Dr. Li is Researcher, Shandong Academy for Environmental Planning.

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Address correspondence to: Dong-qin Zhao, Ph.D., College of Life Sciences, Shandong Normal University, Jinan 250014, Shandong Province, P.R. China (109127@sdsu.edu.cn).

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That's the responses to the reviewer and editor. If you have any questions, please feel free to contact me.

Best regards.

Dong-Qin Zhao

zhaodq@sdsu.edu.cn